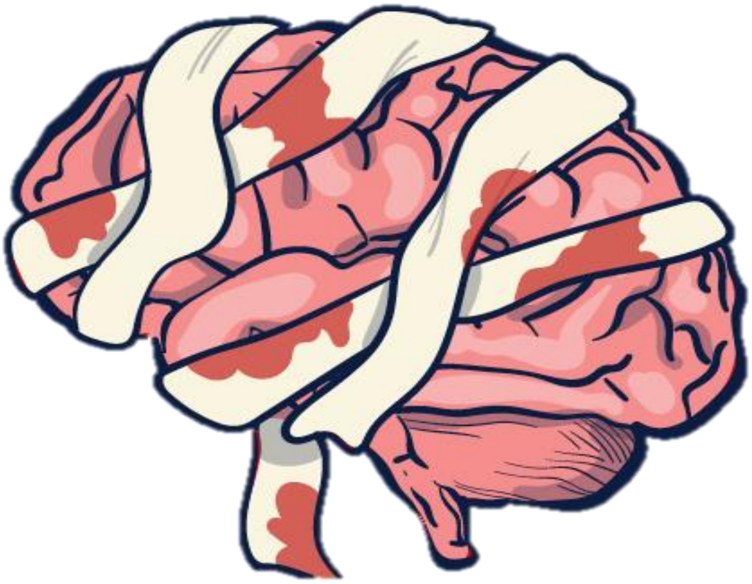
WATCH YOUR HEAD: BRAIN NEUROPHYSIOLOGY AND CONTACT SPORTS

By Michail Ntikas



A thesis submitted to the University of Stirling in partial fulfilment for the degree of Doctor of Philosophy

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“Football is the continuation of war by other means”

“[Football] in other words is war minus the shooting”

George Orwell

Orwell was referring to the hatred that football and war both cause; however, they have one more thing in common, chronic traumatic encephalopathy (CTE) amongst their “soldiers”.

Declaration

I declare that this thesis was composed and that all the data was collected and analysed by myself, Michail Ntikas, under the supervision of Dr Magdalena Ietswaart, Prof David Donaldson, Prof Lindsay Wilson, and Dr Dimitrios Koutris. Neither the thesis, nor my contribution to the original work presented therein was submitted to this or any other institution for a higher degree.

Chapter 3 (Scoping review) was performed in collaboration with a colleague (Ms Liivia-Mari Lember), to ensure scientific rigour. Title/abstract screening, full text screening, data extraction and quality assessment were done by each of us in parallel, followed by a joint consensus process where necessary. The introduction and discussion sections of Chapter 3 were written by the thesis author largely independently, while the write up of methods and results was split equally, thus those part are of shared authorship.

This work was significantly disrupted by the COVID-19 pandemic during which lab experiments at the University of Stirling were not allowed for a period of 18 months. As a result, the research was delayed and the thesis plan was in part revised with regards to experimental work.

Michail Ntikas

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DISSEMINATION AND PUBLICATIONS

Chapter 2 of this thesis has been published:

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Chapter 3 of this thesis is based on the following published protocol:

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Conference presentations:

Ntikas, M., D. Donaldson, L. Wilson, D. Koutris, M. Ietswaart. Repeatedly heading a football diminishes attention and impairs learning: Evidence from an experimental imaging study. *Poster presentation, Scottish Dementia Research Consortium (SDRC) annual conference, Glasgow, Scotland, UK, May 2022.*

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ABBREVIATIONS TABLE

Biofluid markers

CSF = CerebroSpinal Fluid
NfL = Neurofilament Light protein
S100b = S100 calcium-binding protein B
NSE = Neuron Specific EnolaseGFAP = Glial Fibrillary Acidic Protein
BDNF = Brain Derived Neurotrophic Factor
UCH-L1 = Ubiquitin Carboxy-terminal Hydrolase L1
CK-BB = Creatine Kinase isoenzyme BB
NAA = N-acetyl aspartate
Glx = Glutamine
tCR = Creatine-containing compounds
tCho = Choline-containing compounds
Cho = Choline
EV = Extracellular Vesicles

Neuroimaging

EEG = Electroencephalography
ERP = Event Related Potential
CSp = Cortical Silent period
tDCS = transcranial Direct Current Stimulation
MRI = Magnetic Resonance Imaging
DTI = Diffusion Tensor Imaging
MRS = Magnetic Resonance Spectroscopy
TMS = Transcranial Magnetic Stimulation
rTMS = repetitive Transcranial Magnetic Stimulation
fMRI = functional Magnetic Resonance Imaging
qMRI = quantitative Magnetic Resonance Imaging
CT = Computerised Tomography

Data and Statistics

ANOVA = Analysis Of Variance
OP = Observed Power
SPSS = Statistical Package for Social Sciences
BF = Bayes Factor
SD = Standard Deviation
RMS = Root Mean Square
COP = Centre Of Pressure
COM = Centre Of Mass
ApEn = Approximate Entropy
mph = miles per hour
psi = pounds per square inch
HRmax = Heart Rate maximum

Sports

UEFA = Union of European Football Associations
FA = Football Association
MMA = Mixed Martial Arts
GK = Goalkeeper
DC = Central Defender
FB = Full Back
CM = Central Midfielder
CF = Center Forward

Clinical terms & Brain areas

RSHI = Repetitive Subconcussive Head Impacts
TBI = Traumatic Brain Injury
mTBI = mild Traumatic Brain Injury
SR-TBI = Sport Related Traumatic Brain Injury
CTE = Chronic Traumatic Encephalopathy
ALS = Amyotrophic Lateral Sclerosis
PTSD = Post-Traumatic Stress Disorder
ASAPS = American Society of Anaesthesiologists Physical Status Classification System
CENTER-TBI = Collaborative European NeuroTrauma Effectiveness Research in TBI
GCS = Glasgow comma scale
AIS = Abbreviated Injury Scale
ICU = Intensive Care Unit
ER = Emergency Room
A&E = Accident & Emergency
M1 = primary motor cortex
LC = Locus Coeruleus

Neurocognitive tests

ImPACT = Immediate Post-Concussion Assessment and Cognitive Testing
SCAT = Sport Concussion Assessment Tool
GOSE = Glasgow Outcome Scale Extended
PHQ-9 = Patient Health Questionnaire
GAD-7 = Generalised Anxiety Disorder scale
PCL-5 = Post-traumatic Stress Disorder Checklist
SF12v2 = Short-Form-12 version 2
RPQ = Rivermead Post-concussion symptoms Questionnaire
SRTT = Serial Reaction Time Task
PAL = Paired Associates Learning
SWM = Spatial Working Memory
CANTAB = Cambridge Neuropsychological Test Automated Battery

THESIS ABSTRACT

In the world of contact sports there is rising concern about the long-term effects of sport participation on athletes' brains. Apart from concussions, the repetitive subconcussive head impacts (RSHI) in sports have been suggested to be detrimental for brain health. RSHI in football are thought to be linked with the onset of neurodegenerative diseases, like Chronic Traumatic Encephalopathy and Alzheimer's disease. However, to understand how and why the athletes' brains might suffer in the long-term, we should first understand the acute brain changes caused by the potential risk factors for brain damage (concussive and sub-concussive impacts). The experimental studies of this PhD thesis aim to investigate the acute effects of heading, the main source of RSHI in football, on the brain functions of athletes, by using a mixture of sensitive neuroscientific modalities. Secondary data is used in this thesis for method development and to examine the broader problem posed by sport-related head impact. Chapter 1 expands on the aims of the thesis. Chapter 2 presents the current state of the literature on RSHI. Chapter 3 includes a scoping review of the literature on biofluid markers use to assess the effects of RSHI highlighting the high heterogeneity of the existing studies and providing guidelines for future studies. Chapter 4 includes an investigation of the injury characteristics and prognosis of sport-related traumatic brain injury (TBI). It highlights the seriousness of sport-related TBI and specifically sport-related mTBI, whose remaining effects can potentially be worsened by the burden of RSHI during play. Chapter 5 investigated the effects of RSHI on balance in various ways, providing no evidence of an effect, while chapter 6 provides evidence for associative memory changes caused by heading. The following chapters (7 & 8) attempted to further examine the alterations in cognitive functioning post heading and present the first EEG evidence that the cognitive functions of attention, memory and learning are acutely affected by RSHI. After showing that RSHI affect mainly association learning and attention processes and not affect response inhibition, motor control and motor learning, chapter 9 aimed to examine the replicability of RSHI effects on motor cortex inhibition, providing evidence of no effect. The outcome of this thesis is that RSHI have detrimental effects to athletes' cognition, mainly in the functions of learning and attention, while motor control appears to remain intact.

CHAPTER 1. General Introduction

During the early 2000s, the detrimental effects of contact sport participation started receiving more attention from the scientific community. The rising incidence of neurodegenerative diseases in former contact sport players raised concerns about the safety of contact sports and gained media attention, amplified by a Hollywood film, starring Will Smith, titled “Concussion” and with extensive documentaries from mainstream media. This media attention led some governing bodies to take measures to ensure the safety of contact sports athletes. In (European) football specifically, in recent years the governing bodies in the UK and the UEFA have tried to reduce the number of RSHI occurring during training and matches; see Chapter 2 (literature review) for specific information on the steps taken from those governing bodies since 2020.

The problem: long term effects of contact sport participation

Head impacts and multiple concussions have been linked with the increased probability and the earlier onset of neurodegenerative diseases (CTE, ALS, Alzheimer’s disease, etc.). Chronic Traumatic Encephalopathy (CTE) is a syndrome caused by recurrent head trauma which is characterised by cognitive impairment and behavioural disturbance (Stern et al., 2013). Initially, CTE was associated mainly with boxing (Solomon, 2018), and the relevant research was not focusing on the potential long-term effects sport-related concussions in other sports might have. Repetitive subconcussive blows to the head in sports other than boxing started gaining more attention this century with initial speculations about their adverse effects presented as early as 2001 (Rabadi & Jordan, 2001).

Sport related concussions have notable adverse effects on cognition and motor control, however those symptoms wave off after one or two weeks, so for some academics and governing bodies they were considered minor (McCrory et al., 2017). This has changed the last years, in which there is an increased concern about the presence of CTE and other neurodegenerative diseases in athletes who participated in sports where athletes suffer frequent concussions and numerous other, non-concussive, impacts. This shift to the investigation of the long-term effects of concussions begun after several autopsies on American football athletes with a history of multiple concussions showed that those athletes had diffuse amyloid plaques and neurofibrillary tangles, and tau positive neuritic threads all over their cerebral cortex (Omalu et al., 2005, 2006, 2010). This finding opened a serious discussion about the long-term effects of contact sport participation initially in the USA and subsequently in Europe and the rest of the world. Recent evidence from autopsies performed in retired football (soccer) players provided evidence of CTE caused by football participation and football heading specifically (Ling et al., 2017), with some CTE cases in former professional UK football players getting wide media attention (Slater, 2017).

Football (Soccer) for a long time had not been considered a sport that can induce considerable burden to the brain, as the impacts during football play seem less severe compared to the ones in American football or rugby. Somehow ironically, Scottish culture seems to have had unconsciously recognised the consequences of football heading by using the term “heid the baw” (head the ball) to refer to annoying, idiotic people implying cognitive impairment from intensive ball heading. The first evidence for long-term problems caused by football participation was found in Italy during the 2000s, where football players were found to have higher incidence of Amyotrophic Lateral Sclerosis (ALS or motor neurone disease).

The link between ALS and football was examined after an Italian Judge called for the investigation of the incidence of ALS on football players. This investigation found 33 cases of ALS in a pool of 24,000 football players, a number more than 20 times higher than the prevalence in the general population (Piazza et al., 2004). Moreover, the age of onset of ALS in the football players was found to be 40-50 years, lower than that for the general population (60+ years). The same pattern of results was also found by Chio (Chiò et al., 2005, 2009), who examined the ALS prevalence in all Italian professional football players from 1970 to 2001. After excluding players not born in Italy, they identified 15 cases of ALS in a cohort of 7325 football players. For the 15 football players who were playing football after 1970 the mean age of ALS onset was 43.4. Chio (Chiò et al., 2005) found that former football players were 6.5 more likely to develop ALS, and this ALS onset happened earlier than in the general population. They concluded that playing football is a risk factor for the development of ALS and they offer a series of possible hypotheses about which aspects of football playing might be the risk factor for ALS. Those hypotheses include the intense physical exercise, the head impacts and concussions and the performance enhancing drugs. However, the fact that in the ALS prevalence in 6000 professional cyclists was 0 (as cited in Piazza et al., 2004), makes the hypotheses that physical exercise or the drugs abuse less likely to be a risk factor, since cycling is a sport with very intense physical activity and in which the use of performance-enhancing drugs is a common practise.

Interestingly, in Chio’s et al (Chiò et al., 2005) study, none of the players with ALS had a history of major head traumas, family history of ALS or contact with substances suspected to be related to ALS. This finding further strengthens Piaza’s proposal that mild recurrent head injuries rather than severe head trauma might increase the risk of developing ALS and decrease the age of onset in people who are genetically predisposed.

ALS is not the only neurodegenerative disease linked with football participation. A group of 14 former soccer players who developed dementia late in life was followed by a team of researchers (Ling et al., 2017). Out of those 14, six gave them permission to examine their brains post-mortem. All those six cases showed mixed pathologies with four of them been diagnosed with CTE, together with other neurodegenerative pathologies like Alzheimer's disease, TDP-43 pathology and hippocampal sclerosis. Based on the significantly more CTE cases in this cohort of former soccer players compared to the population of elderly individuals with or without neurodegenerative diseases and the absence of a history of recurrent concussions, they stated that mainly the CTE but maybe also the AD and TPD-43 pathologies might be attributed to the repetitive and prolonged exposure to subconcussive head impacts from both heading and head-to-head collisions. CTE was also the most frequent neuropathological finding from the Australian Sports Brain Bank, a biobank accepting brain donations from Australian athletes of any level, with 12 out of the 21 first donated brains having pathognomonic CTE lesions. Strikingly, 20 out of the 21 donated brains presented some form of neurodegeneration (Suter et al., 2022). Furthermore, recent investigations on former Scottish football players found them to be 3 to 5 times more likely to have died from a neurodegenerative disease, like Alzheimer's disease, compared to the general population (Mackay et al., 2019).

Repetitive subconcussive head impacts and football (soccer) heading.

The aforementioned initial studies on former contact sport athletes indicate that sports with repetitive head impacts might be a risk factor for the development of neurodegenerative diseases, mainly but not only CTE. In this thesis the focus is primarily on the effects of the repetitive head impacts on football players. Unlike other contact sports, football has a relatively lower incidence of concussions, especially compared to American football, Australian rules football, rugby and ice-hockey (Clay et al., 2013; Pfister et al., 2016), however, it is unique, since it is the only popular contact sport in which head impacts (heading) are voluntary and integral part of the game. This unique phenomenon makes the investigation of headers more efficient for experimental studies, since in other sports most head impacts are considered accidental and replicating them in a lab raises ethical considerations. Taken together with the popularity of the sport across all ages (especially in Europe), the importance of the potential harmful effects of this integral part of play has on athletes' brain becomes apparent.

So far the literature on the effects of sport-related subconcussive head impact, including heading, on brain functioning is mixed (see Chapter 2, literature review, for a detailed presentation of the current literature). A plethora of studies investigated how heading affects postural stability, motor control, cognitive functioning, biomarkers of neuroinflammation and brain integrity (Mainwaring et al., 2018; Tarnutzer et al., 2017), however they have not yet managed to provide a clear picture on which brain processes are predominantly affected by RSHI. Chapter 2 (literature review) of this thesis expands on the argument that sensitive multimodal techniques are necessary to investigate the aforementioned effects. Since tools used to assess concussion are developed to pick up issues in athletes with explicit symptoms of concussion and not to pick up subtle brain changes that do not manifest in overt symptoms, their use is deemed of limited utility to investigate RSHI.

The necessity for comprehensive approach and sensitive measures.

In this thesis it is argued that there is a lack of studies that use a comprehensive approach, including multiple measures of the brain processes thought to be affected by RSHI. Furthermore, apart from comprehensive and multimodal, research on the effects of RSHI on athletes' brain health should utilise measures that are sensitive enough to detect subtle changes. The use of measures used to detect concussions and TBI should be avoided, as they lack sensitivity and can only perplex and adrift the conservation about any potential effects.

In order to understand how RSHI acutely affect brain processes sensitive neurophysiological and neuroscientific techniques should be used immediately after those RSHI have taken place. Neurophysiological techniques like EEG have been deemed very effective at examining subtle cognitive changes that go undetected on conventional tests in studies that examine mild brain injury (Broglio et al., 2011), however their use so far in RSHI is limited (Ntikas et al., 2022; Tarnutzer et al., 2017). In this thesis, multiple behavioural measures of cognitive function and motor control were used initially in order to identify the which cognitive functions and motor control measures appear more promising at investigating the effects of RSHI. Following this, EEG measures were used to examine those identified cognitive functions further and provide evidence of acute cognitive decline immediately after heading.

A comprehensive approach is necessary to understand the exact brain mechanisms and functions affected by RSHI. In this thesis a wide range of cognitive and motor control tasks are implemented on the same, or similar, cohorts of athletes (young adult football players playing for local clubs in central belt of Scotland) with the aim to provide a much-needed multi-method approach in the field of RSHI. A comprehensive approach allows for direct comparisons between different measures and allows this thesis to provide a parallel investigation of evidence on motor control issues and cognitive impairment, providing evidence only for the later.

Aims & overview of thesis

The primary aim of this thesis was to use sensitive neuropsychological tests and neuroscientific techniques to identify the alterations in athletes cognitive functioning caused by RSHI in football at the acute stage; that is, immediately after heading. To understand better the causes of the long-term problems found in former contact sport athletes, first there is a need to understand what is happening in the brain immediately following RSHI. Finding the cognitive functions and the brain areas showing changes, apart from providing evidence about the mechanisms of injury, will also provide a way to assess the burden athletes have experienced during play, so they can be offered the appropriate support (i.e., rest, advised to refrain from RSHI for a certain period) in order to minimise any further adverse effects. Specifically, in this thesis the effects of RSHI on cognitive functions and motor control/physiological measures were examined, providing supportive evidence for effects on the basic cognitive functions of attention, memory and learning and evidence of no effects on the motor control processes of the athletes.

Notably, this thesis offers the first brain-level electrophysiological evidence of acute attention, memory and learning problems after heading similar to the ones seen following concussion (Chapters 7 & 8, ERPs oddball and Go/No-Go & ERP verbal learning respectively). Before that, a literature review examining the state of the art on the field of RSHI in contact sports is presented (Chapter 2, literature review) in which it becomes apparent that certain neuroscientific techniques can offer valuable evidence about the effects of RSHI on athletes' brain health, however, their use is only limited at this point. Interestingly, apart from neuroimaging techniques, some biofluid markers of brain injury that provide evidence of specific types of brain damage (i.e., structural, axonal, glial) on TBI patients might also be of use in the investigation of RSHI. However, at this point, the literature so far is very diverse (Chapter 2, literature review), so it was decided that a scoping review of the biomarker literature is necessary before their use in any experimental designs. The main aims of this scoping review (Chapter 3) were to identify which biomarkers are most likely to be affected by RSHI and at which time they are more likely to be affected (e.g., 1 hour, 24 hours or 48 hours post heading).

As reported in Chapter 2 (literature review) and seen in Chapter 3 (Scoping review), it can be challenging to discriminate between concussive and subconcussive impacts with many studies failing to do this effectively. In this thesis we argue that concussion can be a major confounding variable when examining RSHI that needs to be effectively controlled. The 4th chapter highlights the persistent effects concussions have on athletes. It focuses on the characteristics and the prognosis of sport related TBI, by using existing data from patients that attended the emergency services of European hospitals with a TBI. This database offers a unique chance to compare the outcomes of sport-related TBI and sport-related mTBI with the outcomes seen in TBI in the general population. The consequences of mTBI can be very serious, causing neurodegeneration which is linked with disability, memory impairments and reduced quality of life. Those impairments increase with both severity (Whiteneck et al., 2016) and frequency of TBI (Wilson et al., 2017). Although there are significant risks in repeated injuries, many athletes choose not to report their mTBI symptoms or wait until the playing season is over before doing so (Asken et al., 2016). This practise stems from players' reluctance to miss training or games, or the pressure put on them from their coaches or family to play through the injury (Kroshus et al., 2015). To safeguard players against the consequences of concussion the 5th consensus statement on concussion in sport developed comprehensive return-to-play (RTP) guidelines (McCrory et al., 2017). However, the analysis reported in Chapter 4 (TBI prognosis) revealed that a considerable percentage of patients with sport related mTBI has not recovered 3- and 6-months post injury, which raises concerns that those RTP guidelines might need to be revised.

So far, in the field of sport-TBI outcomes much of the research has focused on capturing outcomes in defined athlete populations, such as elite athlete or college participants, with little data available of wider, community level sports TBI and outcomes. In this thesis, evidence of persistent symptoms in a considerable proportion of patients with sport-related mTBI is reported even at 6 months post injury. This raises further concerns about the potential effect of RSHI can have especially on this sub-population of athletes that are allowed to return to play, but still have some underlining problems. The effects of RSHI on this sub-population was not in the scope of this thesis, but chapter 4 highlights the importance of previous concussive impacts while investigating the effects of RSHI. Based on this, in all the experiments executed as part of this thesis, all participants were clear of concussions at least 2 years before taking part. Doing so, ensures the safety of the participants taking part in the experiments and provides further confidence that the findings presented in this thesis are not caused by any pre-existing or underlying concussive issues, but are purely due to the heading induced by the experimental protocol.

Chapters 5 through 9 present a series of measurements aimed to identify the mechanisms which are affected by football heading. In concussion diagnosis self-reported symptoms, motor control and cognitive functioning are the main domains assessed in order to identify a suspected concussion. For example, the widely used concussion assessment tool SCAT5 includes an assessment of athletes' symptomatology, cognitive functioning and balance (Echemendia et al., 2017). Here multiple sensitive measures of motor control and basic cognitive functioning are employed. Importantly, all the measures employed were not clinical assessment tools, but measures used to detect subtle and specific brain differences in healthy populations or high-functioning versions of clinical tools (thus not tools aimed to assess cognitive decline, but subtle cognitive differences between groups). This thesis aimed at examining both motor control and cognition with sensitive measures and provide evidence on which cognitive or motor control functions are affected by heading and which are not.

This parallel assessment of motor control processes and the basic cognitive functions of attention, memory and learning provides rich insights into the nature of the deleterious effects of football heading. This thesis provides evidence of attention and working memory alterations (Chapter 7, ERP oddball and Go/No-Go) and learning impairments (Chapter 6, behavioural measures of cognitive functioning & Chapter 8, ERP verbal learning) caused by a session of repetitive headers, while providing evidence of no effects on multiple assessments of motor control (Chapter 5, examining postural stability; Chapter 6, Behavioural measures of cognitive functioning; Chapter 9; TMS and motor learning

To provide further clarity on the findings, and acknowledging the mixed findings in the literature so far as well as the lack of replicability of the effects (Mainwaring et al., 2018; Tarnutzer et al., 2017), most measures that failed to provide evidence of an effect were also analysed with Bayesian linear models in order to investigate if the data collected provides evidence for the absence of an effect. Unfortunately, so far, the vast majority of studies on RSHI that were not able to provide evidence of effects of RSHI on athletes' brain health made strong comments about the absence of those effects, without providing direct evidence that their data provides support for such statements. Here, by using Bayesian analyses a clear distinction is being made between which measures provide evidence of an effect (alternative hypotheses), which provide evidence for no effect (null hypotheses) and which do not provide evidence for neither the null nor the alternative hypotheses, and therefore more research is necessary. Chapter 5: postural stability and Chapter 9: TMS and motor learning provide more information on how Bayesian linear models can provide this information.

Since studies in this field are used to inform clinical implications and recommendations on ways to reduce any adverse effects of RSHI and concussions, it is important that the data used those studies is analysed properly and any conclusions are evidence based and not assumptions or leaps of faith. Therefore, all data collected in this thesis was analysed with the most efficient way and the findings are interpreted with caution while avoiding bold statements, especially when it comes to absence of effects.

CHAPTER 2. Repeated Sub-Concussive Impacts and the Negative Effects of Contact Sports on Cognition and Brain Integrity

This chapter includes a narrative review of the current state of the literature as published in the International Journal of Environmental Research and Public Health.

International Journal of
Environmental Research
and Public Health

Review

Repeated Sub-Concussive Impacts and the Negative Effects of Contact Sports on Cognition and Brain Integrity

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Abstract: *Sports are yielding a wealth of benefits for cardiovascular fitness, for psychological resilience, and for cognition. The amount of practice, and the type of practiced sports, are of importance to obtain these benefits and avoid any side effects. This is especially important in the context of contact sports. Contact sports are not only known to be a major source of injuries of the musculoskeletal apparatus, they are also significantly related to concussion and sub-concussion. Sub-concussive head impacts accumulate throughout the active sports career, and thus can cause measurable deficits and changes to brain health. Emerging research in the area of cumulative sub-concussions in contact sports has revealed several associated markers of brain injury. For example, recent studies discovered that repeated headers in soccer not only cause measurable signs of cognitive impairment but are also related to a prolonged cortical silent period in transcranial magnetic stimulation measurements. Other cognitive and neuroimaging biomarkers are also pointing to adverse effects of heading. A range of fluid biomarkers completes the picture of cumulating effects of subconcussive impacts. Those accumulating effects can cause significant cognitive impairment later in life of active contact sportswomen and men. The aim of this review is to highlight the current scientific evidence on the effects of repeated sub-concussive head impacts on contact sports athletes' brains, identify the areas in need of further investigation, highlight the potential of advanced neuroscientific methods, and comment on the steps governing bodies have made to address this issue. We conclude that there are indeed neural and biofluid markers that can help better understand the effects of repeated sub-concussive head impacts and that some aspects of contact sports should be redefined, especially in situations where sub-concussive impacts and concussions can be minimized.*

Keywords: *contact sports; soccer; heading; traumatic brain injury; concussion; sub-concussion; brain health; dementia prevention; neuroimaging; fluid biomarkers*

1. Introduction.

Sports, especially when performed on a regular basis, are yielding a wealth of benefits for the cardiovascular fitness, for psychological resilience and for cognition. Because of the dose-response relation between physical activity and health, persons who wish to further improve their personal fitness, reduce their risk for chronic diseases and disabilities or prevent unhealthy weight gain may benefit by exceeding the minimum recommended amounts of physical activity. However, sport participation comes with its own risks, especially the risk of injury. Those injuries can be musculoskeletal, or brain related. Repeated concussion has been shown to pose a risk to future brain health (L. Wilson et al., 2017), but the concern is that the low level but repeated nature of routine head impact in contact sport is similarly detrimental to otherwise healthy individuals. This is particularly relevant in the context of the current special issue dedicated to evidence for multifaceted positive effects of exercise and sport on cognitive functions and for prevention of cognitive decline.

In this review we therefore focused on the negative consequences of sport participation on brain health by reviewing the literature on the effects of repetitive sub-concussive head impacts in contact sport participation. The aim of our contribution to this special issue is threefold. First, we aim to draw attention to possible negative effects of sport-related exposure to impacts. Secondly, we want to highlight the most promising avenues for the much-needed increase in research activity on this topic. Lastly, we warn against any complacency within sport governance in light of scientific evidence.

2. Potentially negative effects of sports on brain health

Concern about the danger that sport-related concussion poses to the nervous system has become more accepted, in particular in contact sports such as American football, soccer, rugby, ice-hockey and boxing. The rates of sport-related concussion are high and have been found to be increasing year on year (Selassie et al., 2013). “One population in particular, high school athletes, must be monitored more closely as research indicates that they are neurologically more vulnerable than the collegiate population” (Field et al., 2003). This monitoring is important especially since teenagers have been found to have the highest rates of sport-related concussion amongst athletes (Selassie et al., 2013), with two thirds of concussions involving athletes under the age of 19 (Coronado et al., 2015). There were approximately 800,000 reported concussions suffered by high school athletes in the US between 2005 and 2010, with boys’ American football accounting for 47.8% of this number (Castile et al., 2012). However, the examination of the incidence of traumatic brain injury at scale dated back at least 20 years, until the launch of a recent large-scale international observational study (Maas et al., 2015). Cohort studies like this are critical to examine sport-related brain injury incidence, sport-specific profiles, demographic data such as age and sex, and critically the incidence and outcomes of those incurring multiple brain injuries while participating in sport. While this monitoring of concussions is important for the prevention of further catastrophic damage, in particular that associated with second impact syndrome (Cantu, 1998), a number of studies have also demonstrated that neurophysiological changes can accumulate due to repetitive sub-concussive blows (Bailes et al., 2013; Baugh et al., 2012; Erlanger et al., 1999; Gavett et al., 2011; McKee et al., 2009; Poole et al., 2014; Talavage et al., 2014). As yet, the physiological mechanisms underlying the consequences of sport-related impact in general, and repetitive sub-concussive head impacts (RSHI) in particular, are yet unknown. Below, we consider what the different assessment measures now available reveal about the nature of the effects of RSHI on brain health.

3. What cognitive markers tell us about the consequences of routine impact in sport

Literature on the effects of RSHI on cognitive markers (neurocognitive testing) is mixed, with studies showing some cognitive domains to be affected by RSHI while others failed to find any link between RSHI and neurocognitive performance (Mainwaring et al., 2018). The majority of neurocognitive studies resorting to clinical instruments used to diagnose and monitor full-blown concussion, such as concussion recognition tools SCAT 3-5 and ImPACT, did not find any evidence (Caccese et al., 2019; M Dorminy et al., 2015; J. R. Miller et al., 2007; Poole et al., 2014) with regards to the consequences of routine (sub-concussive) head impacts in sport. However, some researchers identified differences between the reaction time and processing speed of non-concussed players in contact sports with high concussion-incidence compared to players with “low-contact sports” by using ImPACT (Tsushima et al., 2016).

Studies that used cognitive tests assessing specific cognitive domains such memory, attention, learning, and reaction time, also yield mixed results, with some finding RSHI affecting cognition (Di Virgilio et al., 2016; Koerte et al., 2017; Levitch et al., 2018, 2020; T. W. McAllister et al., 2012). Specifically, performing 20 soccer headers, akin to a routine heading drill, was found to impair scores in a pair associative learning task compared to baseline assessment (Di Virgilio et al., 2016). Additionally, the number of recently performed headers was found to be negatively correlated with psychomotor speed while the number of headers performed the past year was negatively correlated with verbal learning and verbal memory performance (Levitch et al., 2018). Moreover, soccer, American football, and ice-hockey players' scores on sensorimotor function and learning have been found to improve less when compared to the score improvement showed in non-contact sports athletes throughout a season of competitive play (Koerte et al., 2017; T. W. McAllister et al., 2012), indicating the hindering of the learning effects commonly found in cognitive tasks.

One major issue about the effects of RSHI on cognition is that any impairment in a cognitive function is a symptom of alterations in a physiological/neurological mechanism that is yet unknown. Establishing a better understanding of what physiological mechanisms are affected by RSHI would provide a better understanding about which aspects of cognition we expect to be affected. Axonal damage, structural alterations, increased cortical inhibition, neurovascular damage, excitotoxicity, oedema, blood flow alterations, are all mechanisms that might play a role, and each of them would result in different symptomatology in the cognitive domain. Without this information at hand, cognitive studies are shooting in the dark, trying to find how cognition is affected by using a wide range of tests that assess different cognitive functions without providing further insights into why those functions are expected to be impaired. Reliable gold-standard cognitive assessment, designed to identify gross cognitive deficits following significant brain damage, may furthermore lack the fine-grained resolution and sensitivity required when documenting subclinical effects and accumulation of subtle consequences of RSHI in relation to each of these potential mechanisms. If any gross deficits were arising from routine impact in sport, we would have known about it. The use of broad-range clinical instruments is therefore questionable. Furthermore, the use of multiple tests in the same studies increases the chances of false positives that can make the literature of the field incoherent and unreliable. Historically, established cognitive testing has been the go-to method of evaluation of the consequences of exposure to RSHI. This may be why only neurocognitive studies are of sufficient numbers to conduct meta-analyses, for example of adverse effects of football heading (Kontos et al., 2017). However, the lack of sensitivity of these measures could lead to misleading claims that there are no consequences to such exposure in sport.

4. Understanding the negative consequences of sport and the current state of neurophysiological biomarkers

4.1 Neuroimaging

One way to gain a more in-depth understanding of the effects of RSHI on athletes' brain health is by using neuroimaging modalities. Neuroimaging biomarkers have proven to be valuable in detecting brain changes that occur before neurocognitive symptoms in contact sport athletes. Unlike cognitive markers, neurophysiological markers provide evidence of the deleterious effects that RSHI have on the athletes' brain (Mainwaring et al., 2018). This relationship between sub-concussive impacts and evidence of altered brain function has been examined using multiple modalities - structural (magnetization transfer imaging, voxel based MRI morphometry, diffusion tensor imaging - DTI), metabolic (magnetic resonance spectroscopy - MRS), functional (fMRI, resting state-fMRI) and electrophysiological (transcranial magnetic stimulation, EEG) - methods. We explore the contribution on the issue from each of these domains below. As part of this, we highlight the most promising avenues in this domain. For example, we highlight the relevance of examining acute changes in the metabolic state of athletes' brains following routine impact using Magnetic Resonance Spectroscopy. We also highlight the potential of more advanced MRI techniques – qMRI (quantitative Magnetic Resonance Imaging) – that offer the potential to quantitatively measure physical parameters such as the relaxation times T1 and T2* and, importantly, water content in brain tissue. Water content reflects the accumulation of oedema at the site of injury in the brain and it is noted that low-grade oedema can be easily overlooked or confused with imaging artefacts arising from imperfect hardware, for example. We also highlight the need for more EEG research, which can offer further insights on the cognitive functions and the brain alterations caused by repetitive head impacts, before we turn to another important emerging field in the context: biofluid marker research.

4.1.1 Structural imaging

A neuropathologic examination of a retired former National Football League player, who experienced several blows to his head but did not have any history of brain trauma or concussion, revealed clear signs of Chronic Traumatic Encephalopathy (CTE) (Omalu et al., 2005, 2006, 2010) . And McKee and colleagues reported 46 neuropathologically diagnosed cases of CTE (90%) among 51 athletes. The most prominent neuronal loss was seen in the hippocampus, entorhinal cortex, and amygdala, with less severe losses in the subcallosal and insular cortex, olfactory bulbs, mammillary bodies, locus coeruleus, substantia nigra, medial thalamus, and cerebral cortex (McKee et al., 2009). These cases kindled the discussion about possible negative effects of contact sports on athletes' brain health, in particular where there is high incidence of concussion. However, the effects of concussion and RSHI are conflated in all contact sports, perhaps with the exception of soccer where sport-related exposure is mainly due to routine head impact. At this time, RSHI was perhaps off the hook somewhat following an earlier conventional structural magnetic resonance imaging (MRI) study that found no correlation between career soccer heading exposure and structural abnormalities (S. E. Jordan et al., 1996). This negative result was confirmed in one well designed prospective study where no conventional MRI changes were discernible in professional soccer players (observation period=5 years) and also no cognitive changes were found (Kemp et al., 2016).

On the other hand in a retrospective study in former professionals, voxel based MRI morphometry demonstrated cortical thinning in the right inferolateral parietal, temporal and occipital cortex and found to be associated with worse performance on 1 of 6 cognitive tests (Rey-Osterrieth complex-figure long-delay recall) while also being inversely correlated with lifetime estimates of number of soccer headers (Koerte et al., 2016). Additionally, in college soccer players, voxel based MRI morphometry showed decreased grey matter density and volume within the anterior–temporal cortex (Adams et al., 2007).

In an earlier study of professional players, DTI indicated widespread white matter abnormalities (albeit no changes in fractional anisotropy) (Koerte et al., 2012). However, Lipton and colleagues found lower levels of fractional anisotropy in parieto-occipital areas which was associated with decreased scores in 1 of 6 cognitive tests (poorer memory) and was inversely correlated with the annual number of headers (Lipton et al., 2013). All those findings are bound to a longer history of header-associated routine head impact and signal additive effects of sub-concussion on the white matter. In a more recent study Manning et al. (Manning et al., 2020) found in a female group of contact athletes with and without concussion over a season an increase in mean axial and radial diffusivity with decreased fractional anisotropy in multiple white matter tracts. Axial diffusivity was significantly lower in the genu and splenium of the corpus callosum in those contact athletes with a history of concussion.

Of note is the potential protective effect of wearing a collar which compressed the jugular vein (Myer et al., 2019). In a group of female athletes, the researchers found that significant white matter changes in DTI-parameters were detectable only in the non-collar group, whereas there were no changes in the collar group. Measures like this may lessen the burden of concussion and RSHI in active contact sports athletes, but more research in this area is necessary to confirm the effectiveness of such protective measures. However, if proving reliable, the efficacy of a blood flow related intervention does also have the potential to reveal which mechanisms are underlying the effects of unprotected sport-related head impact, which would advance neurological understanding.

Perhaps unsurprising, DTI changes do not seem to present reliably, for example in a pilot study on 10 athletes Kenny et al. (Kenny et al., 2019) did not find any changes in fractional anisotropy or mean diffusivity after one round of heading the ball. An informative systematic review of DTI in contact sport athletes can be found in Schneider et al., (Schneider et al., 2019).

In conclusion, despite the lack of sensitivity that MR based structural neuroimaging may present, an association has been found between routine sport-related head impact, and structural abnormalities that appears dissociable from concussion and that is indeed indicative of cumulative negative effects of RSHI in contact sport leading to structural alterations of white matter.

4.1.2 Magnetic Resonance Spectroscopy of the brain

Important information about the metabolic state of athletes' brains comes from Proton (H1) Magnetic Resonance Spectroscopy (MRS) investigations. Poole and colleagues performed a comprehensive and prospective H1 MRS study on a cohort of high school American football athletes (Poole et al., 2014). They studied the following metabolites: N-acetyl aspartate (NAA) - biomarker of neuronal integrity; glutamate and glutamine (Glx) - a neurotransmitter and its precursor, both related to synaptic activity; creatine-containing compounds (tCr) - a mirror of energy metabolism; choline-containing compounds (tCho) - markers of membrane turnover; and myo-inositol (Ins) - an osmolyte involved in glial cell growth. In contact sport athletes, changes in neuro metabolism within the dorsolateral prefrontal cortex and primary motor cortex along with changes in neurocognitive performance were found. A reduction in total creatine (tCr) and in dorsolateral prefrontal inositol (Ins) on the one hand and deviations in the glutamate and glutamine (Glx) concentration in primary motor cortex on the other were observed (Poole et al., 2014). Long-term effects of high exposure to RSHI on neurochemistry in athletes without a history of clinically diagnosed concussion were investigated by Koerte et al., (Koerte et al., 2015). Compared with control athletes, soccer players showed a significant increase in both choline (Cho) and myo-inositol (ml). Additionally, ml and glutathione (GSH) were significantly correlated with lifetime estimate of RSHI within the soccer group. In contrast to Poole et al., (Poole et al., 2014), there was no significant difference in neurocognitive tests between groups. Both studies demonstrate that RSHI can have significant negative effects on brain metabolism, which precede cognitive deficits (Koerte et al., 2015; Poole et al., 2014).

Interestingly, a study by Panchal et al. (Panchal et al., 2018) aimed at examining exposure to concussive and sub-concussive repeated head impact, compared H1-MRS and neurocognitive evaluation before and after the Canadian Inter-university Sports ice-hockey season in male and female athletes. Over the season they found a significant decrease in NAA concentration and a trend towards a decrease of Cho concentration in the Corpus Callosum.

Although in females there was a trend towards a decrease in Glutamine (Glu) and in males an increase in Glu, in both males and females, a negative correlation was observed between changes in Glu and changes in verbal memory. Another more quantitative study was performed by Bari et al. (S. Bari et al., 2019). In this study paired H1-MRS with head impact monitoring was performed to quantify the relationship between metabolic changes and head acceleration event characteristics in high school-aged male American football players and in female soccer players. During the period of exposure to sub-concussive events, asymptomatic male American football players exhibited statistically significant changes in concentrations of glutamate+glutamine (Glx) and total choline containing compounds (tCho) in dorsolateral prefrontal cortex, and female soccer players exhibited changes in glutamate+glutamine (Glx) in primary motor cortex. Neurometabolic alterations observed in the male American football players during the second half of the season were found to be significantly associated with the average acceleration per head acceleration events, being best predicted by the accumulation of events exceeding 50 g-force (S. Bari et al., 2019).

Although to date only few studies exist that have employed spectroscopy to examine metabolic state of athletes' brains, all these findings indicate possible glial dysfunction, alteration of neural signaling and of energy supply to the brain due to cumulative effects of RSHI.

4.1.3 Functional Magnetic Resonance Imaging (fMRI)

Functional neuroimaging also yields important insights into the effects of collision events on brain function. In a prospective study, neurological performance and health following head collision events was assessed in small cohort of 11 high school American football players, using longitudinal measures of collision events (HIT-System), neurocognitive testing (ImPACT), and fMRI (N-back memory task) (Talavage et al., 2014). Athletes with history of concussion, and with cognitive symptoms, had altered activation in the left middle and superior temporal regions in comparison with those without concussion and cognitive symptoms. Interestingly, the group with no history of concussion, but with cognitive symptoms had altered activation in the left dorsolateral prefrontal cortex. The latter group had significantly higher numbers of non-concussive head contact events to the top-front of the head, directly above the dorsolateral prefrontal cortex, a structure important for working memory (Talavage et al., 2014).

A study by Svadi and colleagues prospectively paired functional magnetic resonance imaging with head impact monitoring to track cerebrovascular reactivity changes throughout a season and to test whether the observed changes could be attributed to mechanical loading experienced by 14 female athletes participating in high school soccer (Svaldi et al., 2017). Breath-hold fMRI revealed significant reductions in frontotemporal cerebrovascular reactivity persisting up to 4–5 months after the season had ended. A similar reduction in cerebrovascular reactivity had previously been observed in a large group of patients with actual mild traumatic brain injury who were investigated with functional Transcranial Doppler sonography of the middle cerebral artery (Becelewski & Pierzchała, 2003). Similarly, Len and colleagues found a reduction of cerebrovascular reactivity following sport-induced concussion using Transcranial Doppler of the middle cerebral artery and PETCO2 measurements (Trevor K. Len et al., 2011) (for review see(T. K. Len & Neary, 2011)).

Using resting-state-fMRI, Manning et al. (Manning et al., 2020) followed a group of 73 female non-concussion contact sport (rugby) athletes over a season and found modulated functional connectivity in default mode and visual networks, which was related to changes in white-matter structures.

