Application of multimodal techniques in the identification of biomarkers in mild Traumatic Brain Injury

Ekaterina Lunkova

Integrated Program in Neuroscience, McGill University, Montreal

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Abstract

Concussion, often used synonymously with mild traumatic brain injury (mTBI), is a major public health problem. Ambiguity still exists regarding the pathophysiology and management of concussions, leaving an objective diagnosis a topical problem. Previous studies demonstrated that fMRI is an objective approach that allows consistent, reproducible results in concussion research. However, given the limited availability of fMRI in the clinical setting, an alternative approach is needed. Other forms of brain imaging have been used to assess patients post-concussion, including susceptibility-weighted imaging (SWI), resting-state fMRI (rs-fMRI) and perfusion MRI, but the results have been mixed at best. A multimodal approach incorporating multiple imaging modalities may prove to be complementary and more powerful for diagnosis than each technique alone. In addition, one of the often-reported pathologies in mTBI is a deficit in oculomotor function. A number of brain areas are particularly affected by TBI, such as frontal lobes, portions of the corpus callosum, and the thalamus. These brain regions are made up of several structures that play key roles in oculomotor movement. Due to the neuroanatomical overlap between eye-movement circuitry and mTBI pathophysiology, visual deficits are expected to follow mTBI. One objective of this study is to investigate the possibility of using an automated tool for oculomotor assessment in the diagnosis of concussion. Another aim is to identify, using MRI/fMRI modalities, the structural, metabolic, and hemodynamic changes seen in symptomatic patients post-concussion, ascertain whether these methods can furnish enough information to be used as imaging biomarkers in mTBI, as well as to investigate possible abnormalities in oculomotor functions post-concussion and identify their underlying neural mechanisms.

29 adult concussed symptomatic individuals participated in the study within 1-month post-injury, as well as 29 age and sex-matched healthy controls. All subjects underwent MRI scanning using

task-based and resting state fMRI, susceptibility weighted imaging (SWI), and arterial spin labeling (ASL). Before scanning, testing of oculomotor functions was conducted using eight tasks presented via an automated eye-tracking system (four of these tasks were presented during fMRI scanning to help identify their underlying neural mechanisms). Concussed individuals also completed questionnaires evaluating post-concussion symptoms and were subjected to a neuropsychological assessment.

From the neuroimaging standpoint, only task-based and rs-fMRI showed significant differences between healthy controls and concussed subjects, with concussed subjects predominantly showing an increase in %BOLD signal as well as in functional connectivity. The groups' performances on 2/8 oculomotor tasks were significantly different, with concussed subjects showing lower mean eye velocity when following a target. No significant differences were found in ASL results, and only 3/29 subjects showed abnormalities on SWI.

Rs-fMRI appeared to be the most illustrative of concussion, as all subjects showed alterations in functional connectivity. Concussed subjects demonstrated oculomotor deficits compared to healthy controls, reinforcing the premise that oculomotor assessment is a promising approach in determining mTBI biomarkers, with two of the tasks – Anti-Saccades and Optokinetic Nystagmus – being the most sensitive to concussion. These findings are promising for the development of an objective approach to the diagnosis of concussion.

Résumé

La commotion cérébrale, souvent utilisée synonymement avec le traumatisme crânien léger (mTBI), constitue un problème de santé publique majeur. L'ambiguïté persiste concernant la physiopathologie et la prise en charge des commotions cérébrales, ce qui pose un problème pour un diagnostic objectif. Des études antérieures ont démontré que l'IRM fonctionnelle (IRMf) est une approche objective permettant d'obtenir des résultats cohérents et reproductibles dans la recherche sur les commotions cérébrales. Cependant, étant donné la disponibilité limitée de l'IRMf en milieu clinique, une approche alternative est nécessaire. D'autres formes d'imagerie cérébrale ont été utilisées pour évaluer les patients après une commotion cérébrale, notamment l'imagerie pondérée en susceptibilité (SWI), l'IRM en état de repos (IRM-rs) et l'IRM de perfusion, mais les résultats sont mitigés. Une approche multimodale combinant plusieurs modalités d'imagerie pourrait s'avérer complémentaire et plus puissante que chaque technique seule pour aider à arriver au diagnostic de commotion. De plus, une des pathologies fréquemment rapportées pour le TCC léger est un déficit de la fonction oculomotrice. Un certain nombre de zones cérébrales sont particulièrement affectées par le TBI, telles que les lobes frontaux, des parties du corps calleux et le thalamus. Ces régions cérébrales sont constituées de plusieurs structures qui jouent des rôles clés dans le mouvement oculomoteur. En raison du chevauchement neuroanatomique entre les circuits des mouvements oculaires et la physiopathologie du TCCL, des déficits visuels sont attendus après une commotion cérébrale. Dans cette étude, nous visons à étudier la possibilité d'utiliser un outil automatisé d'évaluation oculomotrice dans le diagnostic des commotions cérébrales. Cette étude vise à identifier, en utilisant les modalités d'IRM/IRMf, les changements structuraux, métaboliques et hémodynamiques observés chez les patients symptomatiques après une commotion cérébrale, à déterminer si ces méthodes peuvent fournir suffisamment

d'informations pour être utilisées comme biomarqueurs d'imagerie dans le mTBI, ainsi qu'à étudier les éventuelles anomalies des fonctions oculomotrices après une commotion cérébrale et à identifier leurs mécanismes neuronaux sous-jacents.

Vingt-neuf adultes présentant des symptômes de commotion cérébrale ont participé à l'étude dans le mois suivant leur blessure, ainsi que vingt-neuf sujets témoins sains appariés en termes d'âge et de sexe. Tous les sujets ont passé une IRM avec des tâches fonctionnelles et une IRM en état de repos, une SWI et une ASL un marquage arteriospinal (ASL). Avant l'examen, des tests des fonctions oculomotrices ont été réalisés à l'aide de huit tâches présentées par un système automatisé de poursuite oculaire (quatre de ces tâches ont été présentées pendant l'IRMf pour identifier leurs mécanismes neuronaux sous-jacents). Les individus commotionnés ont également rempli des questionnaires évaluant les symptômes et ils ont été soumis à une évaluation neuropsychologique.

Parmi les modalités de neuroimagerie, seules les IRM fonctionnelles avec tâches et celles en état de repos ont montré des différences significatives entre les témoins sains et les sujets commotionnés, avec une augmentation prédominante du signal %BOLD et de la connectivité fonctionnelle chez les TCC légers. Les performances des groupes dans 2/8 tâches oculomotrices étaient significativement différentes, les sujets commotionnés présentant une vélocité oculaire moyenne significativement plus faible lors de la poursuite oculaire de la cible. Aucune différence significative n'a été observée dans les résultats de la ASL, et seulement 3/29 sujets ont présenté des anomalies à la SWI.

L'IRMf en état de repos semble être la plus efficace pour identifier la commotion cérébrale, tous les sujets commotionnés présentant des altérations de la connectivité fonctionnelle. Ceux-ci ont également montré un déficit oculomoteur par rapport aux témoins sains, ce qui indique que l'évaluation oculomotrice est une approche prometteuse pour déterminer les biomarqueurs du TCC léger, avec deux des tâches étant les plus sensibles à la commotion (Anti-Saccades et Nystagmus Optokinétique). Ces résultats sont prometteurs pour le développement d'une approche objective dans le diagnostic des commotions cérébrales.

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I am also extremely grateful to Dr. Jen-Kai Chen for patiently listening to my most meticulous questions and always providing me with valuable insights. I owe him the most knowledge I have now in neuroimaging data processing field.

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Contribution to original knowledge

All three articles presented in this thesis represent original scholarship and have made distinct contributions to knowledge. Article one focused on testing novel software for an eye-tracking system, which has the potential to become a screening tool for mTBI in the future. It was the first study to test in healthy controls this oculomotor assessment technique with fMRI and to define the brain areas involved in the implementation of these tasks. The second article was following the first one naturally, investigating abnormalities detected by the eye-tracking system in a group of concussed subjects compared to healthy controls, as well as the differences in BOLD signal change during similar tasks in both groups. It is the first study using this software with an eye-tracking system in concussed subjects, and, to our knowledge, it is the first study using the Optokinetic Nystagmus task in a group of concussed subjects. Article three presents the first multimodal study in the mTBI field to incorporate metabolic (ASL), hemodynamic (rs-FMRI), and structural (SWI) imaging.

It is also important to note that most studies investigating mTBI/concussion are conducted with athletes, and our study was conducted on the general population (excluding professional athletes).

Contribution of Authors

I am the first author of all three articles, with contributions from Dr. Jen-Kai Chen, Sarah McCabe, Joelle Amir, Dr. Alain Ptito, and Dr. Rajeet Singh Saluja. This thesis was written by me, with minor changes made based on the feedback of the individuals mentioned above.

For each part of the project included in this thesis (Articles 1, 2, and 3), Dr. Alain Ptito and Dr. Rajeet Singh Saluja were co-supervisors, with Dr. Alain Ptito being the principal investigator. The original idea was developed by Dr. Alain Ptito, Dr. Rajeet Singh Saluja, and Dr. Jen-Kai Chen. Data for all parts of the project were collected by me and master's student, Sarah McCabe. I took responsibility for planning and implementing data processing and statistical analyses for all three articles, with occasional guidance from Dr. Jen-Kai Chen on data processing procedures. All three articles were revised by Dr. Alain Ptito and Dr. Rajeet Singh Saluja.

Chapter I (Introduction and review of the relevant literature) predominantly consists of the parts of the review paper "Lunkova, E., Guberman, G. I., Ptito, A., & Saluja, R. S. (2021). Noninvasive magnetic resonance imaging techniques in mild traumatic brain injury research and diagnosis. *Human brain mapping*, *42*(16), 5477-5494." This paper was written by myself, with the exception of the part on diffusion MRI, which was written by Dr. Guido Guberman. This part was not included in this thesis, however. The paper was revised by Dr. Alain Ptito and Dr. Rajeet Singh Saluja.

General Introduction

Concussion, often used synonymously with mild traumatic brain injury (mTBI), is a major public health problem with incidence rates of over 600/100 000/year in North America and Europe, occurring in males in two-thirds of cases (Li et al., 2016). Unfortunately, our knowledge of the pathophysiology and management of concussions is staggeringly poor. While we have long known that functional disturbances can be found in the brains of concussed individuals, their underlying mechanisms remain elusive. Newer forms of structural brain imaging have been used to assess patients post-concussion, including susceptibility-weighted imaging (SWI) and perfusion MRI, but the results have been mixed at best. In addition, few studies have attempted to directly correlate the functional changes consequent to concussion with novel imaging techniques.

From the perspective of diagnosis, the assessment of concussion currently relies heavily on subjective clinical symptoms that often are unreliable and/or non-specific. Given the high incidence of this type of injury and the significant medico-legal implications of a proper diagnosis, particularly in the realm of professional sports and insurance claims, there is a tremendous need for objective, clinically useful tools for the assessment of concussed patients. In the past, our team has been able to demonstrate that task-based functional MRI is an objective approach that shows consistent and reproducible results in the field of concussion (Chen et al., 2007; Holmes et al., 2019; Saluja et al., 2015). However, although useful, utilization of this technique is limited as it is mostly unavailable outside research centers due to its complex procedures and time-consuming factors, cost, and additional equipment. While most other imaging techniques, such as those mentioned above, have proven somewhat contributory, a multimodal approach incorporating multiple imaging modalities may prove to be complementary and more powerful for the identification of biomarkers in mTBI than each technique alone.

In addition, according to previous studies, one of the commonly seen manifestations in mTBI is an alteration in oculomotor movements (Rockswold et al., 2019; Suh et al., 2006). Several brain areas are particularly affected by mTBI, such as the frontal lobes, portions of the corpus callosum, and the thalamus (Lunkova et al., 2020). Because of the neuroanatomical overlap between eye-movement circuitry and mTBI pathophysiology, it is expected that oculomotor deficits would follow mTBI. However, most saccadic and pursuit deficits may be missed during a clinical examination. Thus, eye-tracking systems have the potential to serve as a valuable and sensitive tool for screening and monitoring in the context of mTBI; Snegireva et al., 2018). With the ability to potentially function as a biomarker, these systems show promise for future mTBI diagnosis and assessment.

Hence, the objective of this thesis is to explore novel and resource-efficient methods and to ascertain whether one or all these methods can furnish enough information to be used as biomarkers in mTBI and help develop an objective clinical test for mTBI diagnosis and assessment of recovery.

Chapter I. Review of the relevant literature

1. Problem of mTBI diagnosis

MTBI is a serious challenge for society and economics as it can be a risk-factor for such consequences as a decline in cognitive functions, early dementia and mental illness (McInnes, Friesen, MacKenzie, Westwood, & Boe, 2017). The mechanism of injury in mTBI is usually described as a result of a transfer of mechanical energy into the brain from a traumatic event such as rapid acceleration/deceleration or a direct impact to the head (Jeter et al., 2013). The other type of injury is a blast-related mTBI, caused by an explosive blast, which may result in brain damage due to tissue-transmitted shock waves affecting the brain (Cernak, 2015).

From the perspective of diagnosis, the assessment of concussion currently relies heavily on subjective clinical symptom reports that are often unreliable and/or nonspecific. MTBI is distinct from moderate and severe TBI on the basis of the Glasgow Coma Scale (GCS); this scale is used for assessing consciousness level, ranging from 3/15 (deep coma or death) to 15/15 (fully awake; Sternbach, 2000). MTBI patients in the acute phase have GCS scores ranging from 13 to 15/15 (Mena et al., 2011). As defined by the WHO (Greenwald, Ambrose, & Armstrong, 2012; McCrory, Meeuwisse, & Johnston, 2009; Ruff et al., 2009) and ACRM (Head, 1993), LOC in mTBI is less than 30 min and PTA duration less than 24 hr. However, it has been shown that concussions can also occur without LOC (Kelly & Rosenberg, 1997). Physicians also look for somatic symptoms such as dizziness, fatigue, headaches, and disturbed sleep. In addition, a neuropsychological assessment used in the acute phase may show deficits that become less apparent in the chronic phase (Echemendia, Putukian, Mackin, Julian, & Shoss, 2001).

For sport-related concussions (SRC), the Sport Concussion Assessment Tool (SCAT) was introduced; SCAT combines the Standardized Assessment of Concussion (SAC) with the Post-Concussion Symptom Scale (PCSS), modified Maddocks' questions, on-field markers of concussion, and a systematic return-to-play protocol, with the aim to standardize sideline concussion diagnosis during sports events while also serving as a tool for patient education (Yengo-Kahn et al., 2016). The SCAT still does not eliminate the factor of subjectivity, however, and is not fully relevant for general population, focusing on athletes.

Thus, in the field of mTBI, the data are controversial and seemingly involve more diverse aspects than initially thought. Reports of subjective symptoms and results of neuropsychological assessment vary broadly, while LOC and PTA are not always present in mTBI/concussion patients. There is, therefore, a dire need to develop an objective assessment tool of mTBI. New neuroimaging approaches offer this opportunity.

2. Neuroimaging methods in the diagnosis of concussion

Neuroimaging data on mTBI have been controversial. Structural imaging such as computed tomography (CT) and conventional MRI (T1-weighted and T2-weighted) have essentially been inefficient to help with the diagnosis of concussion. Other neuroimaging methods such as functional MRI (task-based MRI and resting state MRI; e.g., Chen et al., 2007; Mayer et al., 2015), diffusion weighted imaging (DWI) (Shenton et al., 2018), susceptibility weighted imaging (SWI; Lu et al, 2015; Studerus-Germann et al., 2016), perfusion weighted imaging (PWI; Andre, 2015; Hamer et al., 2020; Clark et al., 2020), positron emission tomography (PET) (Jensen et al., 2018), single photon emission computed tomography (SPECT;Amen et al., 2016; Romero et al., 2015), and magnetoencephalography (MEG; Peitz et al., 2021) have shown promise, but even then the

results have been mixed with a more common utilization in the research environment and a slower integration into the clinical setting due to cost and a lack of a standardization of the procedures.

These methods are particularly relevant as mTBI can be accompanied by various underlying neuropathological alterations, such as metabolic deviations (Giza & Hovda, 2014), diffuse axonal injury and myelin loss (Johnson, Stewart, & Smith, 2013), cerebral microhemorrhages (Park et al., 2009), and changes in cerebral blood flow (Wang et al., 2016). The presence and extent of these alterations can be discovered using different MRI sequences such as fMRI (both task-based and resting state), dMRI and myelin imaging, SWI, and ASL, respectively. Thus, only the combined use of these techniques can give an extensive and comprehensive diagnosis.

2.1.Task-based fMRI

Task-based fMRI has proven to be an effective tool in the diagnosis of concussion. This technique is based on blood-oxygen-level-dependent imaging (BOLD) methodology, where the difference in the MR signal between deoxyhemoglobin and oxyhemoglobin is picked up and monitored using gradient echo sequences. The main processes examined by fMRI can be described as an activation of the neurons in response to the action of the stimulus and an increase in their metabolic needs which leads to local alterations in blood flow. Thus, these alterations are recorded during scanning as a BOLD signal, which measures the hemodynamic response of the brain in relation to the neural activities (Buchbinder, 2016; Buxton, 2013).

Multiple studies have demonstrated altered BOLD signals during performance of cognitive tasks in patients with concussions compared with healthy controls (HCs). Most of the recent studies have used working memory tasks (N-back) for their ease of presentation in the scanner as well as for their sensitivity to alterations in brain activity. The N-back task has been widely utilized in

previous studies as a working memory paradigm, where participants are presented with a stream of stimuli, and the task is to decide for each stimulus whether it matches the one presented N items before (Jaeggi et al., 2010). Other tasks proven to be useful in studies on the role of fMRI in the diagnosis of mTBI are, among others, the spatial navigation task (Holmes et al., 2019; Saluja et al., 2015), the Flanker task (Sullivan, Hayes, Lafleche, Salat, & Verfaellie, 2018), the Shifted-attention Emotion Appraisal Task (SEAT; Wang et al., 2017) and the visual tracking eye movement tests (Astafiev et al., 2015).

Regarding alterations in brain activity after mTBI, the findings vary and are often controversial (Table 1). Most of the latest studies on the topic have yielded mixed activity patterns during task performance, with an elevation of the activity in some areas and deactivation in others. These results appear in keeping with the explanation that higher activity in certain brain areas reflects a compensation strategy for damaged areas that show diminished activation (McAllister et al., 1999). However, there are studies demonstrating either increased or decreased activity during task-performance by mTBI patients, possibly also due to the cognitive requirements of the task. The main regions showing increased fMRI activation in mTBI patients during task performance are parietal (Chen et al., 2016; Hsu et al., 2015; Mayer et al., 2015; Sullivan et al., 2018), occipital (Holmes et al., 2019; Mayer et al., 2015; Sullivan et al., 2018), and frontal (Holmes et al., 2019; Hsu et al., 2015; Westfall et al., 2015) areas as well as the middle temporal gyrus (Saluja et al., 2015; Westfall et al., 2015) and cingulate cortex (Sullivan et al., 2018; Wylie et al., 2015). Decreased activation has been reported, particularly in the components of the default mode network (DMN). These involve the left (Saluja et al., 2015; Sullivan et al., 2018) and right (Chen et al., 2016) precuneus and the right dorsolateral (Saluja et al., 2015), left dorsomedial (Sullivan et al., 2018), and medial (Van der Horn et al., 2016) regions. It should be noted as well that most

of the aforementioned studies were conducted in patients in the subacute phase of mTBI (<1 year postinjury).

Table 1. Summary of mTBI studies using task-based fMRI.

Author	Participants	Task	Major findings (activity alterations in mTBI patients)
Saluja et al., 2015	15 mTBI pediatric patients, up to 3 months post-injury	Spatial navigation task	Diminished activation in the retrosplenial, thalamic, and parahippocampal areas bilaterally, along with the right dorsolateral prefrontal cortex and left precuneus; increased activation in the left hippocampus and right middle temporal gyrus.
Astafiev et al., 2015	45 mTBI patients, 3 months to 5 years post-injury	Visual tracking tasks	Decrease in the right anterior internal capsule and right superior longitudinal fasciculus.
Hsu et al., 2015	30mTBI patients (1) Within 1 month post-injury (2) 6 weeks after (1)	The N-back task	Increased activation of the bilateral frontal and parietal regions: in mTBI patients, decreased activation in 2-back, 1-back conditions were observed in female patients compared with female control subjects at the initial imaging study; increased activation in 2-back, 1-back conditions were observed in male patients compared with male control subjects. At the 6-week follow-up study, female patients showed persistent hypoactivation, male patients showed a regression of hyperactivation to the level of activation similar to control subjects.
Wylie et al., 2015	27 mTBI patients (1) < 72 hours post-injury (2) 1 week later	The N-back task	Changes from time 1 to time 2 showed an increase in posterior cingulate activation; activation was increased greater in those mTBI subjects without cognitive recovery; in increased workload, activation increased in cortical regions in the right hemisphere.
Mayer et al., 2015	46 mTBI patients within 3 weeks of injury; follow-up examination 4 months post-injury	Multisensory (audio-visual) cognitive control task	Abnormal activation within different regions of visual cortex that depended on whether attention was focused on auditory or visual information streams: increased activation within bilateral inferior parietal lobules during higher cognitive/perceptual loads. Functional abnormalities within the visual cortex and

			inferior parietal lobules were only partially resolved at 4 months post-injury.
Westfall et al., 2015	19 adolescents with mTBI, 3–12 months post-injury	Auditory-verbal N-back task	Increased activation during the most difficult part of the task was observed in Cluster 1 (left sub-lobar insula, left middle temporal gyrus, and left superior temporal gyrus), Cluster 2 (left precentral gyrus and left sub-lobar insula), and Cluster 3 (right frontal lobe sub-gyral region and right medial frontal gyrus).
Van der Horn et al., 2016	55 mTBI patients, 4 weeks post-injury	The N-back task	Reduced activation within the medial prefrontal cortex; post-concussive complaints (PCC)-absent patients showed stronger deactivation of the DMN compared to PCC-present patients and HCs, especially during difficult task conditions; functional connectivity between the DMN and FEN was lower in PCC-absent patients compared to PCC-present patients.
Chen et al., 2016	13 younger (21–30 years) and 13 older (51–68 years) mTBI patients (1) Within 1 month post-injury (2) 6 weeks after	The N-back task	Younger patients: initial hyperactivation in the right precuneus and right inferior parietal gyrus in 2-back > 1-back conditions compared to younger HCs; Older patients: hypoactivation in the right precuneus and right inferior frontal gyrus compared to older HCs.
Wang et al., 2017	44 mTBI patients, within 2 weeks post-injury	Shifted-attention Emotion Appraisal Task (SEAT)	Decreased activation in the response to fearful faces in clusters in the left superior parietal gyrus and left medial orbitofrontal gyrus, and bilaterally in the lateral orbitofrontal gyri.
Sullivan et al., 2018	17 individuals with blast-related mTBI	Flanker task	Incongruent trials: increased activation in the left superior parietal lobe, left dorsal anterior cingulate cortex, right supramarginal gyrus, and right lateral occipital cortex. Error trials: greater deactivation in the areas of the default mode network including the left dorsomedial prefrontal cortex (DLPFC) and left posterior cingulate cortex, precuneus.

Sours et al., 2018	30 mTBI patients during chronic phase	The N-back task	Decreased segregation between the DMN and task-positive networks, elevation in functional connectivity within the DMN regions.
Holmes et al., 2019	27 mTBI pediatric patients, high- and low-symptom groups	Spatial navigation task	Low-symptom group had an elevated activity in the frontal and occipital cortices; high-symptom group had broader increased activity in the frontal region and in the cerebellum.
Khetani et al., 2019	60 individuals with PPCS and 30 recovered after mTBI, ~14 y.o, 38 days after mTBI	Visuospatial N-back task	Children with persistent post-concussive symptoms (PPCS) had decreased activation relative to the children with typical recovery in the posterior cingulate and precuneus during the one-back working memory condition, despite similar task performance.
Ramage et al., 2019	60 individuals, 3–24 months post-concussion	Constant Effort Task (CE)	Hyper-connectivity increased with effort level but diminished quickly when maintaining the effort; connectivity between the left anterior insula, rostral anterior cingulate cortex, and right-sided inferior frontal regions, correlated with effort-level and state fatigue in mTBI participants.
Cook et al., 2020	Meta-analysis of 7 studies: 174 patients, acute to subacute phase	Memory and attention tasks	Reduced activation within the right middle frontal gyrus (MFG).

First, in patients with mTBI compared to HCs, performance on the N-back task has been associated with altered activation in the dorsolateral prefrontal cortex (DLPFC), which has proven in numerous studies to be one of the major components in the working memory network (Barch, Sheline, Csernansky, & Snyder, 2003; Kim, Kroger, Calhoun, & Clark, 2015; Mansouri, Tanaka, & Buckley, 2009). Likewise, alterations in DLPFC activation with additional para-hippocampal involvement have been reported in two studies using the spatial navigation task (Holmes et al., 2019; Saluja et al., 2015). These findings, validated in studies using animal models, have established an involvement of direct hippocampal–prefrontal afferent pathways in the continuous updating of the task-related spatial information during spatial working memory performance.

Thus, direct hippocampal–prefrontal afferents appear essential for successful encoding of task-related cues (Spellman et al., 2015).

Furthermore, the studies described above have shown in patients with mTBI abnormal activity patterns involving the precuneus (superior parietal lobule) in the execution of both the working memory (N-back) and spatial navigation tasks. These results are consistent with indications that the precuneus is associated with executive aspects of working memory (Koenigs, Barbey, Postle, & Grafman, 2009) as well as with working memory capacity (activation increases with an augmentation in task load; Vogel & Machizawa, 2004). Interestingly, patients with right parietal cortex lesions show a decline in spatial working memory (Koenigs et al., 2009).

Even though behavioral performance in most of the studies have not discerned between mTBI patients and HCs, some did show a correlation between brain activation and task performance. In an fMRI study using the spatial navigation task with pediatric mTBI patients, Holmes et al. (2019) revealed a difference in task performance between low-symptom and high-symptom groups and HCs: only performance of the low-symptom group was different from HCs with higher total trial times, while there was no difference between HCs and the high-symptom group. Concurrently, brain activation in the high-symptom group had greater elevation in frontal and occipital cortices compared with the low-symptom group, with additional activity in the cerebellum. These results led to the suggestion by the authors that unique symptom-dependent patterns of altered task-related brain activity occur in mTBI patients (Holmes et al., 2019). Consequently, it can be hypothesized that there is an optimal level of activation that reflects an application of compensatory strategies associated with better performance, at least in the pediatric population. In fact, previous studies have demonstrated different activation patterns in youth with mTBI. For example, Keightley et

al. (2014) showed lower task-related activity in adolescents with mTBI due to an inability to involve compensatory strategies.

Overall, there is a significant number of studies in mTBI patients that use task-based fMRI with a broad variety of findings. The general idea among all of them is the variability of the alterations in the BOLD signal during task performance throughout the recovery process. Thus, elevation in activity postinjury can be perceived as an involvement of compensatory mechanisms to overcome the functional deficit of affected areas until they gradually return to normal.

FMRI has proven to be an effective tool in the diagnosis of concussion, although it has its limitations. Task-based fMRI is still more commonly used in research than in clinical practice due to its complex procedures and time-consuming factors such as task development, personnel required to teach the task to the patient, time inside the scanner, and cost as well as additional equipment (screen, joy-stick, etc.). All the above have encouraged researchers to develop more accessible and simple diagnostic neuroimaging method for the mTBI population.

2.2.Resting-State fMRI

Another type of functional MRI which is broadly used in mTBI research is resting state fMRI (rs-fMRI). In recent years, studies using rs-fMRI in mTBI subjects are exceeding task-based fMRI studies, and they used one of the most promising methods for use in the diagnosis of concussion. Compared with task-based fMRI, rs-fMRI does not require special assistance during scanning, that is, a subject does not have to perform any tasks they had to learn prior to the procedure and no additional equipment is needed. However, significant drawback of this method for clinical use is the complexity of data processing following the scan.

Similar to task-based fMRI, rs-fMRI is based on measuring BOLD signal fluctuations. The rs-fMRI examines synchronous activations between spatially distinct regions to identify resting state networks (RSNs). In contrast to task-based fMRI, this method is focused on activations which are occurring in the absence of a task or stimulus (Lee, Smyser & Shimony, 2013).

A great majority of studies elucidated alterations in RSNs following concussion (Table 2). One of the main RSNs investigated in mTBI research is the default mode network (DMN). DMN is potentially associated with the consolidation of memory, working memory, processing of emotionally salient stimuli, and the interplay between emotional processing and cognitive functions (Mohan et al., 2016). Most of the recent studies showed an increase in DMN connectivity in concussed patients (Churchill, Hutchison, Graham, & Schweizer, 2018; Madhavan et al., 2019; Meier et al., 2020; Van der Horn et al., 2017), although decreased connectivity was also noted by several authors (D'Souza et al., 2020; Iyer et al., 2019).

Table 2. Summary of mTBI studies using rs-fMRI

Author	Participants Time after injury	Major findings
Meier et al., 2020	93 concussed athletes, within 24 h post-injury	Acute increase in local connectivity was observed in a region in the right middle and superior frontal gyri (DMN).
Shafi et al., 2020	80 individuals with post-concussion syndrome, at least 1 month post-injury	Distinct subnetwork components with hyperconnected frontal nodes and hypoconnected posterior nodes across both the salience and fronto-parietal networks were observed.
Palacios et al., 2017	75 adult mTBI patients within 24 h post-injury	Alterations in the connectivity of the most representative RSNs that are associated with cognitive performance at 6 months after injury.
Churchill et al., 2018	35 athletes with acute concussion (<7 days post-injury)	A network of frontal, temporal and insular regions: connectivity was negatively correlated with symptom severity ; A network with anti-correlated elements of the default-mode network and sensorimotor system: connectivity was positively correlated with symptom severity.

Madhavan et al., 2019	91 adult mTBI patients (with first scanning at >3 days post-injury)	Functional connectivity was correlated with symptom severity in several regions of specific networks, including the dorsal attention, default mode, executive control, motor, visual and salience networks. Motor, visual networks and DMN were found to be associated strongly with symptom severity.
D'Souza et al., 2020	65 mTBI patients within 7 days post-injury	Reduced functional connectivity in the anterior default mode network, central executive network, somato-motor and auditory network; a negative correlation between network connectivity and severity of post-concussive symptoms was observed.
Lu et al., 2019	58 mTBI patients, >10 days post-injury	Reduced left substantia nigra (SN)-based functional connectivity with right insula and caudate and increased left SN-based functional connectivity with left precuneus and left middle occipital gyrus, and reduced right SN-based functional connectivity with left insula; abnormal functional connectivity significantly correlated with cognitive function.
Hou et al., 2019	47 mTBI patients, within 10 days post-injury	Alterations in the auditory and visual sub-networks in patients with PCS.
Iyer et al., 2019	110 pediatric mTBI patients, 4-weeks post-injury	Decrease in connectivity within DMN, visual and somatosensory networks, correlated with cognitive and emotional problems; increased connectivity within the limbic network, correlated with poorer sleep quality and higher fatigue.
Li et al., 2019	55 mTBI patients within 7 days post-injury	Significantly decreased network centrality in the left middle frontal gyrus (MFG); decreased inflows from the left MFG to bilateral middle temporal gyrus, left medial superior frontal gyrus, and left anterior cingulate cortex; changes in network centrality and causal connectivity were associated with deficits in cognitive performance.
Van der Horn et al., 2017	30 mTBI patients, 2 weeks post-injury	Minor longitudinal changes in functional connectivity within the precuneus component of DMN.

Additionally, task-related network (TRN), associated with attention activation throughout the task performance, is studied in parallel with DMN, because one of the consequences of mTBI appears to be a disruption between DMN and TRN (Mayer, Mannell, Ling, Gasparovic, & Yeo, 2011).

Alterations in the interaction between DMN and TRN may result in poor long-term memory consolidation (Lefebvre & D'Angiulli, 2019).

Similarly, it was demonstrated that the salience network (SN) which plays a role in mediation of the balance between DMN and the executive network, may be damaged as a result of concussion (Sharp et al., 2014; Sours et al., 2018). The impairment of SN leading to functional imbalances within the network appears to affect cognitive control, and may diminish self-regulation of cognition, behavior and emotion in particular (Peters, Dunlop, & Downar, 2016). Alterations in salience and fronto-parietal networks connectivity in mTBI patients were highlighted in a study by Shafi et al. (2020).

Together with this, alterations in the following other RS networks came to the attention of mTBI researchers: motor, visual, and auditory networks (D'Souza et al., 2020; Hou et al., 2019; Madhavan et al., 2019). Changes in these networks also were considered to be associated with such PCS as sensitivity to light and noise (visual and auditory networks, respectively; Madhavan et al., 2019). In addition, it is noteworthy that most of the recent mTBI studies using rs-fMRI showed positive correlations between connectivity alterations and various PCS, including cognitive and emotional symptoms. Moreover, deviations in connectivity in the acute postinjury stage were considered as a predictive indicator of subsequent difficulties in cognitive performance (Churchill et al., 2018; Iyer et al., 2019; Li et al., 2019; Lu et al., 2019; Palacios et al., 2017).

Thus, rs-fMRI shows promising results in mTBI research, demonstrating functional changes in the brain following concussion, and having a potential for predictive ability of later cognitive decline. Nevertheless, the complex postprocessing procedure and contradictions in findings is a major factor for this sequence to not be as yet included in clinical practice. Together with this, another

significant limitation of the method, is an insufficient level of specificity of rsMRI results. It is a highly sensitive tool to detect functional alterations following concussion, however, those alterations are unspecific. It also should be taken into consideration that studies using rsfMRI and task-based fMRI are both primarily showing the results at the group level as opposed to the individual level. Even though there is a popular suggestion to use these techniques as a biomarker of concussion, it is not used as such at the individual level because it requires a larger scope of normative data.

2.3.Perfusion MRI

Perfusion MRI or PWI is performed by other types of MRI methods: dynamic susceptibility contrast (DSC), dynamic contrast-enhanced imaging (DCE), and arterial spin-labelling (ASL). DSC and DCE are invasive, and a gadolinium-based contrast is used in these sequences. However, in this review we only consider ASL, a non-invasive perfusion MRI sequence which has been used to measure cerebral blood flow (CBF) in mTBI patients and to demonstrate significant alterations related to symptoms in the acute phase (Wang et al., 2016).

One of the major features of ASL is the possibility of broader use given its safety and non-invasive nature. The main mechanism of ASL is magnetic labeling of the arterial blood water protons with the purpose of exploiting it as an endogenous tracer. Cerebral blood flow (CBF) is the variable most typically evaluated in ASL (De Havenon et al., 2017).

CBF alterations during the acute and subacute phases of mTBI were detected in numerous studies using SPECT (Gowda et al., 2006) and perfusion computed tomography (PCT) (Metting, Spikman, Rödiger, & van der Naalt, 2014). Nevertheless, the main benefit of ASL in comparison

to SPECT and PCT is the absence of ionizing radiation and the possibility it offers for repeating scanning and tapping recovery.

ASL measurements can be divided into two groups of resulting values: absolute (aCBF) and relative CBF (rCBF). The aCBF values correspond to the perfusion level of the region of interest (ROI) independently of other regions, while rCBF values show the changes in the ROI relative to other brain regions. Consequently, rCBF is more sensitive to focal CBF abnormalities whereas aCBF values address the brain as a whole (Aracki-Trenkic et al., 2020).

Several studies (Table 3) showed contentious results regarding alterations of CBF in mTBI, questioned CBF as a valid biomarker in concussions and called for further investigation. Most of the studies using ASL demonstrated a decrease in blood flow up to 1 month after concussion (Lin et al., 2016; Meier et al., 2015; Peng et al., 2016; Wang et al., 2015) with a global decrease in aCBF particularly in the bilateral frontal and left occipital cortices (Lin et al., 2016) and in the bilateral frontotemporal lobes (Wang et al., 2015). Decrease in rCBF was demonstrated in the right insular and superior temporal cortices (Meier et al., 2015) and in the bilateral thalami (Bartnik-Olson et al., 2014). Hamer et al. (2020) also showed diminished CBF bilaterally in temporal areas only in males with chronic mTBI (Hamer et al., 2020). The earlier study by Ge et al. (2009) showed decreased CBF within the thalamus in mTBI patients (Ge et al., 2009). The most recent article on the effect of multiple mTBIs using ASL showed that a history of more than three concussions causes a decrease in aCBF throughout the life span and can lead to an increased risk factor for Alzheimer's disease (Clark et al., 2020).

Table 3. Summary of mTBI studies using perfusion MRI (ASL).

Author	Method of CBF detection	Time after injury (Acute vs Subacute vs Chronic phase)	Type of alterations in CBF	Major findings (CBF alterations in mTBI patients)
Ge et al., 2009	ASL-MRI 3T	chronic (~24 months)	Decrease	Reduced CBF in the bilateral thalami.
Bartnik-Olson et al., 2014	PWI	3-12 months*	Decrease	Reduced rCBF in the bilateral thalami.
Doshi et al., 2015	ASL	3 hours-10 days	Increase	Increase in the left striatum, frontal and occipital lobes.
Meier et al., 2015	ASL-MRI	1 day, 1 week, and 1 month	Decrease	Decrease in the right insula and superior temporal cortex resolved by 1 month, decrease in the dorsal midinsular cortex persisted at 1 month postconcussion.
Liu et al., 2016	ASL-fMRI	subacute (within 2 weeks) and chronic (>12 months)	Increase / Decrease	Acute phase: increase in the right middle frontal gyrus and inferior frontal cortex, right inferior parietal lobe, anterior cingulate cortex, left superior frontal gyrus, bilateral basal ganglia, and thalamus; Chronic phase: increase in the anterior cingulate, middle frontal gyrus, and inferior frontal gyrus, and lower CBF in precuneus, extending to PCC, paracentral lobule, and inferior parietal lobule; Decrease in DMN in both phases.
Wang et al., 2016	ASL-MRI	acute (within 24 hours)/Subacute(after 8 days)	Decrease	Decrease in the bilateral frontal and temporal area within 24 hours and greater decrease after 8 days.
Lin et al., 2016	3D pulse continuous ASL-MRI	within 1 month	Increase / Decrease	Decrease in the bilateral frontal and left occipital cortex, in more severe symptoms - higher CBF in the bilateral frontal and left occipital lobes.
Barlow et al., 2017	Pseudo continuous ASL-MRI	40 days	Increase / Decrease	Global CBF was higher in the bilateral inferior frontal and occipital regions in the symptomatic group and lower in the inferior temporal and parietal regions asymptomatic group compared with controls.
Churchill et al., 2017	2D pulsed ASL-MRI	7 days	Increase /	Greater total symptom severity – elevated posterior cortical CBF; greater

			Decrease	cognitive symptoms - lower frontal and subcortical CBF.
Stephens et al., 2018	pseudo-continuous ASL	2 and 6 weeks*	Increase	2 weeks: increased rCBF in the left dorsal anterior cingulate cortex (ACC) and left insula than controls; 6 weeks: higher rCBF persisted in the left dorsal ACC. Elevation of rCBF in the left dorsal ACC was higher in athletes with physical symptoms six weeks post-injury compared with asymptomatic athletes and HCs.
Bai et al., 2019	3-D ASL-MRI	>1 month	Increase	Increased CBF in the posterior parietal cortex, only in males.
Hamer et al., 2019	2D pulsed ASL-MRI	Chronic (and multiple)	Decrease	Lower CBF bilaterally in temporal area, only in males.
Wang et al., 2019	ASL-MRI	within 24-48 hours	Decrease	Decrease in the left inferior parietal lobule (IPL), right supramarginal gyrus (SMG), right middle frontal gyrus (MFG), posterior cingulate cortex, left occipital gyrus, and thalamus.
Brooks et al., 2019	3D pseudo-continuous ASL	chronic (>6 months) *	Increase / Decrease	Increase in anterior frontal/temporal regions, decrease in posterior and inferior regions.

In addition, various studies showed a correlation between CBF alterations and cognitive decline. Bai et al. (2019) demonstrated that concussed males had a decrease in aCBF in the subacute phase of mTBI compared with healthy males; at the same time, lower aCBF in the posterior parietal cortex was associated with worse cognitive performance (Bai et al., 2019). Also, a decrease in CBF correlated with lower cognitive assessment scores in athletes with sport-related concussion (SRC) 24–48 hr postinjury (Wang et al., 2019).

In contrast, other findings showed an increase in CBF in mTBI (Doshi et al., 2015; Liu et al., 2016; Stephens, Liu, Lu, & Suskauer, 2018) as well as an absence of alterations (Militana et al., 2016). Doshi et al. (2015) revealed an increase in rCBF up to 10 days after mTBI in the frontal lobes,

occipital lobes, and left striatum, in contradiction with the studies described above. The study by Stephens et al. (2018) examined teenage athletes 2–6 weeks postinjury. Compared with controls, rCBF was increased after 2 weeks in the left dorsal anterior cingulate cortex (ACC) and left insula. After 6 weeks, higher rCBF persisted only in the left dorsal ACC. Elevation of rCBF in the left dorsal ACC was higher in athletes with physical symptoms 6 weeks postinjury compared with asymptomatic athletes and HCs (Stephens et al., 2018). Interestingly, Stephens et al. (2018) and Brooks et al. (2019) showed elevation of rCBF in the cingulate cortex in concussed youths. It was suggested that an increase in CBF in mTBI patients can represent "a neuroprotective response in an effort to meet the metabolic demands of the tissue during a time of injury" (Williams & Danan, 2016).

In addition, an ASL-fMRI study of mental fatigue during performance of a psychomotor vigilance test by patients in the acute and chronic phases of mTBI showed that those in the acute phase (within 2 weeks after injury) showed greater CBF in "bottom-up" and "top-down" attention areas (PFC, right inferior parietal lobe, anterior cingulate cortex, bilateral basal ganglia, and thalamus) than the HCs, even though the behavioral performances did not differ significantly; at the same time, there was lower CBF in the DMN. A comparison of the acute and chronic phases showed that patients in the chronic phase (12 months postinjury) had higher CBF in the anterior cingulate, middle frontal gyrus, and inferior frontal gyrus, and lower CBF in the precuneus, extending to PCC, paracentral lobule, and inferior parietal lobule, a result consistent with partial recovery (Liu et al., 2016). Of note, there is no uniform definition of acute phase with it ranging from 24h (Doshi et al., 2015) to 2 weeks (Liu et al., 2016; Gravel et al., 2013) depending on the literature.

Interesting recent findings were presented by Churchill, Hutchison, Graham, and Schweizer (2017) and Barlow et al. (2017). In the first study, increased aCBF was initially seen in

the first 3 days post trauma followed by a decrease on days 5–7 (Churchill et al., 2017). The second study showed higher aCBF in symptomatic concussed children and lower aCBF in asymptomatic ones (scanning of both groups was made about a month after concussion; Barlow et al., 2017). A mixed pattern of alterations in rCBF was also demonstrated in the study by Brooks et al. (2019) in concussed youths. The study showed elevations in the anterior frontal and temporal regions, whereas the posterior and inferior regions had diminished rCBF (Brooks et al., 2019).

Even though the findings are conflicting, and no consensus is reached so far, a study involving another method of perfusion MRI, namely dynamic susceptibility contrast-enhanced perfusion-weighted imaging (DSC PWI), showed the results in favor of a decrease in CBF following concussion (Liu et al., 2013). Additionally, a review on various perfusion imaging methods (CT perfusion, MR perfusion, and SPECT) concluded that blood flow and blood volume post-concussion tend to be reduced, mostly in the frontal and temporal lobes (Yuh, Hawryluk, & Manley, 2014).

Overall, the application of ASL has been growing for the past years yielding encouraging results, but its drawbacks remain: lower signal difference (compared, e.g., to BOLD) (Detre & Wang, 2002), poor temporal resolution (Liu & Brown, 2007), and necessity of the complex subtraction procedure (Golay, Hendrikse, & Lim, 2004). Still, these disadvantages can be partially resolved using parallel imaging (Deshmane et al., 2012) and pseudo continuous labeling (Dai, Garcia, De Bazelaire, & Alsop, 2008; Haller et al., 2016). The ASL method offers advantages such as stability over time and less variability across subjects (compared with BOLD), making it particularly useful for longitudinal studies in mTBI and other neurological disorders (Brown, Clark, & Liu, 2007). Considering the numerous mTBI studies with perfusion MRI, ASL used

together with other MRI modalities, carries a high potential and may be part of novel methods of diagnosing concussion in the clinical setting.

2.4.Susceptibility weighted imaging

Along with ASL, SWI is getting increasingly recognized in the diagnosis of mTBI. SWI, being a fully flow-compensated three-dimensional (3D) gradient echo (GRE) sequence, is particularly sensitive to microhemorrhages and venous blood and iron levels. Currently, SWI is considered the most promising technique for microbleeds identification, showing up to five times more precise results than such methods as GRE or echo-planar imaging (EPI;Benedictus et al., 2013; Sepehry, Lang, Hsiung, & Rauscher, 2016). Thus, SWI may be useful in showing cerebral microbleeds following concussion.

The SWI sequence is especially focused on the detection of intravascular venous deoxygenated blood and extravascular blood products (Haacke, Mittal, Wu, Neelavalli, & Cheng, 2009; Reichenbach, Venkatesan, Schillinger, Kido, & Haacke, 1997). The main principle of this technique is an exploitation of the magnetic property of iron, particularly within different states of hemoglobin, which causes magnetic field distortion affecting both T2* relaxation times and phase data (Toth, 2015). Both magnitude and phase information are necessary to generate proper tissue characterization, and they are merged together to create an SWI image (Haacke, Xu, Cheng, & Reichenbach, 2004). Hemorrhages are detected by dephasing of the signal caused by paramagnetic blood products, for example, deoxyhemoglobin in acute hematomas has a strong paramagnetic effect and leads to a significant signal loss on SWI (Kirov, Whitlow, & Zamora, 2018).

Despite being less effective for delineating anatomical structures because of its low contrast (Toth, 2015), SWI appears to be most precise for detection of hemorrhages among other MRI

modalities (CT, etc.). However, there are post-trauma time limitations for SWI diagnosis to be accurate. Hyperacute hemorrhages (<12 hr after the incident) cannot be spotted by SWI as during this period oxyhemoglobin lacks unpaired electrons, which yields to weak diamagnetic characteristics which in turn results in unaffected SWI dephasing. Nonetheless, in the acute phase, oxyhemoglobin is transformed into deoxyhemoglobin when oxygen molecules are released from blood particles, thus causing the paramagnetic effect that affects signal loss (Ong & Stuckey, 2010).

Together with this, SWI is used for quantitative susceptibility mapping (QSM). QSM is an MRI technique for quantifying the spatial distribution of magnetic susceptibility within biological tissues (Liu, Ghimire, Pang, Wu, & Shi, 2015). Specifically, QSM is used to measure cerebral SVO_2 (mixed venous oxygen saturation; Doshi et al. (2015)), as well as to assess deep gray matter iron (Koch et al., 2021) and the role of myelin in white matter after mTBI (Weber et al., 2018). In a study by Chai et al. (2017), the decreased susceptibility of the straight sinus appeared to correlate with PCS, and with a recovery to normal levels of oxygenation over time. Moreover, increased global white-matter susceptibility and decreased global subcortical gray matter susceptibility were observed in mTBI patients (Koch et al., 2021). In addition, the prognostic ability of QSM method was underlined by several authors (Koch et al., 2021; Weber et al., 2018).

We are considering SWI in this review as it proved to be a valid method for detecting one of the signs of mTBI—microhemorrhages, in keeping with reports from multiple studies (Beauchamp & Anderson, 2013; Steenerson & Starling, 2017). Detection of microhemorrhages in the early stages of mTBI can contribute to outcome prediction in the presence of PCS (Beauchamp et al., 2011; Geurts, Andriessen, Goraj, & Vos, 2012). Furthermore, SWI can be performed on conventional scanners, which makes this method highly applicable within the clinical setting.

Microbleeds are a marker of a traumatic axonal injury (TAI), a specific sign of mTBI. TAI has been demonstrated in mTBI patients in post-mortem autopsies (Bigler, 2004), and its extent is an important prognostic factor in the later development of cognitive decline and neuropsychiatric disability (Medana & Esiri, 2003). Einarsen et al. (2019) also showed that signs of TAI lesions remain on SWI scans at 3 and 12 months postinjury (Einarsen et al., 2019).

In one recent trauma study, microbleeds in brain tissue detected by SWI in the acute phase predicted worse cognitive decline and persistent PCS in concussed patients (Studerus-Germann et al., 2018). The same study compared efficacy of SWI and DTI in prediction ability of worse outcome. It showed that exploitation of the SWI technique in the acute phase of mTBI is more effective while, in contrast, DTI would be more practical in later phases (considering that DTI did not reveal any alterations in structural integrity 1 week post injury but demonstrated significant changes after 1 year). Another study (Liu et al., 2015) investigated residual iron deposits as a marker of prior microbleeds; patients with persistent PCS showed a higher rate of microhemorrhages than mTBI patients who completely recovered.