A large-scale prospective study in male and female collegiate American football players, characterized the time course of the acute physiological effects of sport-related concussion as measured with resting-state-fMRI and their relationship with clinical recovery (Meier et al., 2017). Also here, concussed athletes had elevated local connectivity in the right middle and superior frontal gyri at the 24-h visit that returned to normal levels by the asymptomatic and post return-to-play visits. Moreover, elevated local connectivity in the middle and superior frontal gyri at the 24-h visit in concussed athletes was associated with elevated psychological symptoms at the time-point at which athletes were cleared to start their return-to-play progression. These results suggest that sport-related concussion is associated with an acute alteration in local functional connectivity that follows a similar time course as clinical recovery in athletes who ultimately recover (Meier et al., 2017).

In general, functional imaging demonstrates changes in activity and in functional connectivity, which are tightly related to cognitive symptoms and can partially explain them. What is more, significant changes to the cerebrovascular reactivity signal a more global reaction of the central nervous system to RSHI.

4.1.4 Quantitative Magnetic Resonance Imaging (qMRI)

As already noted above, neuroimaging has established itself as a powerful tool for investigating brain-related sports injuries. Nonetheless, in contrast to *qualitative* imaging, as used in most investigations, *quantitative* MRI yields precise and accurate data, which are, for a given field strength, hardware-independent and enable objective interpretation as well as numerical comparison. These methods allow for sensitive detection of even slight concussion related pathologies at early stages after impact.

The development of accurate methods for quantitative mapping of T1, as well as free water content has a long and successful history (H. Neeb et al., 2006; Schall et al., 2018; Nadim Joni Shah et al., 2003). Advanced MRI techniques that enable the quantitative measurement of physical quantities such as water content (oedema) and relaxation times are also being used with increasing frequency (Abbas et al., 2014, 2015; Iordanishvili et al., 2019; H. Neeb et al., 2006, 2008; Heiko Neeb et al., 2006; Oros-Peusquens et al., 2017, 2014; Reetz et al., 2015; Schall et al., 2018, 2020; N. J. Shah et al., 2008; N. Jon Shah et al., 2022; Nadim Joni Shah et al., 2010); (Claeser et al., 2019; N. Jon Shah et al., 2022; Nadim Joni Shah et al., 2003; Steinhoff et al., 2001; Zimmermann et al., 2019). Quantitative measures of T1 have long been used as a surrogate for water content in different pathologies, which can be measured quantitatively (Abbas et al., 2014, 2015; Iordanishvili et al., 2019; H. Neeb et al., 2006, 2008; Heiko Neeb et al., 2006; Oros-Peusquens et al., 2017, 2014; Reetz et al., 2015; Schall et al., 2018, 2020; N. J. Shah et al., 2008; N. Jon Shah et al., 2022; Nadim Joni Shah et al., 2010). Moreover, very recently, the first quantitative water content atlas of the healthy human brain has been published (N. Jon Shah et al., 2022) facilitating future quantitative comparisons of the brains of individuals with suspected brain injury with a normal cohort. Oedema, in the form of elevated water content will thus be visible in such comparisons and, importantly, the progression or regression of oedema can thus be studied which the aim being that, in the future, water content can be used to extract prognostic value of concussion related changes from qMRI.

A recently published method facilitates three-dimensional quantitative imaging of the free water content, T1, and the transverse relaxation time ($T2^*$) (Schall et al., 2018). This method can be easily extended to enable the measurement of the semi-quantitative magnetisation transfer ratio (MTR), a sensitive marker for demyelination. A combination of quantitative MR parameters and MTR increases the specificity of the imaging and might help to provide more insight into the underlying mechanisms and pathology associated with sports-related head impacts.

4.2 EEG studies of repetitive sub-concussive head impacts

Despite being a long-established evaluation method, electroencephalogram (EEG) studies appear to be limited in this field, while some initial efforts decades ago found that active and former soccer players had higher rates of “abnormal” EEG recordings compared to age matched controls (A. T. Tysvaer et al., 1989; Alf T. Tysvaer & Storli, 1989). The aforementioned studies offer only limited insight into the effects of RSHI, as there was no control of the physical exercise between the groups and the EEG recordings were visually inspected by a neuropsychologist, making the findings potentially biased.

Recently, more studies using EEG to investigate if RSHI affect athletes' brains have been reported, mainly recording event related potentials (ERPs) in tasks designed to assess attention. Soccer players who suffered sub-concussive impacts were found to have decreased ERP component P3b and P3a amplitudes compared to a control group, indicating that soccer players had diminished attentional resource allocation and attentional orienting compared to athletes practicing sports with no head impacts (Moore et al., 2017). In another study, third and fourth year collegiate American football players were also found to have diminished P3b amplitudes compared to their first-year co-athletes, indicating a measurable cumulative effect of RSHI, and that the seasons of play lead to changes in brain activation and attentional resource allocation (M. J. Wilson et al., 2015). However in this study a single season of American football was not found to be associated with disenable changes in P3b amplitude (M. J. Wilson et al., 2015).

Although limited in number, the appeal of EEG as an evaluation method is that it offers brain-based measures of cognition. To date, EEG evaluations of RSHI show that there is a potential link between head impacts and attention. But as EEG is particularly well established in the domain of attention, and attention-related ERP components are particularly robust, the first logical step when using EEG to investigate the effect of RSHI on athletes' brains would be to look into any attention decrements caused by RSHI. However, as mentioned in section 3 above, we not actually know at present which aspects of cognition to target when examining the effects of RSHI. Therefore, despite EEG's more limited sensitivity in domains outside attention, EEG methods should also be employed to investigate the consequences of RSHI on other cognitive domains such as learning and memory in future research.

4.3 Transcranial magnetic stimulation studies of repetitive sub-concussive head impacts

Transcranial magnetic stimulation (TMS) is another physiological modality used to assess the effects of concussion and RSHI on athletes' brains. TMS derived intracortical inhibition has been found to be altered after a sport-related concussion in several studies (De Beaumont et al., 2011; LaGree et al., 2019; Livingston et al., 2012; N. R. Miller et al., 2014; Ntikas et al., 2021; Pearce et al., 2015; Scott et al., 2020). Cortical silent period (an indicator of corticomotor inhibition) has been found to be increased both at the acute stage after a concussion (N. R. Miller et al., 2014; Ntikas et al., 2021; Pearce et al., 2015) and several months later (De Beaumont et al., 2011). RSHI have yet to be investigated in depth with the use of TMS with some studies showing increased cortical silence after soccer heading, rugby tackling and boxing bouts (Di Virgilio et al., 2016, 2019; McNabb et al., 2020). Specifically, the cortical silent period was found to be increased after 20 soccer headers compared to baseline (Di Virgilio et al., 2016), after a 3-minute sparring bout compared to a baseline assessment and a non-contact control group (Di Virgilio et al., 2019) and after 15 rugby tackles compared to baseline and compared to a control group (McNabb et al., 2020).

More research is necessary on TMS and RSHI, however, when taken together with the findings from the imaging literature, TMS studies provide additional evidence that RSHI can induce alterations in brain neurochemistry similar to those seen following brain injury and potentially indicative of the mechanism underlying long-term effects of routine repetitive head impacts in sport.

5. Sport-related impact and the current state of fluid biomarkers

A wide range of biomarkers that assess brain chemistry, axonal damage and neuroinflammation have been used to assess sport-related head impact so far such as s100 calcium binding protein b (s100b), tau (either total or phosphorylated), neuron specific enolase (NSE), creatine kinase isoenzyme BB (CK-BB), neurofilament light (NfL) and glial fibrillary acidic protein (GFAP). In the past few years, the field seems to be focusing more on NfL, S100b and tau, which has resulted in mixed findings, with boxing, American football and soccer being the most studied sports (Bernick et al., 2020; Michael R. Graham et al., 2011; Huibregtse et al., 2020; Wirsching et al., 2019; Zetterberg et al., 2006; Zonner et al., 2019).

Specifically, NfL concentrations in blood have been found to be increased in American football players after a practice session (L. H. Rubin et al., 2019) and after a competitive season (J M Oliver et al., 2019; Jonathan M Oliver et al., 2016) compared to baseline measures. In boxers NfL has been found to increase in corticospinal fluid and in blood after a series of boxing bouts (Neselius et al., 2012; Shahim et al., 2017b; Zetterberg et al., 2006). In soccer players few studies have been reported, with the only reported randomised controlled lab study showing that a series of 10 headers significantly increases blood NfL concentrations compared to a control condition (Wirsching et al., 2019).

Neuron specific enolase also appears to be a promising marker, with a handful of studies reporting significant increases after RSHI (Rogatzki et al., 2016; B-M Stålnacke et al., 2006; Britt-Marie Stålnacke et al., 2004). Tau (either total or phosphorylated) on the other hand appears to be showing mixed results (Kawata, Mitsuhashi, et al., 2018; Kawata, Rubin, et al., 2018; Jonathan M Oliver et al., 2017; Wallace et al., 2018); with studies finding no tau increase immediately after 40 soccer headers (Wallace et al., 2018). Similarly, no alteration of tau concentrations was found during a season of American football (Jonathan M Oliver et al., 2017), but increased tau concentrations were found immediately after American football practices compared to baseline assessments; however, this increase was not correlated to the amount and severity of head impacts during the practices (Kawata, Rubin, et al., 2018).

Recent soccer studies are implementing lab designs (Huibregtse et al., 2020; Wirsching et al., 2019). By doing so, the effects of repetitive head impacts on fluid biomarkers can be examined without the effects of exercise or of body impacts which can influence certain biomarkers’ concentrations. Unlike soccer, many studies of American football and boxing, even though they provide promising findings, fail to provide a clear distinction between the effects of RSHI and body impacts or exercise.

Generally, the use of fluid biomarkers to examine RSHI is still in its infancy but is showing increased traction in the last decade with a marked increase in the number of studies aiming to examine the relationship. However, it should be noted here that some of the studies in this field present several limitations especially since some of the biomarkers assessed can also be increased by other aspects of sport participation, for example S100b is also increased after increased physical activity. A recent review concluded that the markers of neuroinflammation seem to be promising and future research should focus on them (Mainwaring et al., 2018). The relevant literature has still not been reviewed in depth; however, efforts to map the literature are being made (Lember et al., 2021). Fluid biomarkers in parallel with neuroimaging biomarkers can provide a clearer picture on the effects of RSHI, explain which brain processes those impacts are inducing and what can be the potential effects of those processes on athletes everyday cognitive functioning as well as the potential long-term effects like neurodegeneration.

So far, the findings from neuroimaging and biofluid marker studies are providing some evidence that RSHI affect the brains of the athletes in ways that can impair their cognitive functioning in the short-term and potentially induce changes that in the long-term can lead to neurodegeneration.

6. What is the way forward? Research avenues and preventive measures

Although it is well established that physical activity and sports participation have specific physical and mental health benefits, there is strong emerging evidence that participation in some specific types of athletic activities, namely contact sports, comes with adverse effects and that there are negative consequences to brain function and brain health.

Of course, the issue of sport-related head impacts intuitively raises concerns at a grassroots level, in particular where it concerns young people. But it is also understandable that there is a resistance at a sport governing level to introduce any changes to the sport without an abundance of research, considering what is at stake. However, recent development in the understanding of the chronic and evolving neurological consequences of traumatic brain damage (L. Wilson et al., 2017), does warrant a potential complete rethink regarding the acceptability of sport-related impact, both at a concussive and a sub-concussive (routine) level. Recent technological advancement means that it is now possible to employ sensitive and informative brain measures, and it is a matter of urgency that support is provided to apply the latest neuroscientific methods to reveal what goes on in the brain as a result of exposure to sport-related head impact. We welcome the trend towards larger, well designed, controlled studies using the newest techniques. The outcome of this is that we get more and more reliable and better interpretable results.

What is also required is a more inter-disciplinary and multi-methods approach, in particular when focused on cause and effect or dose-response. As the results from single measures are easily dismissible, converging evidence instead offers both a compelling evidence-base and offers insight with regards to the mechanisms at play. From the review above we can see that measurable cognitive deficits in sport can be explained through brain damage as represented by findings in structural, functional and metabolic imaging, in electrophysiological measurements and in fluid biomarkers. It is therefore important not to dismiss less traditional research approaches as ‘experimental’ or ‘unproven’, but instead embrace the opportunities offered by cutting-edge neuroscience and technological innovation. We have seen above the important explanatory power of newly applied methods such as TMS, EEG, qMRI and MR spectroscopy, and how these offer a window into the nature of the potentially negative consequences of sport, in particular when employed in combination and relation to more indirect measures such as cognitive function, motor control, or blood biomarkers. Today’s situation with regards to blood biomarkers is that information about the effects of sport-related head impact is still not consistent. One of the reasons is that the older studies were in many cases underpowered and had an exploratory character. The bottom line is, however, that evidence for the negative effects of sub-concussions in contact sports is growing.

Combined evidence suggests that RSHI seems to alter brain activity. In this review we covered the evidence pointing to acute alterations following a series of RSHI and to cumulative effects of RSHI throughout seasons of contact sport participation. The link between those acute and sub-acute (semi-acute) effects with long-term consequences is yet to be made, but research involving retired contact sport athletes presents the case for increased risk of neurodegenerative diseases in populations without history of concussion, indicating the deleterious long-term effects of RSHI (Mackay et al., 2019; McKee et al., 2009).

Raised awareness of the adverse effects of RSHI on athletes' brains has already led to changes in policy. In February 2020, soccer's Football Association (FA) in England, the Scottish FA, and the Irish FA changed their governance policy by: i) issuing updated heading guidance for training; ii) stating that under-11s should no longer practice heading; iii) severely limiting heading practice frequency for 12-16 year olds; iv) reducing practice frequency for 16-18 year olds; along with v) reduced ball pressures; and vi) specific ball sizes for this practice (Football Association (England), 2020a, 2020b; Premier League & Football Association (England), 2021; Scottish Football Association, 2020). In June 2020, UEFA (Europe's governing body of soccer the Union of European Football Associations) issued guideline for heading, to protect the health and safety of youth players, stating that the guidelines are to manage heading in training and in matches aimed at "*limiting the header burden in youth football*", and the UEFA Medical Committee chairman noting the decision was taken "*after debates continued over whether* heading *a football could lead to altering a player's brain*" (UEFA, 2020). We would like to point out, however, that there is an absence of research to inform at which age it is safe to start incurring RSHI, or that reduced ball size or ball pressure mitigates the burden of RSHI. In July 2021, the FA took further action by introducing heading guidance across every level of the professional and amateur game, recommending that a maximum of 10 higher force headers are carried out in any training week. We note that although research from our lab confirmed brain changes after 20 headers, there is an absence of research to show that taking 10 headers is safe. Future soccer studies should aim to assess the effects of 10 e.g. versus 20 headers realistically replicating routine soccer practice, and quantify the magnitude of those headers. The need for this type of studies to investigate the cognitive, biological, structural, neurochemical changes caused by heading was also highlighted in a recent review (Snowden et al., 2021). This is a demonstration of how further research will be required to guide governing bodies seeking to impose restrictions in RSHI in sports, even before we have gained a better understanding of the source and mechanisms underlying any negative consequences of sport-related impact.

In conclusion, we reiterate that it is not currently known how much sport-related impact is safe. It is the task for future studies to work out the dose dependent effects of sport contacts and find out whether there are in fact safe limits in sport-related impact.

7. Conclusions

The emerging evidence reviewed here suggests that routine head impacts in many popular contact sports have adverse effects, in the context of the otherwise positive effects of exercise and sport on cognitive functions and the prevention of cognitive decline discussed elsewhere in this special issue. Considering the potential public health ‘timebomb’ that constitutes these adverse effects of sports such as soccer played by hundreds of millions around the globe (often from a young age), there should be a great sense of urgency in funding the best scientific research that has the power to reveal and explain what the link between sport and poor brain health outcomes is. To date, there appears to be little commitment to generous funding either from sport or from government. The evidence reviewed here shows that the neuroscientific community has stepped to the plate and has turned their innovation and fledgling research programs on the topic into important firsts steps in revealing the dangers of routine head impact in sport. These multi-methods capabilities, when brought together in an orchestrated way and reaching across the disciplines and established traditions, can provide real answers to the public, practitioners, sports governance, and government and offer the solid evidence-base that is urgently needed to take meaningful action in addressing this public health concern.

Account of each authors’ role in producing this published review article

MI and FB were first approached to produce an article for a special issue. It was decided that MN should take the lead and the first author role. MN together with FB reviewed the literature, discussed the evidence-base and jointly wrote a full draft of the manuscript. NJS and MI then reviewed the draft and edited the manuscript. Following peer-review, MN, FB, NJS and MI added further comments and literature sources to the manuscript. All reviewed and approved the final version of the manuscript.

Conflicts of Interest

None declared.

INTERIM DISCUSSION 1

It was clearly stated in the narrative review presented in the previous chapter that there are certain areas in the literature of subconcussive impacts that need to be researched further. Following the outcomes of the review, it was decided that even though biofluid markers might be very useful in assessing the effects of RSHI, there is a need to map the literature before implementing their use into any experimental investigations. Doing so will provide a clearer idea about which biomarkers should be used and the sampling times at which each biomarker is expected to show effects. However, the heterogeneity of the literature evident from the narrative review means that a meta-analysis would limit systematic review. Therefore, a broader scoping review was devised, and peer-review of its protocol was developed (Lember & Ntikas et al., 2021), the study was then conducted and reported in the next chapter (Chapter 3).

Moreover, sport mTBI, sport concussion and sport related subconcussive impacts are very often examined together in the literature. We consider RSHI as a phenomenon that should be investigated separately from the sport related TBI (SR-TBI), thus in all the measurements presented in this thesis (Chapters 5,6,7,8 & 9) participants were clear of SR-TBI of any severity and the heading protocol implemented could not induce a SR-TBI. The importance of effects of sport related concussion and SR-TBI were also highlighted in the review, especially since they are considered to be more trivial than the non-sport related brain injuries with athletes appearing to recover quicker that the rest of the population. Chapter 4 (TBI prognosis) challenges this commonly held position within sport by presenting evidence that a considerable percentage of people with mild SR-TBI have not fully recovered 3 months post injury or even 6 months post injury.

Lastly, even though EEG is a neurophysiological modality widely used in brain research, there was limited amount of evidence using EEG (Chapter 2, literature review), so this modality was used as the core tool to evaluate the effects of RSHI on athletes cognitive functioning and brain health in this thesis, along with other behavioural measures. Before this, a series of behavioural measures were used in order to investigate which cognitive functions are more likely to be affected by heading and thus need to be further investigated by the use of EEG measures.

The following chapters present: a scoping review of the literature on biofluid markers(Lember et al., 2021) examining their utility on investigating the effects of RSHI, a comparison of TBI characteristics and prognosis in athletes and non-athletes followed by a series of measurements implementing EEG and sensitive computerised behavioural tasks to assess the acute effects of RSHI on athletes’ brains.

CHAPTER 3. Effects of sport-related repetitive subconcussive head impacts on biofluid markers: a scoping review

Abstract

Objectives: The aims of this review were to systematically scope the existing body of evidence on the effects of repetitive sub-concussive head impacts (RSHI) on biofluid markers, evaluate research quality and identify research gaps in order to guide future research and determine the clinical utility of biomarkers for assessing the effects of RSHI in sports.

Methods: A comprehensive search strategy was used to retrieve all available and relevant articles in the literature. The following electronic databases were systematically searched: MEDLINE (EBSCO host; from 1809 to 2022); Scopus (from 1788 to 2022); SPORTDiscus (from 1892 to 2022); CINAHL Complete (from 1937 to 2022); PsycINFO (from 1887 to 2022); Cochrane Library (to 2022); OpenGrey (to 2022); ClinicalTrials.gov (to 2022) and WHO International Clinical Trials Registry Platform (to 2022). Only primarily biomedical studies evaluating the biofluid markers following RSHI were considered. Two independent reviewers screened the articles for inclusion using predefined eligibility criteria and extract data of retained articles.

Results: The search identified 79 studies that used biofluid markers to examine the effects of RSHI on brain integrity. The number of studies in the field was found to be increasing exponentially. The main markers used were S-100b, NfL, tau, NSE, GFAP, BDNF, UCHL-1 and hormones. As expected, there was high heterogeneity between the studies, mainly with regards to the sports assessed, sampling times, experimental setting and study design and type (acute, semi-acute, chronic). NfL was the biomarker found to be the most promising utility in assessing the effects of RSHI, along with NSE and possibly S-100b.

Conclusions: In this review the high heterogeneity of the field is highlighted, and a series of steps to reduce it are proposed. Biofluid markers' use to establish the effects of RSHI on the brain was found to be increasing exponentially, indicating that biofluid markers are one of the up-and-coming ways to assess RSHI in sports. Although concrete conclusions about the effectiveness of most biofluid markers could not be drawn, certain biofluid markers like NfL and NSE were found to the have promise.

INTRODUCTION

A growing amount of evidence demonstrates a link between contact sport participation and cognitive impairment and, or neurodegenerative diseases (Chiò et al., 2005; Ling et al., 2017; Mackay et al., 2019; Omalu et al., 2005, 2006; Wilson et al., 2017). It has been reported that as many as 10-20% of professional boxers suffer from chronic neuropsychiatric disorders (Förstl et al., 2010; Jordan, 2000; Jordan et al., 1992). Further, increased mortality from neurodegenerative causes have been observed in professional soccer (Mackay et al., 2019) and National Football League (NFL) players compared to the general population (Lehman et al., 2012). Traumatic brain injury (TBI) is increasingly recognised as a risk factor for later developing neurodegenerative disease (Maas et al., 2017; L. Wilson et al., 2017). Evidence for a link between contact sport and the specifics of chronic traumatic encephalopathy (CTE) is also strengthening (Mackay et al., 2019; Suter et al., 2022), however, it has been found that athletes can develop CTE in the absence of history of TBI however mild (Ling et al., 2017; T. D. Stein et al., 2015). Moreover, years of contact sport exposure have been associated with CTE pathology regardless of the number of mTBIs (Stein et al., 2015). Further, estimated total cumulative exposure to repetitive head impacts has been found to be a stronger predictor of later cognitive and neurobehavioral impairment than concussion history in American football players (Montenigro et al., 2017), raising questions about the safety of routine exposure to non-concussive head impacts in contact sport athletes. Such impacts are termed repetitive sub-concussive head impacts (RSHI) and characterise impacts resulting from either direct (e.g., sparring or heading a soccer ball) or indirect head impacts acquired through full-body collisions between players or between players and objects (Lember et al., 2021). Due to the nature of the contact sports however, the effects of concussive and RSHI on brain health are often conflated.

This review attempts to identify evidence specific to the effects of RSHI. The investigation of the effects of RSHI on brain health is challenging since sub-concussive impacts do not necessarily result in overt symptoms. It is suggested that neurophysiological changes precede discernible neurocognitive deficits (Dubois et al., 2016; Mainwaring et al., 2018). Biofluid markers are an objective measure of axonal, neuronal and astroglial changes and injury (Zetterberg et al., 2013) and therefore, they may have the potential to detect RSHI induced subtle brain changes as they emerge, separate from structural changes, that may otherwise be concealed by factors such as cognitive reserve. Moreover, fluid biomarkers can be valuable objective, inexpensive and efficient tools in aiding the understanding of the progression, regression and outcome of a disease or an injury (Mayeux, 2004). Biofluid markers have been found to significantly increase in response to TBI and be correlated with severity and prognosis (Di Battista et al., 2015; Mercier et al., 2013; Mondello et al., 2020; Shahim et al., 2018). Furthermore, markers such as tau and neurofilament light (NfL) amongst others, have been associated with the progression and the prognosis of several neurodegenerative disorders (Bäckström et al., 2020; Gendron & Petrucelli, 2009; Mattsson et al., 2019). Assessing the functionality of different biomarkers and their ability to detect the effects of RSHI on the brain is thus of great importance as these markers may provide an understanding of RSHI induced brain pathology and give an insight into the link between acute brain changes and chronic neurodegenerative sequelae.

Using biofluid markers for the detection of RSHI induced brain changes is an emerging field of research with significant heterogeneity. Any contrasting or mixed findings may be related to methodological and analytical variability amongst the studies, such as differences in markers assessed, research designs, settings, sampling times, source and so on. Those methodological and analytical differences between studies make the combination and/or comparison of the different findings complicated, and therefore prevent drawing conclusions about the utility of biofluid markers for assessing the effects of RSHI. To date there is no comprehensive overview of studies that use biofluid markers to assess the effects of RSHI on brain health. Hence, the aims of this review are to systematically scope the existing body of evidence, evaluate its quality and identify research gaps in order to guide future research and where possible determine the clinical utility of biomarkers for assessing the effects of RSHI.

METHODS

Protocol and Registration

This scoping review adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) (Tricco et al., 2018) guidelines. The review protocol has been published in BMJ Open (Lember et al., 2021).

Information Sources

The following seven electronic databases were searched: Cochrane Library (to March 2022), MEDLINE (EBSCO host; from 1809 to March 2022), Scopus (from 1788 to March 2022), SPORTDiscus (from 1892 to March 2022), CINAHL Complete (from 1937 to March 2022), PsycINFO (from 1887 to March 2022) and OpenGrey (to March 2022). The following clinical trial registration platforms were also searched for relevant protocols and corresponding full-text publications: ClinicalTrials.gov (to March 2022) and WHO International Clinical Trials Registry Platform (to March 2022). Key descriptors that included terms for subconcussive head impacts, biomarker and contact sport, were used for the search. The full search strategies are available in the published review protocol (Lember et al., 2021). Reference lists of the included studies were also screened to identify additional records. Records were uploaded and screened using web-based systematic review software Covidence (Covidence, Veritas Health Innovation, Melbourne, Australia) (available at www.covidence.org).

Eligibility Criteria and Study Selection

This review included studies that investigated biomarkers (including neuroinjury markers such as S100B, GFAP, NfL, tau etc., cytokines, chemokines and hormones) in blood (serum or plasma), cerebrospinal fluid (CSF), saliva or urine in athletes who were acutely or chronically exposed to sport related RSHI.

This review did not include studies assessing biomarker concentrations following solely sports-related concussion or traumatic brain injury. Post-mortem and non-human examinations were also excluded. No restrictions were placed on methodological standards, analytical platforms, study design and sample size. Studies were included regardless of geographic location and date of publication. We considered reports in the English, French, German, and Italian languages. Detailed inclusion criteria including the PECOS framework applied in this ScR is described in our published review protocol (Lember et al., 2021).

After removal of duplicates, two reviewers (L-ML, MN) independently screened the titles and abstracts against the predetermined eligibility criteria, followed by full-text review of retained articles. Any disputes between reviewers were be resolved through discussion and if necessary, by a third member (SM). A list of excluded articles during full-text screening is provided in supplementary material (Chapter 3 Supplementary Table 1, accessible at https://osf.io/r7t2c).

Data Extraction

Data were recorded independently by two reviewers (L-ML and MN) using a standardized and piloted data collection form (See supplementary material for Chapter 3). Disagreements were discussed until consensus was reached and, if necessary, a third reviewer was consulted for arbitration. Information about study design, aim(s), population, RSHI definition, exposure to RSHI and biofluid marker characteristics (including sampling time, source, analytical platform and concentrations) were extracted. Studies were classified as either lab or field based, depending on whether the RSHI occurred in a controlled environment or in the field (such as during training, games or matches). Further, studies were categorised as acute, semi-acute or chronic. Studies were considered semi-acute if changes in biomarker concentrations were assessed following an extended rest period from RSHI (e.g., ≥2 weeks) or if the aim was to assess the effect of accumulation of RSHI on biofluid markers over a season. Studies that investigated the relationship between history of contact sport participation (years of participation, total number of games or competitions in lifetime) and biofluid marker concentrations were considered to assess the chronic effects.

Risk of Bias and Quality Assessment of Included Studies

A modified version of the ROBINS-I was used to assess the methodological quality of all primary research publications by evaluating (Sterne et al., 2016) four domains: (1) confounding variables, (2) missing data, (3) measurement of outcomes and (4) selection of reported results. Confounding variables were considered factors, other than RSHI, that could influence the concentration of the biofluid markers, such as exercise, history of concussion, peripheral injuries, neurological diseases and so on.

In addition, a modified version of the Subconcussion-Specific Tool (SST) was utilized to assess the quality of the included studies (Comper et al., 2010; Mainwaring et al., 2018). Each study was assessed for the following six criteria: (1) Was there an attempt to define the term ‘subconcussion’? (2) Was the number or magnitude of impacts reported (if recorded but not reported however, used the analysis the criterion was considered attained)? (3) Were subjects who sustained a concussion during the study controlled for or excluded from analyses? (4) Were subjects with a history of concussion controlled for or excluded from the analyses? (5) Was the control group matched on two or more variables (e.g., history of concussion, sex, age etc.) (6) Did the study analyse sex differences, or acknowledge limitations associated with sampling only males or females? Studies were classified as category A, B or C (i.e., high, medium and low quality) depending on how many criteria were fulfilled. Category A studies met five or more criteria, B category studies three or four criteria and C two or less. Question three was not relevant to cross-sectional studies assessing the chronic effects of RSHI in retired athletes and as such, for the purpose of classification, this criterion was considered achieved for these studies. Two of the review authors (L-ML, MN) independently assessed the studies for bias and quality. Disagreements were solved through consensus and if necessary, arbitration by a third reviewer was sought.

Synthesis of Results

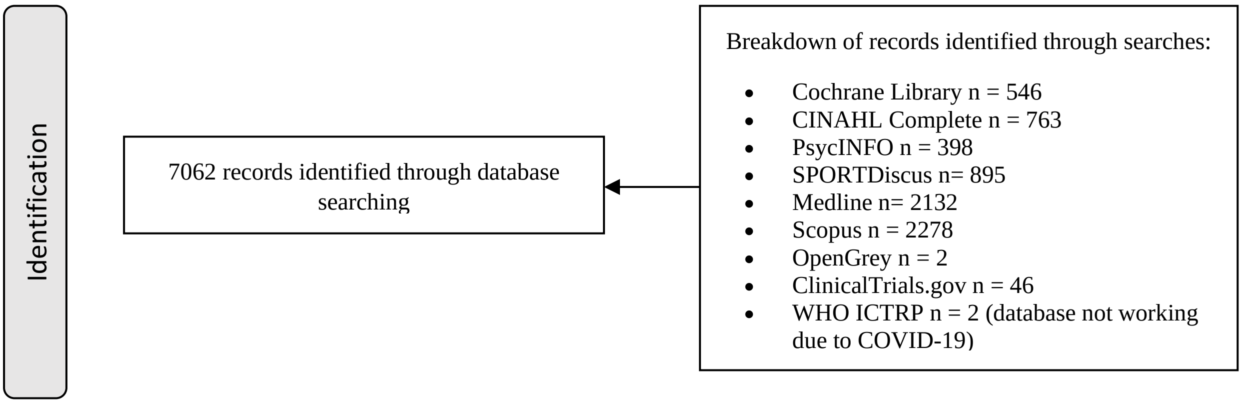
The inter-rater reliability was calculated using Cohen’s Kappa for the inclusion of studies from title/abstract and full-text screening; the agreement values range from poor ($\kappa < 0.00$) to almost perfect ($\kappa = 0.81–1.00$) (Landis & Koch, 1977).

The search results are reported in a flow diagram detailing the review decision process. The synthesis of results includes a narrative and quantitative summary in text and the main characteristics of the included studies are presented in tables. The results are categorized and presented by biofluid markers, type (acute, semi-acute or chronic), sport, setting (lab or field) and sample source (blood or CSF). Risk of bias graphs were generated using robvis web-based software (McGuinness & Higgins, n.d.) (available at https://mcguinlu.shinyapps.io/robvis/).

RESULTS

Description of studies

The systematic search in the nine databases yielded 7062 records from which 4139 abstracts were screened following removal of duplicates. One hundred and thirty-five full-text articles were assessed for eligibility and 79 articles were included in the review (see Figure 3.1; detailed information about the studies can be found in "Chapter 3 Supplementary material", Table 2). Cohen's Kappa agreement strength between reviewers was substantial for title/abstract screening and moderate for full text review (κ = 0.71 and 0.60, respectively).



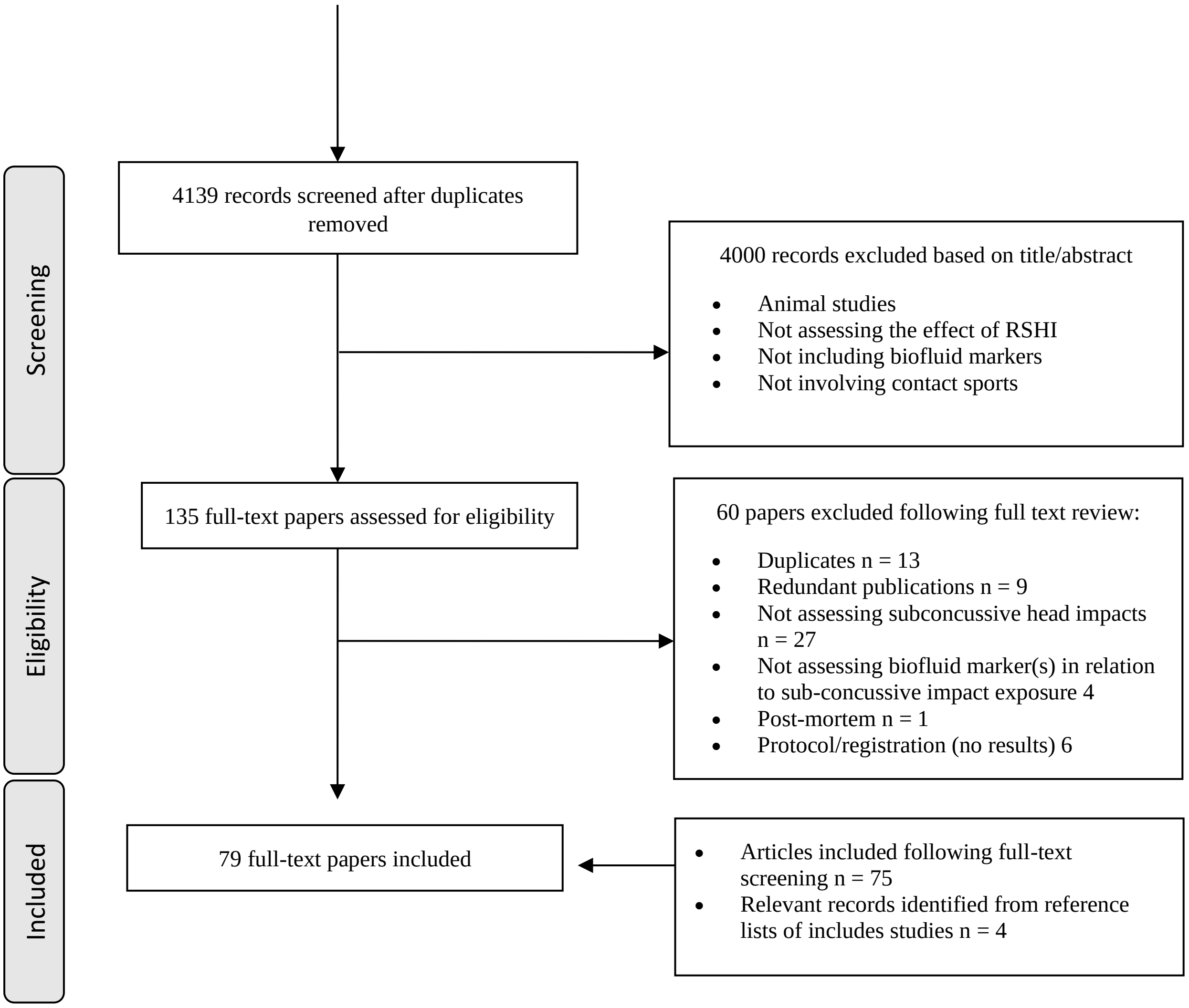


Figure 3.1. PRISMA flow diagram.

The earliest identified record was published in 1982 with the number of studies increasing remarkably the last decade (Figure 3.2).

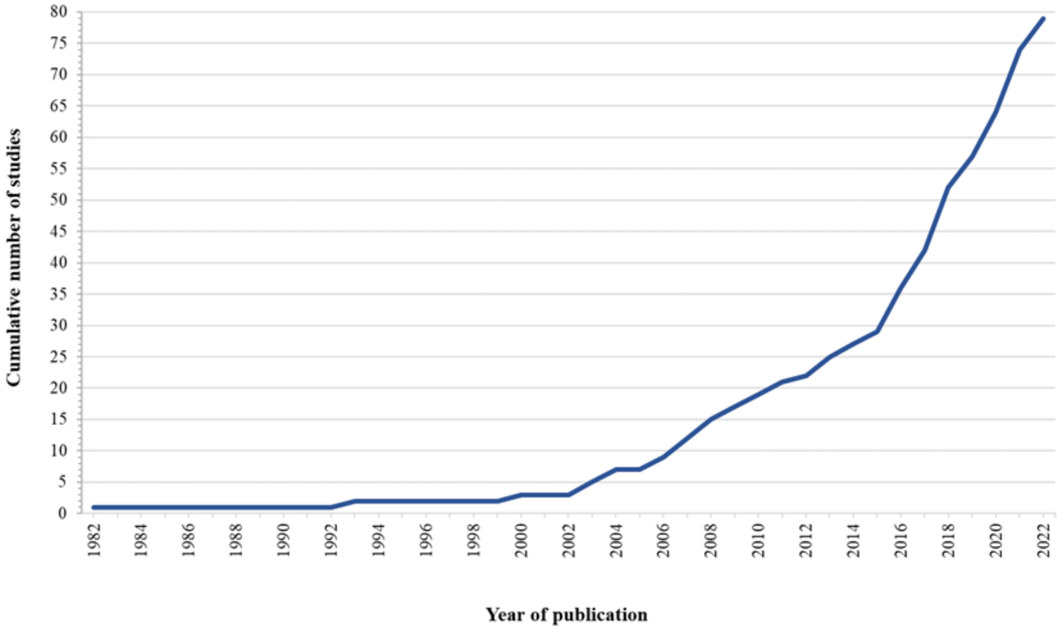


Figure 3.2. Cumulative line graph depicting the temporal trend of all the studies identified in this review. First study identified was published in 1982.

The biofluid markers S100b, NfL and tau were the most studied ones, and the ones receiving the most attention the last decade, along with GFAP (Figure 3.6).

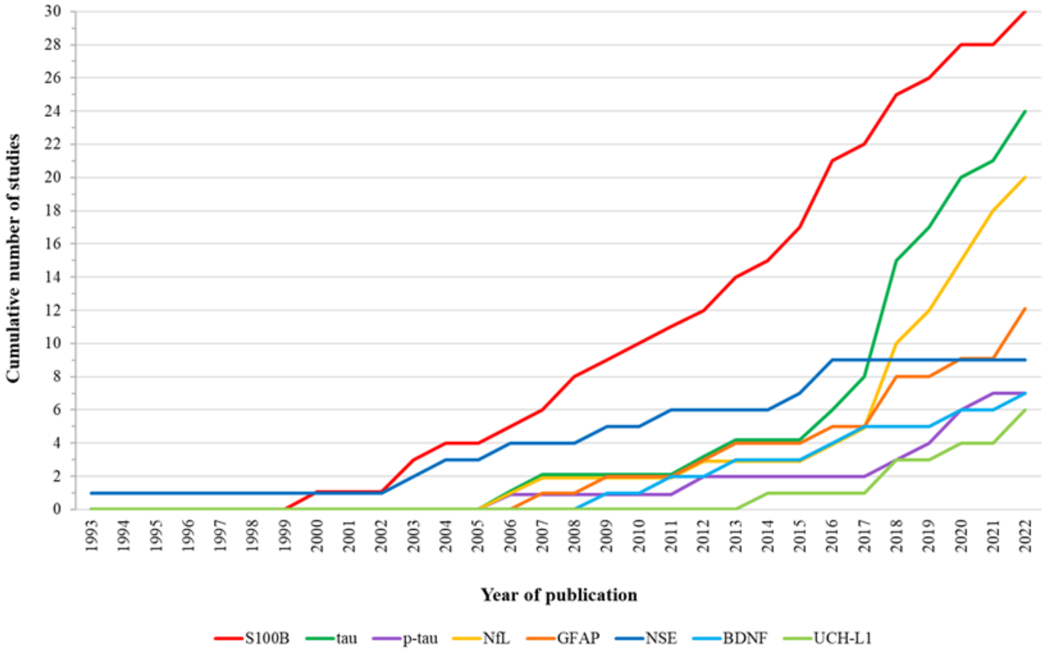


Figure 3.6. Cumulative line graph depicting the temporal trend of the number of studies identified in this review for each biomarker.

The majority (~85%) of the studies employed an observational analytic design with 44 cohort, 19 cross-sectional and four case-control studies. Only 11 studies (~14%) employed an experimental design with seven of them being randomized. We identified just one case report relevant to this scoping review.

Forty-eight studies assessed the acute effects of RSHI, 23 reported semi-acute effects and 26 investigated chronic effects. Of the 79 studies 18 assessed a mix of acute, semi-acute and chronic effects.

Further, 13 studies (~16%) were lab-based and 45 were field-based (~57%). A case report and majority of the chronic studies were not considered lab- nor field-based and were categorized as “other” (22 out of 79, ~28% of studies).

Majority of studies (~53%) assessed the effect of RSHI in a male only cohort (42 out of 79), there were two female only studies(Antonio et al., 2021; Stålnacke et al., 2006) and 20 with mixed population. Sex was not specified in 15 studies. There were only three studies conducted in a juvenile only cohort (age range: ~13-17)(Joseph et al., 2018; Mussack et al., 2003; Zonner et al., 2019). Fifty-two studies employed either a control condition or had a control cohort.

Most studied markers were: S100B (30 studies), tau (24 studies, including 4 studies assessing tau in extracellular vesicles (EVs)), NfL (20 studies), GFAP (12 studies), NSE (9 studies), BDNF (7 studies), phosphorylated tau (p-tau) (7 studies) and UCH-L1 (6 studies). Further, nine studies assessed the hormonal response to RSHI (~10%). The vast majority of studies (n=73) assessed biomarkers in blood (serum and/or plasma); six studies, however, investigated biomarker concentrations in CSF and four studies collected saliva samples. Fifty-five (~70%) studies assessed more than one biomarker simultaneously.

American football was the most studied sport with 26 studies, followed by soccer with 21 and boxing with 18 (including 2 kickboxing) studies (Figure 3.3).

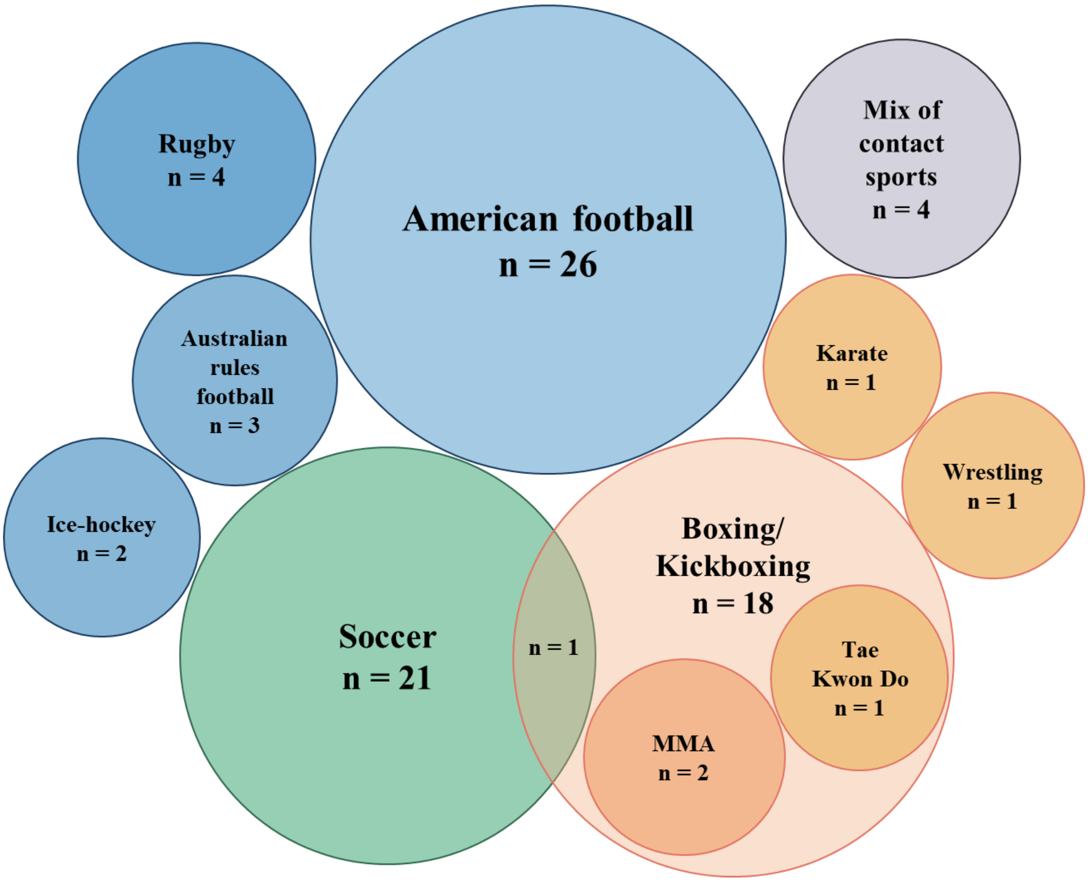


Figure 3.3. Number of identified articles per sport.

Fifteen research reports (~19%) provided a definition for sub-concussive head impacts (definitions provided in Supplementary Table 3). Thirty (~38%) of the studies included an additional outcome measure other than biofluid marker(s) to assess the effects of RSHI. Commonly used measures included brain imaging (in 9 studies) and neurocognitive tests, motor control and/or concussion symptom assessment (in 26 studies); five studies had a multi-methods approach and included brain imaging and neurocognitive tests, motor control and/or concussion symptom assessment.

Methodological quality of evidence

Based on our analysis of the risk of bias, two studies (~2.5%) were scored as critical, 20 (~25%) as serious, 49 (~62%) as moderate and only 8 (~10%) of the studies received a low risk of bias rating. Most studies that received moderate or higher risk of bias did so due to failing to control for confounding variables (Figures 3.4 & 3.5).

Specific subconcussion methodological quality assessment results are displayed in Table 4 of supplementary material; ~46% of studies received a category C (n=36), ~40.5% category B (n=32) and ~14% category A (n=11) rating, meeting almost all of the specific criteria with regards to subconcussion methodological quality. The most commonly unmet criteria were a failure to provide a definition for RSHI or account for sex differences.

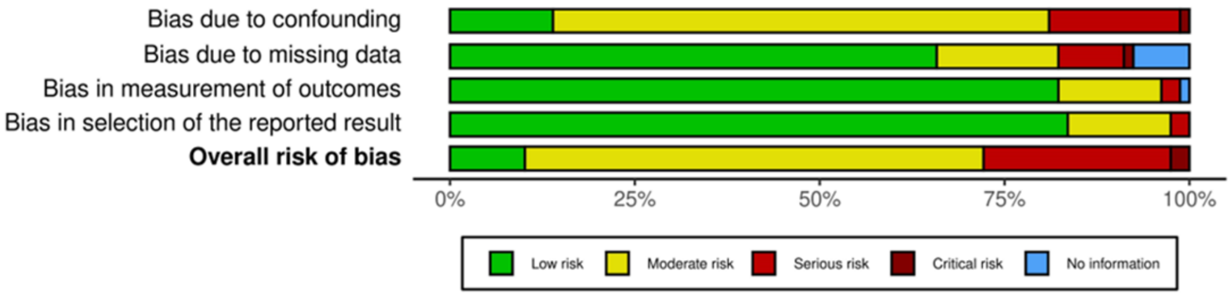
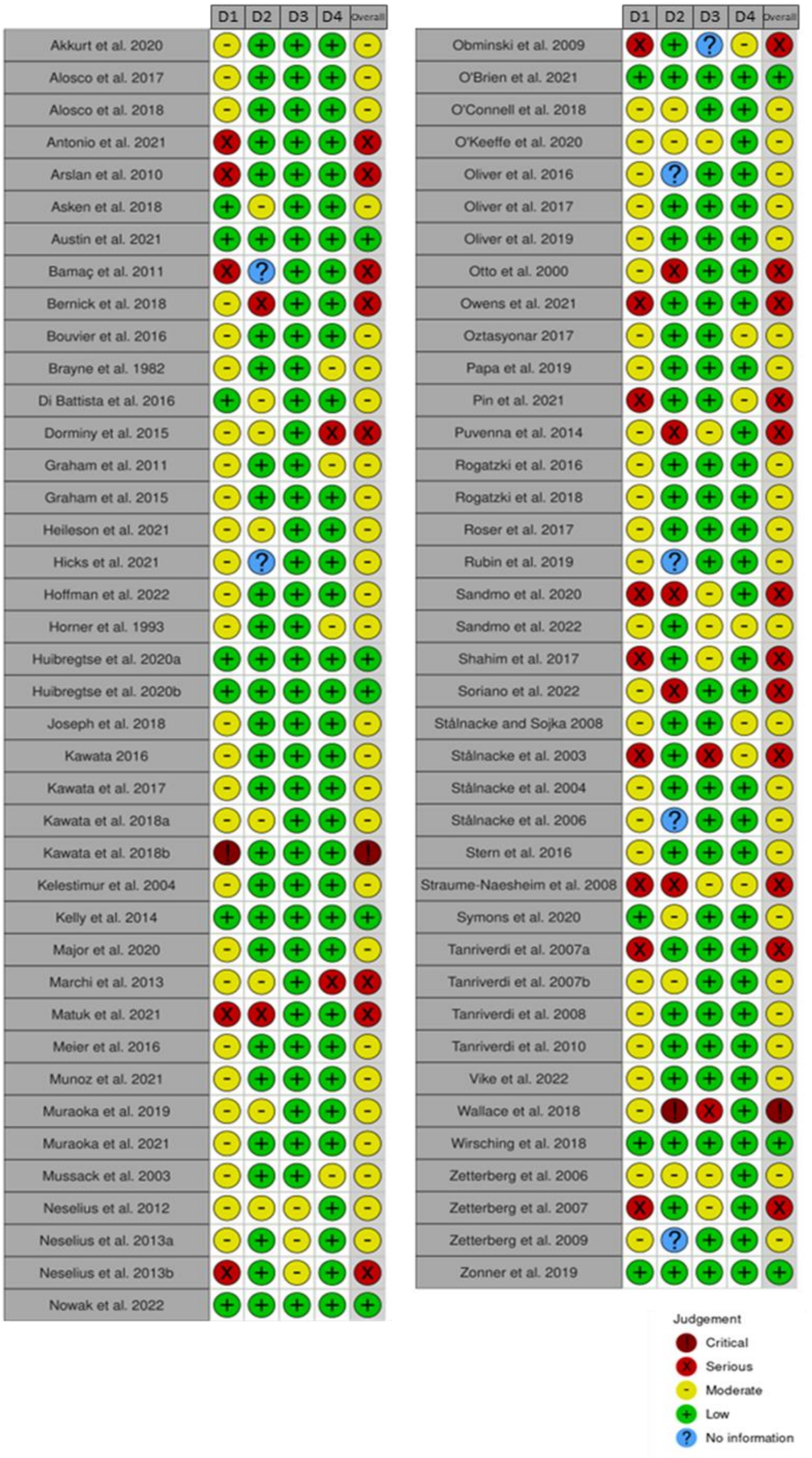


Figure 3.4. Pooled risk of bias score for each domain as a percentage.



	D1	D2	D3	D4	Overall
Akkurt et al. 2020	-	+	+	+	-
Alosco et al. 2017	-	+	+	+	-
Alosco et al. 2018	-	+	+	+	-
Antonio et al. 2021	X	+	+	+	X
Arslan et al. 2010	X	+	+	+	X
Asken et al. 2018	+	-	+	+	-
Austin et al. 2021	+	+	+	+	+
Bamaç et al. 2011	X	?	+	+	X
Bernick et al. 2018	-	X	+	+	X
Bouvier et al. 2016	-	+	+	+	-
Brayne et al. 1982	-	+	+	-	-
Di Battista et al. 2016	+	-	+	+	-
Dorminy et al. 2015	-	-	+	X	X
Graham et al. 2011	-	+	+	-	-
Graham et al. 2015	-	+	+	+	-
Heileson et al. 2021	-	-	+	+	-
Hicks et al. 2021	-	?	+	+	-
Hoffman et al. 2022	-	+	+	+	-
Horner et al. 1993	-	+	+	-	-
Huibregtse et al. 2020a	+	+	+	+	+
Huibregtse et al. 2020b	+	+	+	+	+
Joseph et al. 2018	-	+	+	+	-
Kawata 2016	-	+	+	+	-
Kawata et al. 2017	-	+	+	+	-
Kawata et al. 2018a	-	-	+	+	-
Kawata et al. 2018b	!	+	+	+	!
Kelestimur et al. 2004	-	+	+	+	-
Kelly et al. 2014	+	+	+	+	+
Major et al. 2020	-	+	+	+	-
Marchi et al. 2013	-	-	+	X	X
Matuk et al. 2021	X	X	+	+	X
Meier et al. 2016	-	+	+	+	-
Munoz et al. 2021	-	+	+	+	-
Muraoka et al. 2019	-	-	+	+	-
Muraoka et al. 2021	-	+	+	+	-
Mussack et al. 2003	-	+	+	-	-
Neselius et al. 2012	-	-	-	+	-
Neselius et al. 2013a	-	+	-	+	-
Neselius et al. 2013b	X	+	-	+	X
Nowak et al. 2022	+	+	+	+	+

	D1	D2	D3	D4	Overall
Obminski et al. 2009	X	+	?	-	X
O'Brien et al. 2021	+	+	+	+	+
O'Connell et al. 2018	-	-	+	+	-
O'Keeffe et al. 2020	-	-	-	+	-
Oliver et al. 2016	-	?	+	+	-
Oliver et al. 2017	-	+	+	+	-
Oliver et al. 2019	-	+	+	+	-
Otto et al. 2000	-	X	+	+	X
Owens et al. 2021	X	+	+	+	X
Oztasyonar 2017	-	+	+	-	-
Papa et al. 2019	-	+	+	+	-
Pin et al. 2021	X	+	+	-	X
Puvenna et al. 2014	-	X	-	+	X
Rogatzki et al. 2016	-	+	+	+	-
Rogatzki et al. 2018	-	+	+	+	-
Roser et al. 2017	-	+	+	+	-
Rubin et al. 2019	-	?	+	+	-
Sandmo et al. 2020	X	X	-	+	X
Sandmo et al. 2022	-	+	-	-	-
Shahim et al. 2017	X	+	-	+	X
Soriano et al. 2022	-	X	+	+	X
Stålnacke and Sojka 2008	-	+	+	-	-
Stålnacke et al. 2003	X	+	X	-	X
Stålnacke et al. 2004	-	+	+	+	-
Stålnacke et al. 2006	-	?	+	+	-
Stern et al. 2016	-	+	+	+	-
Straume-Naesheim et al. 2008	X	X	-	-	X
Symons et al. 2020	+	-	+	+	-
Tanriverdi et al. 2007a	X	+	+	+	X
Tanriverdi et al. 2007b	-	-	+	+	-
Tanriverdi et al. 2008	-	+	+	+	-
Tanriverdi et al. 2010	-	+	+	+	-
Vike et al. 2022	-	+	+	+	-
Wallace et al. 2018	-	!	X	+	!
Wirsching et al. 2018	+	+	+	+	+
Zetterberg et al. 2006	-	-	-	+	-
Zetterberg et al. 2007	X	+	-	+	X
Zetterberg et al. 2009	-	?	+	+	-
Zonner et al. 2019	+	+	+	+	+

Judgement

!	Critical
X	Serious
-	Moderate
+	Low
?	No information

Figure 3.5. Reviewers' rating for individual risk of bias domains and the overall score for each study, D1: Bias due to confounding; D2: Bias due to missing data; D3: Bias in measurement of outcomes; D4: Bias in selection of the reported results. As <2% of the risk of bias domains were not available in the publications reviewed and in most of the cases those publications were more than 10 years old, it was decided not to attempt contact with the authors of those publications and limit the review to the information in the public domain.

S100B (s100 calcium binding protein b (s100b) and the acute effects of RSHI

Twenty-four studies assessed acute effects of RSHI on S100B expression in blood (“Chapter 3 Supplementary material” Table 2), of which 17 studies found a significant increase in S100B within two hours of RSHI exposure (range: 1.3 to 5.3-fold, 26%-431% increase). All 17 studies were field based where the effect of physical activity could not be eliminated. This is evident from the control data. Eight of the 17 studies employed a control group or condition investigating the effect of exercise and/or peripheral injuries on S100B expression. Critically, in six of the eight studies, a significant increase in S100B was observed also in the control group or control condition. Overall, S100B increased 1.3-1.8-fold (26-78% increase) following exercise where no head impacts occurred (O’Connell et al., 2018; Rogatzki et al., 2018; Straume-Naesheim et al., 2008); whereas two studies reported no significant changes in S100B following exercise (Graham et al., 2015; Graham et al., 2011). Laboratory-based studies investigating the effects of soccer heading, where physical activity was controlled, reported no effect of RSHI on S100B (Dorminy et al., 2015; Huibregtse et al., 2020; Mussack et al., 2003; Otto et al., 2000; B. M. Stålnacke & Sojka, 2008; Zetterberg et al., 2007).

Increases in S100B were found to be significantly correlated with impact metrics, with studies reporting correlation coefficients ranging from 0.43 to 0.66. Perhaps of note, one study reported a correlation between increases in S100B and the number of jumps in a basketball game (r = 0.71) (Stålnacke et al., 2003).

Two studies measured S100B in CSF following exposure to RSHI. One study reported significantly (~1.2 fold) higher S100B concentrations in CSF (but not in serum) 1–6 days after a boxing bout that normalised after a rest period (≥14 days) compared to the controls (Neselius et al., 2012, 2013a). Whereas S100B levels in CSF and serum were not significantly higher compared to the control group 7–10 days after controlled soccer heading in the other study (Zetterberg et al., 2007). Overall, the evidence-base of S100B as a marker of the acute effects of RSHI is limited due to significant methodological issues, in particular with regards to controlling for the effect of exercise.

S100B and the semi-acute and chronic effects of RSHI

None of the nine studies that assessed semi-acute effects of RSHI found a significant increase in S100B. Also, no relationship between prior contact sport exposure and S100B was found in the three studies that investigated the chronic effects of RSHI in active contact sport athletes following a period of rest (~2-6 months) from contact sport participation

(Asken et al., 2018; Di Battista et al., 2016; Zetterberg et al., 2009). Overall, S100B does not show promise in evidencing the semi-acute and chronic effects of S100B in active players. No studies assessing the chronic effects have been done in retired contact sport athletes.

Tau

Twenty-four studies examining the effects of RSHI on tau were identified, information about study type and design, type of exposure and participant characteristics can be found in “Chapter 3 Supplementary material”, Table 2. Of these, 9 studies examined the acute effects of RSHI on tau concentrations; 6 studies had moderate or low risk of bias, 4 of them reported significant tau increases after impacts incurred in boxing and American football (Joseph et al., 2018; Kawata et al., 2018; Neselius et al., 2012; Zetterberg et al., 2006), the other two did not report significant findings after soccer heading (Nowak et al., 2022) and an American football match (Hoffman et al., 2022). Eight studies investigated the semi-acute effects of RSHI yielding mixed findings, with five of the studies finding no significant differences in tau concentrations (Bernick et al., 2018; Kawata et al., 2018; Neselius et al., 2012, 2013a; Oliver et al., 2017), while one found significant increases (Joseph et al., 2018) and one a significant decrease (Oliver et al., 2018) (one study failed to detect tau in serum (Soriano et al., 2022)). Twelve of the 24 studies examined if RSHI cause chronic tau increases, of which six did not find significant differences (Alosco et al., 2018; Asken et al., 2018; Major et al., 2020; Muraoka et al., 2019; Sandmo et al., 2020; Zetterberg et al., 2006), while four studies found increased tau levels (Di Battista et al., 2016; Muraoka et al., 2021; Stern et al., 2016; Symons et al., 2020). Questions were raised by a study that found a correlation between RSHI career exposure and tau concentrations although those tau concentrations were not significantly different to those of controls (Alosco et al., 2017), and another study that found yearly tau increases in active MMA fighters but not in controls or boxers; in the same study tau concentrations in retired boxers did not differ from controls (Bernick et al., 2018). Therefore, although tau is one of the markers currently receiving the most attention (see Figure 3.6), the utility of this biomarker in evidencing the effects of RSHI in contact sport is uncertain.

Seven of the aforementioned 24 studies also examined phosphorylated tau (p-tau). Two investigated the acute effects of RSHI on p-tau, finding no significant differences (Neselius et al., 2012; Zetterberg et al., 2006). Neselius et al. (2012) also investigated the semi-acute effects again reporting no significant results. The chronic effects of RSHI on p-tau concentrations in active and former athletes were investigated in 6 studies, with 4 of them reporting no significant effects in American (Alosco et al., 2017; Muraoka et al., 2019) and

Australian rules football players (Major et al., 2020), and boxers (Zetterberg et al., 2006) while 2 studies reported significant increases of 20-80% in former and active American football players (Muraoka et al., 2021; Symons et al., 2020). All 7 studies had moderate risk of bias.

Neurofilament light (*NfL)*

Twenty studies examining the effects of RSHI on NfL concentrations were identified; information about study type and design, type of exposure and participant characteristics can be found in "Chapter 3 Supplementary material", Table 2. Eleven studies investigated the acute effects of RSHI on NfL concentrations, with 7 of them reporting a significant increase (Joseph et al., 2019; Neselius et al., 2012; Nowak et al., 2022; Rubin et al., 2019; Shahim et al., 2017a; Wirsching et al., 2019; Zetterberg et al., 2006). Almost all of the acute studies with low or moderate risk of bias showed increased NfL concentrations after RSHI (Supplementary material, Table 2). Significant increases were also reported in the 6 of the 7 studies that examined the semi-acute effects of RSHI in American football, boxing and ice-hockey (Bernick et al., 2018; Heileson et al., 2021; Kawata et al., 2018; Neselius et al., 2012; Oliver et al., 2019; Oliver et al., 2016). Seven studies investigated if RSHI cause chronic NfL increase, with four of them reporting significant findings (Antonio et al., 2021; Bernick et al., 2018; Shahim et al., 2017a; Zetterberg et al., 2006). Irrespective of sport, most studies that were identified as well-designed (based on risk of bias and the QA) provided significant findings. Therefore, of the up-and-coming biomarkers (see Figure 3.6), NfL appears as one of the most promising in demonstrating the effects of RSHI in sport.

Neuron specific enolase (*NSE)*

Nine studies investigating the effects of RSHI on NSE concentrations; information about study type and design, type of exposure to RSHI and participant characteristics can be found in "Chapter 3 Supplementary material", Table 2. All of the 6 acute studies that were identified as well-designed (risk of bias moderate) reported significant findings, with soccer games causing a 1.1-2 fold increase of NSE (Stålnacke et al., 2006; Stålnacke et al., 2004), American football a 1.9 fold increase (Rogatzki et al., 2016) and boxing 1.5-2.5 fold increase (Graham et al., 2011; Horner et al., 1993). The two studies examining the chronic effects yielded mixed results (Di Battista et al., 2016; Zetterberg et al., 2009). Therefore, the studies including NSE to examine the effects of RSHI showed promise in particular demonstrating the acute effects of impact in sport in higher quality studies.

Glial fibrillary acidic protein (GFAP)

Twelve studies assessing the effects or RSHI on GFAP were identified (see "Chapter 3 Supplementary material" Table 2 for details on study type and design, type of exposure and participant characteristics). Six studies investigated acute effects (two in CSF), of which only two studies found a significant increase (2 fold) of GFAP following RSHI in boxers and American football athletes (Hoffman et al., 2022). GFAP was not detectable (in serum) in one of the studies.

Five studies assessing semi-acute effects (one in CSF) with only one reported significant increase. All four chronic studies (carried out in active athletes) found no effect of RSHI on GFAP levels. GFAP was not detectable (in serum) in two studies: one assessing semi-acute and the other assessing chronic effects (Neselius et al., 2013a; Zetterberg et al., 2009).

With regards to methodological constraints, the limit of detection for the assays failing to detect GFAP levels were 150 and 780 ng/L. Overall, GFAP appears not to be affected by RSHI however, this conclusion is subject to limited evidence.

Brain-derived neurotrophic factor (BDNF)

Seven studies assessing the effects of RSHI on BDNF were found ("Chapter 3 Supplementary material" Table 2). The acute effects were assessed in 5 studies yielding mixed results (Bamaç et al., 2011; Hoffman et al., 2022; Neselius et al., 2013b; O'Keeffe et al., 2020; Oztasyonar, 2017). BDNF was found to increase after boxing, American football and taekwondo but not after rugby in the three well designed acute studies (O'Keeffe et al., 2020; Oztasyonar, 2017). The two studies that were identified as having serious risk of bias showed increased BDNF after soccer but no effects after boxing (Bamaç et al., 2011; Neselius et al., 2013b). Two studies investigated the semi-acute effects with the one revealing increased BDNF concentrations after a rugby season (O'Keeffe et al., 2020), and two studies investigated the chronic effects without finding evidence of BDNF alterations (Di Battista et al., 2016; Zetterberg et al., 2009). Therefore, BDNF as a measure appears to have revealed little about the effect of RSHI in sport.

Ubiquitin C-terminal hydrolase L1 (UCH-L1)

Six studies used UCH-L1(Asken et al., 2018; Joseph et al., 2018; Major et al., 2020; Nowak et al., 2022; Puvenna et al., 2014; Soriano et al., 2022) to investigate the effects of RSHI on athletes' brain health (see "Chapter 3 Supplementary material" Table 2 for details). Three studies reported a significant increase in UCH-L1 levels acutely following RSHI exposure (Joseph et al., 2018; Madeleine K Nowak et al., 2022; Puvenna et al., 2014). Two studies also assessed UCH-L1 expression in semi-acute and two in chronic setting. One of the semi-acute studies found a significant increase in UCH-L1 concentrations following a season of American football(Jacob R Joseph et al., 2018) whereas majority of the samples were not quantifiable in the other study(Soriano et al., 2022). Neither of the studies assessing chronic effects of RSHI found UCH-L1 levels affected (Asken et al., 2018; Major et al., 2020). Therefore, UCH-L1 appears to be increased acutely but not chronically following RSHI exposure however, evidence thus far is limited.

Hormonal studies

Nine studies investigated the effects of RSHI on hormonal response, one case study reported acute and semi-acute effects on a kickboxer (Tanriverdi et al., 2007), and seven studies reported the chronic effects (see Chapter 3 “Supplementary material” Table 2). Five studies that examined the chronic effects of RSHI in boxing and American football revealed growth hormone secretory deficiencies (Kelestimur et al., 2004; Kelly et al., 2014; Tanriverdi et al., 2007, 2008), anti-hypothalamus and anti-pituitary antibodies presence (Tanriverdi et al., 2010), insulin-like growth factor 1 (Kelestimur et al., 2004; Tanriverdi et al., 2007) and adrenocorticotropic hormone (Tanriverdi et al., 2007, 2008) deficiency, and hypogonadism (Kelly et al., 2014). RSHI in soccer revealed no long-term effects on hormonal responses (Akkurt et al., 2020; Roser et al., 2018). Overall, sustained exposure to RSHI appears to increase the risk of pituitary dysfunction in contact sport athletes.

DISCUSSION

The primary aim of the review was to map the existing literature, so the inclusion criteria were broad to allow for the identification of all studies that used biofluid markers to assess “subconcussion”. Hence, the review was successful in identifying a large number of studies using multiple biomarkers. Seventy-nine studies examining the effects of RSHI on biofluid marker concentrations were found, a number higher than expected with the number of studies in this field growing exponentially (Figure 3.2). Even though the studies were high in number, they were also very heterogeneous and thus, it was difficult to draw specific conclusions. Furthermore, it became apparent that a potential meta-analysis is difficult due to the wide range of sports investigated, multiple setting, study designs, biofluid markers assessed etc.

A secondary aim of this review was to assess the quality of the literature so far. As it can be seen in Figure 3.4 the quality of studies reviewed was generally poor, with less than 10% of the studies rated as having a low risk of bias. Notably, the majority of the studies had similar limitations that reduced their quality, and those limitations were primarily failures to control for confounding variables. The effect of exercise, recent head impact exposure,

exposure to body impacts and control for past concussions and the exposure to concussive impacts during the experiment were the main confounding variables not controlled in the identified studies. It should be mentioned here that certain sport characteristics can make the control of specific confounding variable difficult, for example replicating the impacts sustained during a rugby match while effectively controlling for the effects of exercise. However, this is crucial to ensure the validity of the findings and there are ways in which such confounding variables like exercise can be controlled (McNabb et al., 2020). In this review, studies performed in a lab setting were found to be of better quality and produce lower risk of bias, therefore the use of lab settings to assess the effects of RSHI on markers of brain injury is recommended were possible.

As it can be seen in Figure 3.6, apart from the finding that interest in this field is growing exponentially, certain biofluid markers seem to concentrate the majority of this interest. S100b, NfL and tau appear to be the biomarkers concentrating the most interest followed by a more recent (since 2017) interest in the biomarker GFAP. In the past few years there has been an increased interest in microRNA and contact sport participation, however the majority of microRNA studies were excluded in the full text review because they were not separating RSHI from concussion. Each of the less than a handful of microRNA studies included in this review found changes in different microRNAs, thus no valuable information could be extracted.

In acute and semi-acute setting NfL showed promise as a biofluid marker that is affected by RSHI. Irrespective of sport, most well-designed studies revealed that RSHI caused significant NfL increases. Hence, of the up-and-coming biomarkers, NfL appears one of the most promising. Unlike NfL, tau yield mixed findings both in an acute/semi-acute and chronic setting. This raises questions about the suitability of this biofluid marker at assessing RSHI effect on brain functioning, especially since tau alterations have been linked to the onset many neurodegenerative diseases.

The recent interest on GFAP seems not to be justified, at least so far, since the majority of studies that employed this biofluid marker did not find it to be affected in any setting (acute, semi-acute and chronic) and sport. Here it should be noted that evidence so far is limited, but in this review GFAP does not appear to show much promise as a biofluid marker able to detect subtle changes caused by RSHI. NSE was the one biomarker, other than NfL, found to be acutely affected by RSHI. Unfortunately, no new studies using NSE have been done since 2015 (Figure 3.6). The findings of this chapter highlight the need for NSE to be examined in future studies aiming to use biofluid marker to assess the effects of RSHI.

The evidence for certain biofluid markers mentioned above, although promising, is nevertheless insufficient as an evidence base for clinical utility or application. NfL is a marker of axonal injury that suggests damage on the long myelinated white matter axons and is affected in mild and severe TBI and several types of dementias (Khalil et al., 2018). NSE is a biofluid marker that has been found to be correlated with TBI severity and prognosis and is consider a marker of brain tissue damage (Herrmann et al., 1999). Further research is necessary to provide more evidence that the aforementioned biomarkers are modulated by RSHI, and, importantly, to what extent, as small increases of biofluid markers can be considered benign or at least not of clinical significance. Another finding of this review was the lack of research on biofluid markers sampled from cerebrospinal fluid (CSF). More studies sampling biomarkers both from blood and CSF are necessary to make sure that the changes seen in blood reflect exclusively brain alterations and/or damage.

One main finding of this review, as mentioned earlier, is the low quality of the studies identified. This finding provides very useful information both to the scientific community and the author of this thesis, since it highlights the importance of controlling specific confounders while aiming to examine RSHI effects on the brain effectively. All the experiments performed as part of this thesis aimed to produce studies of low risk of bias and high quality by controlling the main confounders of exercise, recent head and body impacts and concussion history, as identified here. To do so, a lab setting with a control condition was used, with an absence of unwanted (contaminating) concussive impacts and the effect of exercise could be controlled. History of concussion was recorded and athletes with a concussion less than a year before the testing sessions were excluded; this limit was also informed by the findings of Chapter 4 (TBI prognosis). Furthermore, to ensure that the headers produced subconcussive impacts of non-negligible force, but also not such high impacts that can be classed as concussive, impact sensors were used to monitor impact in-situ and to record the linear and angular accelerations sustained by athletes’ heads during heading.

Finally, although biofluid markers seem to be a promising clinical tool to evaluate the effects of RSHI on athletes’ brains, the identified uncertainty in the research field led the author of the thesis to focus primarily on more sensitive and more neuroscientific tools, like EEG, to try and detect those effects.

CHAPTER 4. Characteristics and outcomes of sports and non-sports traumatic brain injury: a multicentre cohort study

Abstract

Objectives: To compare individuals attending hospital with sports and non-sports traumatic brain injury (TBI) for background characteristics, clinical factors and outcomes.

Methods: The CENTER-TBI study recruited 4509 individuals with TBI, and included information on demographics, pre-injury health, and clinical features in the acute stage. Patients were followed up at 3 and 6 months using the Glasgow Outcome Scale-Extended (GOSE), and patient-reported measures of health-related quality of life and mental health.

Results: There were 285/ 4509 (6.3%) cases with sports-related TBI. Compared to other causes of injury, patients with sports TBI were younger, more likely to have mild TBI, more likely to be previously healthy, less likely to have a psychiatric history, but more likely to have history of concussion. In a regression analysis adjusting for differences between groups return to normal life (GOSE=8) was 1.52 times more likely 3 months post-injury and 1.43 more likely at 6 months for patients with sport-related TBI. Similar differences were observed across patient-reported outcomes. Notwithstanding better outcomes after sports TBI, we observed GOSE scores <8 in 52% at 3 months and 41% at 6 months. The corresponding figures for patients with mild sports TBI were 42% and 33%.

Conclusions: Patients with sport-related TBI attending hospital have lower risk factors for poor outcome and better outcomes than non-sports injuries. Nevertheless, many patients report problems and limitations at 3 and 6 months post-injury. There is a need for effective clinical follow-up and support for patients who attend hospital with TBI in sport, even when the injury is considered “mild”.

INTRODUCTION

There is growing attention to the potential brain health consequences of traumatic brain injury (TBI) and repetitive head impacts, in particular the association with increased risk of neurodegenerative disease (Wilson et al., 2017). Participation in sport is a common cause of TBI: sport is responsible for around 6% of emergency department attendances with TBI (Selassie et al., 2013) and 20% or more of TBIs recorded in the community as a whole (Theadom et al., 2014). Exposure to TBI, both single and repeated, has raised widespread concern over participation in sports, particularly over possible long-term consequences.

Historically the observational research literature on TBI has been divided into studies focusing on sport-related injuries and research that has included mixed aetiologies. Based on this literature, sport-related mild TBI has a better prognosis than non-sport-related injury (Karr et al., 2014; Rabinowitz et al., 2014). However, differences are present between the two populations that complicate comparison: individuals with sports TBI typically have lower risk factors for poor outcome than non-sports injury (for example, younger age, fewer pre-injury health conditions and less severe injuries) (Rabinowitz et al., 2014). Despite an extensive literature on both sports TBI and general TBI in adults, few studies have compared sports and non-sports injury (Brady et al., 2022), and those that have been conducted have focused on symptoms at 90 days (Beauchamp et al., 2021). This may not capture the full range and duration of problems. A recent study of patients with mild TBI who attend hospital found that over half report some limitations in daily life 6 months after injury, even those with no abnormality on early CT imaging (Nelson et al., 2019). It has not been established whether similar persisting disability is observed after mild TBI in sports.

CENTER-TBI is a large-scale, prospective longitudinal observational project that enrolled TBI patients from 19 European countries and Israel (Steyerberg et al., 2019). Information is available in the dataset concerning the injuries, background factors, clinical course and outcomes and allows sports-TBI to be compared systematically with TBI from other causes. The aim of the study is to identify differences in the characteristics of sports and non-sports TBI and compare outcomes up to 6 months post-injury.

METHODS

Data came from CENTER-TBI core study consisting of 4509 patients recruited between December 2014 and December 2017. Ethical approval for the study was obtained for each centre in accord with national and local requirements. Details of ethics approvals are included on the project website (https://www.center-tbi.eu/project/ethical-approval).

Participants

Inclusion criteria were: presentation with TBI within 24 hours of injury, a clinical indication for CT, and availability of informed consent. Patients were excluded if they had a severe pre-existing neurological disorder that would interfere with outcome assessments. Patients aged under 16 were excluded from the current analyses to reduce heterogeneity in the sample. Recruitment was to three strata, differentiated by care path: emergency room (ER) stratum (patients attending the ER and discharged), admission stratum (patients admitted to hospital) and intensive care unit (ICU) stratum (patients admitted to the ICU). For the current analyses, patients were identified as having a sport-related TBI (SR-TBI) if the place of injury was recorded as “sport/recreational”, and the cause of injury was not a “road traffic accident” or “violence/attack”.

Measures

Demographic, socioeconomic and injury-related data were collected in the acute stage (Table 1). Pre-injury physical health status was assessed on the American Society of Anaesthesiologists Physical Status Classification System (ASAPS) class (Mayhew et al., 2019).

The presence of intracranial abnormalities was recorded from the first CT after injury (Vyvere et al., 2020). Severity of injury was assessed by the Glasgow Coma Scale (GCS; Teasdale & Jennet, 1974), and the Abbreviated Injury Scale (AIS) (Baker et al., 1974). Injuries were classified as ‘mild’ (GCS 13 to 15), ‘moderate’ (GCS 9 to 12), or ‘severe’ (GCS 3 to 8).

Outcomes

Outcome was assessed at 3- and 6-months follow-up either face-to-face or by postal questionnaire/ telephone interview.

Global functional outcome:

The Glasgow Outcome Scale – Extended (GOSE) assesses global functional outcome in 8 categories: death, vegetative state, lower severe disability, upper severe disability, lower moderate disability, upper moderate disability, lower good recovery, upper good recovery (Wilson et al., 1998). The GOSE was a composite of interviews and questionnaires that were scored centrally (Horton et al., 2021). Since the vegetative state cannot be identified separately using a questionnaire this category was combined with lower severe disability. We used a cut-off value of lower good recovery or less as an indication of incomplete recovery.

The Short-Form-12 version 2 (SF-12v2) assesses health-related quality of life (Ware, 2000). Two scores from the SF-12v2 were used, the Physical Component Summary (PCS) which provides a measure of functional outcome, and the Mental Component Summary

(MCS) which assesses outcome related to aspects of mental health. Outcomes are expressed as T-scores (mean = 50 and SD = 10) based on normative data from a US sample, with higher scores indicating better quality of life. Scores range from 10 to 65 for the PCS and 8 to 72 for the MCS in the CENTER-TBI sample. T-scores <40 on the PCS or MCS are considered to indicate low health-related quality of life.

Mental health:

Symptoms of post-traumatic stress disorder (PTSD) were assessed with the reliable[2] PTSD Checklist-5 (PCL-5) (Blevins et al., 2015; Weathers et al., 1993). A five-point Likert scale (from 0, ‘not at all’, to 4, ‘extreme’) is used to measure 20 symptoms of PTSD that are based on the DSM-V (“Diagnostic Stat. Man. Ment. Disord. 5th Ed.,” 2013). Scores range from 0 to 80, with higher values indicating greater distress. We used a cut-off value of 33 or more as an indication of probable PTSD (Stein et al., 2019).

The Patient Health Questionnaire (PHQ-9) reliably assesses symptoms of depression, Cronbach’s a ~.89 (Kroenke et al., 2001). The PHQ-9 includes nine items on a four-point Likert scale. Total scores range from 0 to 27, with higher values indicating greater emotional distress. A clinical cut-off value of 10 or more was taken to indicate possible depression (Kroenke et al., 2001; Kroenke & Spitzer, 2002).

The Generalized Anxiety Disorder-7 (GAD-7) was used to assess anxiety symptoms with excellent internal reliability, Cronbach’s a ~.92 (Kroenke et al., 2001; Spitzer et al., 2006). The GAD-7 has seven items on a four-point Likert scale. The total score ranges from 0 to 21 with greater values indicating greater emotional distress. A clinical cut-off value of 8 or more was applied to indicate possible anxiety disorder (Kroenke et al., 2007).

TBI symptoms:

TBI symptoms were assessed with the Rivermead Post-Concussion Symptom Questionnaire (RPQ). The RPQ consists of 16 symptoms commonly reported after mild TBI/ concussion, rated by participants on a five-point scale (King et al., 1995). Total scores range from 0 to 64 with higher scores indicating more severe symptoms. We used a cut-off value of 16 or greater as indicative of clinically significant post-concussion symptoms (Thompson et al., 2016).

Statistical Analysis

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[2] Cronbach’s a ~.94

To ascertain whether background and clinical variables were independently associated with SR-TBI, we performed a binary logistic regression with SR-TBI group membership as the dependent variable and demographic, socioeconomic and clinical factors as independent variables. To examine differences in outcomes between patients with SR-TBI and the rest of the sample while controlling for covariates, binary logistic regressions were performed with each of the dichotomized measures. Covariates included were: age, sex, highest level of education, pre-injury employment status, TBI severity, CT abnormality, major extracranial injury, ASAP class, neurological medical history, psychiatric medical history and history of concussion. For missing data on covariates, we employed multiple imputation (Rubin, 1996) using multivariate imputation by chained equations (van Buuren & Groothuis-Oudshoorn, 2011) in the R statistical software (R Core Team, 2021). The number of imputations was set at 10 and the maximum iterations was set at 5. We controlled for multiple comparisons between the 14 outcome measures by adjusting for the false discovery rate using the sequential Bonferroni type procedure as described by Benjamini and Hochberg (Benjamini & Hochberg, 1995). Statistical significance was set at *p* <.05.

RESULTS

The participant selection process is shown in Figure 4.1: 285 (6.3%) patients were identified as having sport related TBI. Demographic and clinical characteristics of the sports injury and non-sports injury groups are given in Table 4.1, and the distribution of types of sports in Table 4.2. Patients with sports TBI predominantly had mild injuries (79%) and were aged 45 or less (67%). The most common setting of SR-TBI was horse-riding (63 cases), followed by skiing (48) and football (soccer; 34). In cycling 38 cases were identified as sport/recreational, 14 of them were excluded due to also being described as “road traffic accidents” leaving 24 cases with SR-TBI caused by cycling. In the database as a whole, cyclists represented 34% (N=578) of road traffic accidents (N=1682); these cases were not included in the current analyses.

Table 4.1. Demographic characteristics, premorbid and injury information of individuals who were injured in sport/recreation (N=285) and all other individuals (N=4224).

	N (%)	
	Sports / recreation (N=285)	Non-sports / recreational or unknown (N=4224)

Age				
Mean (SD)	36.1	(18.8)	49.8	(21.2)
Sex				
Female	109	(38)	1377	(33)
Male	176	(62)	2847	(67)
Highest level of education				
Primary	34	(14)	516	(16)
Secondary	76	(30)	1185	(36)
College / Training	137	(55)	1527	(46)
Missing / Unknown	38		996	
Employment Status				
Working (full or part time)	143	(53)	1833	(49)
Not working / homemaker	16	(6)	390	(11)
Retired	26	(10)	1086	(29)
Student	83	(31)	403	(11)
Missing	17		512	
Care pathway				
Emergency room	79	(28)	769	(18)
Admission	100	(35)	1423	(34)
Intensive Care Unit	106	(37)	2032	(48)
Missing	-		-	
ASA Pre-injury Physical Health				
Healthy patient	229	(81)	2272	(56)
Not Healthy patient	53	(19)	1819	(44)
Missing	3		133	
Pre-injury neurological condition				
Absent	272	(96)	3697	(90)
Present	11	(4)	392	(10)
Missing	2		135	
Pre-injury psychiatric condition				
Absent	266	(94)	3485	(86)
Present	17	(6)	584	(14)
Missing	2		155	
Previous concussion				

Absent	239	(87)	3544	(91)
Present	37	(13)	365	(9)
Missing	9		315	
GCS score at baseline				
3-8	38	(14)	948	(23)
9-12	19	(7)	370	(9)
13-15	219	(79)	2738	(68)
Missing	9		168	
CT Imaging abnormality				
Absent	139	(51)	1506	(39)
Present	131	(49)	2312	(61)
Missing/ uninterpretable	15		406	
Major extracranial injury[1]				
Absent	217	(74)	2656	(63)
Present	74	(26)	1568	(37)

[1] Any non-head & neck AIS ≥ 3 (serious injury)

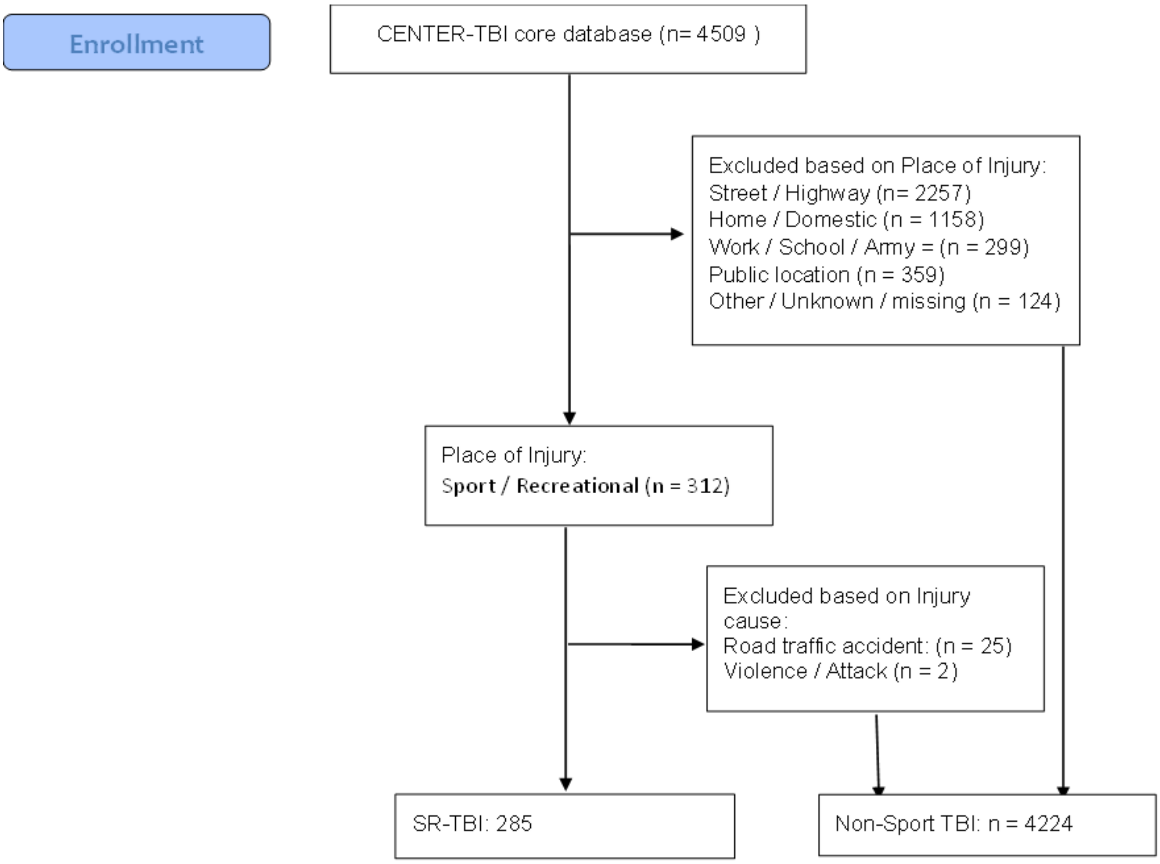
Table 4.2. Sport related TBI: descriptives per type of sport.