Various articles on SWI in mTBI (Table 4) demonstrated that several brain areas typically affected by microbleeds are associated with a negative outcome. Wang et al. showed that microhemorrhages in frontal, parietal and temporal lobes predicted the presence of depression 12 months after the trauma (Wang et al., 2014). De Haan et al. also revealed in their study of the chronic phase of mTBI the presence of microbleeds in frontal and temporal areas. However, the unfavorable functional outcome was correlated with the presence and extent of the microhemorrhages only in temporal cortical areas. The study also showed that no microhemorrhages were found in the thalamus and internal capsule (De Haan et al., 2017). The earlier study showed that microbleeds in mTBI were more frequently found in white matter than

in deep nuclei (Park et al., 2009). Together with this, it was emphasized that traumatic cerebral microbleeds in general were associated with lower scores on the GCS (1 day after injury) and Glasgow Outcome Scale (GOS) (1 year after injury) (Park et al., 2009).

Table 4. **S**ummary of mTBI studies using SWI

Author	Participants, Acute vs Chronic phase	Major Findings
Park et al., 2009	21 mTBI patients without any parenchymal hemorrhage on conventional MRI, within a week after admission	Microbleeds were located more frequently in white matter than in deep nucleus. Lesions were observed in the frontal lobe, occipital lobe, and brain stem.
Hasiloglu et al., 2011	21 amateur boxers	Microhemorrhages were detected only in 2 of 10 patients.
Wang et al., 2014	200 mTBI patients, 2h to 3 days post-injury, with follow-up testing (on presence of depressive symptoms) 1 year after	Depressive group had greater the number and volume of microbleeds than non-depressive group, particularly in the frontal, parietal and temporal lobes.
Liu et al., 2014	63 MTBI patients at least 3 days after injury, and follow-up testing on PCS after 7-15 months	Significant correlation was found between PCS and number of intracranial microbleeds.
Lu et al., 2015	39 patients with mTBI, 6 months after injury	Significantly higher angle radian values were observed in the head of the caudate nucleus, the lenticular nucleus, the hippocampus, the thalamus, the right substantia nigra, the red nucleus, and the splenium of the CC.
de Haan et al., 2017	127 individuals with mTBI (63 with MRI abnormalities and 64 without), chronic phase	Microhemorrhages were predominantly present in the frontal and temporal lobes. Worse outcome was demonstrated in 67% of the group with MRI abnormalities with a significant association of the

		total number of microhemorrhages in the temporal cortical area.
Trifan et al., 2017	180 subjects with persistent neurobehavioral symptoms following head trauma (83% classified as mTBI), chronic phase (~29 months post-injury)	28% of the 180 TBI cases revealed hemorrhages.
Studerus-Germann et al., 2018	30 mTBI patients tested at the baseline and 12 months post-injury	Amount of microbleeds in the acute phase correlates positively with cognitive symptoms such as slowing, difficulty in memory and concentration.
Einarsen et al., 2019	194 mTBI patients, 72 hours, 3 months and 12 months post-injury	TAI lesions in the lobar WM, CC, brainstem, basal ganglia, and thalamus, in 19% of participants after 3 months and in 16% after 12 months.

In contrast, the mTBI study in the chronic phase by Lu et al. showed alterations in angle radian values (which could indicate excessive iron deposition to some extent) predominantly in gray matter (Lu et al., 2015). Angle radian value is calculated with phase image as the quantitative value for cortical iron deposition (Sun et al., 2021). Higher angle radian values were found in the head of the caudate nucleus, the lenticular nucleus, the hippocampus, the thalamus, the right substantia nigra, the red nucleus, and the splenium of the corpus callosum in patients with mTBI. Additionally, performance on the Mini-Mental State Examination (MMSE) by mTBI patients correlated negatively with the angle radian values in the right substantia nigra, suggesting that this area is related to persistent cognitive decline in patients with chronic mTBI (Lu et al., 2015).

SWI is an appealing method in mTBI research, and it has a great number of benefits (some of them are mentioned above). Additionally, SWI is so far the only MRI method which has diagnostic potential, whereas the other techniques have so far only provided results at the group level.

However, there is only a limited number of studies on the topic and findings have raised various questions with contradictory findings. Trifan et al. showed that SWI is not effective with mild TBI, although it shows increased sensitivity for moderate and severe TBI (Trifan et al., 2017). A study by Jarrett et al. (2016) also did not show presence of microhemorrhages in mTBI patients. In addition, SWI is not effective in the hyperacute phase of mTBI (Ong & Stuckey, 2010). Nevertheless, the studies mentioned above still demonstrate promising results. Thus, further investigation using SWI is required to reach a consensus and to identify the best way to apply this technique for diagnosing mTBI in the clinical setting.

The limitations and benefits of the methods reviewed, and the absence of a universal neuroimaging approach underscore the need for a multimodal strategy in mTBI imaging. This involves utilizing diverse imaging techniques and advanced statistical methods to combine modalities and extract pertinent information (Guberman et al., 2021; Manning et al., 2019). While fMRI is effective for diagnosing concussions, its resource-consuming characteristics remain a limiting factor. SWI detects brain microbleeds, and ASL tracks changes in cerebral blood flow as an alternative to the BOLD method. Integrating these methods provides a comprehensive understanding of mTBI mechanisms, enabling a more thorough diagnosis by clinicians.

3. Oculomotor deficits following mTBI

According to previous findings, one of the common mTBI findings is an alteration in oculomotor movements (Rockswold et al., 2019; Suh et al., 2006). Concussed individuals complain of oculomotor symptoms, including blurred vision, convergence insufficiency, and difficulty reading, along with others, which may result in functional difficulties, including impaired academic performance and related cognitive impairments (Capoor & Ciuffreda, 2002; Kontos et al., 2017). The manifestation of oculomotor symptoms following a concussion can be attributed to the

disruption of underlying neurophysiology associated with oculomotor functions caused by the injury (Quintana et al., 2021).

Several brain areas seem affected by mTBI, such as the frontal lobes (especially DLPFC), portions of the corpus callosum, thalamus, visual cortices, caudate nucleus, substantia nigra pars reticulata, as well as parietal and temporal areas (Lunkova et al., 2021). Several structures involving these regions contribute to oculomotor movements including such frontal areas as frontal eye field, supplementary eye fields, and DLPFC; as well as other areas, including middle temporal visual area (MT), parietal eye field, visual association area, thalamus, caudate nucles, substantia nigra pars reticulata, etc. (Johnson et al., 2016). Given this neuroanatomical overlap between eye-movement circuitry and mTBI pathophysiology, visual deficits can be expected following mTBI. Previous findings have demonstrated that patients with mTBI with long-lasting post-concussion symptoms have concurrent oculomotor dysfunction (Rockswold et al., 2019). In addition, mTBI may be a leading cause of clinically impaired smooth pursuit and saccadic eye movements (Cochrane et al., 2019). Literature on the topic is extensively discussed in the Introduction sections of Chapters II and III.

Eye-tracking systems have shown promise as convenient and sensitive tools for screening and monitoring mTBI as was shown in a recent meta-analysis of 21 studies on using these techniques post-concussion (Snegireva et al., 2018). Eye tracking, as an experimental technique, involves recording eye motion and gaze location over time and tasks, serving as a prevalent method for studying the distribution of visual attention (Carter & Luke, 2020). It has the potential to detect saccadic and pursuit deficits, which are often missed during clinical examinations. Recent research

by Zahid et al. (2020) demonstrated the correlation between eye-tracking metrics and concussion symptoms, highlighting its rapid, objective, and non-invasive nature in diagnosing concussions.

Although the specificity and sensitivity of individual oculomotor metrics have been studied with some success, the investigation of clusters of these metrics and their association with specific brain injury areas remains limited. Comprehensive data collection encompassing both sensory and motor circuits is needed to gain a deeper understanding of the underlying mechanisms of oculomotor function and the extent of their susceptibility to mTBI.

Taken together, a multimodal approach, as well as the use of an eye-tracking system for oculomotor evaluation post-concussion, can provide all-encompassing insight into the identification of potential biomarkers of mTBI diagnosis. Finding new reliable biomarkers to facilitate a more definite and precise diagnostic process is essential, especially in the context of rehabilitation and medico-legal cases.

Objectives

1) Identify using MRI/fMRI modalities, the structural (SWI), metabolic (ASL), and hemodynamic (rsfMRI) changes seen in symptomatic patients post-concussion.

2) Compare task-based fMRI activation patterns to the structural (SWI), metabolic (ASL), and hemodynamic (rsfMRI) changes seen in symptomatic patients post-concussion.

3) Ascertain whether one or all these methods can furnish enough information to be used as imaging biomarkers in mTBI.

4) Identify the underlying neural mechanisms of the oculomotor metrics provided by the Neuroflex tool.

5) Determine whether cognitive performance by concussed subjects correlates with an atypical neuroimaging profile.

Hypotheses

1) Symptomatic concussed individuals will show atypical task-based fMRI activation patterns in regions sensitive to oculomotor functions.

2) Symptomatic concussed individuals will show atypical structural (SWI), metabolic (ASL), and hemodynamic (rsfMRI) patterns.

3) One or more of these neuroimaging methods will correlate with symptom severity in concussed individuals.

4) Abnormal performances on the Neuroflex oculomotor tasks will correlate with atypical structural (SWI), metabolic (ASL), and hemodynamic (task-based/rsfMRI) patterns in symptomatic concussed individuals.

5) Abnormal performance on neuropsychological tests will correlate with atypical structural (SWI), metabolic (ASL), and hemodynamic (task-based/rsfMRI) patterns, in symptomatic concussed individuals.

Post-hoc hypothesis: Symptomatic concussed individuals will show atypical results on tasks measuring oculomotor functions.

Chapter II. Evaluation of oculomotor functions in the diagnosis of mTBI: a pilot study with healthy controls using eye-tracking and fMRI

Under review at PLOS One

Ekaterina Lunkova[1], Sarah McCabe[1], Jen-Kai Chen[1], Rajeet Singh Saluja[1,3], Alain Ptito[1,2,3]

[1]Department of Neurology & Neurosurgery, McGill University, Montreal, Quebec, Canada.

[2]Montreal Neurological Institute, Montreal, Quebec, Canada.

[3]McGill University Health Centre Research Institute, Montreal, Quebec, Canada.

1. Introduction

Mild traumatic brain injury (mTBI), often used synonymously with concussion, is a major public health problem. MTBI can be a risk-factor for, among others, for a decline in cognitive functions, early dementia, and mental illness (McInnes et al., 2017), creating serious challenges for society and economics.

Unfortunately, our knowledge of the pathophysiology and management of concussion is insufficient, and the problem of obtaining an objective diagnosis remains topical. In the past, our team has been able to demonstrate that task-based fMRI using working memory (Chen et al., 2004; Keightley et al., 2014) and spatial navigation tests (Saluja et al., 2014) is an objective approach that shows consistent, reproducible results in the diagnosis of concussion. While we acknowledge the limitations of fMRI (it is expensive, non-portable, time consuming, and requires extra personnel), we believe that it can provide valuable insights into the neural basis of tasks, which can inform the development of more efficient and portable diagnostic tool accessible for use in on-the-field setting.

According to previous findings, one of the mTBI pathologies involves an alteration in oculomotor movements (Rockswold et al., 2019; Suh et al., 2006). Concussed individuals often complain of oculomotor symptoms, including blurred vision, convergence insufficiency, difficulty reading, diplopia, headaches, difficulty tracking a moving target, general asthenopia (eye strain), dizziness, nausea, and problems scanning visual information (Kapoor & Ciuffreda, 2002; Kontos et al., 2017). These symptoms may result in functional difficulties including problems reading, impaired academic performance, and related cognitive impairments (Kapoor & Ciuffreda, 2002; Kontos et al., 2017) which can be explained by the fact that concussion may disrupt the underlying neurophysiology of oculomotor functions (Quintana et al., 2021).

Several brain areas such as the frontal lobes, portions of the corpus callosum, and the thalamus seem to be affected by mTBI (Lunkova et al., 2020). Structures within these regions contribute to oculomotor movements and given the neuroanatomical overlap between eye-movement circuitry and mTBI pathophysiology, visuomotor deficits are likely following mTBI. Previous findings have demonstrated that patients with mTBI with long lasting post-concussion symptoms have concurrent oculomotor dysfunction (Rockswold, et al., 2019). In addition, mTBI may be a leading cause of clinically impaired smooth pursuit and saccadic eye movements (Cochrane, et al., 2019). During saccadic eye movements, fMRI activation is observed in frontal eye fields (FEF), supplementary eye fields (SEF), parietal eye fields (PEF) and vermis of the cerebellum, as well as in subcortical areas, such as the substantia nigra pars reticulata (SNpr), caudate nuclei (CN) and superior colliculi (SC) (Sparks, 2002; Berman et al., 1999; Cameron, Riddle & D'Esposito, 2015; Ventura et al., 2016). When performing anti-saccades, additional higher cognitive processes are

required; they involve changes in activity levels within the basic saccade circuitry as well as recruitment of additional areas, such as the prefrontal cortex (McDowell et al., 2008; Cieslik et al., 2016).

Concussed subjects have shown impaired performance in anti-saccades along with hyperactivation in the cerebellum, primary and secondary visual cortex, and visual area V5/MT (Johnson et al., 2014). Moreover, past findings in concussed subjects compared to controls have demonstrated elevated activation in these areas while producing saccadic movements. This suggests that compensatory mechanisms are involved in maintaining functional performance even in the context of minor deficits in the networks (Johnson et al., 2014). In addition, even 3–6 months after mTBI, patients with prolonged post-concussive symptoms are impaired in the production of anti-saccades, memory-guided saccades, and self-paced saccades, when compared to fully recovered patients (Kraus et al., 2007; Heitger et al., 2009; Ventura et al., 2016).

Studies using fMRI during Smooth Pursuit demonstrated involvement of such functional areas as SEF, FEF, MT, the vermis of the cerebellum, and vestibular nuclei (Ventura et al., 2016). Smooth Pursuit eye movements are considered to be one of the components in Optokinetic Nystagmus (OKN). Thus, we suggest that similar patterns of brain activation in mTBI patients as those seen in the smooth pursuit task should be observed during OKN. To our knowledge, no studies to date have used tasks evaluating OKN in fMRI with concussed subjects and pilot data is needed to determine its potential for concussion diagnosis.

In OKN, the circuit for the slow phase of the eye movement is believed to overlap with smooth pursuit, while the fast phase relies on the nucleus of the optic tract (pretectum) (Lencer, Sprenger,

Trillenberg, et al., 2019). After concussion, smooth pursuit tasks are associated with increased activation in regions such as the cerebellum, frontal lobes, and visual cortices in the absence of significant differences in performance compared to healthy controls (Johnson et al., 2014). Increased activation may be explained by compensatory mechanisms in which functional changes in brain resources contribute to correct task performance without permanent alterations in networks.

Most saccadic and pursuit deficits may be missed during clinical examination because of their speed. Thus, eye-tracking systems should be useful and sensitive screening tools in mTBI diagnosis (Snegireva et al., 2018). A recent study showed that eye tracking metrics correlated with concussion symptoms. In addition, they can detect convergence and accommodative abnormalities associated with concussion. Thus, utilization of an eye-tracking system as a rapid, objective, non-invasive tool in the diagnosis of concussion (Zahid et al., 2020) appears warranted.

The specificity and sensitivity of oculomotor metrics have been studied separately and somewhat successfully, although clusters of these metrics and their relationship to the area of brain injury have not yet been extensively investigated. Data collection from a comprehensive combination of sensory and motor circuits will shed light on the underlying mechanisms of oculomotor function and the extent of their vulnerability to mTBI.

In this study, we aimed to:

(1) examine blood oxygen level dependent (BOLD) fMRI alterations corresponding to performances in oculomotor function after mTBI (evaluating saccades, anti-saccades, smooth pursuit and OKN) in healthy control subjects,

(2) investigate the possibility of using virtual reality (VR) goggles with built-in eye-tracker system and a specially developed software system to automatically assess oculomotor functions in complementary tracking tasks.

Our study aims to explore whether eye-tracking metrics can serve as a potential screening tool for concussed patients in acute stage. To validate the use of eye-tracking metrics in concussion diagnosis, we need to establish the neural correlates of oculomotor tasks in healthy controls, which is why we are utilizing fMRI in conjunction with eye-tracking.

The future purpose is validating this test design as a diagnostic tool in concussed patients in the acute stage. Here, we present the results of a pilot study with healthy controls to identify brain regions associated with these tasks and to collect norms for future comparison with concussed subjects.

2. Methods

Ethics statement

We obtained approval for this study from McGill University Institutional Review Board. Written informed consent was obtained from all participants involved in the study. The consent form outlined the purpose of the research, the procedures involved, and the potential risks and benefits.

Participants were informed of their right to withdraw from the study at any time without consequences.

2.1. Participants

A group of 31 adult healthy control subjects (15 males and 16 females) aged 18 to 55 (mean age = 30.6, SD = 9.4) were included in the study. All were screened to be without a history of neurodevelopmental or neurological disorders, or head injuries, ADHD, and/or presence of any significant abnormalities seen on structural MRI scans (assessed by a clinician).

2.2. Oculomotor functions assessment using the VR-goggles eye-tracking system

Oculomotor evaluation was conducted prior to MRI scanning using virtual reality (VR) goggles with NeuroFlex® software equipped with binocular recordings in 3D (horizontal, vertical, and pupil size) and head recordings in 6D (3D angular and 3D linear accelerations). These were recorded concurrently for eye and head angles at a 120 Hz sampling rate. A high-speed laptop computer generated the goggle visual displays and recorded the ocular and head data synchronously. Eye and head movements were evaluated in response to visual and vestibular stimuli, or lack thereof (e.g., to evaluate spontaneous nystagmus) and to detect deviations from the 'normal' eye and head responses of the healthy subjects. Table 1 summarizes the methods and metrics evaluated. The full evaluation consisted of a battery of tests that takes less than 10 minutes to administer, including three head-free conditions (Smooth Pursuit (head-free), Active Visual Vestibulo-Ocular Reflex (VOR, Horizontal), Active Visual VOR (Vertical)) and five head-fixed conditions (Smooth Pursuit (head-fixed), Saccades, Anti-saccades, Optokinetic Nystagmus (OKN), Spontaneous Nystagmus).

Table 1. Eye-Head coordination tests and measured variables with units

	System of interest (protocol)	**Measured aspect of Metrics**
1	Saccades (flashed targets, self-paced)	Delay (ms) Accuracy (degrees)
		Generation rate (S/sec) Main sequence (peak velocity vs. duration)
2	Anti-saccades	Accuracy (degrees)
		Latency (ms)
3	Active head-fixed or head-free passive VOR active VOR, pursuit, OKN	Mean vergence over the whole test period (sac/min)
		Vergence for each phase of movement (saccade and fixation; degrees)
4	Nystagmus during active gaze shifts head-fixed or head-free Spontaneous nystagmus in the dark Vestibulo-Ocular Reflex Optokinetic Nystagmus	Asymmetry of peak response, phase lag (%)
		Full response characterization in both phases with numeric parameters
		Generation frequency
		Tracking error, gaze stabilization (degrees)
5	Head free gaze shifts	Eye vs head contributions
6	2D Target tracking head-fixed or head-free	Accuracy in different initial positions (degrees)
		Corrective saccade rate (S/sec)

	smooth pursuit and corrective saccades	Response symmetry
7	Pupil size	Diameter (mm)

After subjects underwent evaluation using VR goggles, four tasks selected: Smooth Pursuit, Saccades, Anti-Saccades and OKN – were repeated during fMRI sessions to measure brain activation associated with performance of each task. The 4 tasks during fMRI were identical to the tasks during the evaluation using VR goggles and were presented to the subjects via projector in the MRI room.

2.3. Image acquisition

All scanning was performed on a Siemens 3 Tesla MRI system equipped with a 64-channel head coil at the Montreal Neurological Institute (MNI) BIC MRI platform. First, T_1-weighted images were acquired for anatomical reference (3D MP-RAGE, TR=2300ms, TE=2.98ms, 176 slices, slice thickness=1mm, FOV = 256mm, image matrix = 256 x 256, flip angle = 9 degrees, interleaved excitation) for fMRI data. fMRI data was acquired using BOLD activation studies with T2* weighted GE-EPI (TR=3000ms, TE=30ms, 38 slices, slice thickness=4mm, FOV=256mm, image matrix=128x128, interleaved excitation).

2.4. Oculomotor tasks used in fMRI

We used task-based fMRI with 4 tasks evaluating oculomotor functions: (1) **Smooth Pursuit**: subjects were asked to follow a moving target (dot) with their eyes; (2) **Saccades:** subjects were warned that the dot will jump around the screen, and that they had to follow it with their eyes only;

(3) **Anti-saccades**: subjects had to look at the dot at the center of the screen – when a red X appeared, they had to avoid looking at the red X and instead orient their eyes in the opposite field of view in the same location – then follow the dot back to the center; (4) OKN: subjects were asked to pick a dot and follow it until it left their field of view, and to continue in the same manner with each subsequent dot; and, (5) **Baseline condition**: a) Prior to each task, the baseline condition was presented to the subjects (for conditions (1), (2) and (3) it was a fixed dot in the center of the screen for a duration of 12 seconds; for condition (4), it was a fixed field of dots for a duration of 15 seconds). Each of the conditions lasted 30 seconds, while subjects were head-fixed and asked to complete the tasks moving their eyes only. Two identical functional scanning sessions were conducted sequentially. Each scanning session lasted 6 minutes and consisted of two runs of the set of the 4 tasks. The subjects had extensive training prior to the scanning to ensure familiarity with the tasks. These tasks were chosen as participants' head is fixed during MRI scanning and these tasks don't require head movement as other head-free conditions in the screening battery (Head-Free Smooth Pursuit, VOR Vertical and Horizontal). Only one out of five head-fixed tasks wasn't replicated during fMRI – Spontaneous Nystagmus, due to recent addition of this task to the screening and lack of normative data for this task.

2.4. Behavioral analysis (Oculomotor & Gaze assessment)

The VR-goggles in the eye-tracking system automatically processed gathered the data through the Neurolex® software system. The results for each subject included all metrics demonstrated in Table 1, and the deviations of the results (if any) were indicated in the reports. Mean values, standard deviation (SD), and normative range of the results in the group of healthy controls were calculated. The normative range was counted as Mean+/-2SD.

2.5. fMRI processing

All MRI images were preprocessed and analyzed using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). During the preprocessing stage, all the functional images were realigned and unwrapped; slice-time corrected; co-registered to a T1-weighted reference image; structurally and functionally normalized and segmented into gray matter, white matter, and CSF tissue; and smoothed using a 6 mm Gaussian kernel. The preprocessed images for each subject then underwent first-level analysis, where the model was specified and estimated for the two runs of the tasks. The model was specified using the conditions and onset times for each task. To determine an alteration of the level of BOLD signal specific for each task, the contrasts were identified between the task condition (separate for each of the four tasks) and the baseline condition. Afterwards, a second level analysis was conducted to identify BOLD signal changes during each task respectively for the healthy controls. Exploratory brain analysis resulted in whole brain activation maps. All the comparisons were subjected to Family-wise error rate (FWER) correction at $p < 0.05$, and only changes filtered out by the correction were considered significant. The changes were considered significant at $p < 0.05$. Regions of interest (ROIs) were then identified for each task using the MarsBaR toolbox (Brett et al., 2002). Each ROIs was created in a sphere shape with a radius of 5mm. The percent BOLD signal change was calculated for the acquired ROIs.

2.6. Comparison between oculomotor metrics and BOLD signal alterations

Oculomotor and BOLD signal analyses and descriptive statistics were performed using the IBM Statistical Package for the Social Sciences (SPSS) version 28.0.1.1 for Mac OS. A multiple regression analysis was used to determine the existence of a relationship (if any) between level of

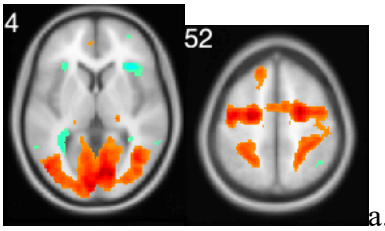
BOLD signal change in each ROI and each oculomotor metric of the four tasks used in fMRI. For each task, multiple comparisons were made, with the BOLD signal change for each ROI of a given task as the independent variables and a metric from a given task as the dependent variable. The same analysis was repeated for each oculomotor metric.

3. Results

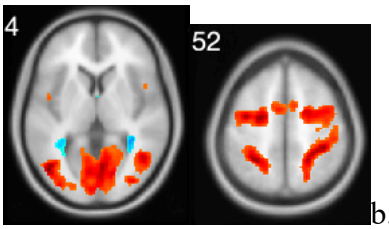
3.1. fMRI results

Analysis of the fMRI data from the group of healthy controls detected various task-related activation foci (Fig. 1). The anatomical location of the activation peaks was identified by superimposing activation maps onto the high-resolution anatomical T1-weighted image in MNI space (Table 2). All the activation peaks showed elevated BOLD signals. During the Smooth Pursuit task, there was an increase in activation in the cuneus, superior parietal lobule, paracentral lobule, cerebellar tonsil, and inferior parietal lobule. In the Saccades task, increased BOLD signals were observed in the middle frontal gyrus, postcentral gyrus, and medial frontal gyrus. In the anti-saccades task, there was an increase in BOLD signal in the precuneus, inferior parietal lobule, and middle frontal gyrus. Finally, in the Optokinetic Nystagmus task, increased activation was seen in the middle temporal gyrus, precuneus, cerebellar tonsil, anterior cingulate cortex, postcentral gyrus, and middle frontal gyrus. These fMRI results were associated with normal performances on the oculomotor tasks as indicated by the data collected with the eye-tracker goggles prior to MRI scanning.