		Number of TBI Cases†	Sex*		Age	TBI severity*		
			Male	Female		mild	moderate	severe
Team Sports	Football	34 (12)	32 (94)	2 (6)	24.5	30 (88)	4 (12)	0
	Rugby	8 (3)	5 (63)	3 (37)	23.5	8 (100)	0	0
	Hockey (ice or field)	6 (2)	4 (67)	2 (33)	29	5 (83)	1 (17)	0

	Other	20 (7)	11 (55)	9 (45)	25	19 (95)	1 (5)	0
	Total	68	52	16		62	6	0
Individual sports	Horse-riding	63 (22)	6 (10)	57 (90)	26	45 (71)	6 (10)	8 (13)
	Skiing	48 (17)	38 (79)	10 (21)	41	33 (69)	5 (10)	9 (19)
	Cycling	24 (8)	19 (79)	5 (21)	44	22 (92)	0	2 (8)
	Off road vehicular sports	8 (3)	8 (100)	0	39	5 (63)	1 (12)	2 (25)
	Rollerblade/scooter/ Skateboard	9 (3)	6 (67)	3 (33)	26	8 (89)	0	1 (11)
	Other	60 (21)	42 (70)	18 (30)	39	41 (68)	1 (2)	15 (30)
	Total	212	119	93		154	13	37

† % out of all 285 cases with SR-TBI in brackets. * % out of the total cases with SR-TBI in each sport.

Figure 4.1. CONSORT 2010 flow diagram.



Factors associated with SR-TBI

Odds ratios for multivariate comparisons of sports and non-sports groups are shown in Figure 4.2. Patients with SR-TBI were found to be younger (Odds ratio: .98[95% CI: .97-.99] (p<.001), they were 1.36[95% CIs: 1.05-1.77] times more likely to be females (p=0.02) and 1.55[1.05-2.25] times more likely to be students (p=0.024). Their injury was 1.65[1.06-2.58] times more likely to be an mTBI (p=.003) and 1.55[1.14-2.11] times less likely to include a major extracranial injury (p=.005). Patients with SR-TBI were found to be 1.76[1.24-2.51] times more likely to be previously healthy (p=.002), 2.3[1.21-3.40] times less likely to have a history of psychiatric disorders (p=.008) and 1.52[1.03-2.24] times more likely to have history of concussion (p=.033).

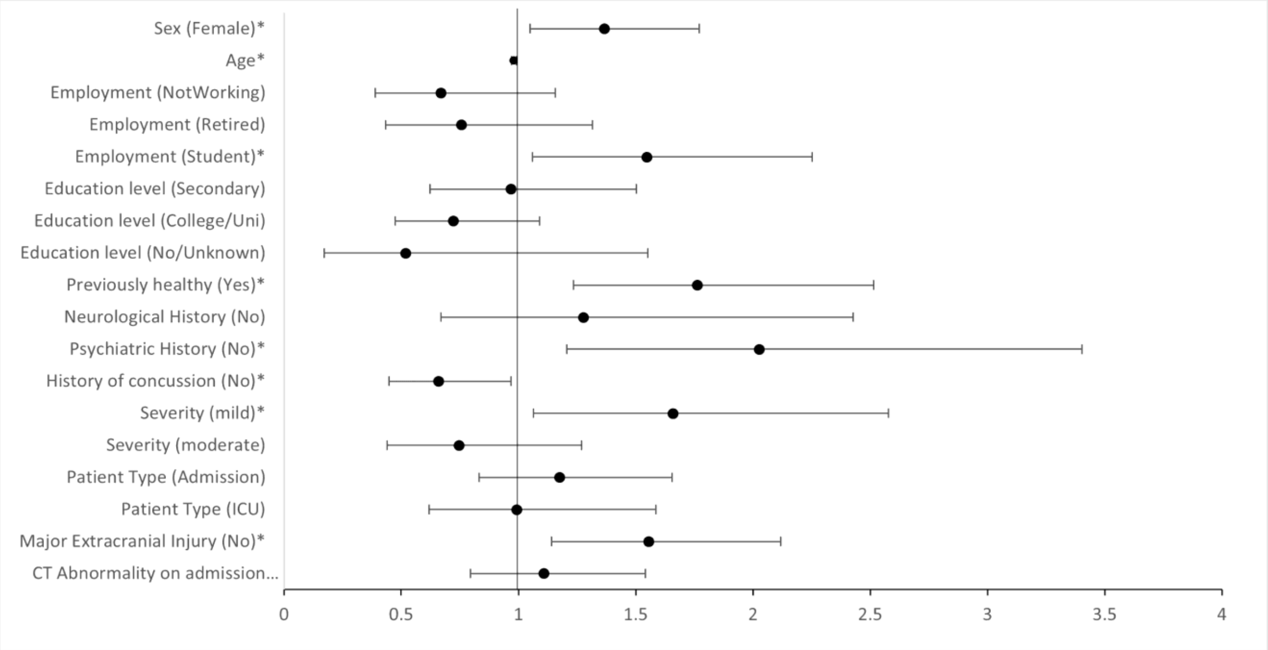


*Figure 4.2. Multivariate association of background and clinical variables with cause of injury. X axis depicting the odds ratio. Odds ratios are given for membership of the SR-TBI group. * Denotes significance at <.05 level. Horizontal lines represent 95% confidence intervals.*

Outcome measures

Percentages of patients with SR-TBI reaching cut-offs for impairment at 3 and 6 months are shown in Figure 4.3. The total sample size (SR-TBI and non-sports TBI samples) of patients with data for each come can be found in Table 1 of "Chapter 4 Supplementary material", accessible at: https://osf.io/z4bsu[3]. A substantial proportion of patients with SR-TBI had not fully recovered at 3- and 6-months follow-up. Specifically, 52% of patients with SR-TBI had incomplete recovery (a GOSE score of 7 or less) at 3 months; this figure was 42% in those patients with mild SR-TBI and 34% in patients with mild SR-TBI and a negative initial CT scan. At 6 months 41% of patients with SR-TBI had incomplete recovery, while for mild SR-TBI this figure was 33%, and 27% for those with mild SR-TBI and a negative CT.

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[3] Password protected, use password: ThesisNtikasChapter4

Twenty-two percent of patients with SR-TBI had significant TBI symptoms (RPQ> 16) at the 3-month follow-up. This figure was similar for the patients with mild SR-TBI (19%) and mild SR-TBI and a normal CT (21%). At 6 months significant symptoms were reported by 14% of patients with SR-TBI, 12% of patients with mild SR-TBI and 7% of patients with mild SR-TBI and normal CT (Figure 4.3).

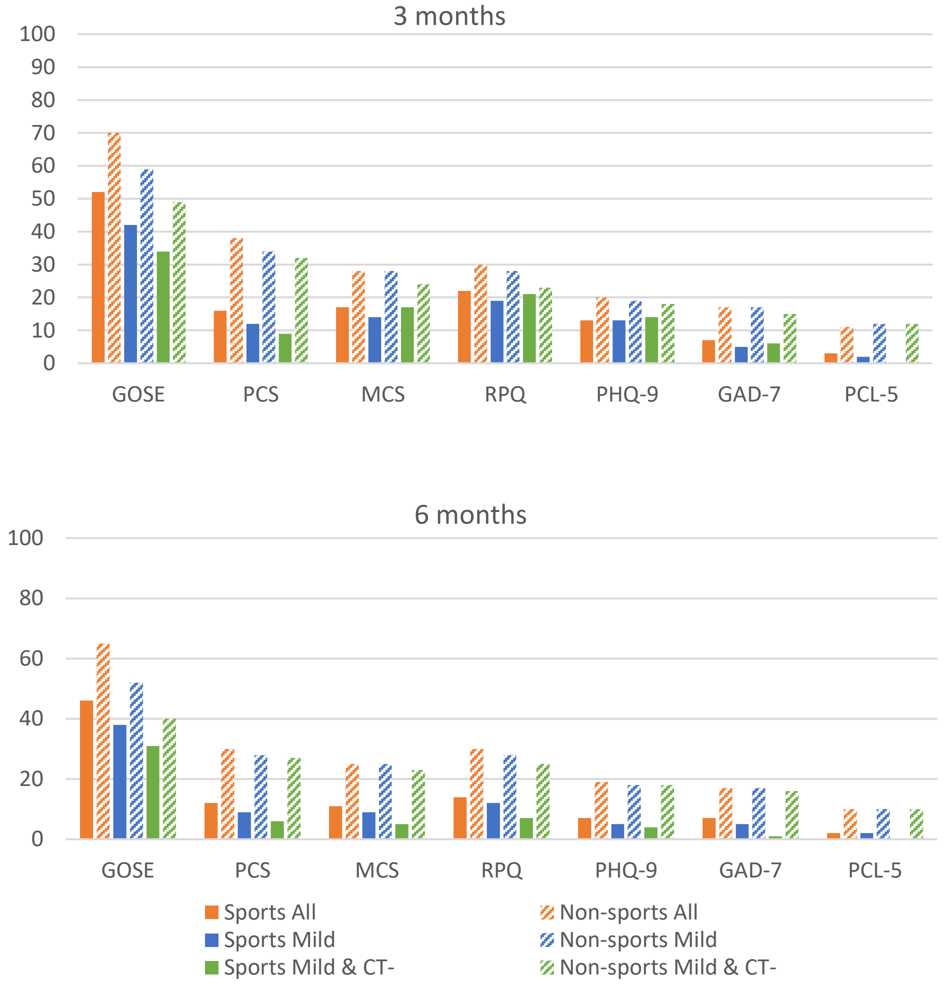

Figure 4.3. Percentages of impaired outcomes at 3 and 6 months for sports and non-sports TBI in three severity groups (all severities of injury, mild TBI, mild TBI with a negative CT). GOSE = Glasgow Outcome Score Extended. RPQ = Rivermead Post-concussion Symptom Questionnaire. PHQ-9 = Patient Health Questionnaire 9. GAD-7 = Generalised Anxiety Disorder 7. PCL-5 = Posttraumatic Stress Disorder Checklist 5. PCS = Physical Component Summary. MCS = Mental Component Summary.

Adjusted comparisons of outcomes between sports and non-sports TBI

Logistic regression revealed differences between the outcomes of patients with SR-TBI and the rest of the sample after adjusting for covariates (Figure 4). In the SR-TBI group return to normal life (GOSE=8) was 1.52 [1.12-2.06] times more likely 3 months post-injury (p=.007) and 1.43[1.01-1.88] more likely at 6 months (p=.042). Normal physical health on the SF-12v2 PCS was 2.15 [1.39-3.34] times more likely at 3 months (p<.001) and was 1.64[1.08-2.49] times more likely at 6 months (p=.02). Similarly, normal mental health on the SF-12v2 MCS was 1.89[1.15-3.10] times more likely at 3 months (p=.012) and 2.26[1.37-3.71] times more likely at 6 months (p=.001).

SR-TBI was not associated with symptoms of concussion on the RPQ at 3 months (*p* =.116), however s at 6 months, patients with SR-TBI were 2.36[1.48-3.75] times less likely to have significant concussion symptoms (p<.001) (Figure 4.4).

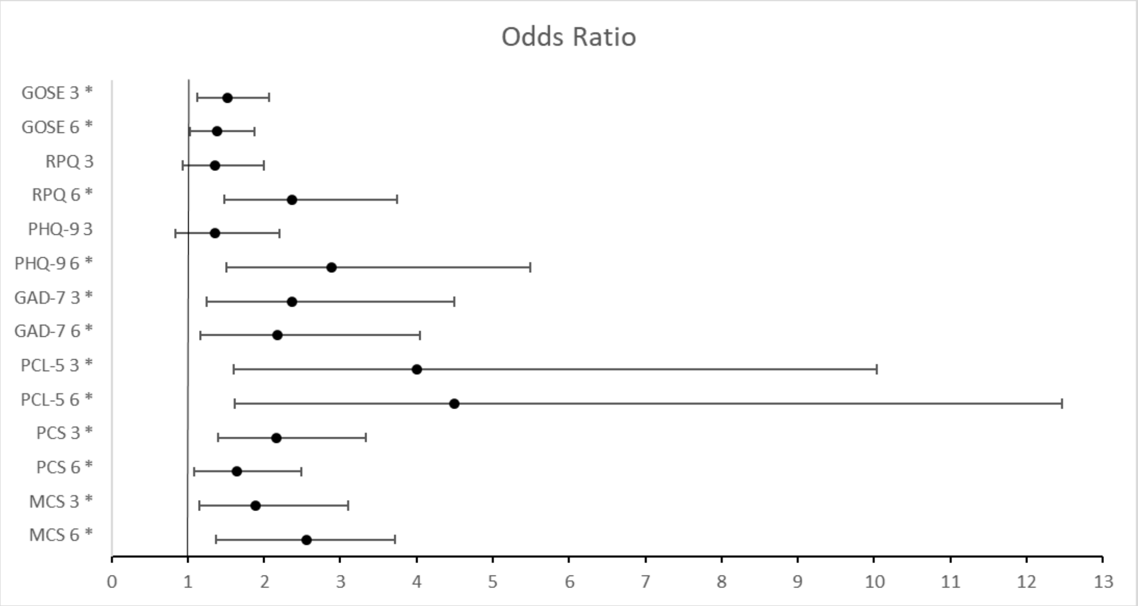

Figure 4.4. SR-TBI outcomes odds ratios at 3- and 6-months post injury adjusted for covariates. Horizontal lines represent 95% confidence intervals. The vertical line represents an odds ratio of 1. Abbreviations: 3 = 3 months, 6 = 6 months. GOSE = Glasgow Outcome Score Extended. RPQ = Rivermead Post-concussion Symptom Questionnaire. PHQ-9 = Patient Health Questionnaire 9. GAD-7 = Generalised Anxiety Disorder 7. PCL-5 = Posttraumatic Stress Disorder Checklist 5. PCS = Physical Component Summary. MCS = Mental Component Summary.

SR-TBI was not associated with depression assessed by the PHQ-9 at 3 months (*p* = .21), but at 6 months post-injury patients with SR-TBI were 2.88[1.51-5.49] times less likely to have clinically significant symptoms of depression (*p*=.001). On the GAD-7, patients with SR-TBI were 2.36[1.24-4.49] times less likely to have possible anxiety disorder at 3 months (p=.009) and 2.17[1.16-4.04] times less likely to have anxiety disorder at 6 months (p=.014). On the PCL-5 patients with SR-TBI were 4[1.6-10.03] times less likely to have probable PTSD at 3 months (p=.003) and 4.49[1.61-12.47] times less likely to have probable PTSD at 6 months p=.004).

All but one *p* values remained below the chosen level of significance (.05) after correction for multiple comparison. GOSE score at 6 months was not significant with an adjusted p-value of .051 (see Chapter 4 Supplementary Material, Table 2).

In a sensitivity analysis we included all patients with outcome measures at both 3 and 6 months (see Chapter 4 supplementary material). Findings for this analysis were similar to that using all cases, and the confidence intervals at 3 and 6 months overlapped for all measures (see Figure 1 in Chapter 4 Supplementary Material). There was a trend for differences on measures of functional outcomes (GOSE and PCS) to decrease over time (non-significantly different GOSE scores at 6 months), whereas differences on mental health outcomes (MCS, PHQ-9, GAD-7, PCL-5) tended to increase over time. As can be seen in Figure 3, functional outcomes remained impaired at 6 months in the SR-TBI group, while, unlike the non-sports group, symptoms and mental health problems appeared to subside.

DISCUSSION

The strength of this study is that it is a cohort study. That said, this study samples a population of TBI cases attending hospital, and therefore as a population may not be entirely representative for the wider population which includes typical “sports concussion”. However, as noted in Chapter 2, the examination of the incidence of traumatic brain injury at scale dated back at least 20 years, until the launch this large-scale international observational study (Maas et al., 2015). Furthermore, previous studies were US based, while it is important to examine a European cohort with regards to sport-related brain injury incidence, sport-specific profiles, demographic data such as age and sex, and critically the incidence and outcomes of those incurring multiple brain injuries while participating in sport in a European context.

The cohort consisted of patients visiting specialised neuroscience centres (equivalent to level 1 trauma centres in the US) for whom it was deemed necessary to conduct a brain scan (e.g., to check for a hematoma or a bleed), which we here refer to as patients ‘triaged to CT’. There are several hundred such specialized centres across Europe and the US; the findings are thus of direct relevance to a large group of patients. Furthermore, the results also have potential implications individuals visiting emergency departments with TBI who are triaged to CT: a figure estimated to be some 2.5 million people annually in the US (Korley et al., 2016). Especially, since the SR-TBI cases in emergency departments are rising consistently the last decades, with sports like soccer, rugby, roller-skates and cycling showing the greatest level of increase (Finch et al., 2013).

The findings confirm that there are systematic demographic, background, and injury-related differences between groups with sports and non-sports TBI. In a multivariate analysis we found multiple differences between groups. Patients with SR-TBI are younger, more likely to be female and college students, and report better health before injury and fewer pre-injury mental health problems. SR-TBI is also likely to be less severe and without concomitant major extracranial injury. The differences described here are consistent with characteristics that have previously been inferred by contrasting studies of sports and non-sports TBI (Rabinowitz et al., 2014). The current analysis demonstrates that these differences are independently associated with sports-related TBI and confirms the view that individuals with sports-related injuries represent a highly selective subsample of individuals with TBI. A noteworthy finding is that a history of concussion was more common in the sports-injury group. As described in previous reports (Selassie et al., 2013; Theadom et al., 2014), the higher prevalence of previous TBI among the sports-injury group is relevant because it may make this group vulnerable to the chronic effects of repeated TBI (Rabinowitz et al., 2014).

The SR-TBI group recovered better than the rest of the sample, even after considering the lower risk factors for poor outcome. They were ~1.5 times more likely to have full recovery at 3 and 6 months on the GOSE, 2.4 times less likely to have significant TBI symptoms at 6 months, and 2.21 to 4.63 times less likely to have mental health problems at 3- or 6-months post-injury. These differences in outcome point to additional differences between the groups that were not controlled in the analyses. For example, individuals attending hospital following sports-injuries may have milder types of TBI, not captured by the measures of severity employed here. Athletes may also receive superior post-injury support from sporting colleagues familiar with concussion. Light controlled exercise the first week after mild TBI has been found to be beneficial for recovery (Lal et al., 2018; Leddy et al., 2019). Patients with SR-TBI are presumably more likely to engage in exercise in the weeks after TBI than other patients, however this rehabilitation exercise might not be well utilised in non-professional athletes. It is also possible that people with better pre-injury health have greater resilience, and therefore recover more completely. The latter interpretation assumes individuals with sport-related injury to better cope with the effects of TBI, according to a benign view of the effects of concussion in sport.

Despite having outcomes that were better than the rest of the sample, more than half of patients with SR-TBI had an incomplete recovery on the GOSE 3 months after the TBI while a substantial proportion were still not fully recovered even at 6 months (41%). Even in those patients with a SR-TBI who incurred injuries that would be considered “mild” this proportion was still high (33%) six months after injury. This is the case despite relatively low risk factors for poor outcome in the sports TBI group. The finding that sports TBI, including mild sports TBI, is associated with persisting limitations is novel and has important implications for management of this type of injury. It specifically warns against assuming a benign view of mild sports-TBI and calls for action to be taken to ensure the well-being of athletes is not compromised by fast-track return-to-play protocols.

Elevated TBI symptoms as assessed on the RPQ, were found in 22% of patients with SR-TBI at 3 months and 14% at 6 months. The proportion found here at 3 months is higher than previously described for college athletes (McCrea et al., 2013), but the prevalence of significant symptoms diminishes by 6 months. Notably, there was little evidence of persisting distress on assessments of mental health in the SR-TBI group. This is consistent with other reports, including, for example, low observed prevalence of mental health problems in former professional soccer players (Russell et al., 2020). The finding that persisting problems in the SR-TBI group are not attributable to emotional difficulties is important because it argues against the idea that limitations reported after mild TBI are a result of pre-existing mental health issues.

Limitations

An important limitation of this study is the low sample size of the SR-TBI group in some comparisons, especially in the comparison of PTSD symptoms (PCL-5 scores). This should be taken under consideration when interpreting those findings as their validity might be compromised by the very low number of TBI patients who developed PTSD the months post injury.

Clinical implications

The study shows that the sample with SR-TBI differs in multiple ways from the non-sports group and is characterised by having lower risk factors for poor outcome. Notably, the risk factor of past concussions was higher in the SR-TBI group. Recovery after SR-TBI is better than the rest of the TBI population, even after considering risk factors in the group. However, in contrast to the idea that recovery after mild/ TBI concussion is generally unproblematic, we found that around one third of individuals with SR-TBI who presented with an initial GCS of 13 to 15 and a negative CT scan had persisting problems at 6 months. The findings have important implications for management of individuals presenting at hospital with SR-TBI and indicate that even among individuals with sports injuries considered to be “mild” many will require systematic follow-up and treatment.

INTERIM DISCUSSION 2

The mapping of the biomarker literature helped to clarify which biomarkers are more promising in assessing the effects of RSHI on athletes' brain functioning. Importantly the scoping review (Chapter 3) identified the certain central problems that the literature on RSHI as a whole has, which result in studies of below average quality and with high risk of bias. Specifically, most studies in the scoping review that failed to achieve a low risk of bias and high-quality score, did so because they fail to efficiently control for some important confounding variables. Those confounding variables were: the potential effect of exercise on biomarker concentration, the effects of recent head impact exposure and the control for past and recent concussions or concussive impacts occurrence during the experiment. All experiments reported in this thesis controlled for those confounding variables by using exercise control conditions, making sure the impacts during the experiments are not concussive while recruiting people with no recent concussions and asking them to refrain for exposure to head impacts the days before participating in the research as these were active football players.

Chapter 4 (TBI prognosis) highlighted the importance of athletes' brain health and the burden of mild TBIs in sports showing that even 3 months after injury a high percentage of people with mild SR-TBI have not fully recovered, with a fair number of those patients having acquired this mTBI while playing football. This finding increases the concerns about the burden of heading in football, both for healthy athletes and for athletes that have a history of concussion and still play football. To investigate how football heading affects athletes brains this thesis aimed to examine how football heading can affect the motor control and the cognitive functioning of athletes. Any findings of altered brain function post heading will raise serious questions about return-to-play protocols. A third of people with mild TBI had not fully recovered 3 months post injury. Return-to-play protocols, however, dictate that if athletes pass the not sensitive concussion assessment protocols (i.e. SCAT5) they can return to play as early as 10 days post injury. If RSHI cause brain alterations, it raises the question whether those athletes might be more at risk of further brain health problems, and it is therefore important to understand the mechanisms at play following exposure to RSHI..

To investigate the effects of RSHI in athletes' brains a series of measurements were implemented, using cutting edge technology and computerised tasks able to pick up subtle differences caused by football heading. Those measures aimed to examine if RSHI cause brain alterations to athletes and if so, what the nature of those alterations is. The measures used examined two different aspects of brain functioning, motor control and cortical inhibition, and learning and memory. As presented in Chapter 2 (literature review) and in the next chapter (Chapter 5, examining postural stability), there is mixed evidence for the effects of RSHI on motor control and cortical inhibition. Similarly, there is mixed evidence on the effects of RSHI on the basic cognitive functions of athletes (attention, memory and learning). In the following chapters an effort is made to understand if and how RSHI affect motor control and cognition.

To do so, a series of experiments were conducted, starting with experiments examining if and how 20 headers affect athletes’ performance in gold-standard behavioural neurocognitive tasks and a series of balance tasks. Following this, neurocognitive function was examined with more sophisticated electrophysiological techniques which complete the picture of the effects of heading.

CHAPTER 5. No effects of RSHI on football players' postural control

Abstract

Objectives: Balance is one of the main ways used by researchers, especially in sports science, to assess the effects of RSHI. The aim of this chapter was to use sensitive methods to collect and analyse balance data to provide concrete answers on whether RSHI affect athletes' motor control.

Methods: Twenty-seven young (~23 y.o.) football athletes were recruited from local clubs. They performed four balance tasks assessing balance at different cognitive loads and visual input before and after 20 headers or a kicking control condition. A custom-built force plate platform was used to record centre of pressure (COP) data while athletes performed the balance tasks. COP data was analysed in respect to regularity (approximate entropy), area of displacement (root mean squared deviation from the mean) and speed of displacement (sway velocity).

Results: Football heading, unlike sport-related concussion, did not affect balance in any of the ways measured here. The expected effects of visual input and cognitive load were found; however, athletes' performance was similar in the heading and kicking control conditions.

Conclusions: The findings of this chapter add to the null findings reported previously from our lab and the null findings found in previous studies. Therefore, balance tasks appear to be an inefficient way to detect any motor control changes caused by RSHI. Future studies should use more sensitive neurophysiological measures of neuromuscular control instead.

INTRODUCTION

The long-term and the acute effects of heading in football (soccer) have long been investigated as a possible cause for brain damage and as a possible cause of impaired performance on cognitive and balance tasks (Di Virgilio et al., 2016; Haran et al., 2013; Levitch et al., 2018; Lipton et al., 2013).

Balance was at first considered an effective way to detect and examine the effects of subconcussive impacts, as it has been a reliable way to detect sport-related concussions and assess the necessary return-to-play periods. Balance measures are an integral part of most return-to-play protocols (McCrory et al., 2017). However, whereas in concussion balance is one of the main ways to clinically detect a concussed individual, in the literature of subconcussive impacts it seems to be an ineffective or unreliable way to measure the effects of those impacts (Broglio et al., 2004; Di Virgilio et al., 2016). This lack of clinical representation of motor control changes after RSHI can potentially be a way to set apart sport-related concussion which is an injury that can affect athletes for a long period of time (see Chapter 4), from the acute effects of RSHI that are more subtle and wave off quickly.

Most of the studies which used the clinical balance tests for concussion failed to find any significant effects of subconcussive impacts in football (Broglio et al., 2004; Caccese et al., 2018; Di Virgilio et al., 2016; Mangus et al., 2004). Broglio et al. (2004) did one of the first attempts to thoroughly examine the effects of heading on footballers' balance. In their study they examined the effects of linear, rotational and simulated rotational headers[4]. All participants in the three experimental groups headed the ball (or did a simulated header) 20 times; the control group did no heading. No differences in the mean centre of pressure and total sway were found across all conditions, so the authors concluded that heading is not associated with any acute risks for football players. A strong claim considering the sensitivity of those measures; however, this study shows that the acute effects of subconcussive impacts are not as severe as the ones from the impacts that cause concussion.

Contrary to the aforementioned studies, the studies that used more sensitive ways to measure differences in postural sway showed some effects of heading on balance and postural control, with specific emphasis on vestibular system malfunctions (Haran et al., 2013; Hwang et al., 2017). Haran et al. (2013) measured the centre of mass (COM) displacement after 10 linear headers. Contrary to Broglio et al. (2004), they used six different

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[4] Participants acted like they headed a football, without actually heading a ball

static and dynamic conditions that exploit different balance systems (vision, vestibular function and proprioception) and they used more sophisticated methods of data analysis; like the root mean square deviation from the mean of the COM. Their results showed that heading affected balance, especially in the condition in which the proprioception and the visual input were manipulated. Since balance was affected more in the condition in which participants were heavily relied on their vestibular system, the results of this study indicate that football heading might be linked with vestibular system malfunctions. Those findings are also supported by a recent study by Hwang et al. (2017) in which it was found that heading leads to transient vestibular systems dysfunctions.

A very recent scoping review found that only ~21% of the studies investigating the short-term effects of RSHI on vestibular function found RSHI to affect vestibular function, however the studies finding effects were more likely to use objective measures and control for confounders (Stephen et al., 2022). Similarly, a recent systematic review on the effects of RSHI on balance found that more than half of the studies identified did not reveal any significant effects of RSHI on athletes' balance (Bonke et al., 2021), however they acknowledge the high heterogeneity between the studies in terms of batteries used, RSHI exposure and sports assessed.

From the studies described above, it is evident that the types of balance tasks used, as well as the way the collected data is analysed, is crucial as it affects the sensitivity of the measures. By using sensitive ways to analyse the data the likelihood of any potential minor changes in athletes' postural control to be identified is increased. Since differences in the centre of mass and the centre of pressure can be analysed in different ways, it is important to further investigate balance with the ways that seem to be the most sensitive. Unfortunately, most of the ways used to analyse centre of pressure data take into account only the amount of movement of the centre of pressure but fail to examine the temporal structure of the signal (Baltich et al., 2014). The conventional methods of analysing the data (as used in DiVirgilio et al. 2016; Broglio et al. 2004) might not be sensitive enough to detect the differences in balance caused by impacts far less severe than the ones who cause concussion.

Haran et al. (2012) analysed their COP data from the force plate by analysing the root mean square deviation from the mean which represents the average spread of a time series distribution relative to its mean. Increased root mean square values indicate decreased postural stability. In another study (Haran & Keshner, 2008) apart from the root mean square deviation from the mean, used the MATLAB function of approximate entropy (ApEn) to analyse the regularity of their data. Approximate entropy is a probability statistic which examines the likelihood that a data sample is remaining in a predefined tolerance window. The approximate entropy has values ranging from 0 to 2, with values closer to 2 indicating greater irregularity and unpredictable motion. Interestingly in the literature of concussion, concussed individuals seem to have more predictable motion (values closer to 0) compared to healthy subjects on balance tasks (Cavanaugh et al., 2005, 2006). Notably, impaired balance systems tend to be less irregular compared to healthy ones and become more and more irregular as they overcome this impairment. For example, patients following a stroke have been found to have more regular centre of pressure compared to healthy people, a regularity which decreases and eventually disappears (signal became more irregular) after rehabilitation or during the administration of an easy dual task (Roerdink et al., 2006).

So far only one study has used approximate entropy to investigate the effects of RSHI on athletes' balance (Caccese et al., 2021). Caccese et al. (2021) used a mix of high-school and collegiate football (soccer) players to investigate changes in balance before and after a series of 12 headers. They found that sway velocity was higher post heading compared to the control group, but they found no differences in the 95% centre of pressure area and approximate entropy.

The difficulty of a task and the presence of a second cognitive task in parallel with a balance task are also found to increase approximate entropy values (Cavanaugh et al., 2007). Interestingly, Cavanaugh et al. (2007) found that the approximate entropy was the only way to analyse the data which was able to detect the decrement caused by the dual task condition. One reasonable assumption for this type of relationship between balance regularity and task condition is that, when individuals have difficulties maintaining their balance and try to act on it, cause irregular balance adjustments that are picked up with this way of analysis. Cavanaugh et al. (2007) also explicitly state that approximate entropy might be useful in Sport Medicine, where the use of Entropy functions can help clinicians to better diagnose subtle impairments in athletes with minor injuries, who appear to have normal performance in the cruder balance measures.

This relationship between dual task and entropy measures has been found in many studies (Cavanaugh et al., 2007; Dai et al., 2018; Donker et al., 2007; Haid & Federolf, 2019; Roerdink et al., 2006), this finding together with the finding that during eyes closed condition the sample regularity increases (Cavanaugh et al., 2007; Donker et al., 2007) indicates that the greater the cognitive involvement on balance is, the more regular the centre of pressure signal becomes. All the aforementioned studies used approximate entropy or a modification of approximate entropy, called Sample Entropy to examine the centre of pressure differences in terms of regularity.

In the studies that used entropic measures to investigate balance, during the eyes closed condition the signal regularity is increased, however, when they do a dual task the normal irregularity of the signal is again present (Donker et al., 2007). Those results indicate an effect of cognitive control and possible attention on balance, when participants are focused on their balance, like in the single task eyes closed condition, there is a decrease in the irregularity of the signal, which is again present when their focus is shifted away from their balance, during a dual task condition.

The existing literature on subconcussive impacts and attention suggests that the classical measures to assess attention are unable to detect any decrements in the attentional processes of football players, but more sensitive ways to measure attention e.g., oddball task while wearing EEG cap, found that attentional resource allocation and attentional orienting were impaired after heading (Moore et al. 2017). Any findings that connect the allocation of attentional resources with balance can add to this finding of problematic resource allocation after heading and force the field to investigate the effects of heading not only on balance but also on attention by using more sensitive and sophisticated measures.

Here the main interest was to examine if balance is impaired due to heading in single and dual task conditions. Greater effects of heading on the balance tasks in which there is less cognitive control would indicate greater damage in the automatic balance processes (possible vestibular system), whereas greater effects of heading on balance tasks which come with greater cognitive control are a possible indicator of a faulty cognitive control system (possibly decrements in allocation of attention).

Based on all the studies mentioned before, the most effective ways to examine balance decrements is by using multiple demanding tasks that exploiting different balance systems, and/or by using more sophisticated ways to analyse the balance data.

This study examined the acute effects of football (soccer) heading on balance with a specific target to use ways of analysing data that have rarely been used in the subconcussive impacts literature. The effects of heading on postural control were compared to a control condition in which players kicked the ball without incurring any head impact. All participants were football (soccer) players, and they performed four different balance tasks; an eyes open single task, an eyes closed single task, an eyes open dual task and an eyes closed dual task. Changes to balance were investigated before and after 20 headers (experimental condition) and before and after kicking (control condition). Unlike Caccese et al. (2021) in this study participants served as their own controls, performed more headers and more tasks.

It was hypothesised that the session of football heading will increase the velocity, deviation from the mean and regularity of postural sway of football players compared to a baseline measure, whereas no change was expected after the kicking session.

METHODS

Participants

The participants of this experiment were active football (soccer) players between the ages of 18-35 who head the ball on a regular basis as part of their training and/or play. Participants had no learning difficulties, no neurological condition or history of brain damage, no psychiatric condition currently treated with psychoactive drugs, they had not use of recreational drugs in the last week and had no history of sports-related concussion in the last year. Because of the nature of balance and procedural learning tasks, participants with current lower or upper limb injuries were also excluded. They were asked to refrain from alcohol 48 hours and avoid caffeinated drinks consumption 8 hours before the study. They were also asked to refrain from head impacts the days before the sessions and inform the researcher if they sustained a high impact during training or games the week before the sessions. Participants were recruited through advertisements (web/poster/leaflet) and direct contact. All athletes were reimbursed on a £8.50/hour rate for their participation. Twenty-seven athletes (14 females) with a mean age of 22.65 (±3.80) were recruited. The sample size was provided by sample size calculations (see appendix 1.1) done on Gpower 3.1 (Erdfelder et al., 2009), using the effect sizes from Di Virgilio et al. study (2016). Two athletes reported that they play at a professional, 9 at a semi-pro and 16 at an amateur/recreational level. All athletes were examined both under the experimental and the control conditions (within-subjects design).

Materials

Impact meter. The impact of each header was measured in terms of linear (G force) and angular acceleration (rad/s^2). This data was provided by a head-mounted accelerometer provided by Protxx Inc. (USA) which provides a set of 6 columns, each of them representing the linear or angular acceleration in each axis (x,y,z).

COP. The centre of pressure was measured using an in-house built force plate platform with a 100Hz sampling rate. Furthermore, a commercially available accelerometer provided by Protxx Inc. was used as additional measure to assess balance differences. This sensor was provided from Protxx Inc. and we were asked to evaluate its efficacy in measuring subtle balance changes. Protxx Inc considers this sensor as a sensitive way to measure differences in postural sway and it has been found to be able to detect subtle balance differences between people who smoke or are under prescribed medication (Grafton et al., 2019).

n-back task. Participants performed an auditory 2-back task while trying to maintain the posture in the dual task condition. N-back tasks are widely used as a way to place continuous demands on working memory in dual task conditions (Owen et al., 2005). Continuous demand on working memory is necessary in the case of this experiment since the temporal characteristics examined by the entropic measures might be influenced by the use of a task that places non-continuous demand on working memory. Participants heard consonants of the English alphabet and they were asked to respond by saying “yes” when the stimulus spoken to them was the same with the one two places back (2-back) from the present stimulus. Reaction times were not recorded during this task, only correct responses (hit/misses). The number of the targeted trials (2-backs) were the same between conditions (6).

Procedure

Familiarisation session. Participants were welcomed in the lab and given the information sheet and the demographics questionnaire to fill in. The first day participants did a familiarisation session, like in DiVirgilio et al. (2016), in which they undertook the balance tasks twice. This procedure was necessary to avoid any learning effects that could potentially mask any effects of heading on balance.

Experimental and control sessions. One week later the athletes came again for the real experiment. After welcoming them back to the lab they were asked to wear the Protxx head-mounted sensor (Figure 4.1) and step onto a force plate platform (Figure 4.2). In both single and dual task conditions participants were asked to stand up straight with their feet pressed together and try to maintain their balance for ~40s; this is the standard procedure suggested by Protxx (Grafton et al. 2019), the company which provided the head-mounted sensor. In the eyes open condition they were instructed to keep their eyes open and look at a fixation point on the wall in front of them. During the single task conditions, they were asked to focus on maintaining their balance, whereas on the dual task conditions they were asked to focus on the cognitive task while they were trying to maintain their balance.

Figure 4.1 Head-mounted impact and balance sensor (Protxx Inc)

Figure 4.2 Force plate platform

After the baseline measures of balance participants were escorted to the heading space location, in which, depending on condition, they either head a football 20 times, a procedure that lasted ~10 minutes, or kick a football for 10 minutes. The ball used was a standard football (70cm circumference; 400g; 8 psi) and it was thrown to participants from a distance of 10 meters from a ball throwing machine (JUGS sports, Tualatin, USA). After the description of the procedure, participants wore a heart rate monitor (Figure 4.3); the balance sensor that were already been wearing in this case served as an impact sensor. The first few ball throws were done in order to find out which speed represents better a routine header from a crossing or a corner kick. Participants were asked to judge the speed by catching the ball in the air. When the appropriate speed (~26-28 mph) was found the heading process begun. Athletes performed 20 rotational headers redirecting the ball to a goal; this process served as the in-lab equivalent of a corner kick. They were instructed to avoid jumping, thus performing only standing headers, and to use their judgement and avoid heading the ball when they feel they cannot head it properly. When a header was missed, or the athlete judged it as “just a scratch” the header was repeated. In the kicking session the exact same football was used, and participants were be asked to kick the ball and redirect it back to the researcher.

Figure 4.3 Heart rate monitor (Polar, UK)

In both conditions athletes' heart rates were monitored in order to make sure that the average heart rate during each condition did not exceed the 63% of maximum heart rate (Hrmax; 220-age). 63% of Hrmax is the maximum heart rate that falls under the light physical activity range (Canning et al., 2014).

After the heading/kicking protocol, athletes were brought back in the lab and the post-heading balance measures were performed in the same way as the baseline measure. Athletes that did first the heading condition came one week later to participate in the kicking condition and vice versa.

Statistical analysis

Data for this experiment was analysed on SPSS and R. Balance data was extracted from the force plate platform in the form of a continuous signal. The first and last 5 seconds of which were removed, resulting in 30-second signals for each participant. These signals were imported into R (R Core Team, 2021) to obtain the approximate entropy, average sway velocity and the root mean squared deviation from the mean values. Balance data from the Protxx sensor was provided by their own developed app. Repeated measures ANOVAs on SPSS (IBM Corp., 2019) were used to identify differences caused by time (pre - post), intervention (heading - kicking), eyes (open – closed) and cognitive load (single task– dual task). The significance value was set at .05. The raw data recorded by the impact sensors was imported to MATLAB (see Appendix 1.2 for the script) and the mean peak linear and angular accelerations for each header were calculated.

RESULTS

Heading protocol. The heading protocol produced headers with mean peak linear accelerations of 17.65 ±2.71g per header and mean peak angular accelerations of 1759 ±629 rad/s^2. None of the athletes' heart rate exceeded the threshold for light medium exercise in the experimental or control conditions, mean bpm: 87 (±9) for kicking session and 85 (±11) bpm for heading session.

Data from the force plate platform was imported on R, in which the approximate entropy values for each participant were computed (see Appendix 1.3.1 for the R script). A 4x2 repeated measures ANOVA to examine the main effects of intervention, time, eyes and cognitive load on balance irregularity on the anterior/posterior plane was performed. There was no main effect of intervention (p = .679) or time (p = .312), but there was a main effect of eyes; F(1,20) = 5.62, p = .028, η^2 = .219, OP: .62 (athletes balance was less irregular during eyes closed condition) and cognitive load; F(1,20) = 6.38, p = .02, η^2 = .242, OP: .67 (athletes balance was more irregular during dual task). There was an intervention*eyes*cognitive load interaction; F(1,20) = 4.57, p = .045, η^2 = .186, OP = .53. No other interaction was significant. This interaction did not included time, but it was investigated further to assess if changes in centre of pressure regularity between eye and task conditions have a different pattern between heading and kicking conditions (especially post intervention). In the pre-intervention measures, there was a main effect of eyes but no main effect of cognitive load, and there was a significant eyes*intervention interaction (p = .028). This interaction is caused by the absence of the normal lower irregularity during eyes closed condition compared to the eyes open condition before heading in the single task condition (Figure 4.4). The effects of cognitive load and eyes were present in the post intervention measures, and no significant interactions were found. By inspecting the Figures 4.4 and 4.5 we see that although the post intervention values follow the expected pattern, eyes closed decreased irregularity, dual task increased the irregularity, in the pre-intervention single task condition this effect is not present (Figure 4.4), pointing to possible inefficient task familiarization.