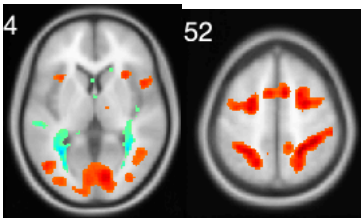
Fig. 1. Activation during the oculomotor tasks. a) Smooth Pursuit; b) Saccades; c) Anti-Saccades; d) OKN.



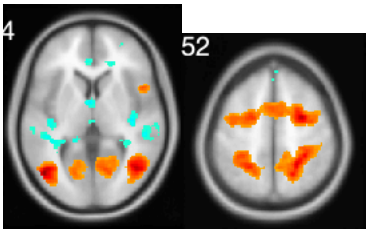
a.



b.



c.



d.

Table 2. MNI coordinates and T- and p-values for each activation peak based on the whole brain analysis results and change in BOLD% for each activation peak according to ROIs analysis.

Task	Peaks	Hemisphere	x	y	z	T value	p value	Average BOLD signal % increase
	Cuneus		-10	-98	14	10.91	0	

Smooth Pursuit	BA7 (Superior parietal lobule)	Left	-24	-54	64	7.59	0.001	0.95
	Cerebellar Tonsil		-18	-52	-48	6.08	0.041	0.23
	Paracentral Lobule (Frontal lobe)		-16	34	50	4.49	0	0.33
	Inferior Parietal Lobule		-48	-36	24	4.32	0.022	0.43
	Cerebellar Tonsil (Cerebellum	Right	16	-54	-48	5.68	0.015	0.23

	Posterior Lobe)							
Saccades	Middle Frontal Gyrus	Left	-36	-4	50	8.11	0.001	0.57
	Postcentral Gyrus	Right	40	-30	42	7.55	0.001	0.43
	Medial Frontal Gyrus		8	-28	68	6.02	0.034	0.26
Anti-Saccades	BA7 (Precuneus)	Left	-20	-62	54	8.34	0	0.87
	Inferior Parietal Lobule	Right	38	-34	40	9.76	0	0.58
	BA6 (Middle Frontal Gyrus)		28	-2	48	8.09	0	0.54
Optokinetic Nystagmus	Middle Temporal Gyrus		-44	-70	8	10.85	0	0.99
	Precuneus		-24	-50	54	7.85	0.001	0.65

	Cerebellar Tonsil	Left	-14	-54	-48	6.67	0.011	0.32
	BA24 (Anterior cingulate cortex)		-14	-18	42	6.41	0.19	0.25
	Postcentral Gyrus		-52	-22	38	3.83	0.024	0.40
	BA 6 (Middle Frontal Gyrus)	Right	28	-2	50	9.14	0	0.67

3.2. Behavioural results

VR eye-tracking tasks

The results acquired using the ® with the VR-goggles eye-tracking system are presented in Table 3. This table includes mean value acquired on each metric with standard deviations as well as range of the values collected on healthy controls in this study in the column 3, and the norms for oculomotor tasks established by the software manufacturer (NeuroFlex). These norms were calculated using a sample size >= 717 healthy adults, ages 18-55. These norms continue to be updated by the software company as more data are acquired.

Table 3. Results on oculomotor tasks using VR system in the group of healthy controls

Task	Mean (±SD)	Range (obtained in this study)	Norms (obtained by Neuroflex©)
Smooth Pursuit (Head Free)			
Mean Vergence (degrees)	-0.10 (±1.05)	-2.2 – 2.0	-7.5 – 7.4
Vergence SD (degrees)	1.46 (±2.10)	-2.74 - 5.66	0 – 7.5
Mean Error (degrees)	2.54 (±0.76)	1.02 – 4.06	0 – 8.8
Number of Saccades (saccades)	14.83 (±7.61)	<30.05	<31
Head Contribution (%)	88.51 (±17.10)	54.31 -122.71	>60
Smooth Pursuit (Head Fixed)			
Mean Vergence (degrees)	0.56 (±0.80)	-1.04 – 2.16	-3 – 3.8
Vergence SD (degrees)	1.76 (±1.10)	-0.44 – 3.96	0 – 7
Mean Error (degrees)	2.17 (±0.60)	0.97 – 3.37	0 – 7.4
Number of Saccades (saccades)	15.16 (±10.75)	<36.66	<31
Vor (Horizontal)			

Mean Vergence (degrees)	0.30 (±1.30)	-2.3 – 2.9	-7.4 – 8.1
Vergence SD (degrees)	1.93 (±1.52)	-1.11 – 4.97	0 – 7.2
Gain Left (%)	92.81 (±7.45)	77.91 – 107.71	67 – 113
Gain Right (%)	93.05 (±7.70)	77.65 – 108.45	66 – 112
VOR (Vertical)			
MeanVergence (deg)	0.09 (±0.72)	-1.35 – 1.53	-6.8-7.1
Vergence SD (degrees)	0.98 (±0.43)	0.12 – 1.84	0 – 5.2
Gain Up (%)	90.94 (±6.69)	77.56 – 104.32	66 – 110
Gain Down (%)	88.95 (±8.23)	72.49 – 105.41	61 – 110
Saccades			
Mean Vergence (deg)	0.03 (±0.64)	-1.25 – 1.31	-5.4 – 5.5
Vergence SD (deg)	1.26 (±0.57)	0.12 – 2.4	0 – 6.6
Acquisition Error (deg)	2.15 (±0.68)	0.79 – 3.51	0 – 9.4
Mean Latency (ms)	244.90 (±26.98)	190.94 – 298.86	0 – 308
Anti-Saccades			
Mean Vergence (degrees)	0.42 (±0.82)	-1.22 – 2.06	-6.4-7.1

Vergence SD (degrees)	1.66 (±1.36)	-1.06 – 4.38	0 – 7.7
Acquisition Error (degrees)	5.71 (±2.85)	0.01 – 11.41	0 – 12
Mean Latency (ms)	464.13 (±103.32)	257.49 – 670.77	0 – 664
Directional Accuracy (%)	70.16 (±23.84)	22.48 – 117.84	>30
<u>OKN</u>			
Mean Vergence (degrees)	-0.13 (±1.84)	-3.81 – 3.55	-8.3 – 6.9
Vergence SD (degrees)	2.15 (±1.04)	0.07 – 4.23	0 – 8.6
Gain Left (%)	77.05 (±10.50)	56.05 – 98.05	49 – 110
Gain Right (%)	77.38 (±13.49)	50.4 – 104.36	46 – 112
Gain Up (%)	68.02 (±12.58)	42.86 – 93.18	39 – 102
Gain Down (%)	69.23 (±12.75)	43.73 – 94.73	36 – 101
<u>SPN</u>			
Mean Tremor Frequency (saccades/second)	0.25 (±0.18)	<0.61	<0.9

Average Drift (degrees/second)	0 (±0)	0	-
Mean Tremor Velocity (degrees/second)	56.10 (±31.78)	<119.66	-

3.3. Regression analysis BOLD signal vs. Oculomotor Metrics

The regression analysis showed a positive correlation between the metric "Gain Down" of the OKN task and three highlighted regions of interest (i.e., activation peaks): cingulate gyrus ($p = 0.019$), cerebellar tonsil ($p = 0.03$) and postcentral gyrus ($p = 0.001$) (Table 4). Other comparisons did not reveal any significant results.

Table 4. Results of regression analysis

Region	Task	Metric	p value
BA24 (Cingulate Gyrus)	OKN	Gain Down	0.019
Cerebellar Tonsil			0.03
Postcentral Gyrus			0.001

4.Discussion

The problem of mTBI diagnosis remains standing, with one of the main deficits being alterations in oculomotor function. In the present study, we have explored the diagnostic potential of a set of oculomotor tasks using VR-goggles, an eye-tracking system, and fMRI. The data acquired from

31 healthy controls aimed to evaluate the range of normal performances on the oculomotor tasks and to determine regions of brain activation related to these tasks for further investigation with concussed subjects. During the oculomotor tasks, the following peak regions of activation in the healthy controls were identified: (1) **Smooth pursuit** – cuneus, superior parietal lobule, paracentral lobule, inferior parietal lobule and cerebellar tonsil, (2) **Saccades** – middle frontal gyrus, postcentral gyrus and medial frontal gyrus, (3) **Anti-saccades** - precuneus, inferior parietal lobule, and middle frontal gyrus, and, finally, (4) **OKN** - middle temporal gyrus, anterior cingulate cortex, postcentral gyrus, middle frontal gyrus, and cerebellar tonsil. These results were all associated with normal performances on the tasks.

4.1. Areas involved in oculomotor functions

The brain regions identified in this study are in line with many of the well-known studies measuring smooth pursuit, OKN, voluntary saccades and anti-saccades (Ruehl et al., 2021, Berman et al., 1999, Sweeney *et al.,* 1996, Koval et al., 2014, Kimming et al., 2001). The coordinates of the activation peaks obtained in this study were compared to those of previous studies (Table 5) to identify if anatomical coordinates indicated in this study were corresponding with functional areas, especially related to implementation of oculomotor movements. Thus, according to previous findings, the following functional areas were involved in tasks implementation: 1) **Smooth Pursuit**: there was increased activation in the left PEF (Berman et al., 1999); 2) **Saccades**: increased activation in the left superior FEF (Luna et al., 1998) and right SEF (Sweeney *et al.,* 1996); 3) **Anti-Saccades**: activations were seen in the PEF bilaterally (Berman et al., 1999, Luna et al., 1998) and the right superior FEF (Luna et al., 1998; Berman et al., 1999; Kimming et al., 2001); 4) **OKN**: increased activation in the left MT (Kolster, Peeter & Orban, 2010), PEF (Berman

et al., 1999), CEF (Koval et al., 2014) and right Superior FEF (Luna et al., 1998; Berman et al., 1999; Kimming et al., 2001).

Table 5. Eye fields corresponding to activation peaks in the current study.

Task	Peaks	Hemisphere	Eye Field	x	y	z
Smooth Pursuit	Cuneus	Left	V1	-10	-98	14
	BA7 (Superior parietal lobule)		PEF (Berman et al., 1999)	-24	-54	64
Saccades	Middle Frontal Gyrus	Left	Superior FEF (Luna et al., 1998)	-36	-4	50
	Medial Frontal Gyrus	Right	SEF (Sweeney *et al.,* 1996)	8	-28	68
Anti-Saccades	BA7 (Precuneus)	Left	PEF (Berman et al., 1999)	-20	-62	54

	Inferior Parietal Lobule	Right	PEF (Luna et al., 1998)	38	-34	40
	BA6 (Middle Frontal Gyrus)		Superior FEF (Luna et al., 1998; Berman et al., 1999; Kimming et al., 2001)	28	-2	48
Optokinetic Nystagmus	Middle Temporal Gyrus	Left	MT (Kolster,, Peeter & Orban, 2010)	-44	-70	8
	Precuneus		PEF (Berman et al., 1999)	-24	-50	54
	BA24 (Anterior cingulate cortex)		CEF (Koval et al., 2014)	-14	-18	42

	BA 6 (Middle Frontal Gyrus)	Right	Superior FEF (Luna et al., 1998; Berman et al., 1999; Kimming et al., 2001)	28	-2	50

Furthermore, other areas less known for their involvement in oculomotor movement were identified as activation peaks. For instance, an activation peak in tonsil complex of the cerebellum was observed during Smooth Pursuit, as well as during the OKN task, where Smooth Pursuit eye movements play a significant role. Previous studies have shown activation in the tonsil complex of the cerebellum, attributing it to primarily high-frequency, transient vestibular responses, and for smooth pursuit maintenance and steady gaze holding (Shemesh & Zee, 2019). In addition, in monkeys, impairment of the tonsil complex homologue (the flocculus/paraflocculus) led to impaired smooth pursuit, and incomplete suppression of an induced but unwanted vestibular nystagmus (Rambold et al., 2002).

Additionally, activation peaks were found in such areas as cuneus (in Smooth Pursuit) and inferior parietal lobule (Anti-Saccades). According to a review by Kobayashi, visual information is processed through the dorsal visual pathway, reaching the inferior parietal lobule, the intraparietal sulcus and the precuneus (Kobayashi, 2016). Pierrot-Deseilligny et al. showed that the inferior parietal lobule is involved in the visuospatial integration used for calculating saccade amplitude

(Pierrot-Deseilligny et al., 1995). In addition, the inferior parietal and ventral occipital cortices are involved in trans saccadic processing of visual object orientation (Dunkley et al., 2016) and the cuneus holds a specific role in spatial frequency processing which contributes significantly to pursuit eye movements (Baltaretu et al., 2021).

Moreover, during the OKN task, an activation peak was identified in BA24 (anterior cingulate cortex), which is attributed to Cingulate Eye Field (CEF). CEF is less discussed in the literature than traditional eye fields such as the FEF, SEF and PEF. It has been suggested that CEF controls early activation of the frontal ocular motor and premotor areas in the brainstem (Gaymard et al., 1998; Ruehl et al., 2021). Earlier fMRI studies provided evidence for an involvement of the anterior cingulate cortex in OKN (Berman et al., 1999; Dieterich, Bucher, Seelos, & Brandt, 1998). In addition, Dieterich et al. have described activations in BA 24 in 10 subjects during OKN (Dieterich et al., 1998), a result in keeping with our findings.

Activation in the postcentral gyrus was detected during the Saccades and OKN tasks and this region has been involved in saccadic movements (Grosbras, Laird & Paus, 2005; Khonsari et al., 2007). In the present study, we saw activation of the postcentral gyrus in both saccades and OKN, which can be explained by the fact that saccadic eye movements are one of the components of OKN.

Finally, the observed increase in BOLD signal in the paracentral lobule is consistent with previous findings by Gurler (2012) and Agtzidis et al. (2020), which detected activation in this area during smooth pursuit and saccadic eye movements.

Overall, these findings contribute to our understanding of the neural mechanisms underlying different types of eye movements and suggest that these movements involve complex interactions between multiple brain regions.

4.2. Areas affected in concussion and future directions

The underlying neurophysiology of oculomotor functions can be disrupted following mTBI (Quintana et al., 2021). Previous studies showed difficulties in smooth pursuit, saccades, and anti-saccades in concussed patients, as well as alterations in BOLD signals in related functional brain areas (e.g., cerebellum, frontal lobes, primary and secondary visual cortex, and visual area V5/MT) (Johnson et al., 2014). Elevated activation during performance of saccadic movements in concussed subjects compared to controls has also been seen, which implies that compensatory mechanisms maintain functional performance when minor deficits in the networks are present (Johnson et al., 2014).

The activation peaks identified in the current study are for the majority overlapping with many of the affected areas in concussion (such as middle temporal gyrus, cingulate cortex, precuneus, middle frontal gyrus, inferior parietal lobule, visual cortex, cerebellum, postcentral gyrus, medial frontal gyrus, superior parietal lobule, etc.) according to previous fMRI studies (Saluja et al., 2015; Westfall et al., 2015; Sullivan et al., 2018; Wylie et al., 2015; Chen et al., 2016; Cook et al., 2020; Van der Horn et al., 2016, Slobounov et al., 2014; Henry et al., 2011, Kawasaki et al., 2015, Mayer et al., 2015). Given that these areas are involved in the implementation of the oculomotor tasks described in this study, we are confident that these tasks can be a sensitive tool in the evaluation of visual functional deficits in the diagnosis of concussion.

Based on ROI analysis and the areas of significant alterations of BOLD signal in the current study, these ROIs can be highlighted for future studies investigating concussed patients. According to the results of this pilot study and based on the findings from previous studies (Ruehl et al., 2021; Luna et al., 1998; Berman et al., 1999; Kimming et al., 2001; Koval et al., 2014; Johnson et al., 2014; Saluja et al., 2015; Westfall et al., 2015; Sullivan et al., 2018; Wylie et al., 2015; Chen et al., 2016; Cook et al., 2020; Van der Horn et al., 2016, Slobounov et al., 2014; Henry et al., 2011; Kawasaki et al., 2015; Mayer et al., 2015), we identify ROIs for each task described in Table 6.

Table 6. Potential ROIs.

Task	**Left**	**x**	**y**	**z**	**Right**	**x**	**y**	**z**
Smooth Pursuit	Superior parietal lobule (BA7)	-24	-54	64	Cerebellar Tonsil	16	-54	-48
	Cerebellar Tonsil	-18	-52	-48				
	Paracentral Lobule	-16	34	50				
	Inferior Parietal Lobule	-48	-36	24				
Saccades	Middle Frontal Gyrus	-36	-4	50	Postcentral Gyrus	40	-30	42
					Medial Frontal Gyrus	8	-28	68
Anti-Saccades	Precuneus (BA7)	-20	-62	54	Inferior Parietal Lobule	38	-34	40

					Middle Frontal Gyrus (BA6)	28	-2	48
Optokinetic Nystagmus	Middle Temporal Gyrus	-44	-70	8	Middle Frontal Gyrus (BA6)	28	-2	50
	Precuneus	-24	-50	54				
	Cerebellar Tonsil	-14	-54	-48				
	Anterior cingulate cortex (BA24)	-14	-18	42				
	Postcentral Gyrus	-52	-22	38				

Our study has the potential to identify novel brain areas involved in eye-movements related to Optokinetic Nystagmus in concussed individuals. To our knowledge, there are no studies using this task with fMRI in such a patient group. As OKN requires multiple components for its execution (Smooth Pursuit eye movements, saccadic eye movements, VOR), it also involves the highest number of brain areas (middle temporal gyrus, precuneus, cerebellar tonsil, anterior cingulate cortex, postcentral gyrus, and middle frontal gyrus) in comparison to other tasks in this study (i.e.., smooth pursuit, saccades, or anti-saccades). It also was the only task which showed

significant results in regression analysis, showing potential involvement of several brain regions (left cingulate gyrus, postcentral gyrus, and cerebellar tonsil) in the process of eye movements in the act of gaining down. Of note, in previous studies, it was demonstrated that cerebellar tonsil is crucial to VOR and pursuit gain (Beh et al., 2017).

Our ROIs coordinates are in keeping with those of previous studies on brain activation in oculomotor tasks using fMRI. The role of these areas in oculomotor function is also highlighted in multiple studies on concussion throughout the past decade. At this point, we predict that concussed individuals will have alterations in BOLD activation when completing oculomotor tasks and that their performances will be altered when executing the tasks through use of the VR goggles. This underlines the clinical potential of these tools in the diagnosis of concussion.

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In Chapter 2, we presented normative data for our study of oculomotor metrics using a system of VR goggles with a built-in eye-tracker and we established potential ROIs for comparison of concussed subjects to healthy controls. It was the first stage of our project, which aimed to determine task-related areas of activation and the nature of BOLD signal pattern changes in these areas, as the stimuli we used were novel, and no previous studies used these versions of the tasks.

Of note is one of the points highlighted in the results which we did not discuss in the manuscript presented in the Chapter II. The regression analysis showed a positive correlation between the metric "Gain Down" of the OKN task and BOLD signal% change in postcentral gyrus, among others (cerebellar tonsil and cingulate gyrus). Postcentral gyrus os the primary sensory cortex, and not typically considered as a structure directly involved in eye movements. However, we see increase in BOLD signal% change during Saccades and Optokinetic Nystagmus. The possible explanation is that while the postcentral gyrus is not directly involved in generating oculomotor movements, it contributes to the overall sensory processing that informs the brain about the position and movement of different body parts, including the head and eyes, which was shown in several studies with monkeys (Wang et al., 2007; Zhang et al., 2008). The integration of sensory information from various regions of the brain, including the postcentral gyrus, is essential for the coordinated control of eye movements.

The ROIs we highlighted in Chapter 1 are in keeping with previous studies using similar tasks, and, according to the literature, all the discussed areas are related to oculomotor functions.

Previous fMRI findings showed that the identified areas activated during the oculomotor tasks used in our study overlap with many of the affected areas in concussion.

These results suggest that the involvement of brain areas susceptible to mTBI in implementing these tasks, taken together with commonly reported oculomotor difficulties post-concussion, may lead to finding objective biomarkers using Neuroflex tasks. Thus, the goal of the next stage of our study, described in the next chapter, is to investigate oculomotor performance and %BOLD signal change during these tasks by concussed subjects and compare these metrics to age- and sex-matched healthy controls. We predicted that concussed individuals would have alterations in BOLD activation when completing oculomotor tasks and that their performances would be altered when executing the tasks through VR goggles with a built-in eye-tracker.

The outcome or natural history of eye movement abnormalities induced mTBI can vary widely among individuals. In some cases, these abnormalities may resolve on their own over time, while in other cases, they may persist over months or even years and contribute to ongoing visual symptoms. Factors influencing the outcome include the severity of the injury, individual differences in healing, and the presence of other concurrent issues (Gasquoine, 1997; Sussman et al., 2016).

Chapter III. Assessment of Oculomotor Functions as a Biomarker in Mild Traumatic Brain Injury: An Integrated Eye-Tracking and fMRI Study

Ekaterina Lunkova[1], Jen-Kai Chen[1], Rajeet Singh Saluja[1,3], Alain Ptito[1,2,3]

[1]Department of Neurology & Neurosurgery, McGill University, Montreal, Quebec, Canada.

[2]Montreal Neurological Institute, Montreal, Quebec, Canada.

[3]McGill University Health Centre Research Institute, Montreal, Quebec, Canada.

1. Introduction

Mild traumatic brain injury (mTBI), often used synonymously with concussion, is a major public health problem. MTBI can be a risk factor for, among others, a decline in cognitive functions, early dementia, and mental illness (McInnes et al., 2017), creating serious challenges for society and economics.

Ambiguity still exists regarding the pathophysiology and management of concussions, leaving an objective diagnosis a topical problem. Previous studies by our team (Chen et al., 2004; Keightley et al., 2014) demonstrated that fMRI is an objective approach that allows consistent, reproducible results as a screening tool for concussion. However, given the limited availability of fMRI in clinical or on-the-field settings, a more efficient and less resource-consuming approach is needed.

Oculomotor dysfunction is a prevalent condition in patients who have suffered a mild traumatic brain injury, with up to 90% exhibiting impairments in the brain's ability to coordinate eye

movements with accuracy and control (Ciuffreda et al., 2016; Cade & Turnbull, 2022). Post-concussion, individuals frequently report experiencing oculomotor symptoms, such as blurred vision, convergence insufficiency, diplopia, difficulty reading, headaches, dizziness, nausea, general asthenopia, and impaired ability to scan visual information. Additionally, they may experience difficulties in tracking moving objects (Kapoor & Ciuffreda, 2002; Kontos et al., 2017). The oculomotor symptoms experienced by individuals post-concussion may lead to functional impairments, such as difficulties reading, decreased academic performance, and cognitive impairments (Kapoor & Ciuffreda, 2002; Kontos et al., 2017). These functional impairments can be attributed to the disruption of the underlying neurophysiology of oculomotor functions caused by concussion (Quintana et al., 2021).

MTBI may be a leading cause of clinically impaired smooth pursuit and saccadic eye movements (Cochrane, et al., 2019). Performance in an anti-saccades task was shown to be impaired in concussed subjects; these subjects also showed hyperactivation in the cerebellum, primary and secondary visual cortex, and visual area V5/MT (Johnson et al., 2014). Furthermore, previous studies with subjects who sustained mTBI compared to controls have demonstrated increased activation in these areas while producing saccadic movements. A study by Hecimovich et al. (2022) with college rugby players tested before and after the season showed significant differences between concussed and non-concussed groups for total saccades, with differences from baseline to follow-up observed for saccade velocity in both groups. Even 3–6 months after mTBI, patients with prolonged post-concussive symptoms demonstrated impaired production of anti-saccades, memory-guided saccades, and self-paced saccades, in comparison to fully recovered patients (Kraus et al., 2007; Heitger et al., 2009; Ventura et al., 2016).

Astafiev et al. (2015) demonstrated that smooth pursuit may exhibit increased variability and be susceptible to disruption under higher cognitive loads, which corresponds to differences in activation of the right inferior frontal gyrus and basal ganglia. Another study by Johnson and colleagues (2014) showed that during smooth pursuit eye movements post-concussion, increased activation was observed in the cerebellum, frontal lobes, and visual cortices, although no significant differences were observed in the performance of concussed group compared to healthy individuals. Increased activation may be explained by compensatory mechanisms in which functional changes in brain resources contribute to correct task performance without permanent alterations in networks (Johnson et al., 2014). Overall, the findings on the smooth pursuit eye movements post-concussion remain ambiguous.

One of the other eye movements commonly evaluated by eye-tracking systems is Optokinetic Nystagmus (OKN). To our knowledge, no studies to date have used tasks evaluating OKN in fMRI with concussed subjects, and its potential for concussion diagnosis is yet to be determined. As OKN consists of two components – (1) Smooth Pursuit and (2) Saccades (Lencer et al., 2019), it can be suggested that similar patterns of brain activation observed in individuals with mTBI during smooth pursuit and saccadic eye movements may also be present during OKN.

Due to the high speed of saccadic and pursuit movements, oculomotor impairments are often missed during clinical examinations, highlighting the need for sensitive screening tools in mTBI diagnosis, such as eye-tracking systems (Snegireva et al., 2018). Recent findings demonstrated that eye-tracking metrics correlate with concussion symptoms and can detect convergence and

accommodative abnormalities associated with concussion. Therefore, the use of an eye-tracking system as a rapid, objective, and non-invasive tool for diagnosing mTBI appears to be warranted (Zahid et al., 2020). Furthermore, considering the highly variable recovery trajectory following mTBI, it is crucial to utilize reliable and objective oculomotor function tests to monitor patient outcomes. Non-invasive eye-tracking experiments using these techniques are widely employed across various research domains, such as vision science, psychology, sport and exercise sciences, automotive sciences, marketing, and the gaming industry (Cade & Turnbull, 2022; Kaae et al., 2022).

Of note, not all concussed subjects complain of eye-movement-related symptoms, which suggests that some of them have preserved oculomotor functions. In everyday life, this deficit can go unnoticed; however, under increased workload condition (i.e., in sports) these subjects' difficulties may surge. Thus, it is of utmost importance to use more sensitive oculomotor tasks that have the potential to reveal more profound deficits. Data collection from a comprehensive combination of sensory and motor circuits will shed light on the underlying mechanisms of oculomotor function and the extent of their vulnerability to mTBI.

In this study, we aimed to:

(1) examine blood oxygen level-dependent (BOLD) fMRI alterations associated with performances in oculomotor function after mTBI (evaluating saccades, anti-saccades, smooth pursuit and OKN),

(2) evaluate the efficacy of the oculomotor assessment in detecting oculomotor and gaze deficits following mTBI

2. Methods

2.1. Participants

29 concussed symptomatic adults within one month (1-5 weeks) post-injury were selected according to the WHO task force criteria (8 males/21 females, mean age = 28.3, SD = 10) and a group of 29 sex- and age-matched adult healthy control subjects (mean age = 29.1, SD = 9.7) without a history of neurodevelopmental or neurological disorders, head injuries, ADHD, and/or presence of significant abnormalities seen on structural MRI scans (assessed by a clinician) were included in the study.

2.2. Oculomotor functions assessment using the VR-goggles eye-tracking system.

The oculomotor evaluation was conducted prior to MRI scanning using virtual reality (VR) goggles equipped with binocular recordings in 3D (horizontal, vertical, and pupil size) and head recordings in 6D (3D angular and 3D linear accelerations). These were recorded concurrently for eye and head angles at a 120 Hz sampling rate. A high-speed laptop computer generated the goggle visual displays and recorded the ocular and head data synchronously. Eye and head movements were evaluated in response to visual and vestibular stimuli, or lack thereof (e.g., to evaluate spontaneous nystagmus), to detect deviations from 'normal' eye and head responses of healthy subjects. Table 1 summarizes the methods and metrics evaluated. The full evaluation consisted of a battery of tests that takes less than 10 minutes to administer, including three head-free conditions (Smooth Pursuit (head-free), Active Visual Vestibulo-Ocular Reflex (VOR, Horizontal), Active Visual VOR (Vertical)) and five head-fixed conditions (Smooth Pursuit (head-fixed), Saccades, Anti-saccades, Optokinetic Nystagmus (OKN), Spontaneous Nystagmus) (Table 1). Afterwards,

four tasks – Smooth Pursuit, Saccades, Anti-Saccades and OKN – were repeated during fMRI to measure brain activation associated with performances on the tasks. Only head-fixed conditions were chosen for use inside the MRI scanner.

Table 1. Eye-Head coordination tests and measured variables with units

	System of interest (protocol)	**Measured aspect of Metrics**
1	Saccades (flashed targets, self-paced)	Delay (ms) Accuracy (degrees)
		Generation rate (S/sec) Main sequence (peak velocity vs. duration)
2	Anti-saccades	Accuracy (degrees)
		Latency (ms)
3	Active head-fixed or head-free passive VOR active VOR, pursuit, OKN	Mean vergence over the whole test period (sac/min)
		Vergence for each phase of movement (saccade and fixation; degrees)
4	Nystagmus during active gaze shifts head-fixed or head-free Spontaneous nystagmus in the dark Vestibulo-Ocular Reflex Optokinetic Nystagmus	Asymmetry of peak response, phase lag (%)
		Full response characterization in both phases with numeric parameters
		Generation frequency
		Tracking error, gaze stabilization (degrees)
5	Head free gaze shifts	Eye vs head contributions
6	2D Target tracking head-fixed or head-free smooth pursuit and corrective saccades	Accuracy in different initial positions (degrees)
		Corrective saccade rate (S/sec)
		Response symmetry
7	Pupil size	Diameter (mm)

2.3. Image acquisition

All scanning was performed on a Siemens 3 Tesla MRI system equipped with a 64-channel head coil at the Montreal Neurological Institute (MNI) BIC MRI platform. First, T_1-weighted images were acquired for anatomical reference (3D MP-RAGE, TR=2300ms, TE=2.98ms, 176 slices,

slice thickness=1mm, FOV = 256mm, image matrix = 256 x 256, flip angle = 9 degrees, interleaved excitation) for fMRI data. fMRI data was acquired using BOLD activation studies with T2* weighted GE-EPI (TR=3000ms, TE=30ms, 38 slices, slice thickness=4mm, FOV=256mm, image matrix=128x128, interleaved excitation).

2.4. Oculomotor tasks used in fMRI

We used task-based fMRI with 4 tasks evaluating oculomotor functions: (1) **Smooth Pursuit**: subjects were asked to follow a moving target (dot) with their eyes; (2) **Saccades:** subjects were warned that the dot will jump around the screen, and that they had to follow it with their eyes only; (3) **Anti-saccades**: subjects had to look at the dot at the center of the screen – when a red X appeared, they had to avoid looking at the red X and instead orient their eyes into the opposite field of view in the same location – then follow the dot back to the center; (4) OKN: subjects were asked to pick a dot and follow it until it left their field of view, and to continue in the same manner with each subsequent dot; and, (5) **Baseline condition**: a) Prior to each task, the baseline condition was presented to the subjects (for conditions (1), (2) and (3) it was a fixed dot at the center of the screen for a duration of 12 seconds; for condition (4), it was a fixed field of dots for a duration of 15 seconds). Each of the conditions lasted 30 seconds, while subjects were head-fixed and asked to complete the tasks by moving their eyes only. Two identical functional scanning sessions were conducted sequentially. Each scanning session lasted 6 minutes and consisted of two runs of the set of 4 tasks. The subjects underwent extensive training prior to the scanning to ensure familiarity with the tasks. Because the participants' heads had to be fixed during MRI scanning, these tasks were chosen because they also had the same requirement. Other head-free conditions in the oculomotor screening battery were therefore left out (Head-Free Smooth Pursuit, VOR

Vertical and Horizontal). One of the five head-fixed tasks was not used during fMRI – Spontaneous Nystagmus – because it was recently added to the oculomotor screening battery and lacks normative data.

2.5. Behavioural analysis (Oculomotor & Gaze assessment)

The data from the VR goggles and the eye-tracking system were automatically gathered and processed through the Neuroflex® software system. The results for each subject included all metrics enumerated in Table 1, and the deviations of the results (if any) were described in individual reports. Mean values, standard deviation (SD), and normative range of the results in the group of healthy controls were calculated. The normative range was counted as Mean+/-2SD.

2.6. Neuropsychological assessment

Neuropsychological assessment was used to identify the correlation between the severity of cognitive functional impairment, the imaging findings and performance on the oculomotor tasks. The domains included in the neuropsychological assessment were selected according to their sensitivity to mTBI as demonstrated in previous studies: attention, working memory, processing speed, and problem-solving (Lunkova et al., 2021). The neuropsychological assessments consisted of the following tests: Verbal Working Memory Task (M. Petrides), Rey Auditory Verbal Learning Test (RAVLT), Trial Making Test (TMT), Purdue Pegboard, Tower of London, WAIS-IV Processing Speed Index Subtests (Symbol Search, Coding), Symbol Digit Modalities Test (SDMT). The neuropsychological tests are conducted to determine if cognitive difficulties are related to structural and hemodynamic alterations identified by MRI sequences and/or to oculomotor problems.

2.7. Questionnaires

Concussed individuals were asked to fill out the following questionnaires prior to participating in the study: Post-Concussion Symptom Scale (PCSS), Beck Anxiety Inventory (BAI), Beck Depression Inventory II (BDI-II), and Dizziness Handicap Inventory (DHI)**.** Concussed subjects were identified as symptomatic/asymptomatic according to their results on the PCSS, and only symptomatic subjects were included in the study (PCSS score>21). BAI and BDI-II scores were used to identify the possible presence of depression/anxiety and its effect on task performance.

2.8. Data Processing and Statistical Analysis

2.8.1. *MRI processing*

Task-based fMRI. All MRI images were preprocessed and analyzed using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). During the preprocessing stage, all the functional images were realigned and unwrapped; slice-time corrected; co-registered to a T1-weighted reference image; structurally and functionally normalized and segmented into gray matter, white matter, and CSF tissue; and smoothed using a 6 mm Gaussian kernel. The preprocessed images for each subject then underwent first-level analysis, where the model was specified and estimated for the two runs of the tasks. The model was specified using the conditions and onset times for each task. To determine an alteration of the level of BOLD signal specific to each task, the contrasts were identified between the task condition and the baseline condition. Afterwards, the second level analysis was conducted to identify BOLD signal changes during each task respectively for the group of healthy controls and concussed subjects. Exploratory brain analysis resulted in whole brain activation maps. Between-group comparison of whole-brain maps was conducted using a 2-sample T-test with FWEr correction for p-values. Subsequently, ROIs

were extracted from whole-brain analysis; prior to this, the sphere of 5mm radius was used to create a mask for each ROI using the MarsBaR toolbox (Brett et al., 2002) (ROIs were based on the results of 31 healthy controls (Lunkova et al., 2023) and previous findings using similar tasks). Thereafter, a comparison of the BOLD signal in each ROI between two groups was performed (data checked on normality – data is normally distributed, hence using of 2-sample T-test). Due to multiple comparisons, Bonferroni-Holm correction was applied. Using the results of healthy controls extracted from ROIs, 95% confidence intervals were determined to establish a "normal range" % of BOLD signal change.

2.8.2. Oculomotor assessment

Oculomotor data was gathered using Neuroflex®, as the VR-goggles eye-tracking system was automatically processed through the Neuroflex® software. The results for each subject included all metrics demonstrated in Table 1, and deviations of the results (if any) were identified in individual reports. Data for each metric of each task was tested on normality; in the case of a normal distribution, 2-sample T-test was used, otherwise an U-test was utilized; due to multiple comparisons, Bonferroni-Holm correction was applied.

2.8.3. Correlation between oculomotor metrics and BOLD signal alterations

Oculomotor and BOLD signal analyses and descriptive statistics were performed using the IBM Statistical Package for the Social Sciences (SPSS) version 28.0.1.1 for Mac OS. A multiple regression analysis was used to determine a relationship (if any) between the level of BOLD signal in each ROI and each of the oculomotor metrics of the four tasks used in fMRI. %BOLD signal change in each ROI was entered as a dependent variable, and oculomotor metrics were entered as independent variables; all values were converted to z-scores prior to the implementation of multiple regression

2.8.4. Neuropsychological assessment and questionnaires

Neuropsychological assessment results were converted to standard scores, and several subjects with scores below the normal range were identified according to the norms (Strauss, Sherman & Spreen, 2006) for each task; the conclusions were therefore qualitative in nature. Results on the questionnaires were assessed according to the individual guidelines for each.

2.8.5. Correlation between neuropsychological assessment and questionnaires results and imaging findings

Additional statistical analyses were performed in SPSS 28.0.1.1. To determine correlations with PCSS, BAI, BDI-II, and DHI scores, as well as scores on the cognitive tests, multiple regression analyses were implemented. %BOLD signal change in each ROI was entered as a dependent variable, and results on neuropsychological tests and questionnaires were used as independent variables. All values were converted to z-scores prior to the implementation of multiple regression.

3. Results

3.1.Oculomotor tasks

Compared to healthy controls, concussed subjects showed significant differences in performance on two oculomotor tasks (Anti-saccades and OKN: Fig. 1). During the Anti-Saccade task, they showed significantly higher mean latency between time of stimuli presentation and time of eyes onto target ($p = 0.012$, $d = 0.68$). During the Optokinetic Nystagmus task, the mean eye velocity relative to target velocity when the target moves up/down was significantly lower (up: $p = 0.003$, $d = 0.826$; down: $p = 0.046$, $d = 0.573$) compared to the control group. The results on all the other

metrics did not indicate statistically significant differences. The number of concussed subjects who experienced difficulties with each of the tasks are shown in Table 2.

A.

B.

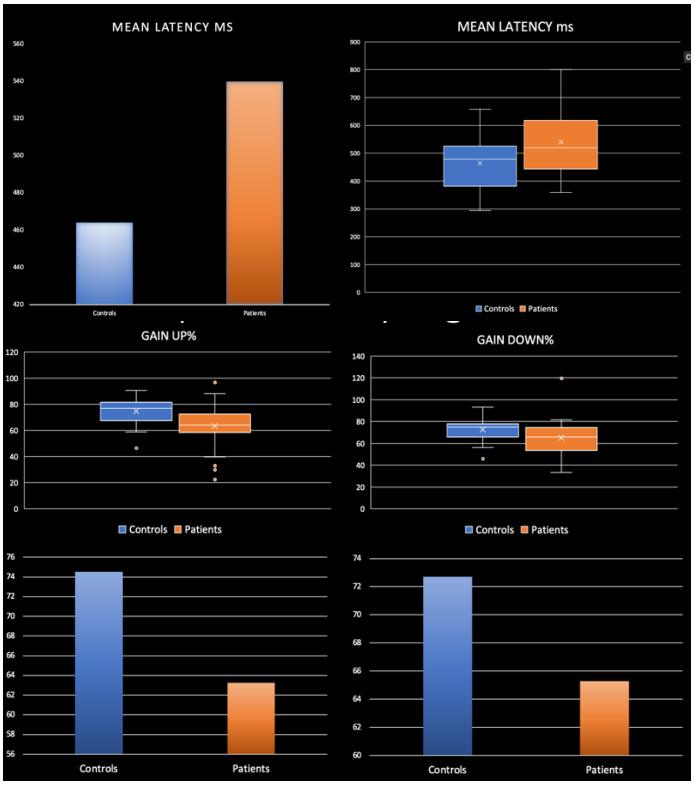


Figure 1. A – Differences between concussed subjects and healthy controls on the Mean Latency metric of the Anti-Saccades task; B - Differences between concussed subjects and healthy controls on the Gain Up/Gain Down metrics of the Optokinetic Nystagmus task.

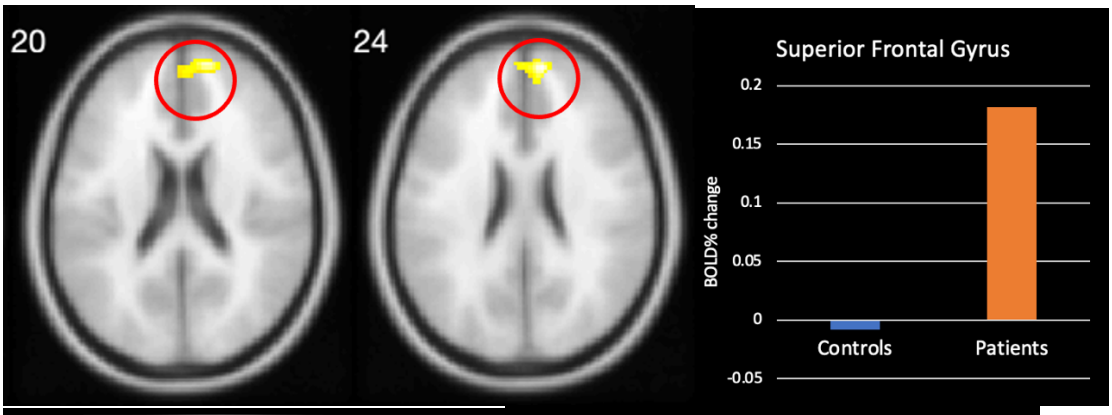
	Patients
SP	1/29
VOR (horizontal)	9/29
VOR (vertical)	8/29
Saccades	9/29
Anti-Saccades	11/29
OKN	15/29

Table 2. Number of subjects outside of the normal range.

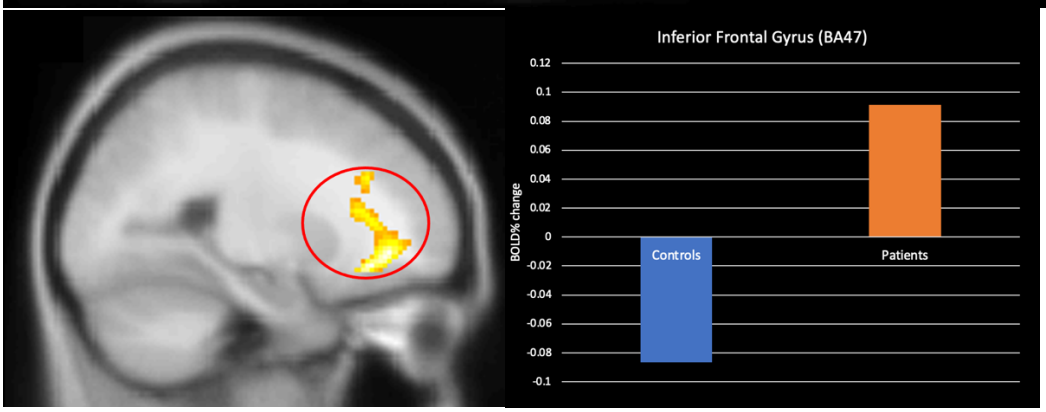
3.2.fMRI results

According to the results of whole brain analysis, the group of concussed subjects appeared to have elevated %BOLD signal change on all four tasks compared to the group of healthy controls. The mTBI group had higher activation in the right superior frontal gyrus (SFG) compared to the control group (Fig. 2A, $p = 0.018$, $d = 1.012$) during the Smooth Pursuit task, in the left inferior frontal gyrus (IFG) during the Saccades task (Fig. 2B, $p = 0.048$, $d = 0.841$), in the right putamen (Fig., 2C, $p = 0$, $d = 1.035$) and left DLPFC (Fig., 2C, $p = 0$, $d = 0.962$) during the Anti-Saccades test, and in the left lingual gyrus (Fig. 2D, $p = 0.005$, $d = 0.937$) and right IFG (Fig. 2D, $p = 0.013$, $d = 0.945$) during the Optokinetic Nystagmus task. ROI analysis, in turn, did not show any significant differences between the groups.

A.



B.



C.

D.

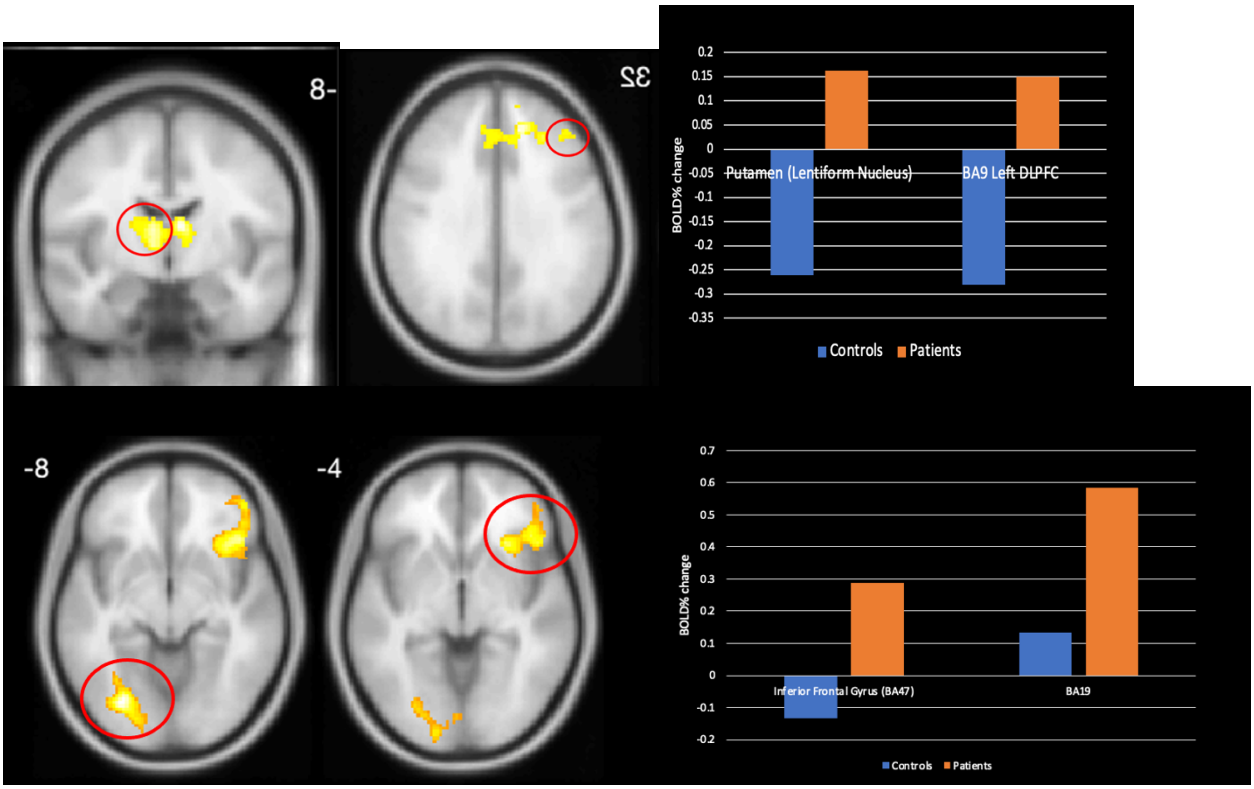


Figure 2. Areas of higher BOLD signal change in concussed subjects compared to healthy controls, with graphs indicating the size of the % BOLD signal change during: A – Smooth Pursuit, B – Saccades, C – Anti-Saccades, D – Optokinetic Nystagmus.

3.3.Neuropsychological assessment

No consistent tendencies in the results of the neuropsychological assessment were observed. Most difficulties experienced by the concussed group were observed on tasks measuring working memory and learning (RAVLT), visual attention and task switching (Trail Making Test), and graphomotor processing speed (SDMT). The number and percentage of concussed participants with results outside of the normal range are indicated in Table 3.

	N of participants with results outside of the normal range	% of participants with results outside of the normal range
RAVLT Learning	13/29	44.8

RAVLT Immediate Recall	6/29	20.7
RAVLT Delayed Recall	7/29	24.1
RAVLT Recognition	7/29	24.1
Pegboard	6/29	20.7
Processing Speed Index	6/29	20.7
Trail Making Test Part A	8/29	27.6
Trail Making Test Part B	14/29	48.3
Tower of London Accuracy	6/29	20.7
Tower of London Speed	3/29	10.3
Symbol Digit Modalities Test	13/29	44.8

Table 3. % of participants with results outside of the normal range.

3.4.Questionnaires

All concussed subjects were symptomatic, and according to the results on the PCSS, the mean score was 44/132 with a SD of 24. The majority (79%) of the concussed subjects showed minimal to mild symptoms of depression, and more than a half of the subjects (55%) showed minimal to mild symptoms of anxiety. In addition, most of the subjects (79%) showed mild to severe symptoms of dizziness and unsteadiness. The means and SDs for all questionnaires are indicated in Table 4. The results of the PCSS, BAI, BDI-II, and DHI are shown in Table 5.

	Mean	SD
PCSS	44.4	23.6
BDI-II	14.1	9.3
BAI	17.3	14.2
DHI	32.6	21.4

Table 4. Mean and SD on each questionnaire.

	Number of concussed subjects	% of concussed subjects

BDI-II		
Normal	18/29	62
“Mild depression”	5/29	17
“Moderate depression”	4/29	13.8
“Severe depression”	2/29	6.9
BAI		
Normal	9/29	31
“Mild anxiety”	7/29	24.1
“Moderate anxiety”	7/29	24.1
“Severe anxiety”	6/29	20.7
DHI		
Normal	6/29	20.7
“Mild handicap”	12/29	41.4
“Moderate handicap”	5/29	17
“Severe handicap”	5/29	17

Table 5. Results of the PCSS, BAI, BDI-II, and DHI questionnaires.