In order to establish that the interactions of interest, intervention*time, show evidence for no effects, rather than no evidence of an effect, 3 Bayes factor analyses for repeated measures were performed in JASP (JASP Team, 2022) for the interaction that were not found to be significant above. The conditions of interest where the (1) eyes closed single task, (2) eyes open dual task and (3) eyes closed dual task. A Bayes factor of >3 was set as an indicator of moderate evidence and a Bayes factor of >10 as an indicator of strong evidence of the presence/absence of an effect. In short, Bayes factor is a value that indicates how much more likely the null hypothesis is compared to the alternative of vice versa.

For the eyes closed single task condition, there was moderate evidence for no effect of intervention (Bayes factor of 6), moderate evidence for the effect of time (Bayes factor of 3.8) and strong evidence of no effect for an intervention*time interaction (Bayes factor of 17.8). The same pattern of results was found in the eyes open dual task condition, no intervention effect (BF = 6.12), no effect of time (BF = 2.9) and no intervention*time interaction (BF = 9.9); and the eyes closed dual task condition, no intervention effect (BF = 6.55), no time effect (BF = 6.6) and no intervention*time interaction (BF = 15.2)

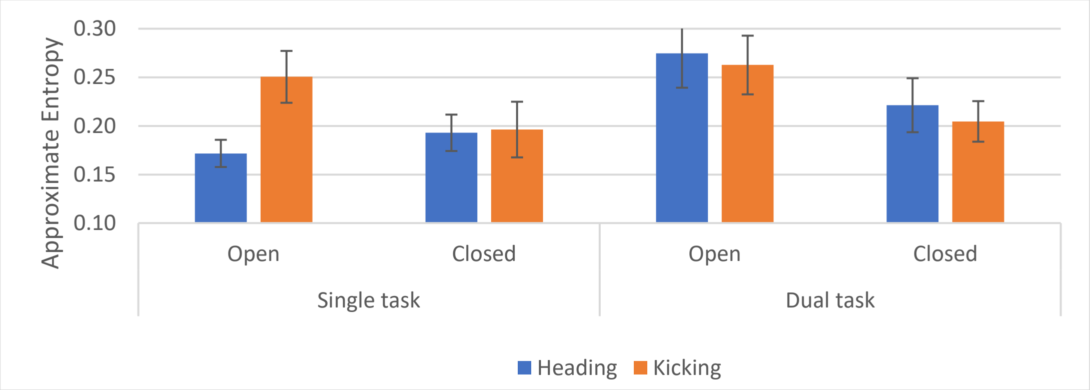

Figure 4.4 Pre-intervention approximate entropy values

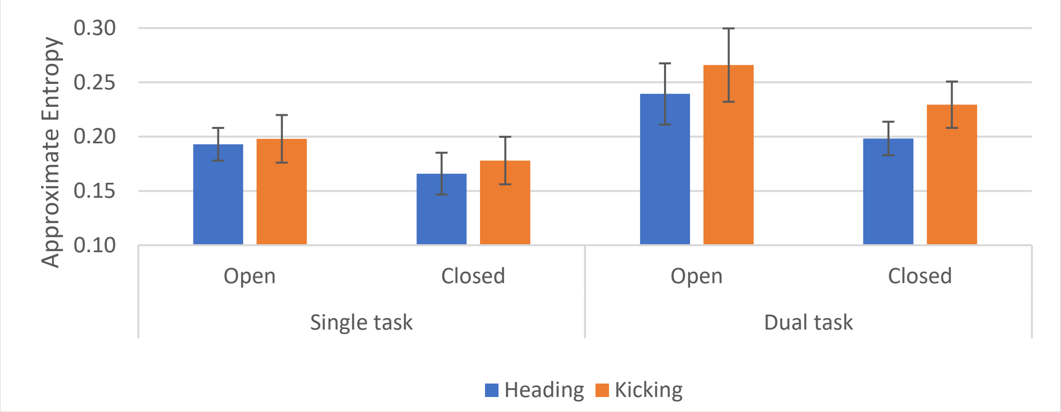

Figure 4.5 Post-intervention approximate entropy values

The same analysis was done for the balance irregularity on the medial/lateral plane. The 4x2 repeated measures ANOVA revealed a main effect of cognitive load, F(1,23) = 13.50, *p* = .001, η^2 = .370, OP = .94, a main effect of eyes, F(1,23) = 28.46, *p* < .001, η^2 = 553, OP = .999, a main effect of intervention, F(1,23) = 4.64, *p* = .042, η^2 = .168, OP = .54, and a main effect of time, F(1,23) = 4.56, *p* = .044, η^2 = .165, OP = .53. There was the expected effect of eyes and cognitive load, with irregularity decreasing when athletes closed their eyes, and increasing when they performed a secondary task (Figure 4.6). Notably, there was an effect of intervention, with athletes' balance being more irregular during kicking sessions and an effect of time with the balance irregularity decreasing significantly post interventions (Figure 4.6). There were no significant interactions between any of the effects.

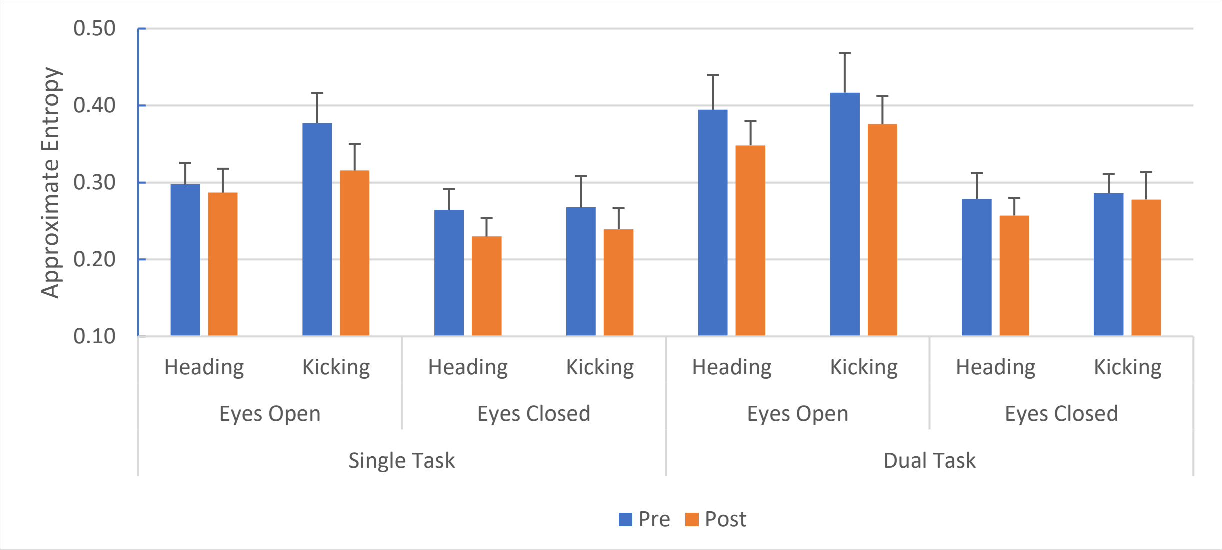

Figure 4.6 Approximate entropy in the medial/lateral plane

The data from the force plate platform on R was also used to calculate were the root mean squared deviation from the mean and sway velocity for each athlete was calculated (see appendix 1.3.2 and 1.3.3 for the R scripts).

A 4x2 repeated measures ANOVA examining the main effects of intervention, time, eyes and cognitive load was conducted. A significant main effect of eyes was found, with athletes having lower root mean squared deviation from the mean with their eyes open; F(1,23) = 25.41, *p* <.001, η^2 = .525. A significant main effect of cognitive load was found with athletes having lower root mean square deviation from the mean during the dual task condition; F(1,23) = 14.19, *p* <.001, η^2 = .386, no main effect of intervention or time was found (p > .40). There was an eyes*time and a cognitive load*intervention*time interaction (p = .028 and p = .029 respectively). By investigating the interactions further, it was evident that the interventions caused an increase in RMS deviation from the mean during the eyes closed single task, however this increase was not present in the dual task. Moreover, there was a different pattern of performance in the single tasks, in which there was a root mean square deviation from the mean increase post kicking compared to pre kicking, but a root mean square deviation from the mean decrease post heading compared to pre heading, pointing to decreased postural stability caused by football passing (Figure 4.7). No interactions that would suggest a detrimental effect of heading on the root mean squared deviation from the mean COP of athletes was found.

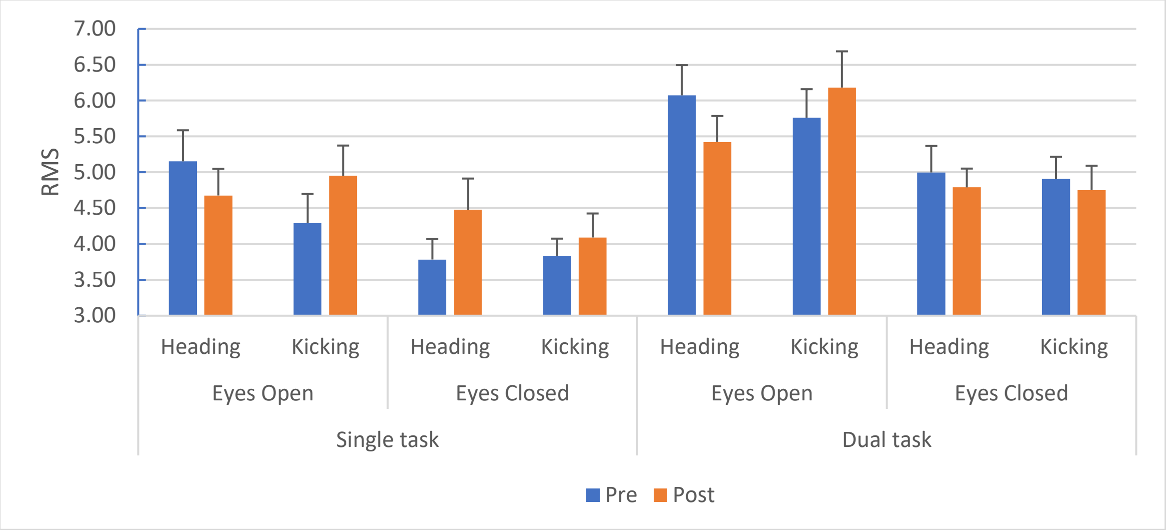

Figure 4.7 Root mean squared deviation from the mean on the anterior/posterior plane per condition.

A 4x2 repeated measures ANOVA examining the main effects of intervention, time, eyes and cognitive load on sway velocity was conducted. A significant main effect of eyes was found, with athletes having lower sway velocity with their eyes open; F(1,23) = 72.36, p <.001, η^2 = .759, OP = 1. A significant main effect of cognitive load was found with athletes having lower sway velocity during the dual task condition; F(1,23) = 17.83, p <.001, η^2 = .437, OP = .98, no main effect of intervention or time was found (p > .25). There was an eyes*cognitive load interaction (p = .003). By investigating the interactions further, it was seen that they are caused by a larger effect of eyes condition during the single task compared to the dual task (Figure 4.8). No interactions that would suggest a detrimental effect of heading on sway velocity of athletes was found.

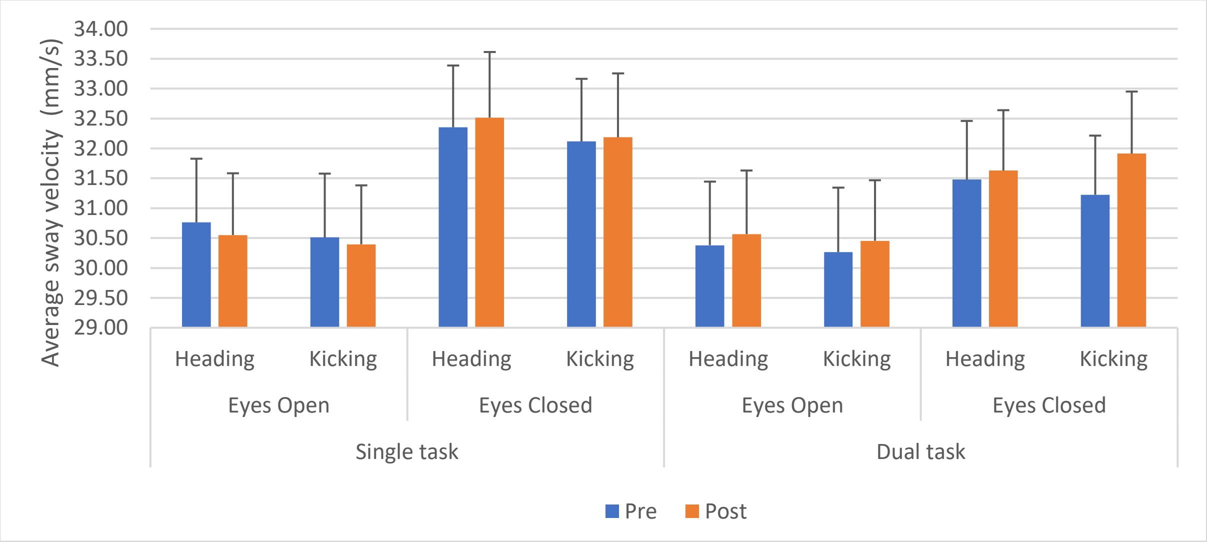

Figure 4.8 Average sway velocity per condition.

The data from the Protxx balance sensor had a lot of outliers and/or missing data (~50%). The outlier values were indicated by the boxplots produced by SPSS with values exceeding 3 SD from the mean being excluded. After the removal of outliers, only 14 athletes had complete data for the analysis. A 4x2 repeated measures ANOVA was conducted and revealed a main effect of eyes (F(1,13) = 18.68, $p < .001$); no other main effects were significant. There was a significant task*eyes*time interaction ($p = .026$), by inspecting the graph we see it is caused by different pre – post patterns during eyes closed conditions in each task (Figure 4.9).

Figure 4.9 Balance power scores as given by the Protxx Balance app per condition.

DISCUSSION

Repetitive subconcussive head impacts did not appear to affect balance in any of the examined conditions. The expected effects of single versus dual task conditions and the eyes open versus closed were found. In line with the previous literature, we found that balance irregularity decreases when participants have their eyes closed compared to when they have their eyes open and increases when participants are performing an additional cognitive task while trying to maintain their posture (Roerdink et al. 2006; Donker et al. 2007; Cavanaugh et al. 2007; Haid et al. 2017). This provides confidence that the procedure and the tasks used were well suited to measure balance changes. Notably, the tasks that caused the postural sway velocity to increase caused an increase in the signal regularity and the postural stability. However, heading did not appear to cause differences neither in the irregularity of the centre of pressure nor its deviation from the mean.

The data from the balance sensor also did not show any effects of heading on athletes' balance, however the data from the Protxx sensor was limited due to high missing/outlier data rate. Irrespective of missing data, the findings do not appear to show any patterns indicating possible differences caused by heading. The Protxx sensor has been found to be very sensitive at picking up differences between individuals e.g., smoker and non-smokers (Grafton et al. 2019), but it this case was not able to pick up any differences caused by heading. This sensitivity of the sensor was probably the reason for the high incidence of outliers as minor movements caused the sway power score to be extremely high (>2) compared to the average sway power in our sample (~.25 - .55 depending on condition). Unfortunately, due to the nature of the measure and the limited flexibility of the application provided by Protxx (sway power values are provided in the application and no access to the recorded signal is provided) we could not process the actual signal recorded so removing the outlier values was the only way to solve this issue.

This finding of unaffected balance after RSHI is in line with the majority of past studies that tried to investigate this topic although less sensitively (Broglio et al. 2004; Di Virgilio et al. 2016; Caccese et al. 2019; Mangus et al. 2003). We used three different ways to analyse the centre of pressure, all three of them representing different aspects of balance. Approximate entropy investigated the dynamic temporal differences of the signal, whereas the root mean square deviation from the mean investigated the area of displacement and the sway velocity investigated the speed with which the centre of pressure moved around this area of displacement. All those measures have been considered as sensitive ways to analyse balance signals. Approximate entropy is sensitive in detecting higher order processing systems and attentional limitations (Buckley et al., 2016; Caccese et al., 2021; Cavanaugh et al., 2007), sway velocity has been proposed as the most sensitive way to detect balance differences (Lin et al., 2008) and root mean square deviation from the mean has been found sensitive enough to detect postural stability in football players (Haran et al., 2013).

Our results indicate that, unlike in sport-related concussion, during repetitive heading there are no subtle balance changes pointing to vestibular or higher order attentional processes malfunctions. The lack of those changes does not guarantee the absence of vestibular or attentional issues after heading, but it indicates that any of those issues, if existing, are not severe enough to manifest in the postural control of athletes. The wide use of balance tasks to detect athletes with concussion influenced the early literature on RSHI which used balance tasks to assess the effects of RSHI on athletes and concluded that heading is safe (Broglio et al., 2004). However, the absence of clinical or subclinical changes in athletes’ balance does not indicate absence of other brain related changes. The absence of balance changes is important as balance and motor control problems in football players might increase their risk of sustaining musculoskeletal or cranial injuries. However, unlike concussion diagnosis, in RSHI sensitive neurophysiological measures should be used, especially since the assessment of acute effects of RSHI on brain health does not aim at a “sub-concussion” diagnosis but aims at understanding the underlying brain processes initiated by RSHI that can lead to long-term brain health problems.

This study does not come without limitations. The sample size was not enough to measure sex differences or cross level (amateur/semi-pro/pro) differences. Due to the nature of the study neither the athletes nor the researchers were blinded to the condition (heading/kicking). It should also be stressed that the findings of this study do not indicate that any amount of heading does not cause balance problems, but indicate that 20 headers, replicating the header players do from a crossing, do not affect players’ balance. Lastly, the sample size for the balance sensor data was very low and the missing data rate very high (~50%), future studies should be careful when using sensors provided by industry partners, especially when the raw data recorded cannot be accessed.

To sum up, it appears that football heading, unlike sport-related concussion, does not affect postural sway velocity, postural stability and signal regularity. This adds to the null findings reported previously from our lab and the null findings found in the majority of previous studies. As a result, it can be concluded that any motor control changes caused by RSHI should be investigated with other more sensitive neurophysiological measures of neuromuscular control and not balance tasks.

CHAPTER 6. The effects of RSHI on football players' associative and procedural learning and spatial working memory.

Abstract

Objectives: As mentioned in previous chapters (1 & 2), the evidence for the effects of RSHI on cognitive functioning is mixed. The aim of this chapter was to use a series of cognitive tasks to investigate which cognitive functions are affected by our in-lab heading paradigm.

Methods: Twenty-seven young (~23 y.o.) football athletes were recruited from local clubs (same participants as in chapter 5). They performed a procedural learning acquisition (serial reaction time task), a paired associations learning and a spatial working memory task both before and after 20 headers or a kicking control condition. Number of errors in the paired associations learning and spatial working memory tasks served as a measure of performance in those tasks. The learning of a sequence, as indicated by improvement in reaction times, served as a measure of procedural learning acquisition.

Results: Number of errors in the paired associations task increased significantly post heading compared to post kicking. No effects were found for the number of errors in the spatial working memory task. Concerning the procedural learning acquisition, performance was similar both after heading and kicking.

Conclusions: The mixed findings of this chapter are in accordance with the previous literature, providing some evidence that heading hinders learning. However, no clear pattern of cognitive impairments could be identified by using solely behavioural cognitive tasks. Future studies should aim to expand on how heading affects learning processes and use more sensitive ways to assess cognition.

INTRODUCTION

Cognitive markers have been found to provide inconclusive evidence for the effects of RSHI in the majority of literature reviews that evaluated their utility (Mainwaring et al., 2018). However, even though cognitive markers provide inconclusive evidence, their use can be beneficial when designed to examine aspects of cognition linked to specific brain alterations found in neuroimaging studies. In this study we aimed to expand on past research that found increased cortical inhibition over the motor cortex (Di Virgilio et al., 2016), by using a cognitive measure of procedural learning, a process that requires the activation of motor cortex.

In addition to the cognitive tasks assessing procedural learning, we used the cognitive measures that showed promising results in previous research (Di Virgilio et at. 2016), while improving the experimental design by adding a control condition and using the "high-functioning" version of those tasks. As mentioned in Chapter 2, since the athletes that sustain RSHI do not belong to a clinical population, the versions of cognitive tasks designed for the general population are a better fit compared to clinical alternatives. Those cognitive measures were the Paired Associative Learning (PAL) and Spatial Working Memory (SWM) test of Cambridge Neuropsychological Test Automated Battery (CANTAB ; Robbins et al., 1994).

Procedural learning

Motor learning is a form of non-declarative procedural learning that requires repetition of an experience that leads to learning; examples of motor learning is learning to ride a bike, play the piano, read etc (Gazzaniga et al., 2009).

Motor learning is a cognitive function that requires the activation of many cortical and subcortical areas including the dorsal premotor cortex, the supplementary motor cortex, the primary motor cortex (M1), the somatosensory cortex, the superior parietal lobe, the putamen, the thalamus, and areas of the cerebellum (Hardwick et al., 2013). In the studies examining the effects of repetitive subconcussive impacts, the M1 was found to show increased inhibition (Di Virgilio et al. 2016; Di Virgilio et al. 2019). M1 is a brain area crucial for motor learning and is part of many circuits that are responsible for both the active learning phase and the phase of memory consolidation immediately after learning, in which the acquired learning becomes resistant to interference (Hardwick et al., 2013; Penhune & Steele, 2012). During the learning phase, it has been suggested that for motor/procedural learning to happen, the GABA levels in M1 must decrease, and this decrease was found to be around 20% (Floyer-Lea et al., 2006). It has also been found that transcranial Direct Current Stimulation (tDCS) induced decrease of GABA correlates significantly with the degree of motor learning (Stagg et al., 2011). More specifically, in that study it was found that the degree of GABA decrease by tDCS was positively correlated with the performance on a Serial Reaction Time Task (SRTT), a task specifically designed to examine procedural learning. Furthermore, tDCS induced GABA reduction on motor cortex was also found to predict individual differences in motor learning and motor memory on a robotic force adaptation task (Kim et al., 2014). A more recent neuroimaging study revealed that GABA decrease on M1 is necessary for procedural learning and that the GABA levels at the beginning of a procedural learning task correlate with the performance on the task, people with higher GABA concentration at the beginning of the task performed worse (Kolasinski et al., 2019). Based on those findings, it can be assumed that any considerable GABA concentration alterations in motor cortex should manifest on cognitive tasks that assess motor/procedural learning.

Only one study tried to examine the effects of football heading on procedural learning (Gallant et al., 2017). Gallant et al. (2017) examined if and how improper football heading affects performance on a procedural learning task. Their study examined the effects of heading on the procedural learning by using a SRTT. More specifically, they examined if participants who had been trained on how to head a ball performed better on the SRTT (e.g., learning the sequence better) after a series of headers compared to untrained participants, without finding any performance differences.

To the author’s knowledge, no other study has tried to investigate the effects of a series of headers compared to another activity of equal physical effort on procedural learning acquisition.

CANTAB

In the literature on subconcussive impacts only DiVirgilio et al. (2016) have used a gold-standard computerised cognitive task assessing paired learning effects like the PAL as a way to measure learning impairments in football (soccer) players before and after a session of headers. After using five tasks from CANTAB battery, they found impairments in the tasks of PAL and the spatial working memory, findings in accordance with the literature on football heading which suggest that the effects of heading on cognition, if any, are more prominent in the functions of learning and memory. Notably, DiVirgilio et al. (2016) used the clinical version of CANTAB, which is mostly used to detect dementia and other cognitive impairments more severe than the effects that repetitive subconcussive head impacts would cause.

The ability to learn spatial associations, like the ones used in PAL, can be linked with the GABA activity on the brain. As such, it was interesting that Di Virgilio and colleagues (2016 & 2019) found the PAL to be affected following RSHI alongside TMS induced corticomotor inhibition thought to reflect increased levels of GABA. GABA receptors antagonists have been found to enhance spatial learning both in animals (maze learning in mice) and humans (Froestl et al., 2004; Mondadori et al., 1996). Learning has also linked with the receptor acetylcholine (Hasselmo, 2006), with studies that infused cholinergic agonists and antagonists in animals showing that decreased acetylcholine decreased memory performance (Tang et al., 1997; Winters & Bussey, 2005).

The PAL task of the CANTAB was created as a tool to detect dementia and numerous studies have used it to validate it as a dementia detection tool and as a tool to evaluate drugs' efficiency as dementia treatments (Barnett et al., 2016). The use of CANTAB's Paired Associative Learning (PAL), instead of other tasks that assess the formation of associations, also allows the evaluation of learning of individuals whose language is not the test's language (Barnett et al. 2015) making it an effective way to measure paired associations learning in multicultural societies like the UK. Participants in this task are asked to remember the location of an object, a process that assess the integrity of the entorhinal and transethorhinal cortex, as well as the hippocampal areas (De Rover et al., 2011). In rats the completion of PAL requires the activation of the glutamate receptors in the parahippocampal area and the activation of the medial prefrontal cortex (K. A. L. McAllister et al., 2015; J. C. Talpos et al., 2009; John C. Talpos et al., 2014). Lesion studies in humans support the findings of the animal studies; patients with lesions in frontal and temporal lobe showed impairments in the PAL task (Owen et al., 1995).

Contrary to DiVirgilio et al (2016) who used the clinical CANTAB version, in this study the high-functioning version of PAL was used with the idea to increase the measure's sensitivity in detecting change. The main difference of which is that it has more stages of difficulty, so it is more challenging and more suitable to the participants of this study (healthy young athletes).

Apart from the PAL, the Spatial Working Memory (SWM) task from CANTAB was used. Errors in SWM were found to be increased post heading (Di Virgilio et al., 2016), however in that study no control condition was used. Spatial working memory is reflecting frontal and prefrontal lobe abnormalities and has been linked with attention, mood and psychotic disorders (Dowson et al., 2004; Piskulic et al., 2007). Di Virgilio et al. (2016) findings need to be further validated before the links between the working memory and associate learning with head impacts can be researched in depth with more sensitive neuroimaging modalities.

Hypotheses

It was hypothesised that a session of football heading will decrease athletes' performance on the two CANTAB tasks, especially the task measuring learning, compared to the baseline measure, whereas no change will be present after the kicking session.

It was also hypothesised that the same session of heading will decrease the procedural learning acquired during the SRTT.

METHODS

Participants

The participants of this study were the same as in the study described in Chapter 5 (examining postural stability). The sample size of 26 was calculated on GPower 3.1 (Erdfelder et al., 2009) by using the effect size of .58 as found in Di Virgilio et al. (2016) on PAL with the aim to produce a study with a power of .80 (see Appendix 1.1 for details).

Materials

SRTT (Nissen & Bullemer, 1987). In this task participants had to respond to the stimuli they see on the screen by pressing the corresponding button. The stimuli were 4 circles, as shown in the picture below (Figure 6.3). Each time a circle lights up blue the participant responds with a button that corresponds to the circle location (Figure 6.4). For the first 5 blocks of trials, those button presses were sequential (pseudorandom); a 12-digit sequence was repeated 8 times at each block. After the first 5 blocks a block of random trials was presented. If participants have learnt the sequence (implicitly) their reaction times in the random block should be increased. After that block, the final block of trial was again a sequential block.

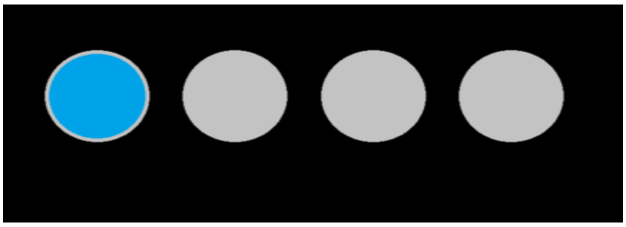

Figure 6.3 SRTT stimulus, in each trial one of the grey circles turned blue as the athletes had to respond with the appropriate response.

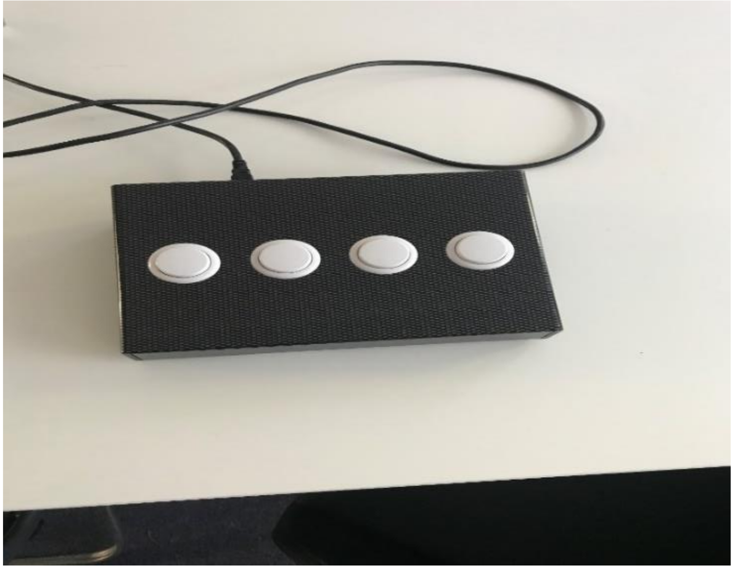

Figure 6.4 The response box used by the athletes to respond to the stimulus shown on Figure 6.3

The procedural learning was defined as the difference in the reaction times of the last blocks with the sequential trials and the block with the random trials.

Paired Associates Learning (PAL). The PAL task of CANTAB (Cambridge cognition, 2019) was used to assess the associative learning of athletes. It is a computerised learning task that runs on an iPad. Athletes were presented with several boxes, ranging from 4 to 12 depending on the trial. Each box included a pattern, those patterns were revealed to the participant at the first stage and immediately after that stage the athletes saw the patterns at the centre of the screen and had to choose the box in which they had seen the pattern (Figure 6.5). The PAL-total errors adjusted for all trials was the measure used in the analyses.

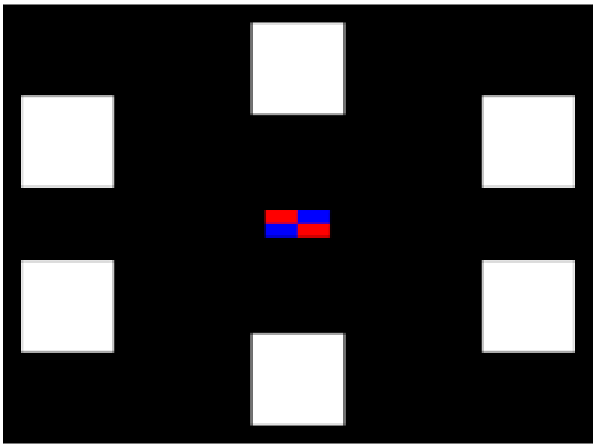

Figure 6.5 Trial example; level 3 of CANTAB paired associates learning task.

Spatial Working Memory (SWM). The SWM task of CANTAB (Cambridge cognition, 2019) was used to assess working memory of athletes. It is a computerised working memory task run on an iPad. During the task several boxes were presented on the screen (Figure 6.6). The task had several trials with increasing number of boxes, from 3 to 12, were presented to the athletes. Athletes task was to search for the tokens by opening the boxes, once a token was found in a box, that box could no longer have a token for the remainder of the trial. The number of between box errors were analysed.

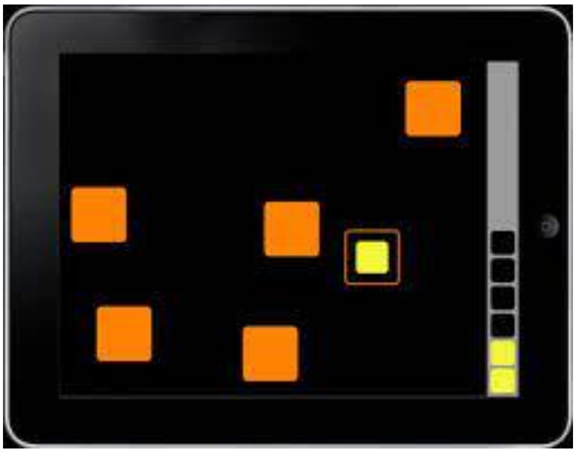

Figure 6.6 Trial example; level 3 of CANTAB spatial working memory task.

Impact and heart rate sensors. The impact of each header and the heart rate values during heading and kicking were recorded as described in Chapter 5.

Procedure

Familiarisation session. Participants were welcomed in the lab and given the information sheet and the demographics questionnaire to fill in. The first day participants did a familiarisation session, like in DiVirgilio et al. (2016), in which they undertook the CANTAB battery tasks twice. This procedure was necessary to avoid any learning effects that could potentially mask any effects of heading on cognitive function.

Heading and kicking sessions. One week later participants came again for the real experiment. After welcoming them back to the lab, athletes were asked to perform the two CANTAB cognitive tasks. First, they completed the PAL task, which took about 8-12 minutes, depending on performance, and then the SWM, a task that took around 6 minutes to complete. Then, athletes were escorted to the heading space location, in which, depending on condition, they either headed a football 20 times, a procedure that lasted ~10 minutes, or kicked a football for 10 minutes. The ball used was a standard football (70cm circumference; 400g; 8 psi) and it was thrown to participants from a distance of 8 meters from a ball throwing machine (JUGS sports, Tualatin, USA). After the description of the procedure, participants wore a heart rate monitor (Figure 4.3), the balance sensor that were already been wearing in this case served as an impact sensor. The first few ball throws were done in order to find out which speed better represents a routine header from a crossing or a corner kick. Participants were asked to judge the speed by catching the ball in the air. When the appropriate speed (~26-28 mph) was found the heading process begun. Athletes performed

20 rotational headers redirecting the ball to a goal; this process served as the in-lab equivalent of a corner kick. They were instructed to avoid jumping, thus performing only standing headers, and to use their judgement on avoiding heading the ball when they felt they cannot head the ball properly. When a header was missed, or the athlete judged it as “just a scratch” the header was repeated. In the kicking session the exact same football and ball throwing machine were used, and participants were be asked to kick the ball and redirect it back to the researcher.

In both conditions athletes’ heart rates were monitored in order to make sure that the average heart rate during teach condition did not exceed the 63% of maximum heart rate (Hrmax; 220-age). 63% of Hrmax is the maximum heart rate that falls under the light physical activity range (Canning et al. 2014).

After the heading/kicking protocol, athletes were brought back in the lab and the post-heading CANTAB measures were performed in the same way as the baseline measures. After finishing those tasks, athletes performed the SRTT for 10 minutes. Athletes that did first the heading condition came one week later in order to participate in the kicking condition and vice versa.

Statistical analysis

Data for this experiment was analysed on SPSS. The number of errors on PAL and SWM were used to assess performance differences in those two tasks and the average reaction time per block of trials was used to compare the procedural learning on the SRTT. Repeated measures ANOVAs were used to identify differences caused by time and intervention for CANTAB tasks, while for SRTT the main effects of block and intervention were assessed by a repeated measures ANOVA. The significance value was set at .05. The raw data recorded by the impact sensors was imported to MATLAB (see Appendix 1.2 for the script) and the mean peak linear and angular accelerations for each header were calculated.

RESULTS

Heading protocol. The heading protocol produced headers with mean peak linear accelerations of 17.65 ±2.71g per header and mean peak angular accelerations of 1759 ±629 rad/s^2. None of the athletes’ heart rate exceeded the threshold for light medium exercise in the experimental or control conditions, mean bpm: 87 (±9) for kicking session and 85 (±11) bpm for heading session.

Missing data and outliers. Not all data from all tasks included the same number of athletes as there were athletes whose data was not recorded properly, or some athletes had extreme values in some measures and therefore their data was excluded from those measures. Data from twenty-six athletes was available for the PAL and SWM measures, while data from 21 athletes was available for the SRTT.

SRTT. A 2x2 repeat measures ANOVA was used to examine the effect of intervention (Heading/Kicking) and sequence (5thSerialBlock/RandomBlock). There was a main effect of sequence type, with random sequence increasing the reaction times compared to the serial sequence; F(1,20) = 49.05, $p < .001$, $\eta^2 = .721$, OP: 1, but no effect of condition ($p > .5$). There was no significant intervention*sequence interaction; F(1,20) = .01, $p = .926$. Therefore, the task produced procedural learning, but heading did not impair this learning (Figure 6.7).

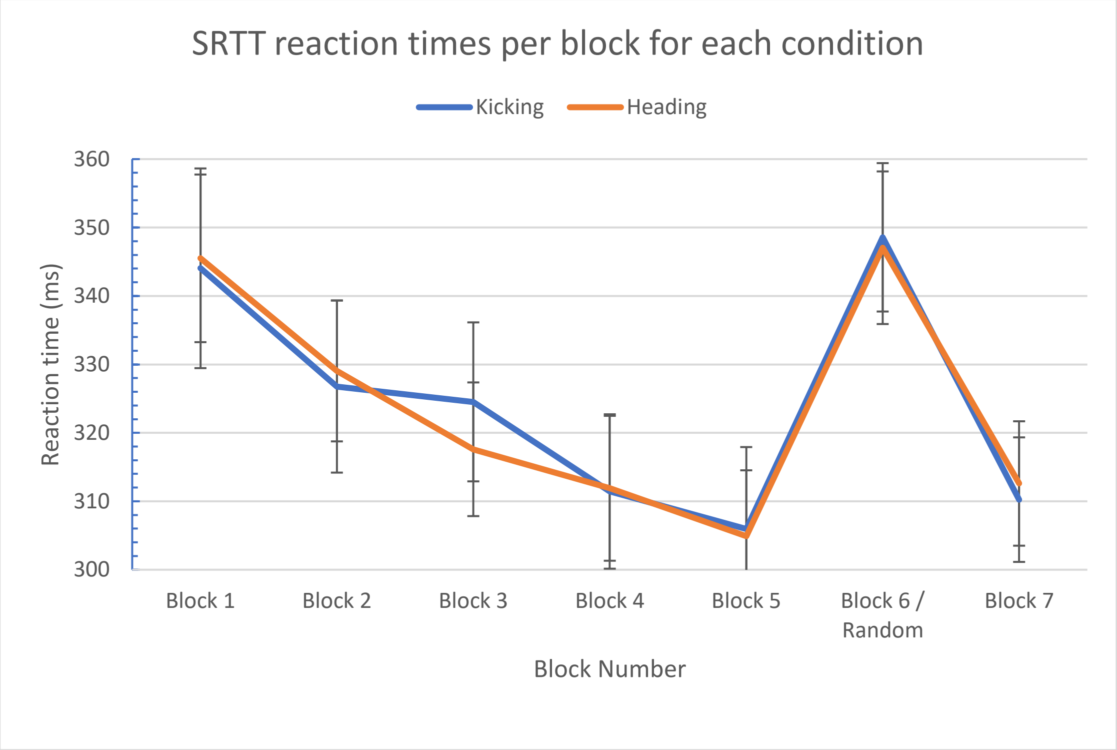

Figure 6.7 Mean reaction time per block of trials in the SRTT. Vertical lines represent standard deviation. Block 6 was the block in which the random sequence was presented. The increased reaction times in block 6 indicate the amount of learning acquired.

PAL. A 2x2 repeated measures ANOVA was used to examine the effects of Condition (Heading/Kicking) and Time (Pre/Post) on the number of errors made in PAL. There was no main effect of intervention (F(1,25) = .07, $p = .796$) and no main effect of time (F(1,25) = .24, $p = .877$). There was a statistically significant Intervention*Time interaction, with errors increasing compared to baseline after the heading condition but not after kicking (Figure 6.8); F(1,25) = 4.31, $p = .048$; $\eta^2 = .147$, OP = .52.

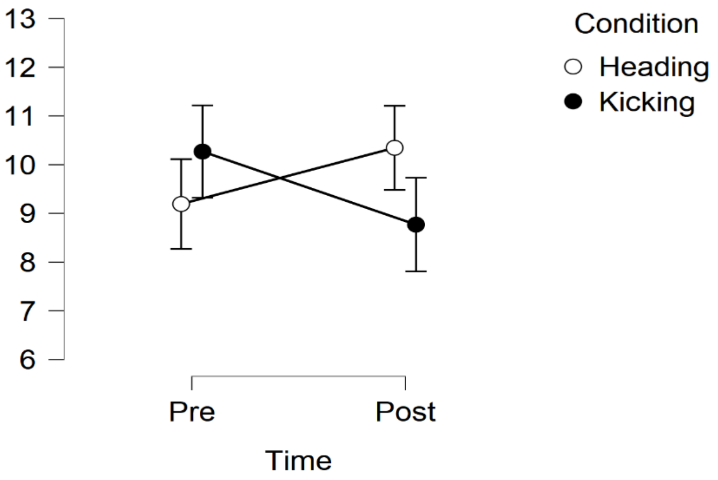

Figure 6.8 Mean number of errors on the PAL task pre- post- heading and kicking. Vertical lines represent standard deviation.

SWM. A 2x2 repeated measures ANOVA was used to examine the effects of Condition (Heading/Kicking) and Time (Pre/Post) on the number of errors made in SWM. There was no main effect of intervention (F(1,25) = .05, *p* = .823) and no main effect of time (F(1,25) = 2.86, *p* = .103). There was also no significant Intervention*Time interaction; F(1,25) = 1.25, *p* = .274.

DISCUSSION

The findings from the SRTT provide further evidence that heading might not cause motor control decrements, similarly to what was found in Chapter 5 (examining postural stability). Procedural learning requires the activation of motor cortex and several other cortical and subcortical areas (Hardwick et al. 2013). The finding of similar performance post heading and post kicking points to the conclusion that heading does not cause, at least observable, alterations to the neural systems involved in procedural learning nor neurochemical imbalances linked with motor learning. However, the lack of decrements in procedural learning acquisition might mean that GABA was not increased at a level that could cause observable alterations in learning, or that 20 headers did not increased GABA on M1. More sensitive measures of neural chemistry might reveal minor GABA or Glutamate changes caused by heading in football.

Out of the two CANTAB tasks, PAL was the one revealing a significant difference caused by heading. This finding provides further support for the previous CANTAB findings (Di Virgilio et al., 2016), and expands on them by adding a control condition and the use of the more sensitive high-performing version of PAL. The finding of decreased ability to form paired associations points to altered activation of the frontal and the hippocampal areas of the athletes' brain (De Rover et al. 2011; Talpos et al. 2009; McAllister et al. 2015). However, other studies found the learning of associations not to be impaired following prefrontal cortex lesions in monkeys, unlike working memory which was impaired by the lesions (Goldman-Rakic, 1992 as cited in Gazzaniga et al. 2014). Although Di Virgilio et al. (2016) examined effects associated with the motor cortex, since this was the brain area suited to TMS assessed effects, there is no reason to believe that RSHI are motor cortex or any other area specific. Making assumptions about the brain location responsible for those associative memory changes is not possible at this point since memory and learning require the activation of multiple brain regions and networks. Further studies using sensitive neuroimaging modalities like fMRI and EEG should be performed to investigate if RSHI affect specific brain networks or areas, or the brain as a whole.