3.5. Correlation between the neuroimaging findings, performance on neuropsychological assessment and oculomotor tests, and results on the questionnaires

Results of multiple regression analyses showed a strong correlation between one of the ROIs of the Anti-Saccades task – the precuneus – and the results on the DHI ($r^2 = 0.672$, $p = 0.037$). There was also a correlation between performance on the Anti-Saccades task (in particular, in the percentage of success in eyes orienting in the correct direction) and the Processing Speed Index of concussed subjects in neuropsychological assessment ($r^2 = 0.454$, $p = 0.019$). There was no correlation between neuroimaging findings and performance on the oculomotor tasks, neuropsychological assessment results, and other questionnaires.

4. Discussion

According to the fMRI findings in our study, concussed subjects demonstrated an increase in % BOLD signal change compared to healthy individuals during all four tasks presented during fMRI: in the right SFG during Smooth Pursuit, the left IFG during Saccades, the right putamen and left DLPFC during Anti-Saccades, and the left IFG and right lingual gyrus during OKN. Of all the oculomotor tasks, only performances by concussed subjects on the Anti-Saccades and Optokinetic Nystagmus tasks were significantly different from healthy controls, with concussed subjects showing significantly lower mean eye velocity when following the target. Overall, the atypical activation patterns observed, taken together with uncharacteristic task performances by the concussed subjects point to functional disruption in the post-mTBI brain.

4.1.Smooth Pursuit and Saccades

In concussed individuals, all tasks showed increased activation patterns in frontal areas, which could be associated with either an enhanced cognitive effort invested in task implementation and/or the engagement of compensatory neural resources to support eye movements production post-injury. More important involvement of broader and additional areas during the oculomotor tasks post-concussion is in keeping with previous findings (Zhang, 2014; Johnson et al., 2015). During Smooth Pursuit, an increase in % BOLD signal change in the right SFG, the area which is suggested to be related to the regulation of spatial attention and visuospatial processing (Seok et al., 2021), is observed. During Saccades – the increase in the left IFG is thought to monitor errors, control inhibition, and regulate attention (Chong et al., 2007). Concurrently, there was no apparent deficit in task performances during these tasks suggesting that the increased activation associated with these tasks may be due to utilization of compensatory mechanisms where transient alterations of brain resources ensure proper task performances without permanently altering functional

networks (Pico-Perez et al., 2017). It has also been proposed that eye movements are so fundamental that they could be resilient to concussion (Zhang, 2014), hence the "normal" performances on the tasks requiring these eye movements. The study by Zhang (2014) also documented a lack of difference in performances post-concussion during smooth pursuit or saccades production, in addition to showing additional activations, especially in frontal areas.

4.2.Anti-Saccades and OKN

In contrast, subjects with mTBI had difficulties during the Anti-Saccades and OKN tasks, showing not only higher mean latency between the time of stimulus presentation and time to eyes on target (Anti-Saccades) and lower mean eye velocity relative to target velocity when the target moves up/down (OKN), but also elevated % BOLD signal changes compared to healthy controls in broader areas than during the Smooth Pursuit and Saccades tasks. Such a distinction could be related to Anti-Saccades and Optokinetic Nystagmus being more challenging than smooth pursuit or basic saccadic eye movements (Johnson, Hallett, & Slobounov, 2015). During Anti-Saccades, increased activation was found in DLPFC, the area which was commonly shown to have altered activation patterns in concussed subjects, especially during verbal and visual working memory tasks (Chen et al. 2004, Keightley et al., 2014).

The involvement of the DLPFC in decision-making processes is well-established. Anti-saccadic eye movements, which require voluntarily redirecting gaze away from a target, necessitate the engagement of various cognitive processes, including inhibitory and attentional control, cognitive flexibility, and the suppression of reflexive eye movements. These cognitive processes are closely

linked to executive functions, underlining the significant contribution of the DLPFC in executing these complex oculomotor tasks. In fact, concussion could impair inhibition and executive control 30 days post-injury, despite the brain's attempt to implement compensatory strategies for achieving task goals (Zhang, 2014). A study by Slobounov and colleagues on concussed patients also showed increased activation in DLPFC during spatial encoding, indicating greater effort compared to healthy controls (Slobounov et al., 2010).

Increased activation during Anti-Saccades was also observed in the putamen, a novel finding not discussed in previous studies with concussed subjects tested on oculomotor functions. The putamen plays a role in motor planning and coordination and contributes to selecting and inhibiting specific eye movements, integrating sensory information related to eye position and movement, and adjusting gaze based on cognitive and motor demands (Phillips & Everling, 2012). Increased activity in the DLPFC and putamen could reflect a heightened cognitive effort to support the inhibition of reflexive eye movements and the execution of anti-saccades.

Only during the execution of OKN did we see a higher % BOLD signal change in concussed subjects relative to healthy controls in both frontal and occipital areas. There was an increase in activation in the right IFG, the area opposite the one activated during Saccades production, as well as in the left lingual gyrus. The right IFG is thought to control premature or no longer appropriate motor response inhibition (Swann et al., 2012; Aron et al., 2014, 2016). A possible explanation for the types of patterns we observed could be related to the fact that OKN is the last task of the set we used, as well as the most different from the others (there are multiple moving dots on a black background (Fig. 3A) as opposed to single dot and blue background, Fig. 3B) and concussed

subjects struggled when adapting new strategies for task implementation. A similar explanation could be applied to the increase in % BOLD signal change in the lingual gyrus, which plays a role in higher-level analysis and interpretation of visual stimuli, including motion processing and integration of visual information with other cognitive processes (Palejwala et al., 2021).

The Optokinetic Nystagmus (OKN) task is known to impose higher visual demands compared to other tasks. It necessitates a more intense suppression of irrelevant stimuli, such as other moving dots, which concussed subjects reported as triggering physical symptoms like brief vertigo and light-headedness. Consequently, these observations suggest that heightened activity in both frontal and occipital areas during eye movements in concussed individuals may indicate increased cognitive effort in supporting the coordination and execution of saccadic and smooth pursuit eye movements, which are fundamental components of the OKN task.

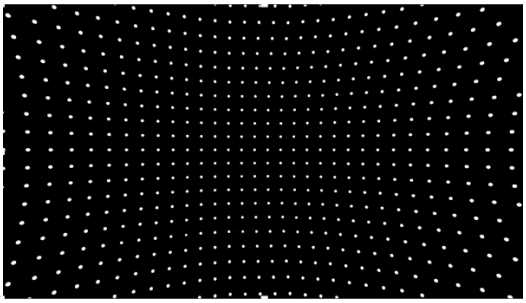
A.  B. 

Figure 3. A – The interface during the Smooth Pursuit, Saccades, and Anti-Saccades tasks implementation; B – The interface during the OKN task implementation.

5. Conclusions

Overall, we can highlight three main findings from our study. First, concussed subjects showed oculomotor deficits compared to healthy controls: their eye velocity was significantly slower relative to target velocity. Secondly, areas with atypical activation patterns in subjects with mTBI during task-based fMRI are primarily associated with regulation and top-down control of the

oculomotor movements, suggesting that concussion, in fact, disrupts oculomotor functions. Finally, our results suggest that oculomotor assessment is a promising approach for determining mTBI biomarkers, with two tasks most sensitive to concussion – Anti-Saccades and Optokinetic Nystagmus. Using these two tasks with an eye-tracker as a potential oculomotor biomarker of concussion could have significant implications for the future of concussion diagnosis. Such an approach could be used for establishing return to work, study or play guidelines and potentially help prevent premature return to activities that could lead to slower recovery and increased vulnerability to repeat injury.

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In Chapter 3, we identified the differences in % BOLD signal change between concussed subjects and healthy controls during task-based fMRI oculomotor tasks and performances on these tasks. We determined that concussed subjects had altered activation patterns during all the tasks. However, performances differed significantly only during the Anti-Saccades and Optokinetic Nystagmus tasks suggesting that those are the two most sensitive tasks to concussion (out of the tasks used in this study). The oculomotor metrics of these tasks to be used as a potential biomarker of mTBI could be a first step towards concussion diagnosis, although it appears insufficient as a standalone method to identify the presence of concussion, but sufficient as a complimentary tool to neuroimaging. To make this tasks more applicable for clinical use, they should be tested on a group with other conditions (e.g., stroke, epilepsy, more severe TBIs,etc.) to determine how specific the tasks are to the deficits seen in concussion.

Even though task-based fMRI using oculomotor tasks showed significant and promising results in the mTBI group, it cannot be fully considered a clinical biomarker due to the complexity of the procedure and its resource-consuming nature. Thus, as we mentioned previously, a more clinically applicable approach is needed. Previous studies have shown various neuropathological alterations post-concussion, which can differ from patient to patient, so there is a need to ideally cover all these pathologies in one all-encompassing approach. In the next chapter, we are investigating the potential of a multimodal neuroimaging approach, including resting state fMRI, ASL and SWI, in defining the biomarkers of mTBI. We also qualitatively compare these findings to the results of task-based fMRI using the oculomotor tasks and the working memory task in the same group of concussed subjects.

Chapter IV. Multimodal Approach in the Identification of Biomarkers of Mild Traumatic Brain Injury: resting state fMRI, ASL, and SWI.

Ekaterina Lunkova[1], Joelle Amir[1], Jen-Kai Chen[1], Rajeet Singh Saluja[1,4], Alain Ptito[1,2,3]

[1]Department of Neurology & Neurosurgery, McGill University, Montreal, Quebec, Canada.

[2]Montreal Neurological Institute, Montreal, Quebec, Canada.

[3]McGill University Health Centre Research Institute, Montreal, Quebec, Canada.

1. Introduction

Concussion, often used synonymously with mild traumatic brain injury (mTBI), is a major public health concern. Yet, our understanding of the brain's functional disturbances in concussed individuals remains limited. From the clinician's perspective, the diagnosis heavily depends on patients' subjective symptoms, the approach which is often unreliable and lacks specificity. Given the frequency of these injuries and their significant legal and sports-related consequences, there is an urgent need for objective assessment tools to evaluate individuals with concussions.

Newer forms of brain imaging techniques have been used to assess patients post-concussion, including functional MRI (fMRI; Chen et al., 2007; Mayer et al., 2015), diffusion-weighted imaging (DWI; Shenton et al., 2018), susceptibility-weighted imaging (SWI; Lu et al., 2015; Studerus-Germann et al., 2016), perfusion MRI (Andre, 2015; Hamer et al., 2020; Clark et al., 2020), positron emission tomography (PET; Jensen & Lauritzen, 2019; Raji & Henderson, 2018), and single photon emission computed tomography (SPECT; Amen et al., 2016; Romero et al., 2015); however, the findings have been inconclusive at best. In the past decades, our team has

been able to demonstrate that task-based functional MRI is an objective approach that shows consistent and reproducible results in concussion screening and allows for an indication of potential biomarkers in concussion (Chen et al., 2007; Holmes et al., 2019; Saluja et al., 2015). Unfortunately, these techniques are seldom available outside research centers due to their resource-consuming nature. Although other imaging modalities, as mentioned above, have shown some utility, a multimodal approach that integrates multiple imaging techniques may offer complementary and synergistic benefits in enhancing diagnostic capabilities, surpassing the limitations of individual techniques alone.

The use of a multimodal approach is particularly relevant as mTBI can be accompanied by various underlying neuropathological alterations, such as metabolic deviations (Giza & Hovda, 2014), diffuse axonal injury and myelin loss (Johnson et al., 2013), cerebral microhemorrhages (Park et al., 2009), and changes in cerebral blood flow (Wang et al., 2016). The presence and extent of these alterations can be identified using different MRI sequences such as fMRI (both task-based and resting state), dMRI and myelin imaging, SWI, and ASL, respectively (Lunkova et al., 2021). Thus, the combined use of these techniques can facilitate the identification of mTBI imaging biomarkers for potential use in a comprehensive diagnosis of concussion.

A brief overview of findings (Lunkova et al., 2021) from using various MRI modalities in concussed subjects demonstrates that rs-fMRI primarily shows alterations in the default mode network (DMN; Churchill et al., 2018; Madhavan et al., 2019; Meier et al., 2020; Van der Horn et al., 2017; D'Souza et al., 2020; Iyer et al., 2019), the task-related network (TRN; Mayer et al., 2011), the salience network (SN; Sharp et al., 2014; Sours et al., 2018), as well as the motor, visual,

and auditory networks (D'Souza et al., 2020; Hou et al., 2019; Madhavan et al., 2019), which are associated with post-concussion symptoms (PCS; Madhavan et al., 2019). Studies using non-invasive perfusion fMRI (Arterial Spin Labeling; ASL) showed changes in cerebral blood flow (CBF) in frontal and left occipital cortices (Lin et al., 2016; Doshi et al., 2015), the frontotemporal lobes (Wang et al., 2015), insular and superior temporal cortex (Meier et al., 2015), thalami (Bartnik-Olson et al., 2014) and temporal areas (Hamer et al., 2020). SWI findings revealed microbleeds in concussed subjects predominantly in frontal, temporal and occipital lobes, brain stem (Park et al., 2009; De Haan et al., 2017), caudate nucleus, hippocampus, thalamus, substantia nigra, and corpus callosum (Lu et al., 2015). The diversity of locations identified with imaging as well as the nature of alterations of activation patterns (e.g., increase/decrease in connectivity/CBF), point to a variety of effects following concussion. Several studies also showed that post-concussion patients could show alterations in one modality while appearing intact in another (Lunkova et al., 2021). Thus, there is a dire need for a multimodal approach to revealing concussion biomarkers, as well as a need for collecting multimodal data to validate norms for potential future use in the diagnosis of concussion. Identifying biomarkers and validating norms will facilitate an individual approach to identifying each patient's concussion neuroimaging profile with objective and reliable methods.

In this study, we investigate the use of a multimodal approach, including resting state fMRI, ASL, and SWI in concussed subjects, to ascertain whether incorporating all or one of these approaches into a screening protocol post-concussion will contribute to the identification of mTBI biomarkers. We opted for the utilization of MRI modalities instead of neuroimaging alternatives such as PET and SPECT for several reasons. MRI offers the advantage of being non-invasive, eliminating the

need for ionizing radiation exposure. Additionally, MRI scans can be conducted serially and relatively quickly, enabling their application in a wide range of individuals, and facilitating assessments of recovery progress. Another benefit of MRI is its capacity to explore various pathophysiological mechanisms using different MRI modalities, all within a single scanner.

2. Methods

2.1.Participants

29 concussed symptomatic adults within one month (1-5 weeks) post-injury selected according to the WHO task force criteria (8 males/21 females, mean age = 28.3, SD = 10) and a group of 29 sex- and age-matched adult healthy control subjects aged between 18 and 54 years (mean age = 29.1, SD = 9.7) without a history of neurodevelopmental or neurological disorders, head injuries, ADHD, and/or presence of significant abnormalities seen on structural MRI scans (assessed by a clinician) are included in the study.

2.2.MRI sequences

All scanning was performed on a Siemens 3 Tesla MRI system equipped with a 64-channel head coil. Each scanning session consisted of the following sequences:

- T_1-weighted acquisition for anatomical reference and cortical thickness analysis (3D MP-RAGE, TR=2300ms, TE=2.98ms, 176 slices, slice thickness=1mm, FOV = 256mm, image matrix = 256 x 256, flip angle = 9 degrees, interleaved excitation).

- Resting-state fMRI (rs-fMRI) using T2* weighted gradient echo echo-planar image (GE-EPI, TR=2000ms, TE=30ms, 42 slices, slice thickness=3.5mm, FOV=224mm, image matrix=64x64, flip angle=90 degrees, interleaved excitation, 240 measurements).

- Dual-Echo Pseudo-Continous Arterial Spin Labeling (DE-PCASL) for CVR, BOLD and CBF quantification (TR: 4s, TE1/TE2=10/30ms, Post-Label Delay 900ms, FA: 90°).

- Susceptibility weighted imaging (SWI) (TR = 270ms, TE = 20ms, flip angle: 20°, slice thickness = 0.75mm, in-plane resolution = 0.75 x 0.75 mm, FOV = 220 mm, 176 slices).

2.3.Questionnaires

Concussed individuals were asked to fill out the following questionnaires prior to participating in the study: Post-Concussion Symptom Scale (PCSS), Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI-II), and Dizziness Handicap Inventory (DHI)**.** Concussed subjects were identified as symptomatic/asymptomatic according to their results on the PCSS, and only symptomatic subjects were included in the study (PCSS score>21). BAI and BDI-II were used to identify the possible presence of depression/anxiety and their effect on task performance.

2.4.Neuropsychological assessment

Neuropsychological assessment was used to identify the correlation between severity of cognitive functional impairment and imaging findings. The domains included in the neuropsychological assessment were selected according to their vulnerability to mTBI, as described in the literature: attention, working memory, processing speed, and problem-solving (Lunkova et al., 2021). The neuropsychological assessments consisted of the following tests: Verbal Working Memory Task (M. Petrides), Rey Auditory Verbal Learning Test (RAVLT), Trial Making Test (TMT), Purdue Pegboard, Tower of London, WAIS-IV Processing Speed Index Subtests (Symbol Search, Coding), Symbol Digit Modalities Test (SDMT). The neuropsychological tests are conducted to

determine if cognitive difficulties are related to structural and hemodynamic alterations identified by MRI sequences.

2.5.Data processing and Statistical Analysis

2.5.1. Rs-fMRI

Rs-fMRI data was preprocessed using SPM12 within the Functional Connectivity (CONN) toolbox. A seed-based correlation approach was used to evaluate the BOLD signal associated with the functional connectivity of the networks as well as separate seeds derived from the Harvard-Oxford Cortical atlas, and ROIs (ROIs were based on the significant differences between patients and healthy controls during the task-based fMRI; Lunkova et al., 2023). Functional Connectivity (FC) measures were extracted from areas with significant differences between groups using REX based on results of seed-to-voxel and ICA analyses to use as dependent variables in regression analysis with oculomotor metrics/neuropsychological assessment results/questionnaires results as independent variables; sex and age were added as compounds. Using the results of healthy controls extracted using REX, 95% confidence intervals were determined to establish a "normal range" for FC measures.

2.5.2. ASL

27 out 29 concussed subjects (19 females, 8 males, mean age = 30.3, SD = 12.4) and 27 age- and sex-matched healthy controls were included in the analysis of ASL data (2 subjects were excluded due to: 1) incorrect data acquisition; 2) claustrophobia (scan was not completed)). The images were preprocessed, and CBF (mL of arterial blood/100g of tissue/minute) was calculated using the Bayesian Inference for Arterial Spin Labeling MRI (BASIL) toolbox for FSL. Regional CBF was

calculated based on ROIs from parcellations from wfupickatlas as well as ROIs based on the task-based fMRI results (Lunkova et al., 2023). The average CBF for each region of interest (ROI) and global CBF were calculated. Comparison between CBF in each ROI and global CBF between two groups were performed using Mann-Whitney U-test (chosen as the data were not normally distributed). Using the results of healthy controls, 5^{th}- and 95^{th} percentiles were determined to establish a "normal range" for CBF; upon statistical advice, these percentiles were chosen because the data were not distributed normally.

2.5.3. SWI

The cerebral microbleed (CMB) number analysis was performed on SWI minimum intensity projection images and susceptibility maps using the Microbleed Anatomical Rating Scale (MARS) (Gregoire et al., 2009). All SWI scans were evaluated by two neurosurgeons with experience in this field, blinded to the clinical details and to the other scans obtained during this study. The number and location of microbleeds were identified.

2.5.4. Neuropsychological assessment and questionnaires

Neuropsychological assessment results were converted to standard scores, and the number of subjects with scores below the normal range was identified according to norms (Strauss, Sherman & Spreen, 2006) for each task; the conclusions were qualitative in nature. Results on the questionnaires were assessed according to the guidelines for each of the questionnaires.

2.5.5. Correlation between neuropsychological assessment and questionnaires results and imaging findings

Additional statistical analyses were performed in SPSS 28.0.1.1. To determine correlations with PCSS, BAI, BDI-II and DHI scores, as well as scores on the cognitive tests, Multiple Regression analyses were implemented. For rs-fMRI, functional connectivity in each network with significant differences between groups was entered as a dependent variable, and results of neuropsychological tests and questionnaires were used as independent variables. For ASL, CBF in each region with significant differences between groups was entered as a dependent variable, and results of neuropsychological tests and questionnaires were used as independent variables. All values were converted to z-scores prior to the implementation of multiple regression analyses.

3. Results

3.1. Resting-state fMRI

3.1.1. Seed-to-Voxel analysis

Hyperconnectivity was observed in concussed subjects compared to healthy controls between the nodes of DMN (Fig. 1), posterior cingulate cortex and medial rPFC, areas as well as other areas, namely right fusiform gyrus and left DLPFC, respectively. They also showed elevated connectivity between the node of SN (Fig. 2), lateral rostral PFC, and left angular and right medial temporal gyri. In addition, there was higher activation between the LPFC nodes of FPN and inferior and superior temporal gyri (Fig. 4, 5). In contrast, hypoconnectivity was found in concussed subjects compared to healthy controls between the FEF node of DAN (Fig. 3) and the left angular gyrus.

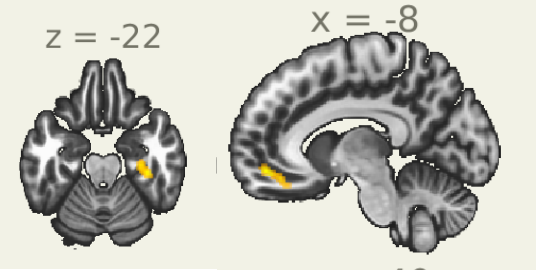

Figure 1. DMN.

1) Connectivity between the node of the DMN, the Posterior Cingulate Cortex, and the Right Fusiform gyrus, p = 0.006; 2) Connectivity between the node of the DMN, the Medial RPFC, and the Left DLPFC p = 0.024.

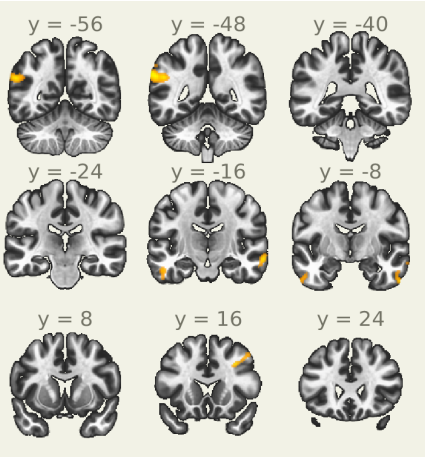

Figure 2. SN.

1) Connectivity between the node of the SN, the Lateral Rostral PFC, and the Left Angular Gyrus, p = 0.000002, 2) Connectivity between the node of the SN, the Lateral Rostral PFC, and the Right Medial Temporal Gyrus, p = 0.0177.

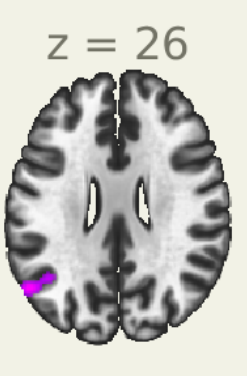

Figure 3. DAN.

Connectivity between the node of the DAN, the Left Frontal Eye Field, and the Left angular gyrus, p = 0.012.

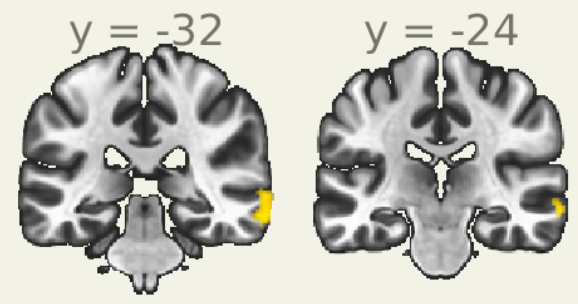

Figure 4. FPN.

Connectivity between the node of the FPN, the Left LPFC, and the Right Superior Temporal Gyrus, p = 0.0019.

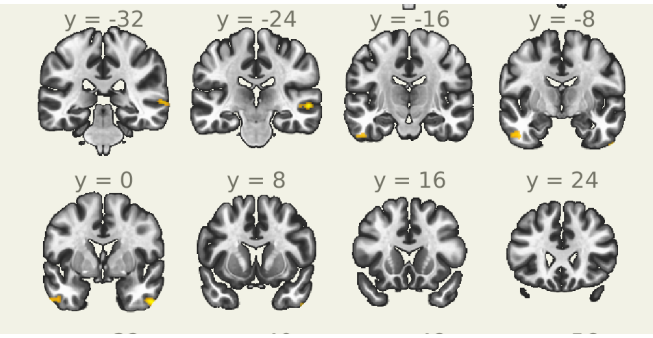

Figure 5. FPN.

1) Connectivity between the node of the FPN, the Right LPFC, and the Left Inferior Temporal Gyrus, p = 0.003, 2) Connectivity between the node of the FPN, the Right LPFC, and the Right Inferior Temporal Gyrus, p = 0.0132, 3) Connectivity between the node of the FPN, the Right LPFC, and the Right Superior Temporal Gyrus, p = 0.039.

3.1.2. ROI-to-ROI analysis

In concussed subjects in comparison to healthy controls (Fig. 6), an increase in connectivity between two ROIs based on task-based fMRI results conducted in the same run as the other tasks was found between the left occipital cortex and right pars orbitalis (the ROIs which had elevated % BOLD signal changes during the Optokinetic Nystagmus task in task-based fMRI in the same cohort; Lunkova et al., 2023). No significant results were found between other regions.

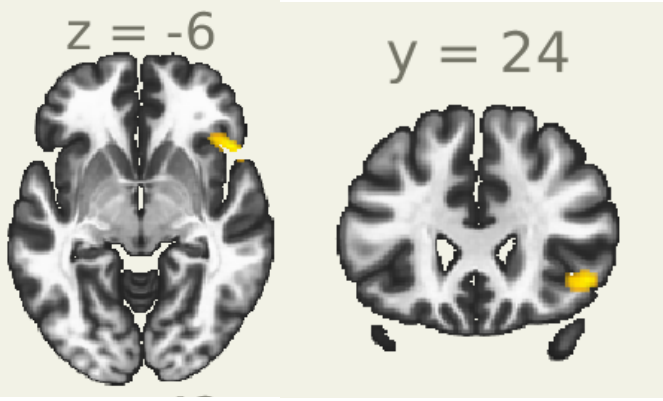

Figure 6. Connectivity between the Left Occipital cortex (BA19) and Right Pars Orbitalis.

Patients>Controls p = 0.0015

3.1.3. ICA results

Results from the ICA showed abnormal mTBI functional connectivity in regions within one out of eight brain networks identified by CONN (p-FDR corrected < 0.05, p-uncorrected < 0.001). Decreased functional connectivity was found in mTBI patients compared to controls between the DMN and right BA4 and BA19 (Fig. 7).

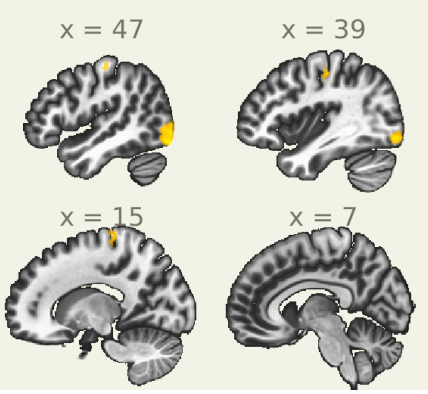

Figure 7. DMN.

Right side: Connectivity between the DMN and the Right BA19 p = 0.000003; Left side: Connectivity between the DMN and the Right BA4 p = 0.029

A summary of the functional connectivity between each pair of regions in each group for Seed-to-Voxel, ROI-to-ROI, and ICA analyses can be found in Table 1 and Fig. 8., indicating that the lower connectivity in concussed subjects compared to healthy controls was found only between

the node of the DAN, the Left Frontal Eye Field, and the Left angular gyrus. Connectivity between every other pair of nodes with significant results was heightened in concussed subjects compared to healthy controls.

Figure 8. Summary of the functional connectivity between each pair of regions in each group.

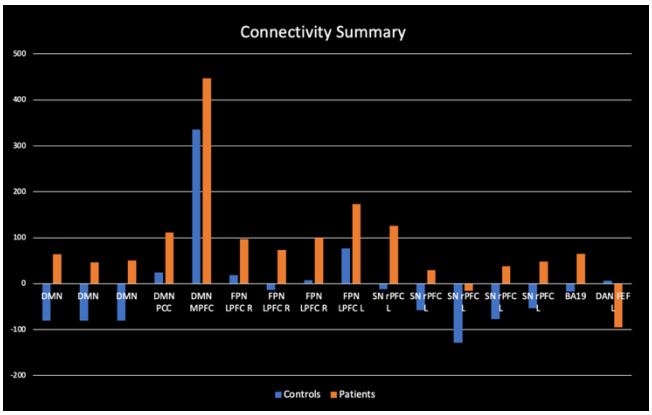

Table 1. Summary of the functional connectivity between each pair of regions in each group for Seed-to-Voxel, ROI-to-ROI, and ICA analyses.

Seed	Target	Voxels	Peak MNI coordinates	p (FWE)	Cohen's d	Direction of FC change
Seed-to-Voxel						
DMN						
Medial Rostral PFC	left DLPFC (BA9)	132	-8 48 -20	0.024	0.959	Hyperconnectivity
Posterior Cingulate Cortex	Right fusiform (BA37)	173	36 -28 -24	0.006	1.09	Hyperconnectivity
SN						
Lateral Rostral L PFC	Left angular gyrus (BA39)	491	-56 -52 28	0.000002	1.151	Hyperconnectivity
	Right Med Temp Gyrus (BA21)	145	64 -16 -12	0.0177	0.97	Hyperconnectivity
DAN						
Left Frontal Eye Field	Left angular gyrus (BA39)	162	-52 -64 26	0.012	-1.8	Hypoconnectivity
FrontoParietal Network						
Left LPFC	Superior Temporal Gyrus R (BA22)	216	62 -28 -2	0.0019	0.629	Hyperconnectivity
Right LPFC	Left Inferior Temporal Gyrus (BA20)	205	-46 -6 -34	0.003	0.691	Hyperconnectivity
	Right Inferior Temporal Gyrus (BA20)	155	54 0 -40	0.0132	0.792	Hyperconnectivity
	Superior Temporal Gyrus R (BA22)	123	56 -24 -2	0.039	0.773	Hyperconnectivity
ROI-to-ROI						
Occipital cortex (BA19)	Pars Orbitalis	211	46 22 -8	0.0015	0.636	Hyperconnectivity
ICA						
DMN	BA19	475	42 -86 -12	0.000003	0.71	Hyperconnectivity
	BA4	104	40 -20 50	0.029	0.567	Hyperconnectivity
	BA4	103	18 -30 72	0.029	0.565	Hyperconnectivity

3.2. ASL

Results of ASL analyses were not significantly different between groups (patients vs. healthy controls); however, the data showed high variability (Table 2), and most patients (23/27) showed results outside the normal range in at least one ROIs (which was established as 5^{th}-95^{th} percentiles from the group of healthy controls).

Table 2. Summary of ASL findings

	Controls		Patients					
	Mean CBF	SD	Mean CBF	SD	Number of subjects with altered CBF	% of patients who showed deviations in CBF in each ROI	% below	% above
Left Frontal	46.9	8.3	43.6	14.5	8/27	27.6	13.8	13.8
Right Frontal	46.4	8.8	43.6	14.1	7/27	24.1	13.8	10.3
Left Anterior Cerebellum	42.1	8.9	46.3	41.9	8/27	27.6	10.3	17.2
Right Anterior Cerebellum	43.2	9.6	43.0	26.9	8/27	27.6	13.8	13.8
Left Limbic	47.4	7.3	44.0	15.0	11/27	37.9	24.1	13.8
Right Limbic	48.4	7.4	44.6	15.3	10/27	34.5	20.7	13.8
Left Medulla	28.0	14.7	25.7	20.9	6/27	20.7	10.3	10.3
Right Medulla	28.4	15.7	25.6	19.5	6/27	20.7	10.3	10.3
Left Midbrain	40.8	8.3	36.8	13.4	5/27	17.2	13.8	3.4
Right Midbrain	40.8	7.6	36.3	12.6	6/27	20.7	17.2	3.4
Left Occipital	50.3	10.6	47.3	21.5	10/27	34.5	20.7	13.8
Right Occipital	49.4	10.5	46.1	20.8	10/27	34.5	20.7	13.8
Left Parietal	54.6	9.8	51.5	20.0	9/27	31.0	17.2	13.8
Right Parietal	54.4	9.9	51.5	18.3	8/27	27.6	17.2	10.3
Left Pons	32.1	7.4	33.3	22.3	8/27	27.6	13.8	13.8
Right Pons	32.8	7.6	33.4	18.4	8/27	27.6	13.8	13.8
Left temporal	44.4	7.2	42.3	14.7	8/27	27.6	10.3	17.2
Right Temporal	44.7	7.5	42.7	14.5	7/27	24.1	10.3	13.8
Left Putamen	47.5	7.9	42.6	15.7	8/27	27.6	20.7	6.9
Right Putamen	46.9	8.0	41.8	15.5	8/27	27.6	17.2	10.3
Left Globus Pallidus	39.7	7.0	34.3	12.2	5/27	17.2	13.8	3.4
Right Globus Pallidus	42.9	7.8	38.2	13.3	4/27	13.8	10.3	3.4
Left Caudate Nucleus	42.8	8.0	39.0	13.6	7/27	24.1	20.7	3.4
Right Caudate Nucleus	42.0	8.2	39.4	13.9	6/27	20.7	17.2	3.4

Left Thalamus	46.7	9.7	40.8	14.3	8/27	27.6	20.7	6.9
Right Thalamus	45.5	9.9	40.2	15.1	6/27	20.7	13.8	6.9
Superior Frontal Gyrus	48.6	12.5	51.2	24.0	5/27	17.2	6.9	10.3
Inferior Frontal Gyrus	41.6	8.2	39.1	16.4	9/27	31.0	20.7	10.3
Putamen	52.8	8.5	50.8	15.5	9/27	31.0	17.2	13.8
Left DLPFC	39.9	9.5	33.7	14.8	10/27	34.5	27.6	6.9
Inferior Frontal Gyrus2	59.3	12.5	51.5	18.5	6/27	20.7	20.7	0.0
BA19	41.1	10.1	37.4	20.2	9/27	31.0	20.7	10.3
Global	44.3	7.5	41.7	14.6	11/27	37.9	27.6	10.3

3.3. SWI

Among the 29 subjects examined, only three (Subject #1, Subject #22, and Subject #26) exhibited microbleeds on the susceptibility-weighted imaging (SWI) scans. Subject #1 displayed a microbleed in the right primary somatosensory cortex, Subject #22 showed a microbleed in the right precuneus, and Subject #26 had microbleeds in the left temporal lobe (Fig. 9).

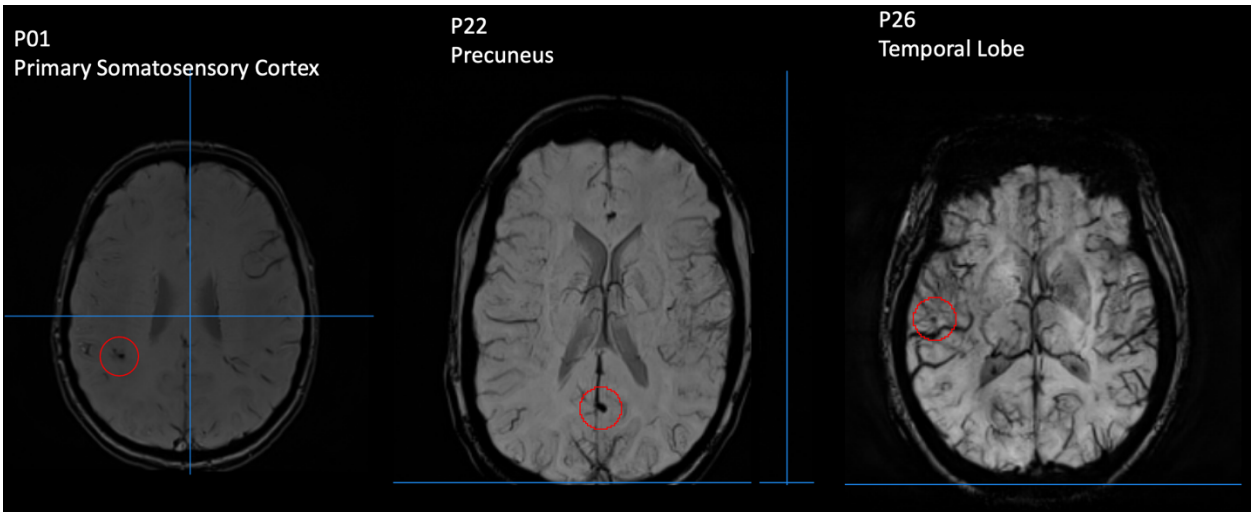

Figure 9. Microbleeds on the SWI images.

3.4. Neuropsychological assessment

No consistent tendencies in the results of the neuropsychological assessment were observed. The most difficulties experienced by the concussion group were observed in tasks measuring working memory and learning (RAVLT), visual attention and task switching (Trail Making Test), and graphomotor processing speed (SDMT). The percentage of subjects and their distribution outside of the normal range are summarized in Table 3 and Fig. 10.

Table 3. Number and percentage of concussed subjects who showed results outside the normal range on each test.

	No. of participants with results outside of the normal range	% of participants with results outside of the normal range
RAVLT Learning	13/29	44.8
RAVLT Immediate Recall	6/29	20.7
RAVLT Delayed Recall	7/29	24.1
RAVLT Recognition	7/29	24.1
Pegboard	6/29	20.7
Processing Speed Index	6/29	20.7
Trail Making Test Part A	8/29	27.6
Trail Making Test Part B	14/29	48.3
Tower of London Accuracy	6/29	20.7
Tower of London Speed	3/29	10.3
Symbol Digit Modalities Test	13/29	44.8

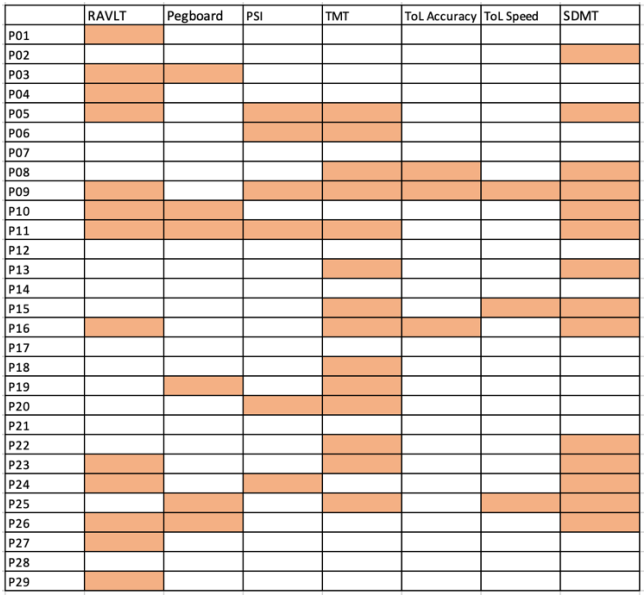

Figure 10. Distribution of concussed subjects with results outside of the normal range across the cohort. Subjects with the results outside of the normal range on each test are marked in orange.

3.5. Questionnaires

All concussed subjects were symptomatic according to the answers on the PCSS, (mean score = 44/132, SD = 24). The results of the PCSS, BAI, BDI-II, and DHI are shown in Table 4 & 5.

Table 4. Mean values and standard deviation in the group of concussed subjects according to their results on the questionnaires.

	Mean	SD
PCSS	44.4	23.6
BDI-II	14.1	9.3
BAI	17.3	14.2
DHI	32.6	21.4

Table 5. Summary of results of concussed subjects on the questionnaires.

	Number of concussed subjects	% of concussed subjects
BDI-II		
Normal	18/29	62
"Mild depression"	5/29	17
"Moderate depression"	4/29	13.8
"Severe depression"	2/29	6.9

BAI		
Normal	9/29	31
“Mild anxiety”	7/29	24.1
“Moderate anxiety”	7/29	24.1
“Severe anxiety”	6/29	20.7
DHI		
Normal	6/29	20.7
“Mild handicap”	12/29	41.4
“Moderate handicap”	5/29	17
“Severe handicap”	5/29	17

3.6. Correlation between the neuroimaging findings and other metrics

Concussed subjects showed a correlation between the imaging results of the rs-fMRI and ASL with the results on the DHI questionnaire. In particular, increased connectivity in the fronto-parietal network and increased CBF in DLPFC and inferior frontal gyrus correlated strongly with dizziness and unsteadiness problems.

Out of all the modalities, only rs-fMRI results correlated with performances on neuropsychological tests. Connectivity in the fronto-parietal and salience networks was linked to performances on the SDMT and Tower of London. Rs-fMRI results also correlated with number of days post-injury and extent of PCS, particularly connectivity in fronto-parietal, default mode, and salience networks (Table 5).

Table 6. Results of multiple regression analyses between neuroimaging findings and neuropsychological tests results, questionnaires scores and days post-injury.

Only significant results with $r^2 > 0.5$ and $p>0.05$ are included in the table.

Dependent	**Independent**	**r sq**	**p**
Rs-fMRI			
ICA_DMN_3	Tower of London accuracy	0.715	0.014
DMN_MPFC			0.015
FP_LPFC_R_3			0.032

SN_rPFC_L_4			0.03
FP_LPFC_L	SDMT	0.586	0.028
DMN_MPFC	PCSS	0.702	0.048
FP_LPFC_L			0.036
FP_LPFC_L	DHI	0.552	0.048
ICA_DMN_2	Days post injury	0.772	0.05
FP_LPFC_R_2			0.029
SN_rPFC_L_2			0.011
SN_rPFC_L_5			0.044
ASL			
LDLPFC	DHI	0.59	0.002
InferiorFrontalGyrus2			<0.001

3.7. Individual profiles

All subjects showed alterations in rs-fMRI in at least one ROIs compared to the confidence interval established with healthy controls. 23/27 subjects showed alterations on both rs-fMRI and ASL. Two subjects showed alterations on both rs-fMRI, ASL, as well as microbleeds on SWI. Comparison to other results can be found in Fig. 11.

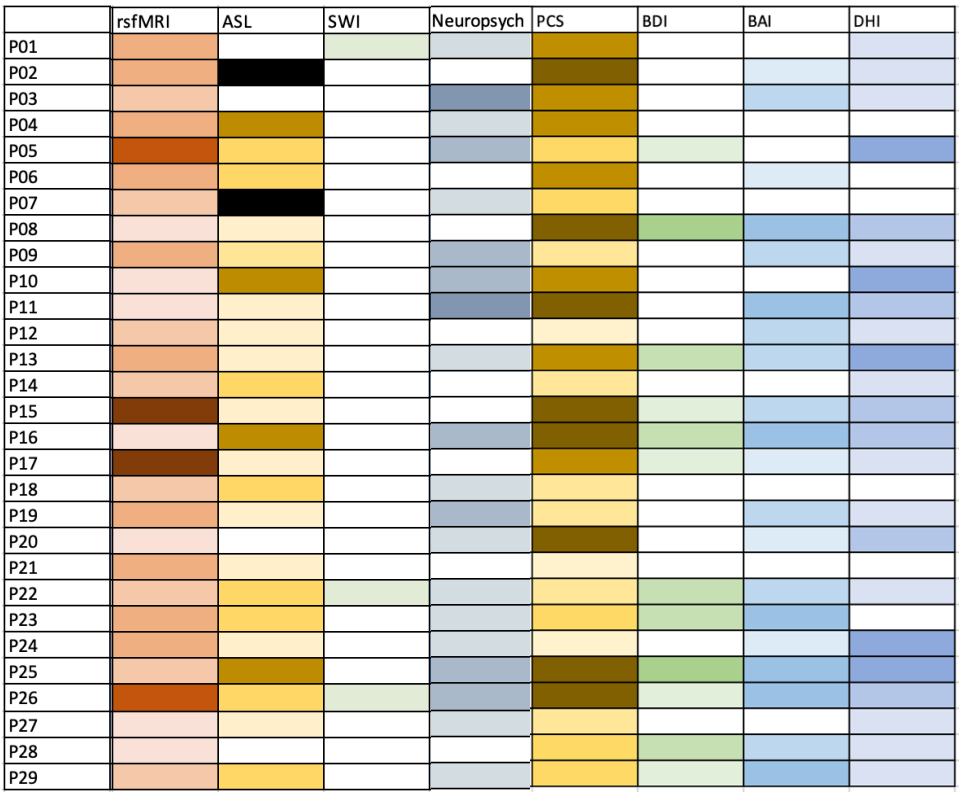

Figure 11. Alterations on neuroimaging modalities in each of the concussed subjects and severity of PCS, anxiety, depression, dizziness and unsteadiness problems, and cognitive deficit on neuropsychological assessment. *

*The extent of neuroimaging alterations/severity of the results on the questionnaires and neuropsychological tests are reflected using color intensity, where white color means "normal" to the darkest color meaning "the most altered"/ "the most severe". In black are subjects who had to be excluded from the particular measurement (in the table, two concussed subjects did not complete the ASL part of MRI scanning).

4. Discussion

This study investigated the potential of a multimodal approach as well as each modality separately to determine biomarkers in concussion. Significant results were obtained only in the rs-fMRI modality. ASL results did not reveal any statistically significant differences at a group level. SWI results, in turn, showed microbleeds in only 3/29 subjects.

4.1. Rs-fMRI results

RS-fMRI results showed predominantly higher connectivity in concussed subjects compared to healthy controls (except for hypoconnectivity between DAN and the left angular gyrus). Previous findings using rs-fMRI in mTBI patients have been inconsistent, showing both increases and decreases in FC post-injury (Lunkova et al., 2021; Amir et al., 2021). The underlying pathophysiology of increased connectivity remains unclear. Literature on the topic suggests several explanations, including the possibility of an underlying diffuse impact of mTBI on axons (Johnson et al., 2013; Shultz et al., 2017) or microvasculature (Werner & Engelhard, 2007), neuroinflammation, edema, disruption of the blood-brain barrier, reduced cerebral blood flow, and other biochemical or neurometabolic responses which potentially could affect connectivity (Giza & Hovda, 2014; Marchi et al., 2013; Kaushal et al., 2019). Even though we were unable to examine the majority of these underlying issues in the framework of our study, we did observe that most subjects showed CBF alterations on ASL, changes which could be related to FC abnormalities.

Alternatively, increased connectivity may reflect compensatory mechanisms in the brain. After a concussion, the brain may reorganize its networks to compensate for injury-related disruptions. The increased connectivity could thus be an adaptive response to mitigate the effects of cognitive deficits and maintain functional performances (Iraji et al., 2016; Shumskaya et al., 2012; Wong et al., 2023).

At the cognitive level, increased connectivity between DMN, SN, FPN and other areas of the brain, which were discussed in the results, as well as a decrease in DAN, can be associated with a deficit in executive functions, especially in the regulation of attention, which corresponds to the correlation between alterations in most of these networks and performances on neuropsychological

tests measuring graphomotor processing speed and planning and problem-solving. Several studies suggest that increased connectivity in the DMN could reflect increased alertness in monitoring further external stimuli or shifts of axonal ions and neurotransmitters along neurons (Giza & Hovda, 2001; Buckner et al., 2008; Zhu et al., 2015). Finally, alterations in the DMN can be related to post-concussion symptoms, such as hypersensitivity to light and noise (Vaughn et al., 2022), as we observed a strong correlation between DMN alteration and PCS, namely dizziness and unsteadiness problems.

One of the interesting findings in the present study is that concussed subjects showed an increase in connectivity between the occipital cortex and pars orbitalis, both areas which showed increased % BOLD signal change during oculomotor tasks with the same cohorts using task-based fMRI (Lunkova et al., 2023). This finding shows that abnormality in these regions can be appreciated using both task-based and rs-fMRI. Such observations could be the first step in the comparison of these modalities and the start of the conversation as to which could be a more reliable biomarker in concussion, with rs-fMRI being a less resource-consuming method. Further research is needed to elaborate on these findings.

4.2. ASL and SWI results

ASL results did not reveal any statistically significant differences at a group level; however, a closer look into the results points to high inhomogeneity of the data, which may be related to either different mechanisms of injury in the group or the need to look at other ROIs. One of the most recent findings in our lab (McCabe et al., 2023) is the significant functional differences between individuals who had an mTBI with loss of consciousness (LOC)/post-traumatic amnesia (PTA),

and those without LOC/PTA. A look at the data in our study also points to distinguishing between these two groups. Further investigation on this differentiation may shed light on the reason there is CBF inhomogeneity in the group of concussed subjects. Furthermore, it is noteworthy that when compared to our previous studies with the same cohorts using task-based fMRI (Lunkova et al., 2023, McCabe et al., 2023), 22/23 subjects who had alterations in CBF also showed altered activation compared to healthy controls during task-based fMRI, either on oculomotor tasks (Lunkova et al., 2023) or on working memory tasks (Petrides et al., 2000; McCabe et al., 2023), or both. Overall, it can be concluded that altered CBF in most concussed subjects indicates cerebral metabolic disruptions post-injury.

SWI images were evaluated only qualitatively, and only 3/29 subjects showed single microbleeds, with microbleed locations being inconsistent ((1) primary somatosensory cortex, (2) precuneus, (3) temporal lobe), which is in line with previous findings (Wang et al., 2014, De Haan, de Groot et al., 2017) and could also be related to differences in injury mechanisms. When comparing to our previous studies with the same cohorts using task-based fMRI (Lunkova et al., 2023, McCabe et al., 2023), it is evident that 2/3 of subjects with microbleeds on SWI also showed alterations in every other MRI modality we used, with alterations on task-based fMRI, rs-fMRI, and ASL, of the other subject showing alterations only on task-based fMRI and rs-fMRI.