Similarly, memory and learning impairments can be linked to a wide range of neurotransmitters like acetylcholine, GABA and dopamine (Froestl et al., 2004; Mondadori et al., 1996; Tang et al., 1997; Winters & Bussey, 2005), so neuroimaging techniques specialising at neurochemistry changes, like MRS, should be used to provide an answer to the neurochemical changes induced by heading in football. As mentioned in Chapter 2 (literature review), changes in cognitive test performance represent a symptom of a brain health issue that is still unknown.

However, the findings of similar performance on procedural learning acquisition after kicking and heading but reduced paired associations learning when taken together can provide further insights on the cognitive outcomes of heading on learning processes. This distinction has important practical implications, particularly for minors and young adults, as it provides evidence that athletes, or any individual who attends training, that includes head impacts will be able to learn new procedural skills, for example during subsequent training, but will have difficulties learning new associations which might pose a challenge for academic attainment.

Limitations

The use of the familiarisation session was necessary to minimise any learning effects on the CANTAB tasks, however as seen in our findings, it was not possible to entirely eliminate the learning effects. This highlights the importance of including a control condition when using cognitive tasks prone to learning effects. One more limitation was the lack of baseline measure for the procedural learning. The fact that athletes had to implicitly learn a sequence meant that different sequences should be used before and after the intervention, resulting in a total of four different sequences, thus considerably increasing the chance of athletes noticing that the stimuli presented to them were in a sequence. Moreover, the effect of adding the sequence learned during baseline could interfere with the learning of the post-intervention sequence.

Conclusion

In this study twenty headers, as a number that is representative of headers players routinely perform during training or football games, was found to cause decreased performance in associative leaning processes, while leaving working memory and motor skill learning unaffected. Those findings are important as they point to the presence of selective memory and learning problems caused by heading that should be investigated further. The lack of procedural learning problems, together with the lack of effects on balance reported in Chapter 5 (examining postural stability), suggests that heading at training or games does not impair the motor system of athletes. Hence the argument that sub-concussive heading can increase the chances of musculoskeletal injury caused by reduced motor control decrements seems weakened.

INTERIM DISCUSSION 3

The experimental findings so far (Chapter 5 & 6) indicate that 20 headers are not causing changes in athletes balance and motor learning systems, and the main differences found were on the associative learning task (PAL). Therefore, it was deemed important to further investigate the effects of heading on learning of associations, and further examine associated cognitive processes such as attention and memory that may be affected following heading. For this purpose, tasks on verbal associations learning and tasks examining attentional resources were employed.

To investigate any effects on athletes' attention and learning effectively, sensitive neuroimaging methods should be used. EEG was chosen as a sensitive way to measure cognitive functioning (see the conclusions of Chapter 2 based on the literature review and for information of previous EEG studies). The aim of the EEG assessment was twofold. First aim was to examine the effects of RSHI on established EEG paradigms and tasks so any effects found would be clearly linked with specific cognitive functions. Second aim was to investigate the cognitive functions most likely to be affected, based on previous findings, which in this case were learning and attention. A response inhibition task was also included to assess any presence of inhibition irregularities on athletes' brain. By using sensitive EEG methods, we can provide stronger evidence on which cognitive functions are affected. EEG can provide brain-based evidence of RSHI effects on athletes' brain health and has the potential to reveal deficits which may not manifest in less sensitive behavioural cognitive measures. Effects in the response inhibition tasks can indicate a whole brain inhibitory response building on the findings of increased inhibition over the motor cortex found using TMS, whereas effects in the attention and learning tasks can indicate a widespread cognitive dampening similar to a minor concussion.

CHAPTER 7. Repeatedly heading a football diminishes attention and memory but not response inhibition: Evidence from neural recordings of brain function

Abstract (for chapters 7 & 8)

Background: There is growing evidence that heading causes acute brain alterations and is a risk factor for neurodegenerative diseases. Information about the effects of repetitive head impacts on cognitive functioning, irrespective of sport, is currently mixed. The aim of these two studies was to use sensitive neurophysiological methods to identify which cognitive processes are affected by RSHI.

Methods: EEG recordings were acquired while 20 young athletes (~ 23 year of age) were performing cognitive tasks assessing their attention, response inhibition and verbal learning. The neural markers of interest were the event related potentials P3b, a marker of attention (Chapter 7), the neural markers of response inhibition N2 and P3a (Chapter 7) and the neural marker of semantic associations and learning, N400 (Chapter 8).

Results: The EEG data revealed that the attention and learning of young athletes was compromised by heading, as indicated by the electrophysiological markers of those cognitive functions. It was found, in a controlled lab setting, that the amplitude of the neural markers of attention and verbal learning, event related potentials P3b and N400, were negatively affected by 20 headers. No effects were found on the neural markers of response inhibition.

Conclusion: Verbal learning and attention were found to be compromised, with the effects on attention were of a similar magnitude with findings from athletes with history of concussion. Importantly, the effects found here were selective, with electrophysiological evidence indicating that other cognitive functions, like response inhibition, remain unaffected. The results demonstrate, for the first time at a brain level, that basic cognitive functions are compromised immediately after heading. This finding raises concerns about the consequences a diminished ability to focus on and learn new information can have for the academic life and success of this otherwise healthy group of young people.

INTRODUCTION

In the existing literature on post concussive symptoms and sub-concussive impacts many behavioural tests that assess cognitive functions like attention, working memory, cognitive flexibility and learning failed to detect any effects of repetitive sub-concussive impacts or remote concussion on athletes cognitive functioning (Kaminski et al., 2007, 2008; Ozen et al., 2013; Rieder & Jansen, 2011; Straume-Naesheim et al., 2005). In the case of remote concussion[5], whereas behavioural measures seem unable to detect the subtle effects of concussion on athletes that remain weeks or months following concussion, the more sophisticated EEG measures have been found able to detect the differences between previously concussed athletes and athletes with no history of concussion (Broglio et al., 2009; Dupuis et al., 2000; Ozen et al., 2013; Segalowitz et al., 2001). A review of the literature on the use of EEG on athletes with a history of concussion concluded that brain related potentials are very well-suited to examine aspects of cognition that are undetected by standard neuropsychological tests (Broglio et al., 2011). Findings like that indicate that event related potentials can also be a very well-suited way to assess the effects of RSHI on the cognitive functioning of athletes. However, as mentioned in chapter 2 (literature review), EEG studies in the field of RSHI are a rare find.

Differences in the EEGs of athletes after a concussion, accompanied with no differences in the behavioural performance on the task, have also been found in studies that examined EEG spectral power differences while athletes undertook the immediate post-concussion assessment and cognitive testing (ImPACT) battery, indicating that they find the tasks more demanding than people with no history of concussion (Teel et al., 2014).

Oddball. Ozen et al. (2013) examined the cognitive functioning of athletes with remote concussion by using an n-back task with 4 levels: 0-back (oddball task variation), 1-back, 2-back, 3-back. Their main result was that participants in the remote concussion group had significantly lower P300 ERP amplitudes compared to the non-concussed athletes, while their performance in terms of reaction times and hit rates was not significantly different. The P300 (or P3) is a positive ERP component that appears 300-500ms after the onset of a stimulus that reflects attentional processes. This ERP component is one of the most intensively research ERP components and it is elicited by the occurrence of an infrequent stimulus amongst another frequent stimulus (Dinteren et al., 2014; Sutton et al., 1965).

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[5] A concussion that happened at least 6 months before the study

Segalowitz et al. (2001) had the same pattern of results when compared the performances of students with remote concussion (mean = 6.4 years before the study) with students with no history of concussion on 4 oddball tasks with varied difficulty. The performance of both groups in terms of reaction times was comparable for the easy oddball tasks, with the students with a history of concussion performing worse only in the difficult oddball tasks. Interestingly, in the ERP measures students with a history of concussion had significantly lower P3 amplitudes in all tasks, both easy and difficult. Again, in this study it was highlighted that the ERPs provide a more sensitive way to detect any subtle remaining impairments in subjects with a history of concussion.

In the subconcussive impacts literature only one study examined how heading affects the P3 ERP component. Moore et al. (2017) examined the P3 evoked by an oddball task on university athletes divided in 3 groups. The first group included athletes with a history of concussion, the second group included athletes that their sport is known for the presence repetitive subconcussive impacts (soccer) and the third group was consisted of non-contact sports athletes. Their results showed that participants in the first and second group had significantly lower P3 peak amplitudes compared to those in the third group. In accordance with the studies the behavioural measures of cognitive functions were not able to detect those subtle differences and only the first group, participants with concussion, seemed to have a few impairments in those tasks.

P3 is known to reflect the attentional resources allocation, so this decrease in its amplitude long after concussion might indicate chronic problems of resource allocation and/or fewer cognitive resources (Ozen et al. 2013). Recent evidence, however, suggests that the parietal P300 (P3b) is also linked with memory updating processes that facilitate memory storage following attending sensory information, while the anterior P3a reflects the attention processes (see figure 7.1; (Debener et al., 2005; Folstein & Van Petten, 2008; He et al., 2001; Polich, 2007). From a neuropharmacological perspective it is suggested that the parietal P300 is associated with the locus coeruleus (LC) noradrenaline system (Nieuwenhuis, 2013; Nieuwenhuis et al., 2005). LC contains many noradrenaline neurons that project to multiple brain areas, especially frontal and parietal regions, and has a major role in arousal, attention and stress response (Benarroch, 2009). Imaging studies, intracranial recordings, and lesion studies have showed that the brain areas linked with P3b are spread across the brain (Soltani & Knight, 2000). A finding that is consistent with the projections deriving from the LC to several brain areas (Nieuwenhuis, 2013). Furthermore, the timing of P3 activity matches the trajectory of LC projections, which initially project to frontal and subcortical areas and then swerve backwards to innervate posterior brain regions (Nieuwenhuis, 2013). The link between P3 and the nor-adrenaline system of LC is manifested in pharmacological and lesion studies, which provide evidence that nor-adrenergic agents and LC lesions modulate the amplitude of P3, as produced by an auditory oddball task, in monkeys (Pineda et al., 1989; Swick et al., 1994). Interestingly, the degeneration of LC is present at the early stages of neurodegenerative diseases like Alzheimer's and Parkinson's (Bari et al., 2020; German et al., 1992; Rommelfanger & Weinshenker, 2007; Weinshenker, 2008).

The fact that P3 reflects attentional and working memory processes together with the findings that as the difficulty of a task increases, the P3 amplitude decreases and the peak latency becomes longer (Kok, 2001; Ozen et al., 2013), might suggest that people with a history of concussion and people who suffer many subconcussive head impacts have attention and working memory impairments and find the same tasks more demanding compared to control groups. Evidence of changes in P3 amplitude could also indicate alterations in the functioning of the nor-adrenergic system of athletes.

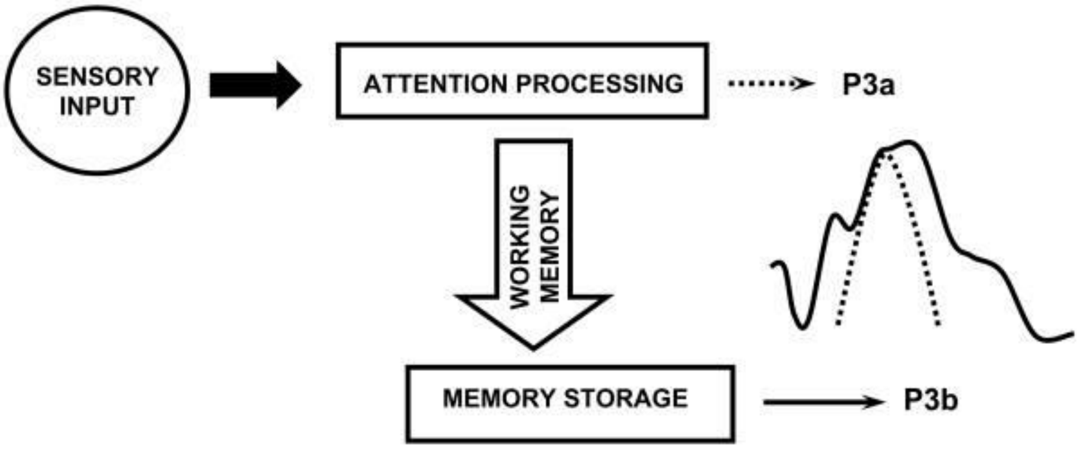

Figure 7.1 How P300 is generated (Polich 2007)

Go/No-Go. A successful completion of a task requires task-relevant information to be enhanced while information irrelevant to the task to be suppressed or inhibited. The Go/No-Go task is designed to assess this type of response inhibition which requires the activation of the anterior cingulate cortex, the pre-supplementary motor area, the insula, the thalamus and the right inferior parietal lobe (Bekker et al., 2005; Hester et al., 2005). Improvements in response inhibition have also been found to be strongly related with higher GABA concentrations (Cheng et al., 2017; Quetscher et al., 2015). Specifically, higher levels of GABA in the striatum are linked with better performance in response inhibition tasks (Quetscher et al., 2015), while higher levels of GABA in right superior temporal gyrus were found to correlate with better performance in the same tasks (Cheng et al., 2017).

In the Go/No-Go task there are three ERPs of interest the frontal N2 and the frontal P3 (P3a) during the No-Go trials and the parietal P3 (P3b) during the Go trials. N2 is the second negative peak in the ERP waveforms ~200ms post stimulus presentation, which in the Go/No-Go task reflects the inhibition of a planned response (Folstein & Petten, 2008). N2 has been found to be increased when participants are under pressure to produce a quick response (Jodo & Kayama, 1992) and is larger in amplitude in participants who do less mistakes during the task (Falkenstein et al. 1999).

The anterior P3 (P3a) in the Go/No-Go tasks is the third positive peak in the ERP waveform and, similarly to N2, is linked with inhibitory processes. However, the timing of the P3a, peaking later than the behavioural response during the Go trials, indicates that P3a is unlikely to be reflecting real-time inhibition, with evidence indicating that it might be reflecting the evaluation of the inhibitory process (Gajewski & Falkenstrain, 2012).

To sum up, both N2 and P3a reflect inhibitory processes, with N2 reflecting the inhibition of the planned response and the P3a reflecting the evaluation of this process (whether the response was successfully inhibited). Increased N2 and P3 reflect successful response inhibition while decreased N2 and P3 indicate unsuccessful inhibition or increased difficulty to inhibit the response.

In the previous experiment (Chapter 6; behavioural measures of cognitive functioning) it was found that heading does not affect the acquisition of a procedural memory, a process linked with GABA concentrations in M1 (Stagg et al. 2011). This absence of an effect raises questions about previous findings (Di Virgilio et al., 2016) in which heading caused prolongation of cortical silent period which is an indirect measure of GABA concentration in M1 (Di Virgilio et al. 2016). Since tasks that assess the response inhibition, like the Go/No-Go task, are a good way to investigate the inhibitory processes and are linked with GABA levels, the use of a response inhibition task to investigate the effects of heading on athletes’ cognition seems promising.

The aim of this study was to utilise two very common tasks in EEG literature to assess which of the cognitive functions of attention, working memory and response inhibition are affected by RSHI.

Based on the aforementioned studies, in this study the acute effects of heading on the allocation of attentional resources, working memory and response inhibition were examined. It was hypothesised that after heading a football the parietal P300 (P3b) ERP component during an oddball task will be decreased in amplitude when compared to the same task being done after a kicking control condition. It is also hypothesised that the performance on the behavioural level (reaction times and hit rates) will be the similar after heading and kicking. Concerning the response inhibition, it is hypothesised that any GABA increases caused by heading will manifest as changes in the performance on the Go/No-Go task both in the electrophysiological and behavioural level.

METHODS

Participants

The participants of this study were 20 football players aged 23.5 (±3.99) years old (18 males) recruited from local teams; (Table 7.1 for participant information). Participants had no learning difficulties, no neurological condition or history of brain damage, no psychiatric condition currently treated with psychoactive drugs and no history of sports-related concussion in the last year. Athletes were asked to refrain from recreational drugs the week before the sessions and from alcohol and caffeinate drinks 24 hours before the sessions. All athletes were reimbursed at a £8.50 per hour rate for their participation. The targeted sample size was n = 20 participants, examined both under the experimental and the control condition. The targeted sample size was decided based on a pilot study that was performed, aiming to produce a power of .80. Information about the pilot study can be found on Appendix 2.

Table 7.1. Demographic characteristics of athletes in the sample.

Participant	Age	Sex	Race	Level	Position
1	20	Male	White	Semi-Pro	CM
2	30	Male	Asian	Semi-Pro	DC
3	25	Male	Asian	Amateur	CM
4	20	Male	White	Semi-Pro	GK
5	20	Male	White	Amateur	DC
6	20	Male	White	Semi-Pro	CM
7	23	Male	Asian	Amateur	CM
8	19	Female	White	Semi-Pro	FB
9	26	Male	Asian	Amateur	DC
10	27	Male	Asian	Amateur	Winger
11	19	Male	Black	Semi-Pro	CM
12	21	Male	White	Pro	Winger
13	25	Male	Asian	Amateur	DC
14	23	Male	Asian	Semi-Pro	Winger

15	28	Male	White	Amateur	FB
16	27	Male	Asian	Pro	DC
17	24	Female	White	Amateur	DC
18	19	Male	Asian	Pro	Winger/CF
19	31	Male	Black	Semi-Pro	CM
20	20	Male	White	Semi-Pro	DC

Materials

Impact meter. The impact of each header was measured in terms of linear (G force) and angular acceleration (rad/s^2). This data was provided by the head-mounted accelerometer provided by Protxx Inc. described in previous chapters. The maximum linear and angular acceleration for each header were derived with the use of a MATLAB script that takes as input the signal from the device and returns the maximum linear or angular accelerations (Appendix 1.2).

Attentional resources allocation and working memory. The visual oddball task is a widely used task in ERP research, used to assess the attention and working memory of participants (Polich, 2007). Athletes during this task were presented with two different stimuli, a red circle and a blue square and were asked to press the space bar of the keyboard when seeing the red circle. The stimuli were presented pseudo-randomly, red circles were presented at 20% of the time (rare) and blue squares at 80% of the time (frequent). The interval between the stimuli was set to be random between 800-1200ms and the stimuli were presented for 80ms (Figure 7.2). The task included 200 stimuli presentations and lasted around 5 minutes. There was also a brief trial with a presentation of 40 stimuli, so the athletes will familiarise themselves with the task.

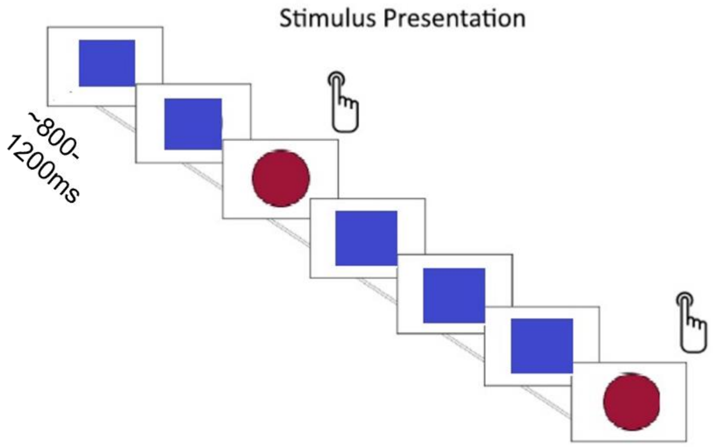

Figure 7.2 Graphic representation of 7 consecutive trials of the oddball task.

Response inhibition (Go/No-Go). Athletes were presented with a word stimulus in the middle of the screen for 100ms, the words used were the word “PRESS” and “STOP”. Words were presented in a random order, with a fixed ratio, the “PRESS” word was presented at 80% of the trials and the “STOP” word 20% of the trials (Figure 7.3). Athletes were asked to press the space bar when presented with the “PRESS” word, the stimulus was on the screen for ~1000ms, but athletes were instructed to be as quick as possible and that there is a 500ms deadline for their response. When they failed to give a response in less than 500ms, a sound indicated that they were too slow, this induced time pressure to the task; no response was required when the “STOP” word was presented, however, if a response was given then the same sound was indicating the incorrect response.

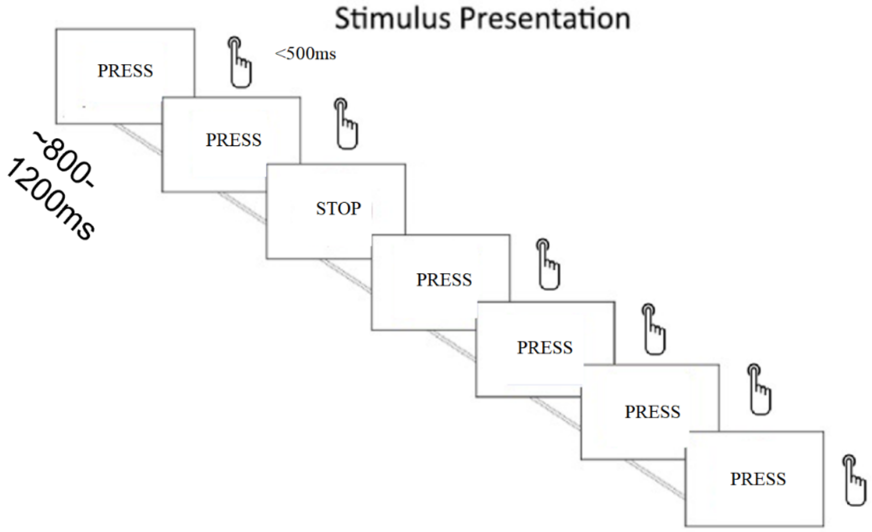

Figure 7.3 Graphic representation of 7 trials of the Go/No-Go task.

Procedure

Athletes came in the lab for the experiment without any previous familiarisation with the tasks. After welcoming them back to the lab, athletes were asked to perform the RTI task on CANTAB which took about 2 minutes. Then, athletes were escorted to the heading space location, in which, depending on condition, they either head a football 20 times, a procedure that lasted ~10 minutes, or kick a football for 10 minutes. The ball used was a standard football (70cm circumference; 400g; 8 psi) and it was thrown to participants from a distance of 8 meters from a ball throwing machine (JUGS sports, Tualatin, USA). After the description of the procedure, participants wore wear a heart rate monitor and the balance sensor that were already been wearing in this case served as an impact sensor. The first few ball throws were done in order to find out which speed represents better a routine header from a crossing or a corner kick. Participants were asked to judge the speed by catching the ball in the air. When the appropriate speed (~26-28 mph) was found the heading process begun.

Athletes performed 20 rotational headers redirecting the ball to a goal; this process served as the in-lab equivalent of a corner kick. They were instructed to avoid jumping, thus performing only standing headers, and to use their judgement on avoiding heading the ball when they feel they cannot head the ball properly. When a header was missed, or the athlete judged it as “just a scratch” the header was repeated. In the kicking session the exact same football and ball throwing machine were used, and participants were be asked to kick the ball and redirect it back to the researcher.

In both conditions athletes’ heart rates were monitored in order to make sure that the average heart rate during teach condition did not exceed the 63% of maximum heart rate (Hrmax; 220-age). 63% of Hrmax is the maximum heart rate that falls under the light physical activity range (Canning et al. 2014).

After the heading/kicking protocol, athletes were brought back in the lab and the post-heading CANTAB RTI measure was performed in the same way as the baseline measure. After finishing this task, athletes capped with a 64 channel EEG cap, a procedure that lasted 30-45 minutes. After the capping, athletes first performed the oddball task for ~5 minutes and following that they performed the Go/No-Go task for ~12 minutes. For both tasks they were asked to respond as quick as they could

Athletes that undertook the heading session on day 1 came a week later for the kicking session and vice versa.

EEG measures and statistical analyses

The electrophysiological data was recorded using a Synamps amplifier (www. neuroscan.com) connected to a 64-channel Ag/Ag/Cl Cap (NeuroMedical Supplies). Electrodes on the cap were arranged based on the extended version of the 10–20 system, we also placed two additional electrodes on left and right mastoid bones, no eye electrodes were used to decrease close contact with athletes’ face (due to COVID). Data was recorded at a rate of 1kHz, filtered online with a band pass between 0.01Hz and 200Hz, using a reference electrode located between Cz and CPz and one between Fz and FPz as ground. Impedance of all electrodes was kept below 5 KOhm. Offline EEG data was imported on Brain Vision Analyzer 2.0. Data was filtered using a low-pass filter at 30 Hz. The continuous data was re-referenced to the average of the mastoid electrodes, eye blinks were removed using ICA, and data was epoched from -200 to 1000ms relative to stimulus onset. Epochs with artifacts were removed using semi-automatic artifact rejection. Epochs were baseline corrected over the pre-stimulus interval (-200-0ms).

For the oddball task data from 320-400ms post stimulus presentation was exported. For the Go No-Go task data from 250-320ms post stimulus presentation was exported to investigate the N2 effect and data from 340-440ms was exported to investigate the P3a effect. The behavioural data used was the reaction times for the rare trials of the oddball task and the errors (space bar presses) athletes did during the No-Go trials of the Go/No-Go task. 2x2 repeated measures ANOVAs were used to investigate the main effects of intervention (heading/kicking) and stimulus type (frequent/rare for oddball, Go/No-Go for Go/No-Go task) and their interactions. Statistical analyses were performed on SPSS v28. The behavioural data was analysed for differences between interventions with paired samples t-tests. Single trial EEG data from the oddball task was also extracted and imported into the MLwin software to perform multilevel modelling. Using single trial data, the effect of time during the oddball task can also be examined.

RESULTS

Impact metrics.

The headers performed by athletes produced impacts with a mean peak linear acceleration of 18.3 ±3.2g and a mean peak angular acceleration was 1611 ±654 rad/s^2. See table 7.2 for impact information for each athlete. None of the athletes' heart rate exceeded the threshold for light medium exercise in the experimental or control conditions, mean bpm: 85 (±10) for kicking session and 86 (±12) bpm for heading session.

Table 7.2. Impact metrics per athlete

Participant	Linear Acceleration per header (g)	Angular Acceleration per header (rad/s^2)
1	17.1	1081.5
2	22.52	2198.8
3	19.87	2285.2
4	16.77	1629.28
5	24.17	2151.78
6	17.86	1344.15
7	15.71	728.46
8	19	2484.35

9	19.71	1751.35
10	13.7	1218.01
11	14	904.25
12	24.7	2135.19
13	15.93	889.75
14	14.49	746.08
15	19.82	2010.03
16	18.04	1725.63
17	16.63	901.8
18	16.64	917.94
19	19.34	2801.95
20	22.71	1204.5

Behavioural findings

Oddball. The paired samples t-test reveal no differences in reaction times between the heading (*Mean* = 303.07, *SD* = 37.76) and kicking (*Mean* = 299.58, *SD* = 29.23) interventions; t(19) = -.677, *p* = .507.

Go/No-Go. The paired samples t-test reveal no significant differences in errors at NoGo trials (false alarms) between the heading (*Mean* = 6.35, *SD* = 5.50) and kicking (*Mean* = 4.75, *SD* = 3.39) interventions; t(19) = 1.747, *p* = .097.

EEG findings

Oddball. The repeated measures ANOVA revealed a main effect of stimulus type ($p < .001$) with rare stimuli eliciting higher amplitudes than the frequent stimuli; no effect of intervention was found. There was a statistically significant stimulus*intervention interaction (F(1,19) = 4.50, p = 0.047, η^2 = .192, OP: 0.52); mean amplitude for the rare stimulus was decreased (Mean = 14.23μV) post heading compared to post kicking (Mean = 15.97) revealing reduced P3b post heading compared to post kicking; see Figure 7.4 for a scalp map showing the P3b amplitude differences (P3b for rare trials minus P3b for frequent trials) this amplitude difference will henceforth be referred to as *P3b effect* and Figure 7,5 for the ERP waves.

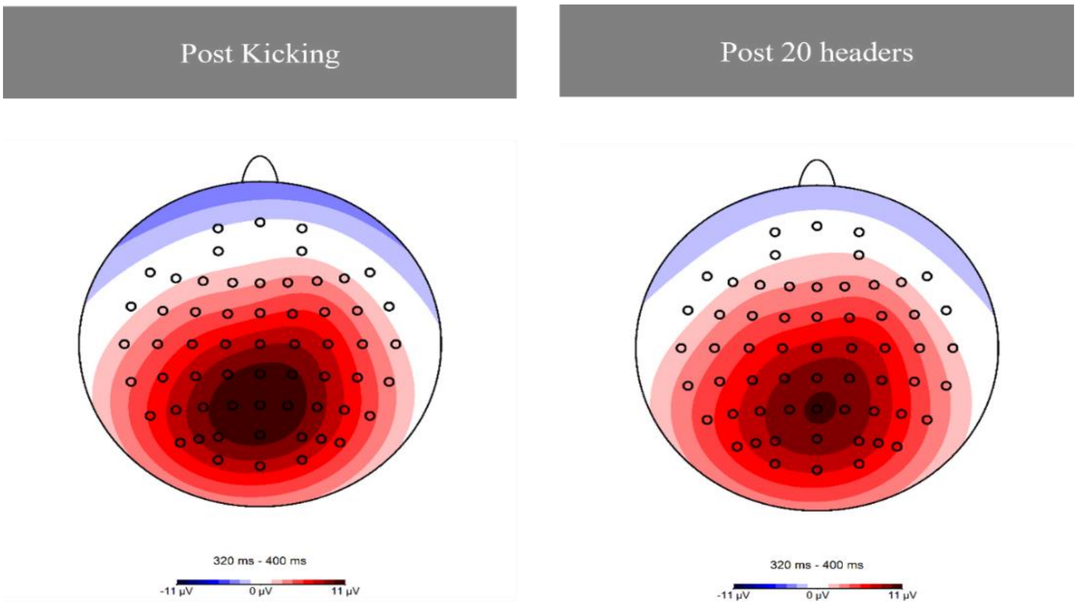

Figure 7.4. Scalp maps at 320-400ms showing the P3b effect produced by the oddball task. As expected, the activity is larger in the parietal areas surrounding Pz. Red represents positive amplitude differences and blue negative. Activity (P3b effect amplitude) was lower post heading compared to the control condition.

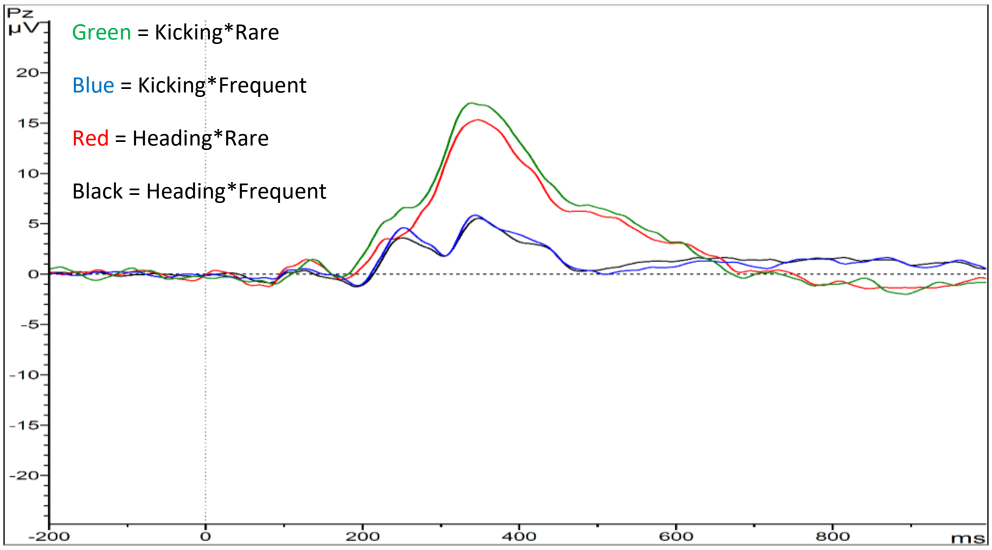

Figure 7.5 ERP wavelengths of the mean amplitude, in μV, for oddball task after heading and kicking. Blue and Black lines depict the brain activity following the presentation of frequent stimuli, at time 0, for kicking and heading intervention respectively. Green and red lines depict the brain activity following the presentation of the rare stimuli for kicking and heading interventions respectively.

Go/No-Go. N2 (250-320ms): The repeated measures ANOVA revealed a main effect of stimulus with the No-Go trials eliciting a higher amplitude over the Fz electrode compared to the Go trials (F(1,19) = 33.60, p < 0.001). There was no main effect of intervention (F(1,19) = 2.43, p = .113) and no significant stimulus*intervention interaction (F(1,19) = .86, p = .366).

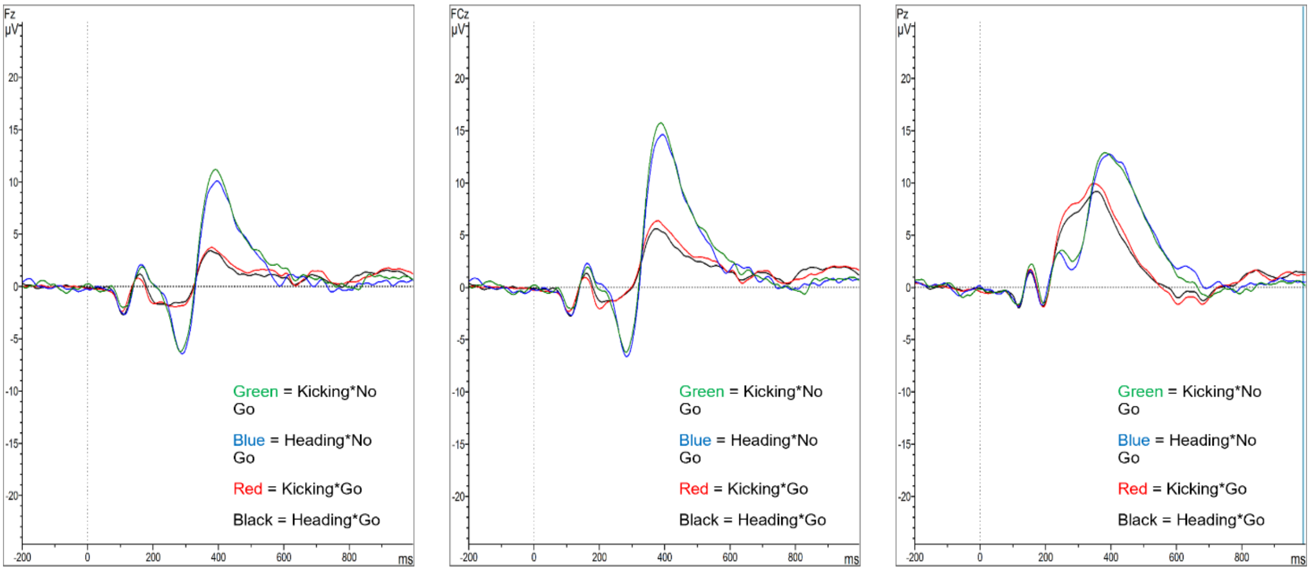

Figure 7.6. ERP wavelengths of the mean amplitude, in µV, over the Fz, FCz and Pz electrodes. Red and black lines depict the brain activity following the presentation of the Go stimuli, at time 0, for kicking and heading intervention respectively. Green and blue lines depict the brain activity following the presentation of the No-Go stimuli for kicking and heading interventions respectively.

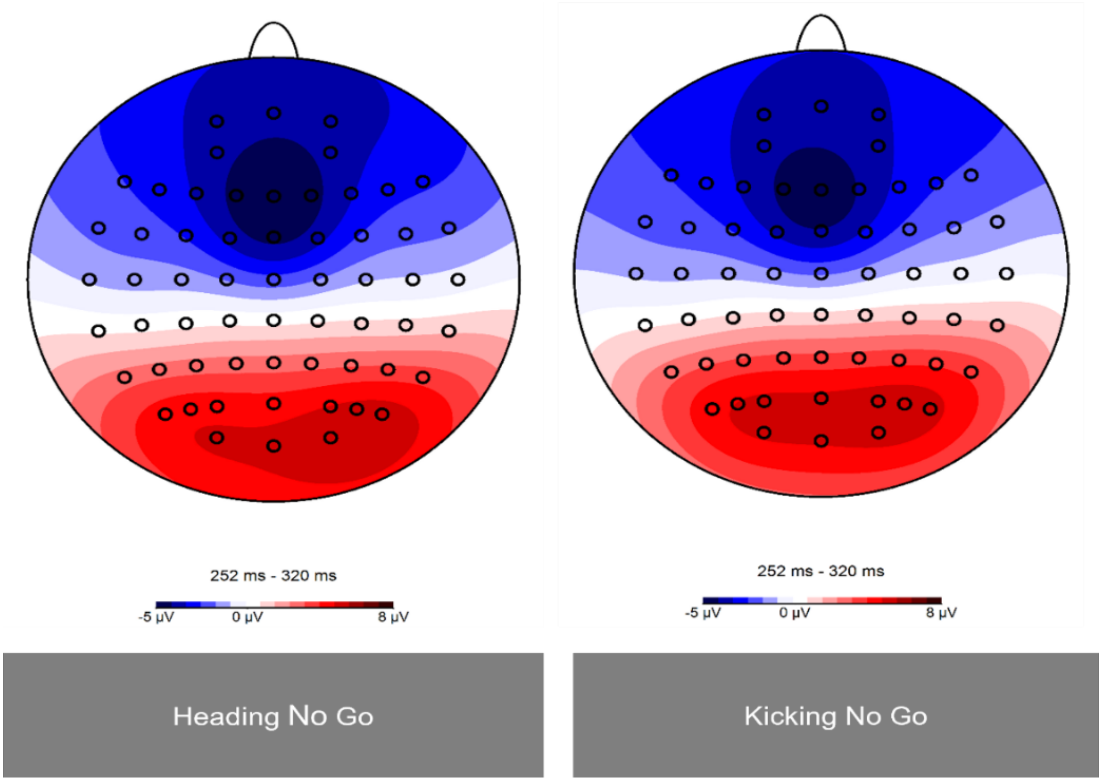

Figure 7.7 Scalp maps for the No-Go condition from 252-320ms.The expected decreased activity in the frontal brain areas (N2) was present in both interventions. Red represents increased and blue decreased activity. Both interventions (heading & kicking) produced similar activity.

P3a (340-440ms): The repeated measures ANOVA revealed a main effect of stimulus with the No-Go trials eliciting a higher amplitude over the Fz electrode compared to the Go trials (F(1,19) = 33.46, $p < 0.001$). There was no main effect of intervention (F(1,19) < .001, p = .993) and no significant stimulus*intervention interaction (F(1,19) = .62, p = .443). The frontal N2 and P3a were not different between the conditions (Figure 7.7 and 7.8).

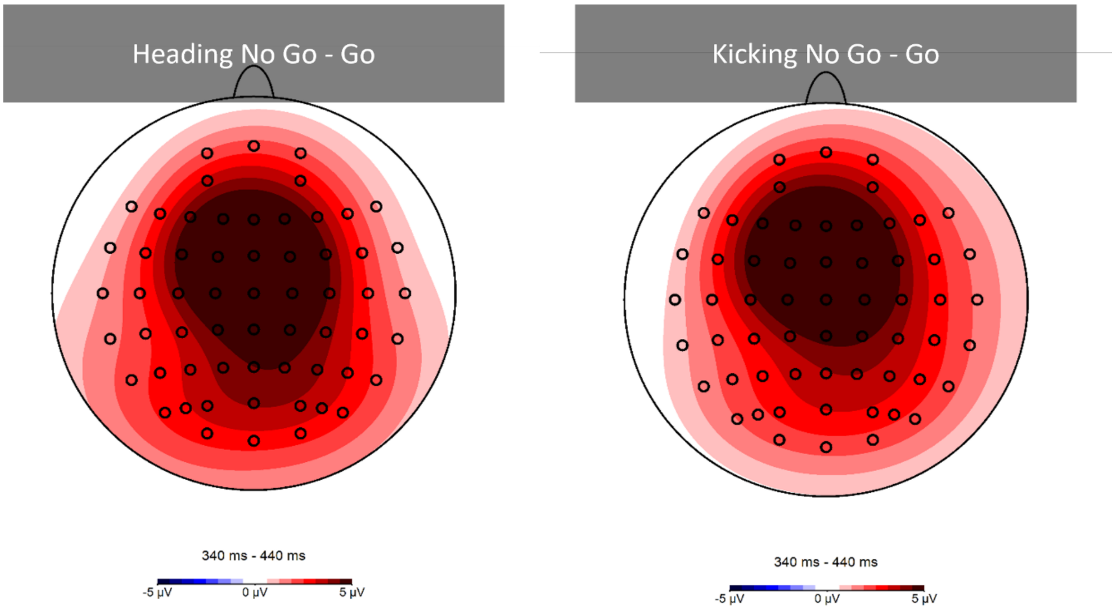

Figure 7.8 Scalp maps showing the P3a ERP component over the frontocentral areas from 340-440ms. The expected increased activity in the frontal brain areas (P3a) was present post both interventions. Red represents increased and blue decreased activity. Both interventions (heading & kicking) produced similar activity.

Post-hoc analyses

Oddball. The multilevel analyses revealed an effect of stimulus frequency, an intervention*stimulus frequency interaction and provided evidence of an effect of time. the best fit model can be seen in Figure 7.9. Rare stimuli produced greater amplitudes over the Pz electrode compared to the frequent stimuli, notably there was a significant random effect, indicating presence of individual effects. Amplitudes over Pz were lower after heading compared to kicking for the rare stimuli, heading reduced the amplitudes by 1.50(.73) µV, individual effects were also present in this interaction (Figure 7.10). There was also a random effect of time, indicating that amplitudes were decreasing by time, -.06(.02) µV per stimulus presentation.

$$\mathrm{PzMean}_{ij} \sim \mathrm{N}(XB, \Omega)$$

$$\mathrm{PzMean}_{ij} = \beta_{0ij}\mathrm{cons} + \beta_{1ij}\mathrm{Rare}_{ij} + \beta_{2j}\mathrm{Heading.Rare}_{ij} + \beta_{3j}\mathrm{Time}_{ij} + \beta_{4j}\mathrm{Time\hat{}2}_{ij} + \text{-}0.0000(0.0000)\mathrm{Time\hat{}3}_{ij} + \beta_{6j}\mathrm{Heading.Time}_{ij}$$

$$\beta_{0ij} = 6.7279(0.7535) + u_{0j} + e_{0ij}$$

$$\beta_{1ij} = 11.1431(1.2540) + u_{1j} + e_{1ij}$$

$$\beta_{2j} = \text{-}1.5023(0.7337) + u_{2j}$$

$$\beta_{3j} = \text{-}0.0638(0.0229) + u_{3j}$$

$$\beta_{4j} = 0.0006(0.0003) + u_{4j}$$

$$\beta_{6j} = \text{-}0.0024(0.0031) + u_{6j}$$

*Figure 7.9 Equation of the best fit model. PzMean = Mean amplitude over the Pz electrode at each trial, Cons = a constant variable equal to 1, Rare = dummy variable having the value 1 for rare stimulus and 0 for frequent, Heading.Rare = dummy variable for the intervention*stimulus interaction being 1 for rare stimulus during the* *heading condition, Time = the continuous variable of time with a range equal to the number of trials. β_x = the coefficient of each variable.*

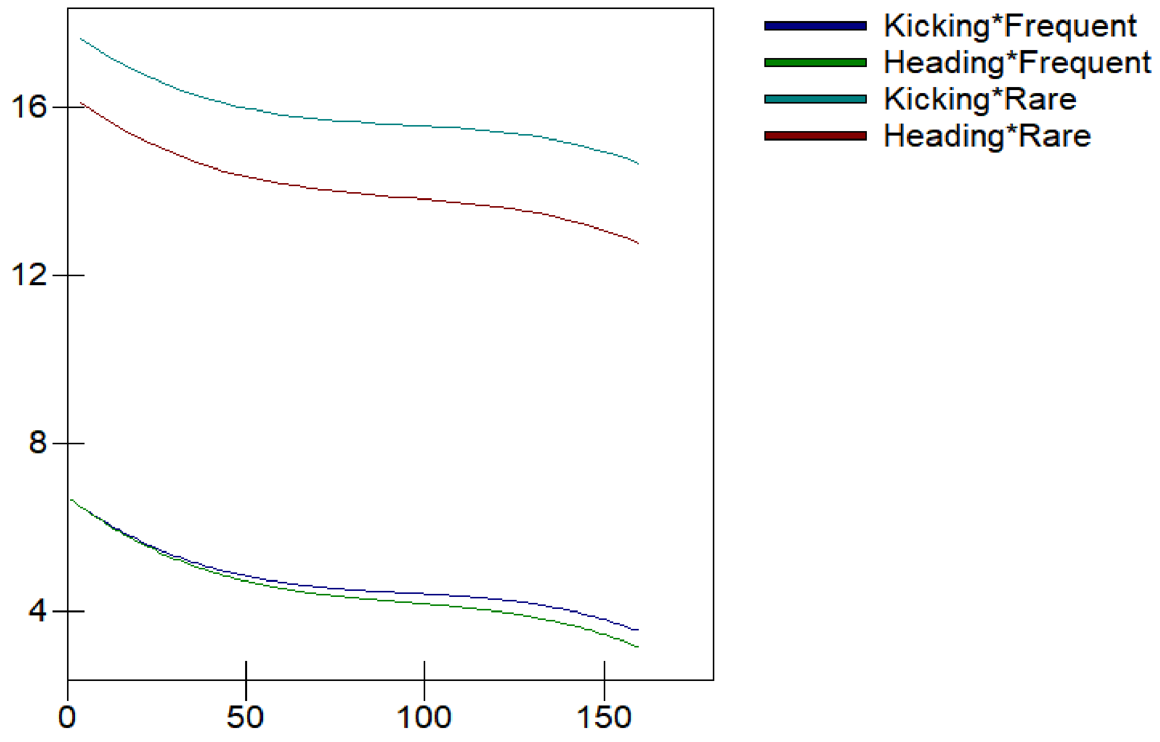

Figure 7.10 Best fit lines for each intervention and stimulus type showing a gradual decrease of activity by time. Y axis depicts amplitude over the Pz electrode in μV.

Go/No-Go. By inspecting the ERPs on Figure 7.6 it was revealed that the parietal P300 during the Go trials might be different between interventions. The parietal P300 of the Go trials was compared between the heading and the control intervention. A paired samples t-test was performed on the amplitudes over Pz at 240-400ms post stimulus presentation. There was a significant P300 amplitude decrease post heading (Mean = 7.73, SD = 4.18) compared to post kicking (Mean = 8.62, SD = 4.31; Figure 7.10); t(19) = -2.46, *p* = .024, ES: .55 [.07-1.01]. Figure 7.11 shows the magnitude and topography of the effect.

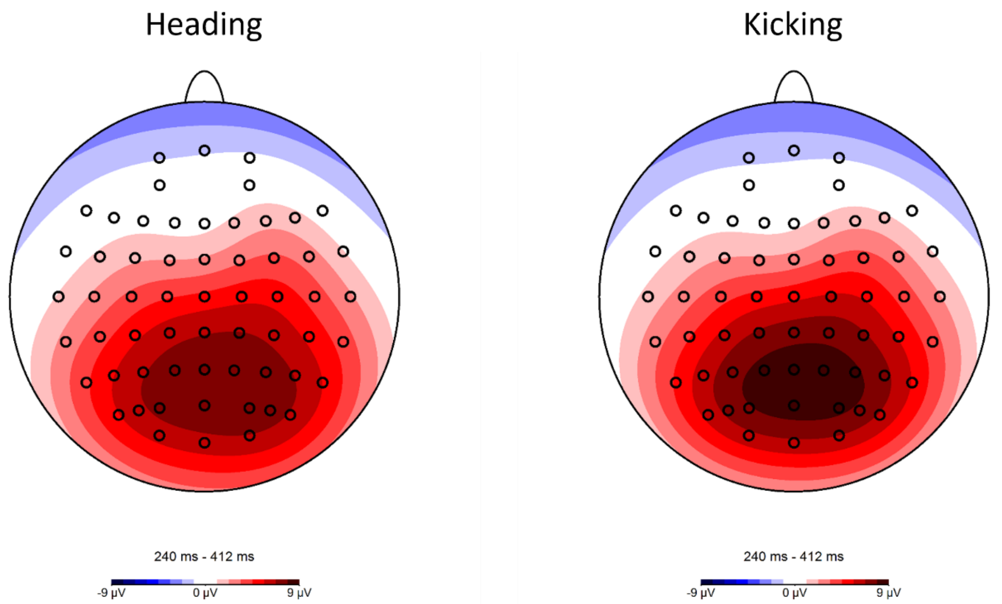

Figure 7.11 Magnitude of activity during the Go trials of the Go/No-Go task 240 - 410 ms post stimulus presentation, showing the expected P3b effect after both interventions. Red represents increased and blue decreased activity. Activity post kicking was higher than post heading.

Predictors of heading effects and correlations between measures

The electrophysiological data shows that the parietal P300 was affected both in the oddball and the Go/No-Go task. Therefore, it is reasonable to assume that those effects should correlate with each other. After the heading session there was a significant positive correlation between the *P3b effect* during oddball and P3b during Go trials; r(19) = .64, *p* = .003 (Figure 7.12). After the kicking session the same effect was found between the two variables of interest; r(19) = .59, *p* = .006 (Figure 7.13). In both cases the correlation was considered high (Cohen, 1988; Cohen 1992). Similarly, the decreases in the ERPs cause by heading (computed as: (ERP amplitude post kicking) – (ERP amplitude post heading)) were correlated positively; r(19) = .49, *p* = .027 (Figure 7.14), showing that athletes who had their *P3b effect* during oddball substantially affected by heading they had also their P3b during the Go trials of the Go/No-Go task affected substantially.

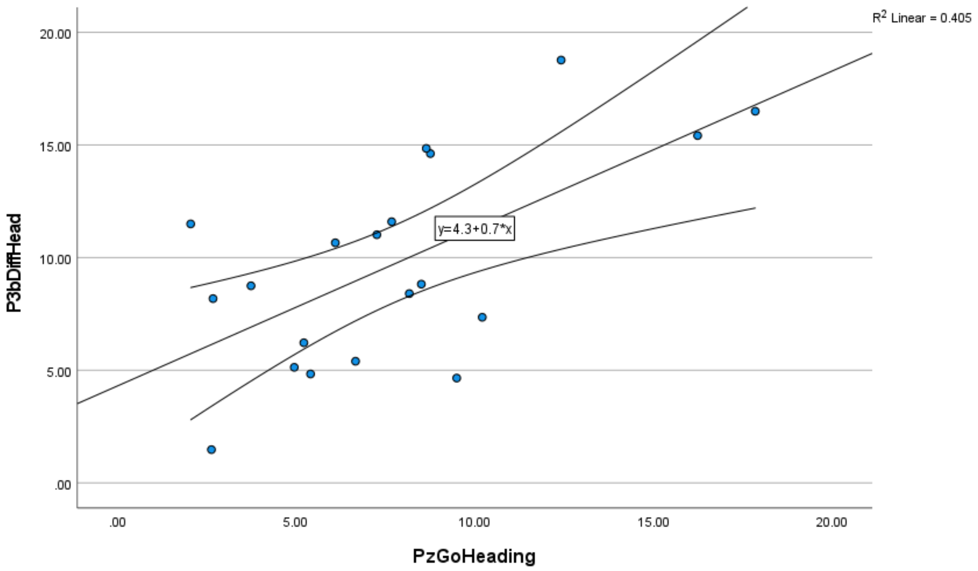

Figure 7.12 Scatterplot with best fit line for the mean amplitudes over the Pz electrode during the oddball (P3b effect) and the Go/No-Go task (Go P3) post heading.

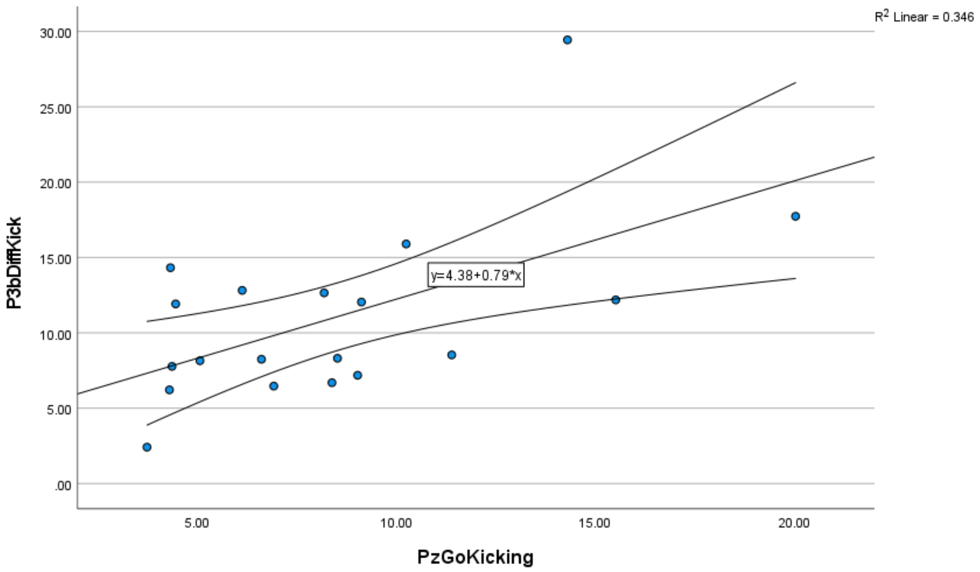

Figure 7.13 Scatterplot with best fit line for the mean amplitudes over the Pz electrode during the oddball (P3b effect) and the Go/No-Go task (Go P3) post kicking.

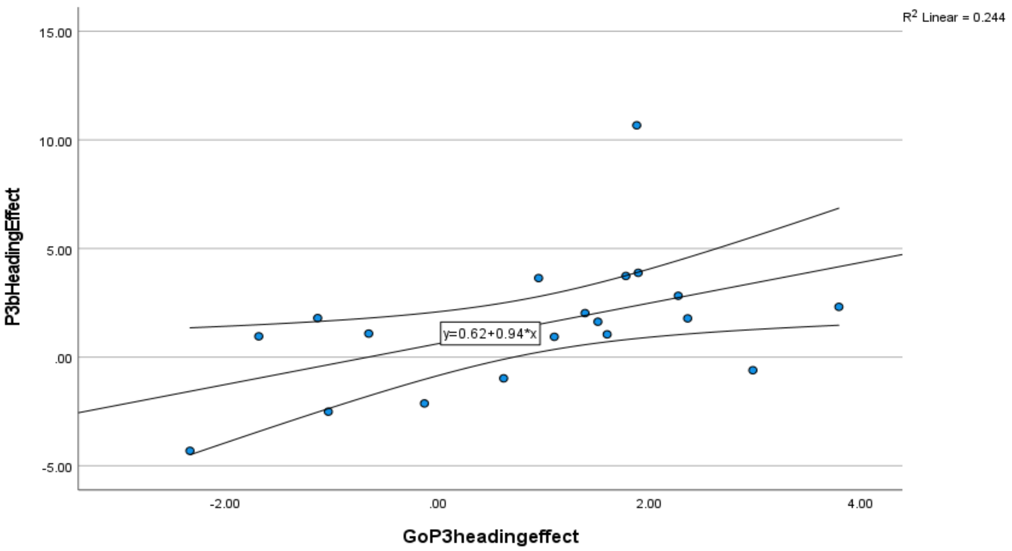

Figure 7.14. Scatterplot with best fit line for the mean difference caused by heading (ERP amplitudes post kicking – ERP amplitudes post heading) in amplitudes over the Pz electrode during the oddball (P3b effect) and the Go/No-Go task (Go P3).

Based on the findings of the multilevel modelling suggesting that athletes’ response to the effects of heading is different between them (intervention*stimulus interaction being a random effect), the next step was to examine which factors might affect this response. The first candidate for this was the peak acceleration at impact during heading, as recorded by the impact sensors, so the correlation of the linear and angular accelerations with the amplitudes of P3b effect was examined. There was no significant correlation between the amplitudes over Pz post heading and the linear or angular accelerations of the impacts; r(19) = .225, *p* = .34 and r(19) = -.07, *p* = .756 respectively (Figure 7.15 & 7.16). There was also no significant correlation between the magnitude of the effect of heading (computed as: P3b effect post kicking – P3b effect post heading) and the linear and angular accelerations; r(19) = -.18, *p* = 45 and r(19) = .29, *p* = .218 (Figure 7.17 & 7.18). This suggests that acceleration at impact does not affect the response to heading, so this effect should be because of other factors.

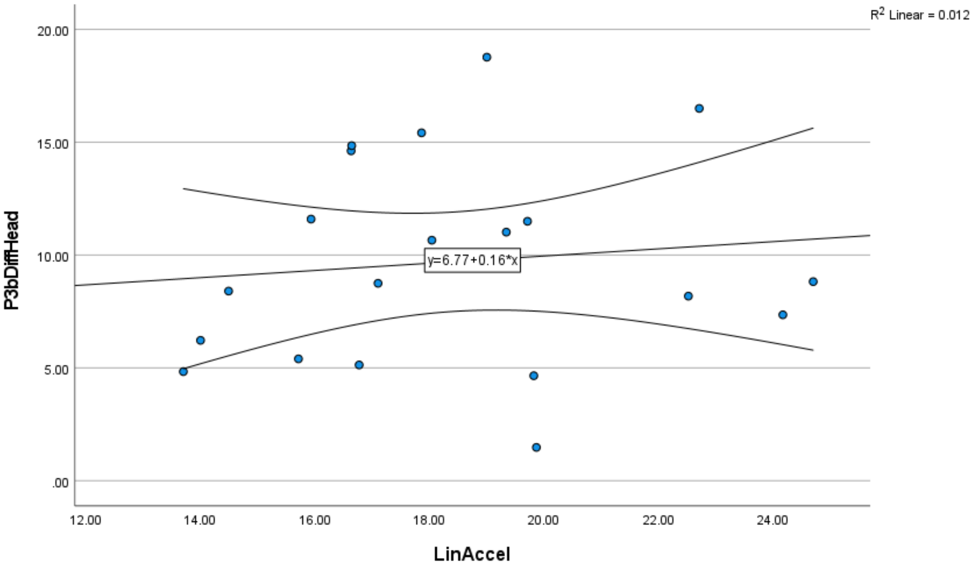

Figure 7.15. Scatterplot, with best fit line, for the mean in amplitudes over the Pz electrode during the oddball (P3b effect) post heading and the mean peak linear acceleration, showing no correlation pattern.

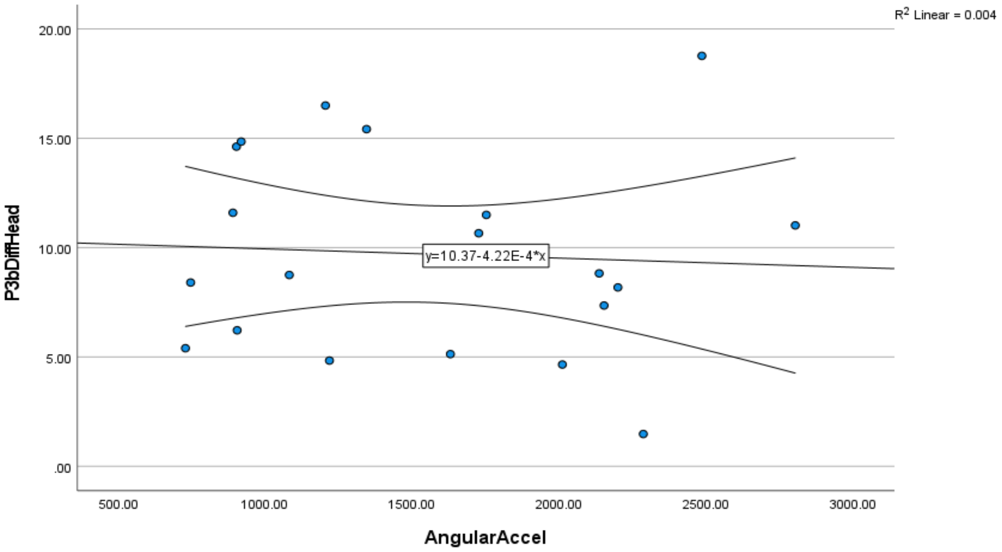

Figure 7.16. Scatterplot, with best fit line, for the mean amplitudes over the Pz electrode during the oddball (P3b effect) post heading and the mean peak angular acceleration, showing no correlation pattern.

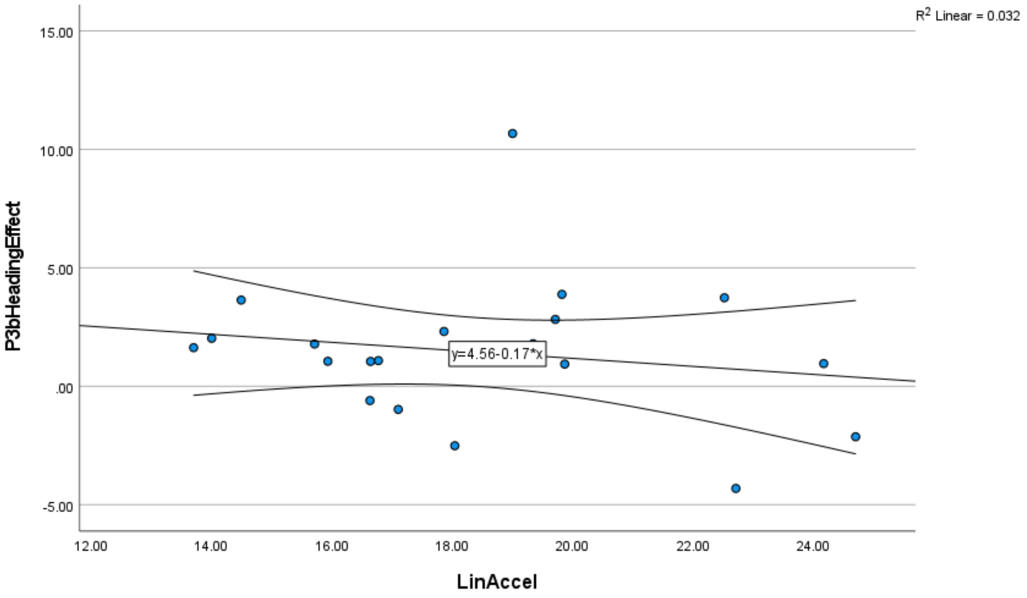

Figure 7.17. Scatterplot, with best fit line, for the mean difference caused by heading (ERP amplitudes post kicking – ERP amplitudes post heading) in amplitudes over the Pz electrode during the oddball (P3b effect) and the mean peak linear acceleration, showing no correlation pattern.

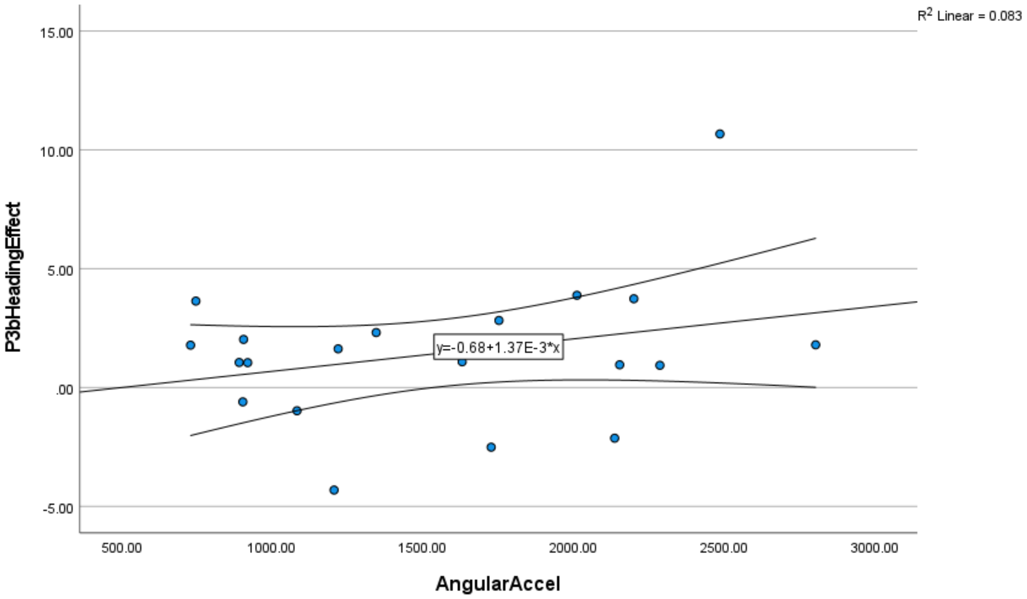

Figure 7.18. Scatterplot, with best fit line, for the mean difference caused by heading (ERP amplitudes post kicking – ERP amplitudes post heading) in amplitudes over the Pz electrode during the oddball (P3b effect) and the mean peak angular acceleration, showing no correlation pattern.

To investigate which factors affect the size of the heading effect, multiple regressions were performed, having the effect of heading as an outcome and age, position, level of play, years of play, grip strength and angular and linear acceleration as predictors. A significant regression equation was found (F(1,18) = 14.19, *p* = .001, with an R^2 of .44. Only grip strength of the non-dominant hand remained in the equation with a coefficient B = -.24, 95% CI: [-.40,-.07] and *p* = .009; The equation is presented below:

$$\mathit{P3bEffectDifference} = 10.54(3.15) - .24(.08)*\mathit{GripNonDom}$$

None of the other possible predictors remained in the model. Thus, only grip strength influenced the P3b effect size difference, with athletes with greater grip strength having less decrease in their P3b effect caused by heading. From this it can be argued that stronger and better trained athletes are less affected by RSHI. However, there could also be an alternative explanation for this effect. Athletes who are stronger and better trained might have sustained more RSHI throughout their career and the few weeks prior to the sessions of this experiment, so the effect of RSHI might appear lesser compared to the rest of the athletes, mainly because their cognitive functions are already affected prior to those sessions. To assess this the data from the control condition was used, to examine if people who are physically stronger have reduced P3b effect amplitudes compared to the rest of the sample.

To examine this a multiple regression was performed with the same predictors as before, but with the amplitude of the P3b effect post kicking as the outcome, in order to investigate what factors affect the P3b effect in our sample of young athletes. A significant regression equation was found (F(18,1) = 10.10 p = .001, with an R^2 of .75. Grip strength and age were the predictors in the equation with B = -.63, 95% CI: [-93, -.33], p < .001 for grip strength and B = -.85, 95% CI: [-1.43, -.29], p = .005 for age. The equation is presented below:

$$\mathit{P3bEffectKicking} = 55.51 -.63(.14)*\mathit{GripNonDom} -.85(.27)*\mathit{Age}$$

None of the other possible predictors remained in the model. Thus, only grip strength and age influenced the size of the P3b effect, with stronger and older athletes having smaller P3b effect amplitudes. The effect of age on P3b amplitudes is well-known, with amplitudes decreasing by age after the age of 20 (Dinteren et al., 2014). The finding that stronger and more trained athletes have reduced P3b effect amplitudes cannot be easily explained, it can be assumed that stronger and better trained players have more exposure to RSHI and thus the size of their P3b effect is affected by it. Unfortunately, data about the recent exposure to RSHI was not collected.

DISCUSSION

The first hypothesis was supported by the data. After heading a ball 20 times, the P3b ERP component amplitude difference (*P3b effect)* during the oddball task was decreased when compared to the P3b effect amplitude difference after kicking. However, this decrease was only present in the neurophysiological data; the performance on the behavioural level (reaction times) were similar post heading and post kicking. Concerning the response inhibition, the hypothesis that any GABA increases caused by heading will manifest as changes in the performance on the Go/No-Go task was not supported neither at the electrophysiological nor at the behavioural level.

The findings of this study provide the first EEG evidence of acute brain changes immediately after heading, similar to the decreases of EEG markers of attention and working memory found in samples of athletes with history of concussion (Ozen et al., 2013; Segalowitz et al., 2001) and athletes with chronic exposure to RSHI (Moore et al., 2017). Our study provides evidence that heading causes a decrease of the parietal P300 (P3b) in response to target stimuli in athletes clear of concussion at a similar level to the P3b effect decrease to target stimuli seen in people with a history of concussion (Ozen et al., 2013). This is of importance, as it shows the level of the effect repetitive headers can have on athletes' brain electrophysiology. By inspecting the significant intervention*stimulus type interaction, and inspecting the evidence from the multilevel modelling analyses, we see that similarly to the previous concussion and RSHI literature, the P3b differences are caused by decreased amplitudes during the rare stimulus presentation, indicating that athletes had difficulty identifying and storing information about the rare stimulus, and not decreased EEG activity in general. Taken together with the findings on Go/No-Go task which showed no differences on any response inhibition measures, we can conclude that the effect on oddball task is selective and reflects attention and working memory updating problems caused by heading.

As mentioned in the introduction of this chapter, the parietal P300 is associated with the locus coeruleus (LC) noradrenaline system, and its amplitude was found to be affected by LC lesions and noradrenaline agents (Nieuwenhuis, 2013; Nieuwenhuis et al., 2005). So, the finding that football heading is decreasing the amplitude of P3b can be an indicator that heading has adverse effects on LC function. Taken together with evidence that suggests that the degeneration of LC is present at the early stages of Alzheimer's and Parkinson's disease (Bari et al., 2020; German et al., 1992; Rommelfanger & Weinshenker, 2007; Weinshenker, 2008), this study provides a possible mechanism of injury that should be investigated further in future studies.

The multilevel modelling analysis provides further insights into the effects of time and the individual differences during the oddball task in our sample. Notably, an effect of time was found, with athletes P3b amplitudes decreasing during the task irresectable of intervention (heading or kicking). This decrease had a 3rd order polynomial pattern (Figure

6.10), indicating greater decreases at the start and towards the end of the oddball task. The fact that the decline was similar in both heading and kicking shows that heading did not boost the rate of decrease caused by fatigue during the task, but the detrimental effect of heading was present and consistent throughout the task. Importantly, the multilevel analysis showed that most of the effects that were found to be significant were random effects, showing that allowing for differences between the athletes improved the model. Notably, those individual differences were also found in the interaction of interest (intervention*stimulus frequency), indicating that the effect of heading is stronger in some athletes and weaker in other. Based on the findings from Moore et al., (2017), which show that players who sustained RSHI during their sport participation had decreased P3b compared to athletes from sports with no RSHI exposure, we can assume that athletes with higher exposure to RSHI might already be under strain from the RSHI exposure they have the days or weeks before the sessions. Based on that, there is a possibility that they will show lower P3b decreases post heading since their working memory mechanisms are already under strain from their recent RSHI exposure. This possibility is supported by the findings of the multiple regressions presented in the results section, in which athletes that are stronger, and more likely to be more “athletic”, have a decreased P3b effect in the control condition. No information on recent and long-term exposure to heading was recorded from the athletes, future studies should make sure to acquire this information as it can provide further insights on the effects examined. A larger scale study would have also been able to examine the P3b effect differences between the level of play and the play positions of the athletes, however, here with a sample of 20, such analyses are not feasible.

Concerning the Go/No-Go task, both the behavioural and the electrophysiological data showed no effects of heading. Response inhibition of a planned response, as indicated by the N2 amplitude, and evaluation of the inhibitory processes, as indicated by P3a amplitude, were unaffected by heading. The task selected in this study was specifically designed to put time pressure on the athletes to perform as good as they can, making the task challenging. However, even if the task was difficult, any adverse effects of heading were not present, showing that the response inhibition processes are unaffected. Notably, even though the ERPs reflecting response inhibition were unaffected by heading, in this task, the ERP indicating working memory updating processes, the P3b during the Go trials, was found to be decreased after heading. This provides further confidence the effects on working memory revealed in the oddball task are real and not a false positive, as both the ERPs designed to assess working memory processes (P3b) were found to be affected by heading.

Further studies are needed to examine the source of this effect in the brain. As mentioned earlier, parietal P300 was found to be associated with the locus coeruleus noradrenaline system (Nieuwenhuis et al., 2005). Showing alterations in locus coeruleus functioning caused by RSHI during attention and working memory tasks might be able to provide a link between RSHI and neurodegenerative diseases, as degeneration of locus coeruleus is evident in early stages of Alzheimer’s and Parkinson’s disease (Weinshenker, 2008; German et al., 1992). Following the findings of this study, an EEG oddball task will be included in a new collaboration with the Institute of Neurosciences and Medicine in Julich, Germany, in which EEG measures will be paired with magnetic resonance neuroimaging methods recorded before and after heading, as well as blood biomarkers. Here we should highlight the importance of using multimethod approaches in neuropsychology. In this case, EEG was used as an effective way to identify the cognitive processes affected by heading before the use of expensive neuroimaging equipment is implemented to examine the brain topography and mechanisms of those effects.

In the following chapter an EEG marker of learning (N400) was used on the same cohort of athletes.

CHAPTER 8. Football heading impairs associative verbal learning in amateur and professional athletes.

INTRODUCTION

The purpose of this study was to test with sensitive measures the neurocognitive function of verbal and associative learning. Associative learning was found to be impaired in previous studies, by implementing a lab-based heading evaluation (like Di Vigilio’s et al, 2016; Chapter 6, behavioural measures of cognitive functioning).

In the EEG literature of semantic associations and verbal learning, it is known that a word that is incongruent with the meaning of its previous context in a sentence or its preceding word elicits a negative wave (N400) with a peak around 400ms after the stimulus onset (Nobre & McCarthy, 1994). In other words, the amplitude of the N400 is inversely related with the semantic match between the target word that elicits the N400 and its previous word or sentence. This negative-going activity is stronger in the central and parietal areas (Kutas & Federmeier, 2000, 2011). Interestingly, this N400 effect has been found to be present even after a single exposure to the novel/to-be-learnt word (Borovsky et al., 2010, 2012).

Perfetti et al. (2005) managed to differentiate between skilled and less skilled “comprehenders” by using ERPs and especially by examining the differences on the N400 ERP component. The participants who learned the words better showed reduced N400, whereas participants who have not learned the words that well exhibited more negative (greater) N400 (Perfetti et al., 2005). Based on that, the ERPs of participants in both interventions (heading and kicking) was examined with an emphasis on the N400 component. Here it should be noted that the N400 might not be negative as a numerical value in absolute terms, but it is a more negative slope 300-500ms after the target stimulus onset that appears when a word is incongruent or unrelated to its previous context, compared to the same response when the word is related with the context of the previous words/sentence. This difference in amplitudes between the related and unrelated target words was the variable of interest.

The N400 as an indicator of learning has been used widely in the existing literature and is considered an effective and reliable way to examine almost every aspect of linguistic processes (Kutas & Federmeier, 2011). In clinical studies it has been used to show that certain clinical populations have impairments in linguistic processes. For example, patients with a history of paediatric TBI were found to have decreased N400 when presented with words with meaning unrelated to the previous context (Knuepffer et al., 2012). N400 is also used as a marker of lexical access in studies that accessed verbal learning and lexical consolidation (Bakker et al., 2015). The term “marker of lexical access” refers to the ability of a novel/newly learnt word to activate a lexical network of another target word with related to the novel word’s meaning, due to the establishment of a meaningful lexical relationship between the novel/learnt word and the target word. The fact that the novel/learnt word causes an N400 amplitude decrease for the target word indicates that the learnt word has been integrated into an existing network of words who are semantically associated.

The sensitivity of the N400 as a measure of sematic associations between words is highlighted by the finding that in most of the studies this effect on the ERPs of the subjects was not accompanied with changes in the behavioural measures. This indicates that those initial neural responses to the newly learnt information are very subtle and thus are not present in measures that assess accuracy of responses and reaction times (Bakker et al. 2015).

In the case of subconcussive impacts, no previous study has examined verbal learning by using the N400 effect on newly learned words. This effect’s sensitivity and the fact that previous studies have found that heading probably has aversive effects on athletes’ learning processes, makes the N400 effect a great way to investigate in depth the effects of heading on athletes’ verbal learning processes.

In this study we implemented an altered version of the verbal learning task used by Perfeti et al. (2005), in which participants had to learn a series of very rare English words that were not included in the Kucera and Francis corpus of more than a million English words (Kučera & Francis, 1969). After the learning phase participants’ learning was examined by a semantic memory task in which they were presented with pairs of words. The first words were the words which they had studied, and the second words were either words related to the meaning of the first word or unrelated words. Participants were asked to report if the second word has a meaning related or unrelated to the first word. The effectiveness of a newly learnt word to serve as a prime for a semantic learning task is a way to measure whether this word was effectively learnt (Perfetti et al. 2005; Borovsky et al. 2012). Therefore, the N400 here served as an indicator of words’ relationship, with increased N400 differences between related and unrelated words demonstrating better ability to identify the words’ relationship (Kutas & Federmeier, 2011), caused by a greater learning during the learning phase.

Based on the previous studies on heading and verbal learning, and N400 sensitivity as a verbal learning measure, it was hypothesized that the verbal learning of participants will be impaired after a heading session compared to after a kicking session. This decline in learning will be evident as a lower number of hits in a semantic memory task and as a relative difference in the amplitudes of the N400 ERP component (the N400 effect).

METHODS

Participants

The participants of this study were the same athletes that took part in the experiment described in Chapter 7 (ERP oddball and Go/No-Go). However, only the athletes where English was their first language were able to take part in this experiment (n = 13). The thirteen athletes who identified themselves as native English speakers had a mean age of 22.23 (±3.63), eleven were male and the majority of them compete at a semi-pro level; see participants number 1,4,5,6,8,11,12,13,14,16,17,19 and 20 in table 7.1 for more information.

Material

Verbal Learning. The verbal learning task consisted of 2 phases. The first phase was the *Learning phase* in which participants were presented with and had to learn 50 very rare English words (Appendix 3). The to-be-learnt words used in this study were the very rare words used by Perfetti et al. (2005). Those words are old English words, mostly used in old English literature. At this phase each word was presented for 42s; on the screen participants viewed the to-be-learnt word, its meaning, and an example sentence (Appendix 3).

After this phase, participants proceeded to the *Testing phase*. In this phase they were presented with pairs of words; the first word was either a very rare English word they have just learnt, a very rare English word they did not learn or an English word of medium rarity. The following word, the '*target'* word, was a word with meaning either related or unrelated to the previously presented word. The target words have been carefully selected based on their frequency of use. Each first word was paired with two *target* words of related and unrelated to it meaning. The two *target* words were of the same frequency of usage. The frequencies of each word were acquired from the iWeb corpus, a corpus of 14 billion words; the biggest online database. Participants saw the 50 words that they had learnt paired with 50 words with related and 50 words with unrelated meaning in a random order. The extra sets of 35 English words of medium rarity were paired with 35 *target* words with related and 35 words with unrelated to them meaning, and 25 very rare 'non-learnt' English words were paired with 25 words with related and 25 words with unrelated to them meaning.

During the *testing phase* participants saw 220 pairs of words in total. The word groups formed with the aforementioned procedure were counterbalanced across the two conditions: heading or kicking a football. In this *semantic memory task* participants saw a fixation point for a duration of 400-550ms and then the first word appeared for 1200ms. Then the second, ‘target’ word was presented and stayed on screen until a response was provided. Participants had to respond either by pressing the ‘R’ button when the first and the second word are related in meaning or by pressing the ‘U’ button when they are unrelated. After each pair presentation there was a gap of 1s until the fixation point for the next trial is presented.

Impact meter. The impact of each header was measured the same way as in the Chapter 7.

Heart rate monitor. See Chapter 7.

Recordings. Same procedure described in Chapter 7.

Procedure

Participants were welcomed to the lab and asked to read the study’s information sheet and sign the consent form. After that, they were taken to the place in which the ball heading/kicking took place.

Heading/Kicking paradigm. This paradigm was described in Chapter 7.

EEG testing. In the psychology imaging lab, the EEG cap was fitted on their scalp from the researcher. During this procedure participants saw a list of the *to-be-learnt* words (alone without their meanings) and asked if they can recognise any of them, if so, they were asked to provide their meaning. If they knew the meaning of a listed word, this word was excluded from the rest of the experiment. After the capping procedure, participants sat in front of a computer and proceed to the *learning phase* of the experiment, which lasted ~32 minutes. Before the beginning of this process, they were told that they have to learn the meaning of the words presented to them and advised to use the learning strategies that think are more efficient for them; there was no need for them to press any key or engage in any other activity other than trying to learn the meaning of the words. They have also been made aware that there will be a *testing phase* following the word presentation. During the whole learning and testing process they were in a soundproof lab, alone, without any distractions and stimuli other than the ones in the computer screen. In the testing phase, the researcher described the task to participants; written instructions were also present at the beginning of the task as well as a brief trial of the procedure. Then the semantic memory task begun. In this task participants viewed the pairs of words and they had to press either the ‘R’ or the ‘U’ key after the presentation of the ‘target’ word. After the presentation of the 220 pairs of words, the task was over and participants were thanked for their participation in this task, debriefed and receive their payment in cash.

Statistical analysis

Details for the EEG equipment used, and the online and offline EEG data analysis were provided in Chapter 7.

The number of correct responses, that is the correct identification of a word as either related or unrelated to the previous one, were recorded and the athletes' accuracy score was calculated as a % of the correct responses in the total responses given by each athlete and compared between intervention with paired samples t-test. The EEG data exported from the central and parietal-central electrodes was analysed using repeated measures ANOVA to examine the main effects of intervention (heading, kicking), word relationship (related, unrelated) and their interaction; significance level was set at .05. Based on previous studies on N400, the ERP amplitudes (in μV) 300-500ms post stimulus presentation were used in the analysis. Due to the small sample size a Bayesian linear model was also used to investigate the presence of an effect of heading on N400 amplitude. Small sample sizes are commonplace in concussion and "subconcussion" research. Bayesian linear models provide direct probabilistic comparisons between groups/treatments, and are suited to studies involving athletes, as they provide more defence against the over-confidence present in small sample sizes (Borg et al., 2018; Mengersen et al., 2016) compared to frequentist techniques. We used the R package RStanarm (Goodrich et al., 2020) to carry out the Bayesian analysis described above and used the posterior distributions to examine the probabilities of the presence of an effect (Swinton et al., 2018). The RStanarm package provides an interface to the Stan probabilistic programming language, which uses Hamiltonian Markov Chain Monte Carlo (MCMC) method to generate the MCMC chains used to characterise the posterior distribution (M. D. Hoffman & Gelman, 2014). After defining the model and priors, the other values set in the call to RStanarm were 4 MCMC chains with 2,000 iterations each and a burn-in period of 200 iterations.

RESULTS

Impact metrics. All athletes performed 20 rotational headers, with a pick mean linear acceleration of 18.52 (±3.43) g and peak mean angular acceleration of 1589 (±676) rad/s^2. Information about individual impact metrics can be found in Table 7.2, in this experiment participants number 1,4,5,6,8,11-14,16,17,19 and 20 from Table 7.2 were tested. None of the athletes' heart rate exceeded the threshold for light medium exercise in the experimental or control conditions, mean bpm: 83 (±9) for kicking session and 85 (±10) bpm for heading session.

Behavioural findings. The paired samples t-test reveal no significant differences in the accuracy of athletes responses, however a trend was present with athletes post kicking having ~5% increased accuracy scores compared to after kicking (75% vs 79.6%); t(12) = 1.88, *p* = .084 (one-sided *p* = .042).

EEG findings. The 2x2 (interventionXword) repeated measures ANOVA was conducted for electrode Cz. Data extracted was the mean amplitude at 300ms to 500ms. There was a main effect of words relationship, with words with related meaning to the previous word producing more positive amplitudes F(1,12) = 7.31, p = .019) and no effect of condition (F(1,13) = 3.68, p = .079). There was a significant condition*word interaction F(1,19) = 8.99, p = .011, η^2 = .428, OP: .79, with increased learning effects post kicking compared to post heading; as it can be seen in Figures 8.1 and 8.2. No differences were found for the known words, apart from the expected main effect of word relationship ($p<0.05$), with unrelated words producing lower amplitudes over the Cz electrode compared to the related words (Figure 8.3).

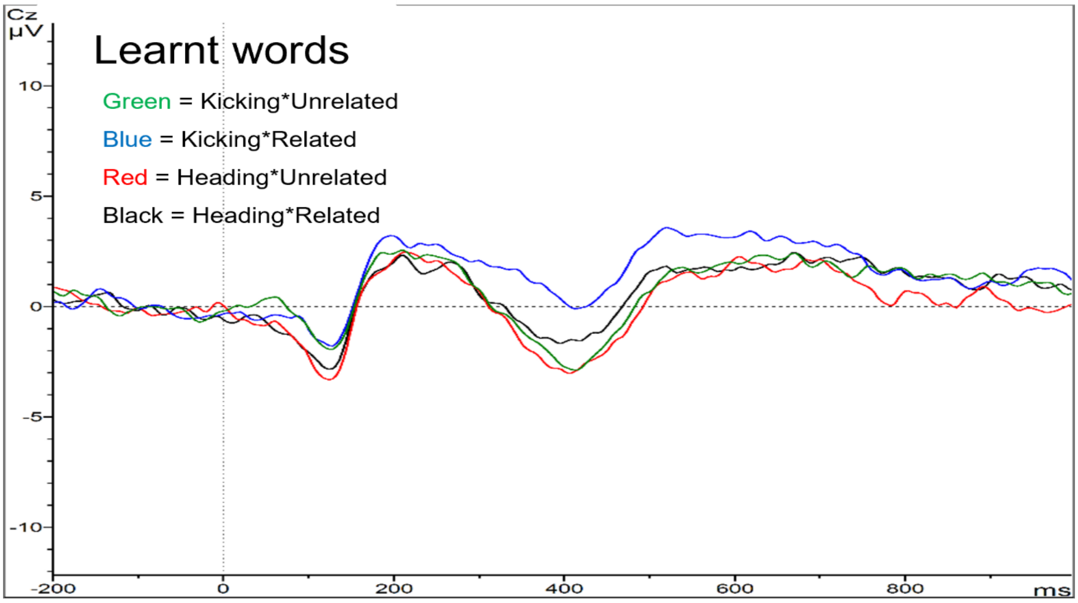

Figure 8.1. ERP wavelengths for the mean amplitude over Cz after the presentation of the second word of the word pair for the learned-words condition. X axis represents time pre and post stimulus (stimulus presentation at time 0).