4.3. Individual profiles of patients

Rs- and task-based fMRI results, and ASL results, showed a correlation with dizziness and unsteadiness problems. Only rs-fMRI results showed a correlation with PCS, as well as with days post-injury, and the results of the neuropsychological assessment (processing speed and problem-

solving), showing that this modality appears to be the most indicative of post-concussion outcomes.

Analysis of the individual profiles of each patient (where results were compared to normal ranges based on healthy controls; Fig. 12) showed that all had results outside of the normal range on rs-fMRI. Most subjects did not show consistent abnormalities in the other modalities, including task-based fMRI using oculomotor tasks (Lunkova et al., 2023) and the working memory task (McCabe et al., 2023; Petrides, 2000). Most participants had abnormalities on task-based fMRI and/or ASL and/or SWI. Only three subjects had microbleeds seen using SWI, and 2/3 of subjects with microbleeds also showed abnormal results on all other MRI modalities used in the current study and previous studies on the same cohort using task-based fMRI (Lunkova et al., 2023, McCabe et al., 2023).

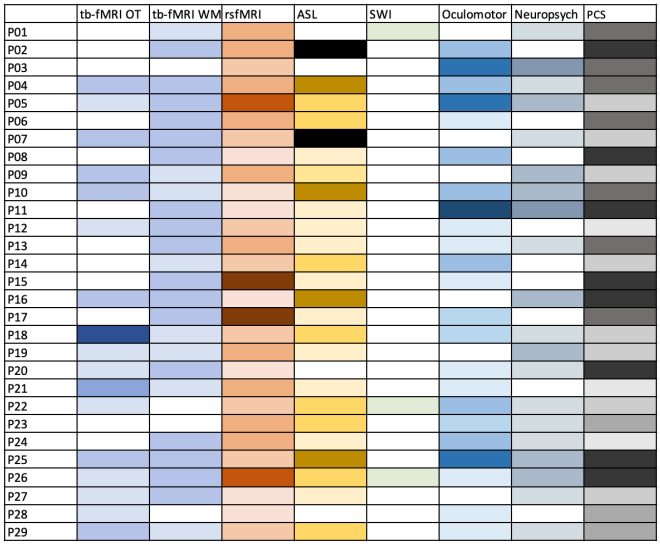

Figure 12. Alterations on neuroimaging modalities in each of the concussed subjects and severity of PCS, oculomotor deficit evaluated by VR goggles (Lunkova et al., 2023), and cognitive deficit on neuropsychological assessment. *

*The extent of neuroimaging alterations/severity of the results on the questionnaires and neuropsychological tests are reflected using color intensity, where white color means “normal” to the darkest color meaning “the most altered”/ “the most severe”. In black are subjects who had

to be excluded from the particular measurement (in the table, two concussed subjects did not complete the ASL part of MRI scanning).

5. Conclusions

Concussed subjects showed abnormal neuroimaging findings in one or more MRI modalities used in this study. RS-fMRI appeared to be the most sensitive in concussion as all the subjects showed alterations in functional connectivity, and it was the only modality which correlated with post-concussion symptoms. While SWI may not be as sensitive to mTBI, it still showed its usefulness in small numbers of the cohort where microbleeds were one of the possible pathologies following concussion. ASL results, with their inhomogeneity and alterations in CBF in many concussed subjects, indicated metabolic disruptions in the brain post-injury. Thus, each of the modalities shows possible post-concussion pathology. Taken together, a multimodal approach can provide all-encompassing insight into the diagnosis of concussion. Finding new reliable biomarkers to facilitate a more definite and precise diagnostic process is essential, especially in the context of rehabilitation and medico-legal cases. This study is considered a steppingstone in defining imaging biomarkers in mTBI.

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Chapter V. Overall Discussion

In this thesis, we addressed the question of the identification of biomarkers in mTBI in the framework of neuroimaging methods and oculomotor function. The following key findings can be highlighted: (1) from the neuroimaging modalities, only task-based and resting state fMRI showed significant differences between healthy controls and concussed subjects, with concussed subjects having predominantly an increase in %BOLD signal change and functional connectivity; (2) Out of all the oculomotor tasks, only performances on Anti-Saccades and Optokinetic Nystagmus were significantly different between healthy controls and concussed subjects, with concussed subjects showing significantly lower mean eye velocity when following the target, (3) No correlations were found between task performances and fMRI/ASL results.

1. Revisiting hypotheses

Our findings were in line with hypothesis #1, namely that "symptomatic concussed individuals will show atypical task-based fMRI activation patterns in regions sensitive to oculomotor functions." In Chapter 3, we demonstrated that the majority of regions with altered BOLD signal in concussed subjects compared to healthy controls were attributed to oculomotor functions in either primary or secondary roles. During Optokinetic Nystagmus and Anti-Saccades, the concussed subjects showed altered activation in the lingual gyrus and putamen, respectively. Both are known for their direct involvement in oculomotor processes, with the lingual gyrus being responsible for the primary and higher-level visual processing (Palejwala et al., 2021) and the putamen being involved in motor control (Ghandil & Munakomi, 2019). There was also altered activation in frontal areas during all four tasks (DLPFC, pars orbitalis), all of which play a role in top-down control of the execution of these movements.

The results of the study were also in keeping with hypothesis #2, "Symptomatic concussed individuals will show atypical structural (SWI), metabolic (ASL), and hemodynamic (rsfMRI) patterns." Indeed, the findings revealed that all concussed subjects who participated in our study had results outside the confidence intervals of healthy controls in rs-fMRI. Many of the subjects also had alterations on ASL, which was not the case for the SWI modality, with only three subjects showing microbleeds. However, one should be cautious with interpretations due to the following limitations. First, we are looking at the "normal range" established with healthy controls only within the framework of our study; 29 subjects represent a limited sample for establishing normative data. In addition, the cohorts consisted of an uneven number of males and females, with a significant prevalence of female subjects. Nevertheless, these findings are promising and confirm the existence of metabolic and hemodynamic disruptions post-concussion, bringing us closer to establishing mTBI biomarkers.

In hypothesis #3, we suggested, "One or more of [these] neuroimaging methods will correlate with symptom severity in concussed individuals." Our results point to a correlation between post-concussion symptoms (PCS) and functional connectivity (FC) in the rs-fMRI modality, especially in the default mode network (DMN) and the frontoparietal network (FPN). Churchill and colleagues' (2018) study also showed that higher FC in DMN was associated with symptom severity. However, we did not find evidence for a correlation between PCS severity and the FPN FC, which could potentially be a novel finding in our study. Further investigation could look into specific PCS (sensory, cognitive, etc.) and how they correlate with FC in different networks.

Our hypothesis #4 which was rejected according to our results, stated that "Abnormal performance on the Neuroflex oculomotor tasks will correlate with atypical structural (SWI), metabolic (ASL), and hemodynamic (task-based/rsfMRI) patterns in symptomatic concussed individuals.". However, even looking at the data qualitatively, we can observe that two tasks that revealed an oculomotor deficit in mTBI subjects also showed broader alterations in % BOLD signal change in more areas compared to other tasks. At the same time, our multiple linear regression results did not show a correlation between the imaging findings and performance on the oculomotor tasks. This lack of correlation could be attributed to the non-linear relationship between them, highlighting the necessity for further in-depth investigation.

Finally, our last hypothesis #5, "Abnormal performance on neuropsychological tests will correlate with atypical structural (SWI), metabolic (ASL), and hemodynamic (task-based/rsfMRI) patterns, in symptomatic concussed individuals," was partially in line with our results. Two neuropsychological tests correlated only with rs-fMRI results, and no other correlations between tests and modalities were found. The Tower of London (number of moves and correct answers) correlated with FC in DMN, FPN, and SN, and the SDMT correlated with FC in FPN. It is important to note that rs-fMRI was also the only modality showing a correlation with days post-injury and PCS, which might point to the overall higher sensitivity of this modality to mTBI outcome.

During the data collection process, an additional post-hoc hypothesis was formulated that states, "Symptomatic concussed individuals will show atypical results on tasks measuring oculomotor functions." While this hypothesis was implicitly embedded within the initial set of hypotheses, it

required explicit articulation for clarity and precision. As discussed above, two oculomotor tasks showed significant differences in performance between concussed subjects and healthy controls – the Anti-Saccades and Optokinetic Nystagmus tasks. While two different metrics showed the deficit, the underlying processes are similar. During Anti-Saccades, the mTBI group had significantly higher mean latency between the time of stimuli presentation and the time of eyes to a target. During Optokinetic Nystagmus, their mean eye velocity relative to target velocity, when the target moved up/down, was significantly lower compared to the control group. Decreased performances on both these metrics describe a delay in targeted eye movements in concussed subjects, pointing to an overall slowing of this function post-concussion. A study by Murray et al. (2020) with sport-related concussion patients showed slower smooth pursuit eye movements in this group compared to healthy controls.

Furthermore, another recent study revealed that mTBI survivors took longer (longer latencies) to react to simple environmental stimuli. Reaction times for auditory and visual stimuli, as well as for saccades in particular, were found to be significantly longer in individuals with a history of mTBI when compared to healthy individuals (Danna-Dos-Santos et al., 2018). In addition, Caplan et al. (2015) conducted a study examining the components of volitional saccadic eye movements and observed that individuals who had experienced mTBI demonstrated slower gaze-shifting speeds. Since smooth pursuit and saccadic eye movements are the underlying components of these two tasks (Anti-Saccades and OKN), they are expected to affect performance as a whole.

2. Neuropsychological assessment

Neuropsychological assessment results were varied across the concussed group which could be related to time post-injury (1-5 weeks), individual dynamics of recovery of each subject, as well

as the mechanism of injury; however, RAVLT, Trail Making Test, and SDMT were the most indicative of cognitive deficits post-injury, pointing at problems with working memory, visual attention, task-switching, and processing speed. Previous studies in mTBI patients are in keeping with these results as they have shown a decrease in processing speed, motor speed, information processing speed, attention, and working memory post-injury (Kim et al., 2015; Clausen et al., 2021; Shi et al., 2021),

Relatively normal neuropsychological results and a lack of correlation with neuroimaging findings could be related to the fact that educational level could have been considered when collecting the data and processing the results, especially with regard to the cognitive tasks. However, recruitment took place primarily within the McGill University community (for both healthy controls and concussed subjects), and their baseline intellectual level was potentially and likely above average (individuals doing their undergraduate degree at the time of their participation or individuals who completed their undergraduate degree or above). In addition, we collected neuropsychological data only from the concussed group due to the availability of normative data for these tests. So, subjects were essentially matched according to age and sex. Thus, it is possible that even if results were technically within the normal range (e.g., 50th percentile), they could still be indicative of a post-concussion deficit compared to baseline. In hindsight, this issue could be resolved with the recruitment of not only age- and sex-matched control groups but also matched by educational level.

Of note, the literature indicates that a neuropsychological assessment used in the acute phase shows deficits that become less apparent in the chronic phase (Echemendia et al., 2001). As we are looking at mTBI within a month post-injury and the time post-injury varies between one and five

weeks, the variability of the results could be due to everyone's recovery pace and measures they have taken to recuperate (e.g., some of the subjects took longer time off work/studies after injury while other returned almost immediately).

3. Dizziness and unsteadiness problems

Rs- and task-based fMRI and ASL results all correlated with dizziness and unsteadiness problems (self-reported by the DHI). These symptoms are commonly reported post-concussion (Szcupak et al., 2016; Kane et al., 2019). Following an mTBI, the affected individual may sustain damage to the vestibular and balance system. Vestibular therapy and rehabilitation have demonstrated positive outcomes for patients with mTBI, specifically regarding cognitive function and the ability to resume daily activities and return to work.

Given that dizziness plays a significant role in both short- and long-term disability following mTBI, its accurate diagnosis and effective treatment are paramount (Szcupak et al., 2016). These symptoms are most apparent, among others, even weeks post-concussion (and, sometimes, months). Thus, their degree of severity strongly correlates with functional, metabolic, and hemodynamic disruptions in the brain post-injury. Their correlation with task-based fMRI using oculomotor tasks is of utmost interest as the evaluated eye movements are also closely related to vestibular problems.

4. Future directions

4.1.LOC/PTA vs. no LOC/PTA: mild simple vs. mild trivial TBI

In North America, mTBI is a "traumatically induced physiological disruption of brain function in the presence of one or more of the following symptoms: loss of consciousness (LOC), post-traumatic amnesia (PTA), alteration in mental state and focal neurological deficit(s)" (ACRM, 1993). However, in Quebec, mTBI has three subtypes: mild trivial, mild simple, and mild complicated. Mild complicated TBI, together with traditional ACRM and WHO criteria, includes a positive CT scan; this category was not included in our study as we relied on WHO criteria for mTBI. However, both mild trivial and mild simple TBIs can be included under the aforementioned definition accepted across North America. The difference between the two is that the latter necessarily includes LOC/PTA for diagnosis, while a mild trivial TBI does not, and both show normal conventional structural neuroimaging. The literature (Amir et al., 2023) also discusses the differences between mTBI and sport-related concussion (SRC); however, so far, both terms "mTBI" and "concussion" have been widely used interchangeably.

Recent findings from our lab revealed that there are, in fact, functional differences between the group of mild trivial and mild simple TBI's. A study by McCabe et al. (2023) showed that a group with mild trivial mTBI and a group with mild simple mTBI have differences in % BOLD signal change; essentially task-based fMRI (using Petrides (2000) working memory task) reflects a higher severity of the latter. This study by McCabe et al. was conducted last year with the same cohort of concussed subjects used in the present study. Because the current study was designed prior to these findings, the results by McCabe were not included in our hypotheses.

Thus, further investigation is required in this cohort of concussed subjects to reveal possible differences between groups in each of the discussed MRI modalities as well as in the oculomotor tasks. A brief look at the data revealed differences in %BOLDsignal change between groups showing a similar trend towards our previous study (McCabe et al., 2023), where subjects with mild simple TBI showed an increase in BOLD signal change in broader areas compared to subjects with mild trivial and healthy controls. In addition, on oculomotor tasks, patients with mild simple TBI showed slower eye velocity and less accuracy in eye movements when following the target compared to patients with mild trivial TBI and healthy controls. Moreover, during rs-fMRI, patients with mild trivial TBI compared to patients with mild simple TBI and healthy controls showed increased functional connectivity in DMN and SN. In addition, further analysis of ASL data showed that the variability in CBF alterations across the mTBI group was possibly related to different recovery patterns in patients with mild simple and mild trivial TBIs. Patients with mild simple TBI showed a decrease in CBF in most of the ROIs, and patients with mild trivial showed an increase in CBF in most of the ROIs (both compared to percentile range established with healthy controls). However, those are only preliminary findings and need further in-depth investigation, which we plan to address outside this thesis.

4.2.Multimodality

Analysis of the individual profiles of each patient (where results were compared to normal ranges based on healthy controls) showed that each patient had results outside of the normal range on rs-fMRI. Many subjects didn't show consistent abnormalities on all other modalities; most had abnormalities on task-based fMRI (with oculomotor tasks or/and working memory tasks) and/or ASL and/or SWI. To understand individual underlying pathologies of concussion in each subject,

we need to obtain a complete picture which is provided by the multimodal approach adopted in the present study. The initial design of this study also included diffusion MRI (dMRI). However, it was advised to exclude it from the framework of this thesis and to investigate it separately due to the thesis being overloaded. Diffuse axonal injury and myelin loss (Johnson et al., 2013) are one of the neuropathologies at the root of mTBI, along with metabolic deviations, cerebral microhemorrhages, and changes in cerebral blood flow. Thus, for the all-encompassing neuroimaging approach covering the reported underlying mTBI pathologies, it is necessary to look at all these modalities: fMRI, ASL, SWI, and dMRI. We plan to investigate this as the next step in our lab, as we have collected the dMRI data with the same cohorts.

Nevertheless, when looking for efficient biomarkers which could be clinically applicable, rs-fMRI is the most promising based on the results of the present study. Extensive normative data collection would be needed for establishing this method as a biomarker as well as clinical longitudinal investigation of subjects post-concussion in the acute stage as well as in subsequent stages of gradual recovery (e.g., within 72h, 1-month and 3-month marks). This modality has the most potential to have clinical implications, due to its relatively easy implementation and consistent abnormalities in mTBI throughout studies.

Of note, the issue with using rsfMRI modality in current stage of research is variability in results described in the literature we discussed throughout this thesis. The only thing remains consistent – abnormal results in concussed individuals compared to healthy controls, weather it is increase or decrease in discussed networks. The root of this variability may lay in the mechanisms of injury, severity and location of the impact, each subject's individual history, etc. The resolution of this

problem and making this modality ready for clinical application could potentially be in subtyping of mild TBIs. Similar approach could be applicable to other modality we discussed – ASL – which shows drastic variability among concussed subjects, with majority of them showing alterations in CBF, but not having a uniform pattern of abnormalities.

Other factors also could affect this variability in the literature, as not all of the studies have uniform procedure for accounting for history of previous injuries, psychiatric and neurological conditions, taking medications, etc. As we stated earlier, all participants with mTBI in the Chapter II, III, and IV were recruited according to specific exclusion criteria – having previous history of neurodevelopmental or neurological disorders, prior head injury within the past year or continually suffering symptoms from a previous head injury at the time of injury, history of ADHD, anxiety and depression (according to BAI and BDI-II questionnaires), and presence of significant abnormalities seen on structural MRI scans (indicative of more severe TBI). All of the subjects with mTBI also were symptomatic according to PCSS and non-athletes, when the majority of the studies with mTBI recruited athletes, often asymptomatic, which also could contribute to variability in findings. In addition, it is more likely for general population group to have more variability in mechanisms of injury then in a group of athletes (which are also often recruited from same sport).

It is important to note that all healthy participants in the Chapter II, III, and IV. Had no previous history of neurodevelopmental or neurological disorders, prior head injury within the past year or continually suffering symptoms from a previous head injury at the time of injury, history of ADHD, anxiety and depression (according to BAI and BDI-II questionnaires), and presence of significant abnormalities seen on structural MRI scans.

4.3.Sex differences

Even though in this thesis we did not observe significant differences between groups of males and females, we still cannot exclude this possibility, considering uneven distribution of two sexes in studies presented in Chapters III and IV (21 females vs. 8 males). Most previous finings focused on sports-related concussions, with limited research on civilian or military-related concussions in females, although incidence of concussions in females is significantly higher. While females tend to report more overall symptoms than males, specific symptom patterns vary. Neuropsychological studies often indicate poorer visual memory performance in females post-concussion, but findings are still inconsistent (Merritt, Padgett, & Jak, 2019). We see as a possible continuation of these studies the recruitment of more male concussed subjects to perform more accurate sex-based analysis in concussed group.

5. Practical implications

It is important to note that this study was conducted with a cohort from the general population and non-athletes, distinguishing it from other studies in the mTBI field. In addition, to our knowledge, it is one of few studies that exclusively included symptomatic individuals.

This study contributes to the development of valid norms for fMRI, which will eventually facilitate its use in a clinical setting and identify tools that could be used interchangeably with fMRI to save resources. It also contributes to gathering normative data for diagnostic and rehabilitation purposes (NeuroFlex) for patients with mTBI and oculomotor deficits; however, more investigation is required before this tool can be considered a “diagnostic” instrument. So far, it has shown its

potential to complement concussion screening for a more in-depth comprehension of the impact of concussion on oculomotor functions.

Eventually, we expect this to lead to the development of mTBI biomarkers with the aim to develop an objective approach to the diagnosis of concussion. Identification of biomarkers is essential to determine when patients are truly ready to return to work, study, or sports. These biomarkers also can apply to a more effective assessment of recovery, which can have important medico-legal implications.

6. Limitations

While we have addressed numerous limitations in the Discussion section, it is essential to acknowledge that a few remain unexplored or unaddressed. First, this study included subjects who suffered mTBI through different mechanisms, including direct impact and acceleration/deceleration: motor vehicle accidents, skiing/snowboarding, household accidents, etc. The mechanism of injury may affect the variability we see in structural/hemodynamic/metabolic changes in this study as well as may explain the wide range of findings in the literature. Secondly, task-based fMRI cannot be directly quantitatively compared to resting state modalities – rs-fMRI, ASL and SWI. This is the reason this study used qualitative comparisons. Thirdly, our study did not account for the LOC and/or PTA, and our recent preliminary look at the data showed possible differences with regards to this. We will address it in the subsequent studies. Forthly, recruitment took place primarily within the McGill University community (for both healthy controls and concussed subjects), and their baseline intellectual level was potentially and likely above average

(individuals doing their undergraduate degree at the time of their participation or individuals who completed their undergraduate degree or above).

In addition, our study also looked only on PCS score only post-concussion, and we did not acquire baseline score from the subjects prior to the injury, which could be a potential limitation. Another limitation of this thesis is the absence of another clinical group to account for specificity of the abnormalities shown in concussed subjects. Finally, we talked about the differences in BOLD signal% change in lingual gyrus between concussed subjects and healthy controls during oculomotor tasks. Dysfunction in the lingual gyrus may impact cortical visual acuity and visual fields (Palejwala et al., 2021); thus, to control for this in the future studies all the subjects are recommended to see an ophthalmologist as well as undergo both visual acuity and formal visual field testing.

Overall Conclusions

Concussed individuals showed abnormal neuroimaging findings in one or more MRI modalities used in this study, showing functional, metabolic, and hemodynamic disruptions, with in rare cases structural findings (microbleeds). All concussed subjects showed abnormalities on resting-state fMRI, and it was the only modality that correlated with post-concussion symptoms and number of days post-injury, suggesting that it could be the most efficient in identifying concussion and the modality that has the most potential to become a reliable biomarker of concussion in the future.

Even though ASL results varied across the cohort, most of the subjects had atypical cerebral blood flow, such that patterns could be carefully investigated for each mechanism of injury and mTBI subtype. While SWI did not uncover consistent structural differences within the concussed group, it is important to note that this does not rule out this possibility entirely. It is worth mentioning that three subjects did exhibit microbleeds following their injury, indicating that such structural variations may be specific to individual characteristics.

Areas with atypical activation patterns during task-based fMRI were associated with oculomotor dysfunctions, and concussed subjects showed oculomotor deficits compared to healthy controls, which aligns with the literature on oculomotor deficits following mTBI. These findings further support the notion of overlapping oculomotor circuitry and the underlying pathophysiology of mTBI. Hence, oculomotor assessment holds promise as a practical approach for identifying biomarkers of mTBI, with Anti-Saccades and Optokinetic Nystagmus emerging as the two tasks most sensitive to concussions.

The findings mentioned above provide encouraging insights for the advancement of an objective approach to the diagnosis of concussion.

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List of abbreviations

2D-pCASL – two-dimensional pseudo-Continuous Arterial Spin Labeling

ACC – Anterior Cingulate Cortex

ACRM - American Congress of Rehabilitation Medicine

ASL – Arterial Spin Labeling

BAI – Beck Anxiety Inventory

BDI-II – Beck Depression Inventory II

CBF – Cerebral Blood Flow

CEF – Cingulate Eye Field

CI – Confidence Interval

CT – Computed Tomography

DAN – Dorsal Attention Network

DHI – Dizziness Handicap Inventory

DLPFC – Dorso-Lateral Prefrontal Cortex

DMN – Default Mode Network

dMRI – diffusion Magnetic Resonance Imaging

DWI – Diffusion-Weighted Imaging

FC – Functional Connectivity

FEF – Frontal Eye Field

fMRI – functional Magnetic Resonance Imaging

FPN – Fronto-Parietal Network

FWEr – Family-Wise Error

GCS – Glasgow Coma Scale

HCs – Healthy Controls

IFG – Inferior Frontal Gyrus

LOC – Loss Of Consciousness

MFG – Middle Frontal Gyrus

MRI – Magnetic Resonance Imaging

mTBI – mild Traumatic Brain Injury

OKN – Optokinetic Nystagmus

PCS – Post-Concussion Symptoms

PEF – Parietal Eye Field

PET – Positron Emission Tomography

PFC – Prefrontal Cortex

PTA – Post-Traumatic Amnesia

PWI – Perfusion-Weighted Imaging

ROI – Region of Interest

rs-fMRI – resting state Magnetic Resonance Imaging

RSN – Resting State Network

SAC - Standardized Assessment of Concussion

SCAT - Sport Concussion Assessment Tool

SEF – Supplementary Eye Field

SFG – Superior Frontal Gyrus

SN – Salience Network

SPECT - Single-Photon Emission Computerized Tomography

SRC – Sport-Related Concussion

SWI – Susceptibility Weighted Imaging

TRN – Task-Related Network

WHO – World Health Organization

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