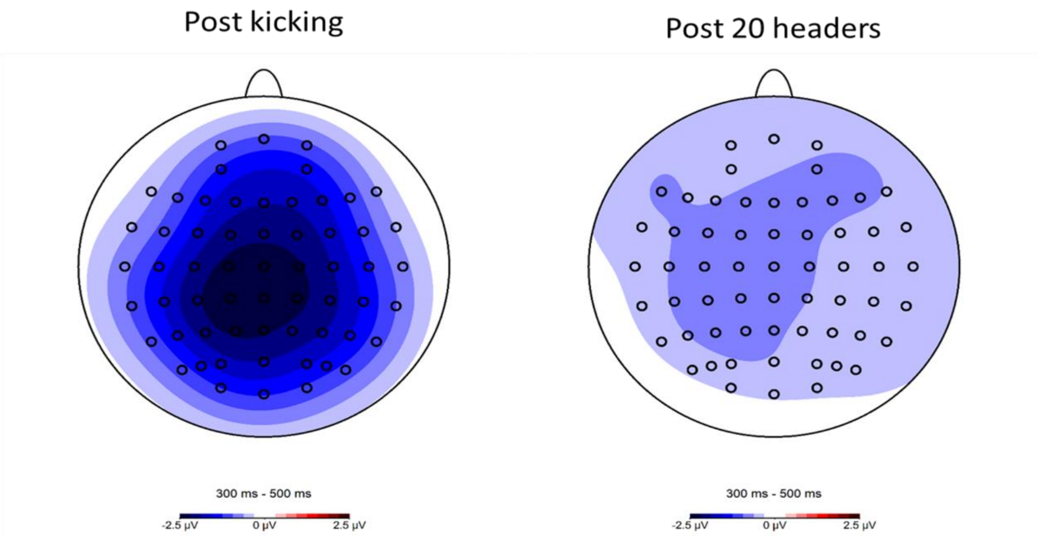

Figure 8.2 Scalp maps for amplitude differences 300ms to 500ms post stimulus presentation (related – unrelated) for the learned-words condition after kicking and after heading. Both maps are scaled at ±2.5μV. Blue areas represent decreased activity and red areas increased activity. The expected N400 effect over the central brain areas was present only post kicking.

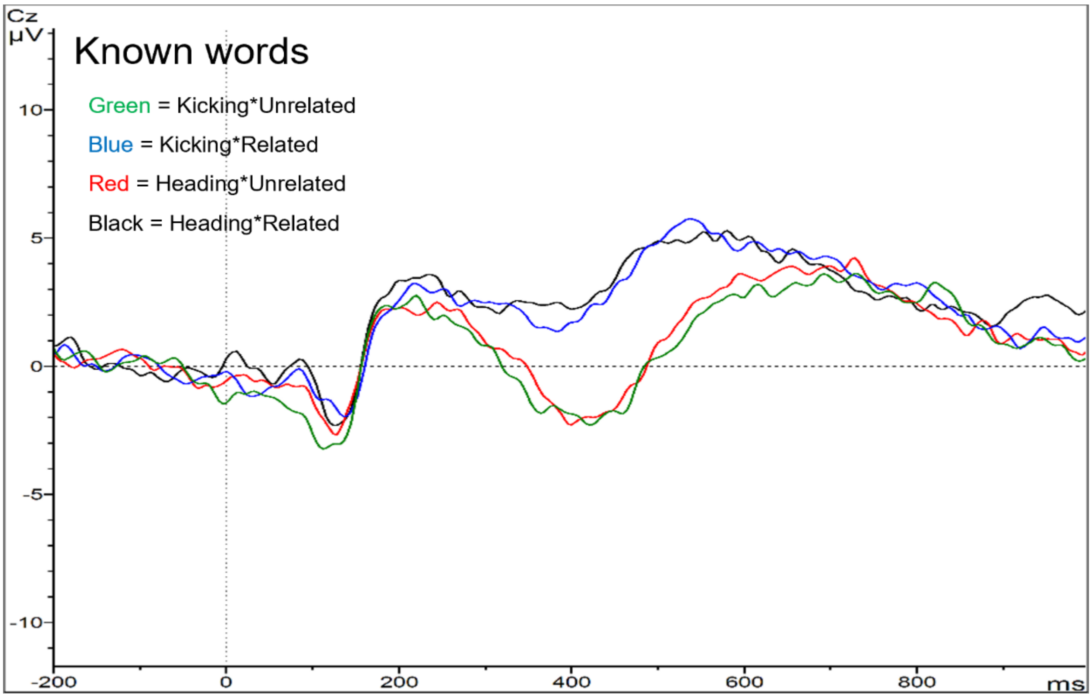

Figure 8.3. ERP wavelengths of the mean amplitudes over Cz after the presentation of the second word of the word pair for the known-words condition. X axis represents time pre and post stimulus (stimulus presentation at time 0).

By visually inspecting the head maps it seems that the effect is more prominent over the CPz electrode and not the Cz as expected. So, a similar to the previous 2x2 ANOVA this time for the amplitudes over the CPz electrode was performed. The 2x2 ANOVA revealed a significant main effect of word relationship (F(12,1) = 6.34, *p* = .027), with words with related meaning having more positive amplitudes than the ones with unrelated meaning. There was also a main effect of intervention, mainly caused by the big difference for the related words post-kicking (Figure 8.4); F(1,12) = 6.84, *p* = .023. The interaction between intervention and word relationship was also significant; F(1,12) = 5.56, *p* = .036, η^2 = .317, OP = .58.

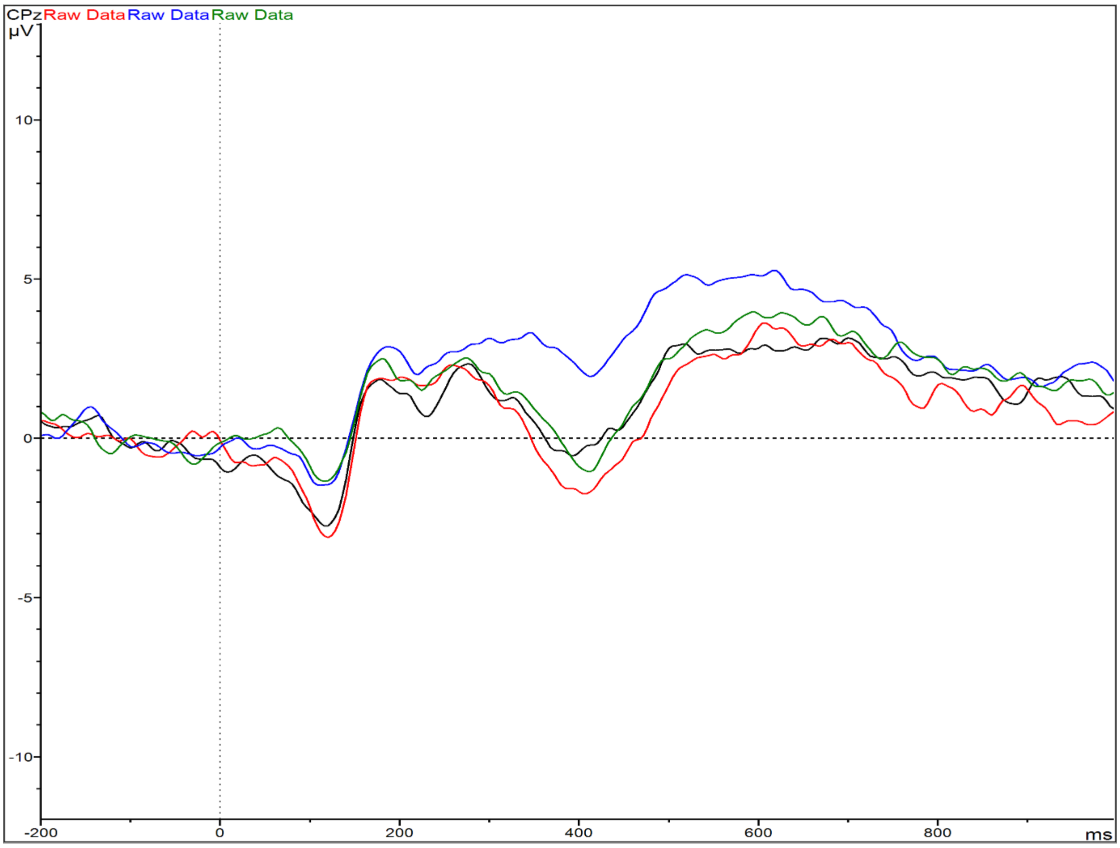

Figure 8.4. ERP wavelengths for mean amplitudes oves CPz after the presentation of the second word of the word pair for the learned-words condition. X axis represents time pre and post stimulus (stimulus presentation at time 0).

Bayesian analyses. A Bayesian linear model was used to analyse the EEG data. Since no previous information of the effect was available an uninformative prior was used. The posterior distributions provided further evidence that N400 effect was decreased post-heading compared to post-kicking; 95% credibility interval: [-2.7, -.3], see Figure 8.5 for the posterior distribution with a 95% credible interval.

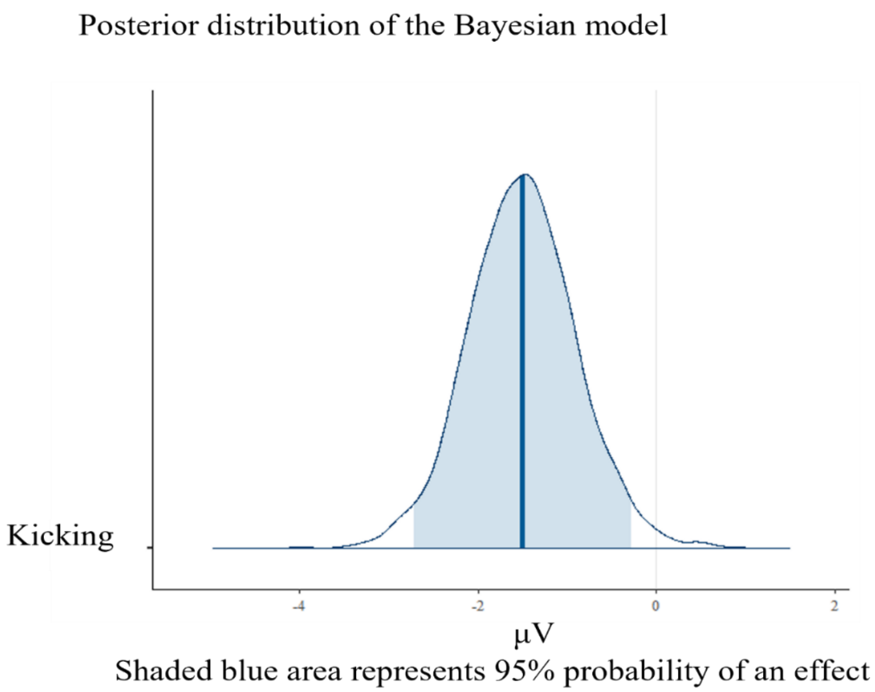

Figure 8.5. Posterior distribution of the Bayesian linear model. The blue area indicates that there is a higher than 95% probability that N400 was greater (more negative) post-kicking compared to post-heading.

Similarly, the behavioural data was analysed with the same Bayesian model, with a non-informative prior. The posterior distributions provided evidence of a trend that correct word identifications were decreased post-heading compared to post-kicking; 95% credibility interval: [-.8, 10.3], see Figure 8.6 for the posterior distribution with a 95% credible interval. By investigating the effect further, it was revealed that there is a 91% probability of an effect; 91% credibility interval [0, 9.3], Figure 8.7.

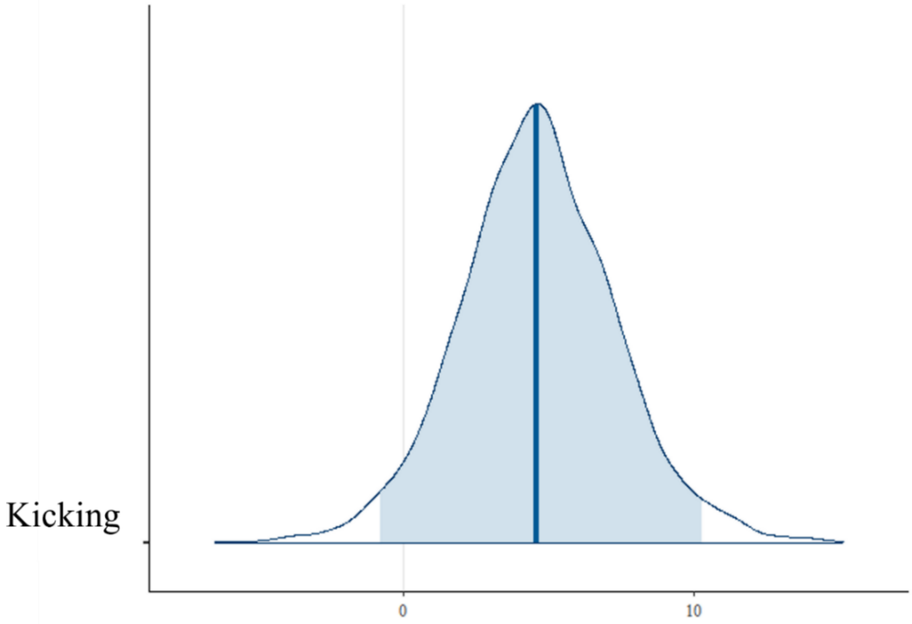

Figure 8.6. Posterior distribution of the Bayesian linear model. The blue area indicates that there is not a higher than 95% probability that athletes gave more correct responses post-kicking compared to post-heading.

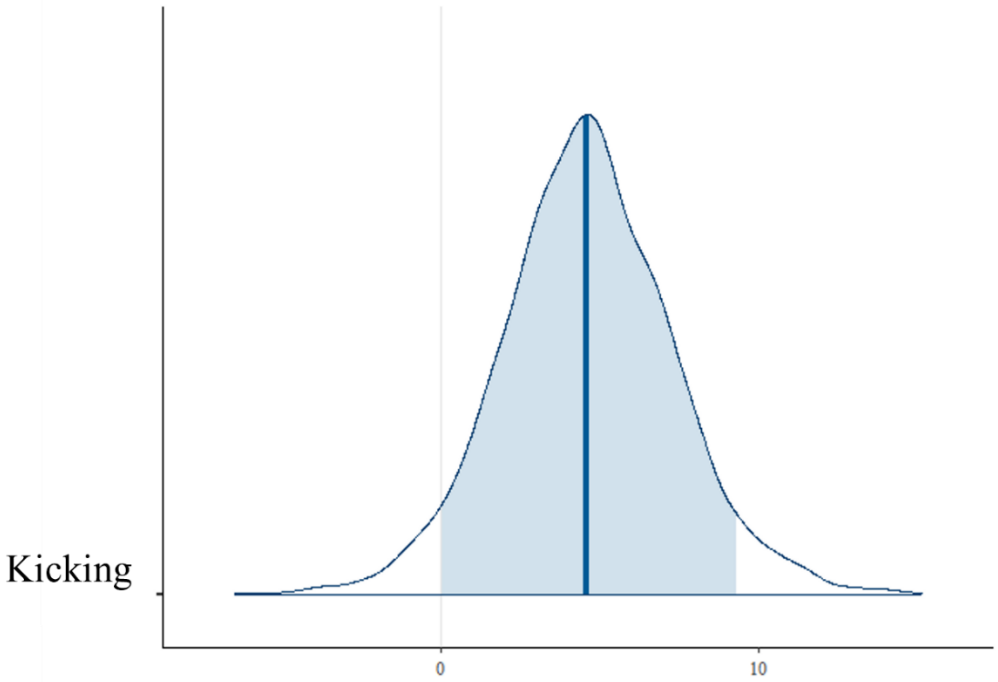

Figure 8.7. Posterior distribution of the Bayesian linear model. The blue area indicates that there is a higher than 91% probability that athletes gave more correct responses post-kicking compared to post-heading.

DISCUSSION

In this study we provide evidence that the N400, a neurophysiological marker of learning, was markedly reduced following heading, providing brain-based evidence for impaired learning. This conclusion of impaired learning was also born out through the trend towards significance for the (non-sensitive) behavioural data. This is important new knowledge in the way it demonstrates that normal learning is not possible after heading.

To the best of our knowledge, this was the first time that athletes' ability to learn new verbal information was examined with a sensitive way like EEG, showing that 20 headers impaired the learning of new verbal information. Previous studies have examined the verbal learning processes by using behavioural tasks like the California Verbal Learning Test (McAllister et al., 2012) or by using the N400 effect to examine if RSHI affect the cognitive processing of already known words (Fickling et al., 2021, 2022), however none has used the N400 to examine the efficacy of verbal learning processes post heading. It should be noted here, that unlike previous findings on N400 for known words (Fickling et al., 2021, 2022), in this study no differences were found when athletes were presented with words they already knew. This indicates that the cognitive processing of already known words was not impaired in the athletes in our sample, on the contrary, it was the inability to learn the new words what caused the absence of the N400 effect post heading. The absence of N400 post heading shows that the words athletes had to learn after the heading were not able to activate the lexical network of the target word, meaning that they were not integrated into the network of words that are semantically associated with them (Bakker et al., 2015), at least not in the same amount as the words learned during the control condition. In simple terms, the learnt words after heading were not learned well enough to be integrated into a lexical network with the words with which they have similar or closely associated meaning, hence the presentation of those words did not activate the appropriate “neurolexical”[6] network, so no N400 effect was produced.

Due to the small sample size, we employed a Bayesian linear model to further investigate the effect of heading on N400 amplitude and provide stronger evidence of its presence. Our Bayesian linear model provided evidence that there is an effect, that is heading hindered learning. This gives us further confidence for the effect since Bayesian linear models provide direct probabilistic comparisons between the measures and provide more defence against the over-confidence present in small sample sizes (Mengersen et al., 2016; Borg et al., 2018) compared to frequentist techniques, like in our case where only 13 athletes were able to take part in this task due to the linguistic constrains of needing to be a native speaker of the language. Here I would like to highlight the importance of using the appropriate statistical techniques based on the research design. In cases when researchers are expected to face small sample sizes, having tools like Bayesian linear models can be beneficial as they can provide additional and more objective evidence for the presence or absence of an effect. The findings in this chapter are in accordance with the findings of Chapter 6 (behavioural measures of cognitive functioning), in which athletes’ ability to form new paired associations was found to be affected by heading. In Chapter 6, athletes had to perform the paired associatives learning (PAL) task from CANTAB, a task that examines participants ability to form non-verbal paired associations, here we also provide evidence that verbal paired association learning is also hindered by heading, evident at a brain level through the virtually decimated neural signature of learning. This converging evidence of the effect on learning processes in separate samples of athletes, strengthens the concerns that RSHI have detrimental effects on athletes cognitive functioning, and especially in learning processes.

When taken together with the effects seen on attentional and working memory processes (Chapter 6), this thesis provides evidence that heading causes widespread cognitive dampening. It can also be argued that the difficulties reported in the learning task were an

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[6] Word made up by the thesis author to describe a network of neurons that is activated by lexical information

aftereffect of difficulties in attentional and working memory processes. Attentional resources are argued to be a predictor of second language learning and specifically the memory of the verbal information learnt (Robinson et al., 2013). The P3b, as induced by a visual oddball task, like in our case, has also been found to be efficient for the prediction of memory recall processes (Amin et al., 2015), showing that attention and learning are interlinked processes. Unfortunately, the sample size of the verbal learning experiment (n = 13) was not large enough to provide reliable correlations between the amplitude of the P3b effect (as found in chapter 6) and the behavioural and electrophysiological data of this chapter, the scatterplots however show a trend for an effect (see Appendix 2.2).

To date, the literature on RSHI was more focused on examining functions widely seen to be affected after sport-related concussion, like motor control, pain, concussion symptomatology, and crude measures of reaction time and memory, and only recently has shifted its focus on more sensitive measures of cognitive function (Ntikas et al., 2022). Here we provide evidence that learning is affected and further recommend that researchers wishing to examine the effects of RSHI on athletes' brains should be aware that they are investigating subtle effects and should implement sensitive measures if they wish to provide useful information to the field.

Based on the findings of this chapter, it is also recommended that in the future functional neuroimaging studies should be implemented to examine the topography of the N400 effect found here. Neuroimaging modalities that can examine the levels of neurotransmitters in the brain could also be of use, as they can provide evidence about which neurotransmitter imbalances are causing those effects on learning. Especially since learning processes can be linked with neuroplasticity and the Glutamate and GABA neurotransmitters (Riedel et al., 2003; Spurny et al. 2020; Kolasinski et al. 2019) and cholinergic processes (Hasselmo, 2006; Tang et al.,). Providing more concrete neuroscientific evidence about the adverse effects of heading on athletes' brain integrity is necessary in order to raise awareness amongst the sports community and the governing bodies. This evidence will also be crucial for linking the acute brain alterations caused by heading with the existing evidence of long-term neurological problems in ex-football players (Mackay et al., 2019).

INTERIM DISCUSSION 4

So far, the majority of the measures employed to investigate motor control issues caused by RSHI yield no significant results. This raises questions about previous evidence that shows motor control alterations caused by RSHI (Di Virgilio et al., 2016, 2019). In those publications the GABA concentrations were highlighted as important adverse effects of RSHI. However, all the measures specifically designed to assess GABA changes in this thesis did not provide any evidence of an effect (SRTT, Go/No-Go task). All GABA measures so far in this thesis were indirect measures of GABA, so no conclusions could be made about whether GABA concentration in the brain of athletes is affected by heading or not. As mentioned earlier in this thesis, one of the aims is to provide evidence for absence or presence of effects and avoid bold statements, especially when it comes to absence of effects, based on limited evidence. Therefore, to avoid the dismissal of the GABAergic response as a mechanism of injury without adequate evidence, it was decided to investigate it further.

Unfortunately, at this point, no measures to assess GABA concentrations directly where available to the researcher (e.g. by using high-cost, specialist, and still emerging MR Spectroscopy as described in Chapter 2, literature review). The next best option to attempt answering this question was to try and replicate Di Virgilio et al. (2016) findings by using TMS induced corticospinal period, the indirect measure of GABA employed in their study, which so far presents the only evidence of GABA alteration caused by RSHI in football. Lengthening of the TMS induced cortical silent period has been previously reported in rugby players (McNabb et al., 2020), however in that study the sample size was very small.

Due to limited availability of time (the COVID-19 pandemic had stopped face-to-face research for 18 months of the PhD at Stirling) and research resources, as well as the aims of this experiment which were to see if Di Virgilio findings replicate, it was decided that the recruitment of athletes will be completed when there is sufficient evidence of the absence or presence of an effect. To do so, Bayesian linear models in R (as described in previous chapters) and Bayes factor analysis in JASP (JASP Team, 2022) were used. Apart from the TMS measure, it was decided to examine the integrity of motor cortex with a task that assesses procedural memory consolidation, a process different than the procedural memory acquisition examined in Chapter 6 (behavioural measures of cognitive functioning), which has been found to be affected when motor cortex is disrupted (inhibited).

CHAPTER 9. Cortical silent period and procedural memory consolidation after 20 headers.

Abstract

Objectives: So far, the evidence from the previous chapters suggest that RSHI do not affect motor control and cortical inhibition. The aim of this study was to investigate the effect of heading on motor control in the same way that has previously shown cortical silent period, a measure of motor control, to be increased after heading.

Methods: Nineteen young male athletes from local football clubs were recruited and came to the lab twice to perform a session of heading and a (control) session of kicking. TMS induced cortical silent period and a procedural learning consolidation task were performed before and after each session. Data was analysed both with a Frequentist and a Bayesian linear model.

Results: Heading was not found to affect cortical silent period nor procedural memory consolidation. The Bayesian linear model provided moderate evidence for no effect of RSHI on cortical silent period.

Conclusions: By using a Bayesian linear model we provided evidence that heading does not affect cortical inhibition as measured by cortical silent period. Cortical silent period has been proposed as a sensitive way of measuring small changes of motor control, however, here it was not sensitive enough to detect any differences. A re-evaluation of the way cortical silent period is recorded in our lab is advised, especially in cases where the examined effects are subtle in nature.

INTRODUCTION

In the previous chapter all measures aimed to investigate cortical inhibition and GABA level fluctuations in the brain provided no evidence for alterations caused by RSHI. This raises concerns about the findings of Di Virgilio et al. (2016) study, in which cortical inhibition was found to be increased post heading. Taking also into account the low power of that study (~.50) and the lack of correction for multiple comparisons (both excitability and inhibition were examined in this study) concerns are raised about the replicability of those findings. Based on this, it was decided to examine if the findings of Di Virgilio et al. (2016) replicate. As in the experiments in Chapters 5 to 8, a kicking condition was added as a control for the effects of time, and exercise on cortical inhibition as measured by the cortical silent period (CSp) duration. We also included a task to measure procedural learning consolidation, a function in which the motor cortex plays a crucial role (Muellbacher et al. 2002).

Cortical inhibition as measured by TMS induced CSp reflects inhibitory circuits in the cortico-cortical and corticospinal tract (Kobayashi & Pascual-Leone, 2003). Those mechanisms have been found to be mediated by the GABA (γ-aminobutyric acid) neurotransmitter (Inghilleri et al. 1993; Kobayashi & Pascual-Leone, 2003; Scott et al. 2020). Increased CSp and cortical inhibition has been found to be increased post-concussion (Ntikas et al., 2021; Christyakov et al., 2001; De Beaumont et al., 2011; Miller et al., 2014; Scott et al., 2020), however in the field of RSHI it has been used less than a handful of times, showing significant CSp increases post RSHI exposure in football, boxing and rugby (Di Virgilio et al., 2016; Di Virgilio et al., 2019; McNaab et al., 2020).

Increased GABA on the brain post-injury is considered beneficial for the self-healing brain processes, however, if this increase is consistent and repetitive can create a toxic environment affecting normal brain development and functioning (Demirtas-Tatlidede et al., 2012). Therefore, given the importance of GABA for brain functioning, the previous findings that RSHI increase GABA, and the findings from the previous chapters showing impaired learning and working memory, but no changes in the more specific measures of GABA (SRTT) and inhibition (Go/No-Go), an investigation of the CSp levels before and after heading is crucial to identify if he focus of future research should be on the GABAergic processes post heading or how heading affects the working memory and learning processes.

Paired with the CSp measure, an additional SRTT was used, this time to investigate if heading affects the procedural memory consolidation. Memory consolidation is the process in which a newly acquired motor skill/memory gradually becomes more stable and solid after an initial stage of instability during which it is subject to interference (Brashers-Krug et al., 1996). During this consolidation period M1 has been found to play an important role, since its disruption causes impairments in the consolidation of the newly acquired motor memory (Muellbacher et al., 2002).

A series of studies have found that the disruption of M1 immediately after the implicit acquisition of a motor sequence (SRTT performance) impairs the consolidation of the newly acquired memory and causes a decrease in performance when the same SRTT is administered again (Brown & Robertson, 2007; Censor & Cohen, 2011; Cohen et al., 2005; Muellbacher et al., 2002; Richardson et al., 2006; Robertson et al., 2004). Most of those studies used repetitive Transcranial Magnetic Stimulation (rTMS) in order to depress the motor cortex excitability.

Robertson et al. (2005) disrupted the M1 activity by using rTMS right after the acquisition phase of the new procedural memory, i.e. the first session that included the completion of an SRTT alteration, and found that this M1 disruption blocked the off-line improvements usually seen when the same SRTT sequence is presented again to participants, hours after the first session. This impairment in the off-line learning was evident when no sleep has taken place between the first and the second session. Similarly, Muellbacher et al (2002) found that the consolidation of a motor memory was impaired by applying rTMS over the M1 area. In this study they conducted several experiments in which rTMS was applied over M1, dorsolateral Prefrontal cortex (DLPFC), occipital cortex. Only rTMS over M1 caused impairments in the motor memory consolidation, a finding that illustrates the importance of M1 on the process of motor memory consolidation.

As mentioned in a previous section, in the literature of repetitive subconcussive impacts M1 was found to be affected (DiVirgilio et al. 2016). More specifically, the M1 excitability was found to be depressed. Since the depression of cortical excitability (cortical inhibition) of M1 was found to impair motor memory consolidation, in this study it will be examined if the consolidation of a newly learnt procedural memory can be impaired by a series of headers right after the procedural learning task (SRTT). Evidence of disrupted motor memory consolidation would serve as indirect but converging evidence that football heading causes the cortical inhibition of M1.

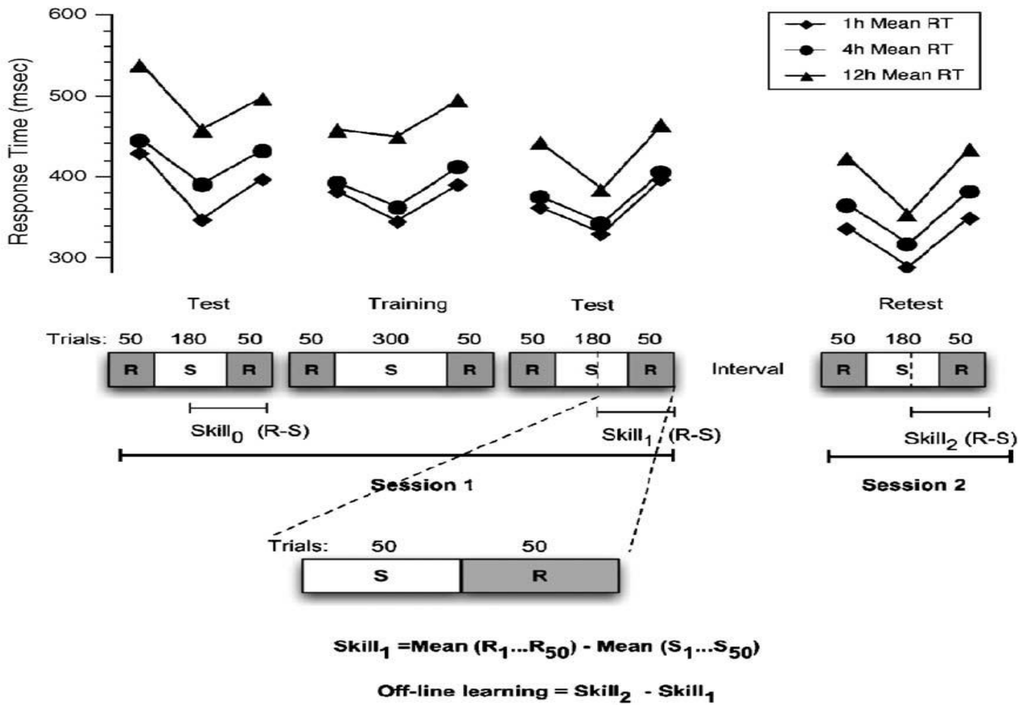

Figure 9.1 Off-line learning as indicated by increased Skill$_2$ compared to Skill$_1$ (Press et al. 2005). R = random trials, S = trails with the sequence.

The value of interest in this study will be the off-line learning as it is shown in Figure 9.1. Participants will be asked to do the SRTT before heading or kicking the ball and 8 hours later they will be presented again with a test block of the SRTT.

Based on the information presented above, it is hypothesised that athletes' off-line learning will be lower, or even absent in the heading condition, whereas in the kicking condition off-line learning will be evident. Concerning the cortical inhibition, it is hypothesised that any cortical inhibition increase should manifest as a prolongation of CSp post-heading compared to a baseline measure, this CSp prolongation is expected to be condition specific, thus absent post-kicking.

METHODS

Participants

The participants of this study were 19 male football players aged 24 (±3.41) years old recruited from local teams; (Table 9.1 for participant information). Participants had no learning difficulties, no neurological condition or history of brain damage, no psychiatric condition currently treated with psychoactive drugs and no history of sports-related concussion in the last year. Athletes were asked to refrain from recreational drugs the week before the sessions and from alcohol and caffeinate drinks 24 hours before the sessions. They were also asked to have the same breakfast and amount of sleep prior to the testing sessions. All athletes were reimbursed at a £8.50 per hour rate for their participation.

Table 9.1. Athletes' characteristics.

Participant	Age	Sex	Race	Level	Position
1	20	Male	White	Semi-Pro	CM
2	30	Male	Asian	Semi-Pro	DC
3	25	Male	Asian	Amateur	CM
4	23	Male	Asian	Semi-Pro	CM
5	26	Male	Asian	Amateur	DC
6	19	Male	Black	Semi-Pro	CM
7	25	Male	Asian	Amateur	DC
8	24	Male	Asian	Semi-Pro	FB
9	23	Male	Asian	Amateur	Winger
10	27	Male	Asian	Pro	DC
11	19	Male	Asian	Pro	Winger
12	31	Male	White	Black	CM
13	24	Male	Asian	Amateur	FB
14		Male	White	Semi-Pro	Winger
15		Male	White	Amateur	DC
16	26	Male	White	Amateur	DC
17	22	Male	White	Amateur	DC
18	23	Male	Asian	Amateur	Winger/CF
19	21	Male	White	Semi-Pro	DC

Materials

EMG. EMG data was recorded using a wireless system (Biopac Systems, Inc. Goleta, CA, USA) and disposable Ag/AgCl surface electrodes (Vermed, Devon, UK).

TMS. Monophasic single pulse magnetic stimulations of 1ms duration were applied using a magnetic stimulator and 110 mm double cone coil (Magstim 2002 unit, The Magstim Company Ltd., Whitland, UK).

SRTT. Athletes in this task performed the implicit version of SRTT. They had to respond to the stimuli they see on the screen by pressing the corresponding button. The stimuli were 4 circles in a horizontal array, as shown in the picture below. Each time a circle lights up blue the participant responds with a button that corresponds to the circle location.

The stimulus remained present until the correct response was made, and the athlete's response time (RT) was the total time from presentation to correct response. The task was presented to the athletes as reaction time task. Without them knowing, a 12-item sequence was introduced, allowing them to acquire skill at the sequence implicitly. Before the intervention (heading or kicking) athletes performed the SRTT in three blocks.

The session began with the first testing block consisting of 48 random trials followed by 180 sequence trials consisting of 15 repetitions of the sequence and ending with 48 random trials. A longer training block followed, also with 48 random trials at the beginning and end, sandwiching 300 sequence trials, consisting of 25 repetitions of the sequence. The session concluded with the final test block identical to the first test block. The 8-hour follow-up session consisted solely of one test block. Three separate measurements of skill were extracted. Skill 0: the skill acquisition from first test block, skill 1: the skill acquired at the second testing block, skill 2: the skill acquired at the follow-up session. Off-line learning was defined as the increase in learning from test 2 (Skill 1) to the retest block (Skill 2; Figure 9.1).

Skill was defined as the difference in RT between the mean of the final 48 random trials in the test block and the mean of the preceding 48 sequential trials, representing the improvement in RT that was achieved due to the sequence. Response times that were longer than two standard deviations greater than the mean RT for the session were excluded. Following the final session, participants' awareness for the underlying sequence was assessed and those with explicit knowledge of the sequence were excluded using both subjective.

Procedure

Pre-stimulation. Athletes were asked to come to the lab early in the morning. Athletes were seated in a customised load cell. Electromyography (EMG) was used to measure surface amplitude of the rectus femoris and vastus lateralis muscles. Previous work (Di Virgilio et al., 2016, 2019) has shown that the rectus femoris is the best muscle in the quadriceps femoris for the elicitation and recording of inhibitory/excitatory responses, therefore recording from the lateralis muscle was merely a backup that will not be analysed if the femoris recording is not compromised. Following the surface EMG for non-invasive assessment of muscles (SENIAM) recommendations (Hermens et al., 2000), electrode positions were selected, shaved, and cleaned with an alcohol wipe. Electrodes were then positioned 2 cm apart.

Transcranial Magnetic Stimulation. Athletes were instructed to extend their leg at ~20% of their maximum voluntary contraction guided by visual feedback of force production. Optimal coil location for generating motor evoked potentials (MEPs) was determined by measuring the sculp of participants from the nasion to the inion and placing the coil in the middle of this distance and contralaterally to the leg of interest. Athletes were stimulated multiple times around this area and the coil position producing the largest MEP was marked with ink and used for the rest of the study (Goodall et al., 2009). Active motor threshold, the lowest TMS stimulation able to produce MEP, was identified by asking participants to contract their leg to 20% of their maximum voluntary contraction, whilst single pulse TMS stimulations were applied. Stimulation intensity started at 25% of the stimulator's maximum output, increasing by 5% increments if four out of five stimulations did not elicit a visible MEP (Wilson et al., 1995). To assess corticomotor inhibition, participants were instructed to perform a 5s maximum voluntary contraction, during which a stimulation of 130% of the active motor threshold was applied. Athletes were stimulated 3 times with a rest period of 30 s between contractions.

SRTT. Following this, athletes were asked to sit in front of a computer and perform the SRTT which took 10 minutes to complete.

Immediately following this task, they were guided to the heading location in which they performed either 20 headers or 10 minutes of kicking as described in the previous chapters. After the intervention, the cortical inhibition of athletes was assessed again the way as before. Six to eight hours following that, athletes came to the lab again to perform the follow-up session of the SRTT. All athletes were asked to refrain from sleeping or consuming alcohol during this time. Athletes who took part in the heading session first were asked to come one week later to do the kicking session and vice versa.

Data analysis plan

EMG data was recording from the Biopac system (Biopac Systems, Inc. Goleta, CA, USA) and sampled at 2 kHz and filtered using 500 Hz low and 1.0 Hz high band filters. Data recorded from the Biopac system was imported to the Spike 2 (Cambridge Electronics Design Limited) where it was rectified. The cortical silent period (CSp) duration was manually extracted and was defined as the time from the end of stimulus artifact to the resumption of normal voluntary EMG activity (figure 9.2). A 2x2 repeated measures ANOVA was used to examine the main effects of intervention (heading – kicking) and time (pre-post) and their interaction. A Bayesian repeated measures ANOVA was performed in JASP statistical software, to examine the effects of time, intervention and time*intervention interaction. A Bayes factor of >3 was set as an indicator of moderate evidence and a Bayes factor of >10 as an indicator of strong evidence of the presence/absence of an effect. Data was also analysed with Bayesian linear models in R using the rstanarm package as described in previous chapter. Data from the SRTT was analysed with a 2x3 repeated measures ANOVA to examine the main effects of intervention (Heading, Kicking), Skill (Skill 0, Skill 1, Skill 2; figure 9.1) and their interaction.

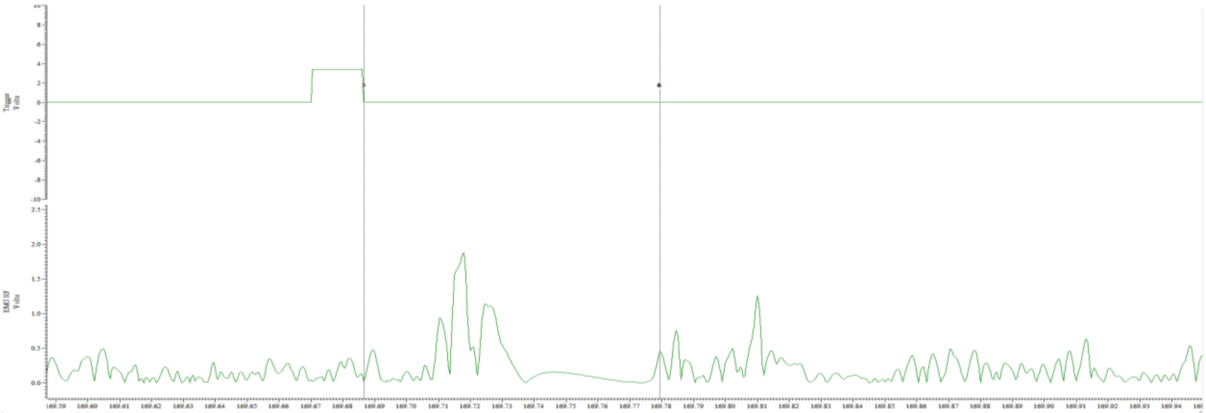

Figure 9.2. Rectified EMG signal from Spike software. Top line represents the signal from the trigger and the bottom the EMG signal from the rectus femoris muscle. The vertical dotted lines indicate the start and the end of the CSp following visual inspection. The x axis represents time in milliseconds and the y axis the amplitude of in Volts of the muscular activity.

RESULTS

CSp. The repeated measures ANOVA reveal no effects of intervention (*p* = .126) and no effects of time (*p* = .254). There was also no significant intervention*time interaction; F(17,1) = .283, *p* = 599 (Figure 9.4).

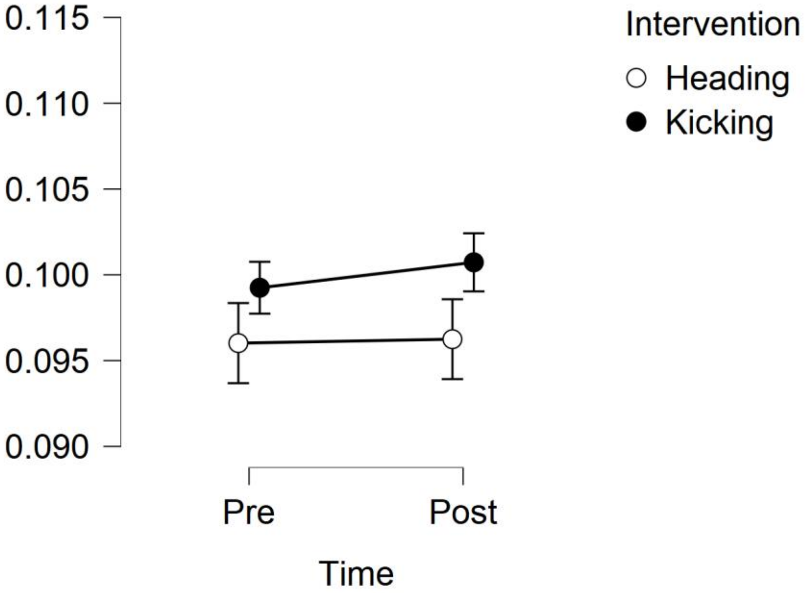

Figure 9.4 CSp duration in seconds is depicted on the y axis, and time (pre post heading or kicking) on the x axis. The error bars represent the standard error of the mean values.

The data was also examined by using a Bayesian linear model. The posterior distributions of the Bayesian linear model revealed no effect of Time; credibility intervals CI: [-2.52, 3.89], a slight possibility of effect of intervention; CI: [-5.12, 1.14] (Figure 9.5) and evidence for no time*intervention interaction; CI: [-4.80, 2.95], by inspecting the posterior distribution (Figure 9.6) it can be seen that there is a minor chance that there is an interaction, however, this will mean that CSp was increased in the control condition and not during heading. Thus, providing evidence that heading does not prolong CSp.

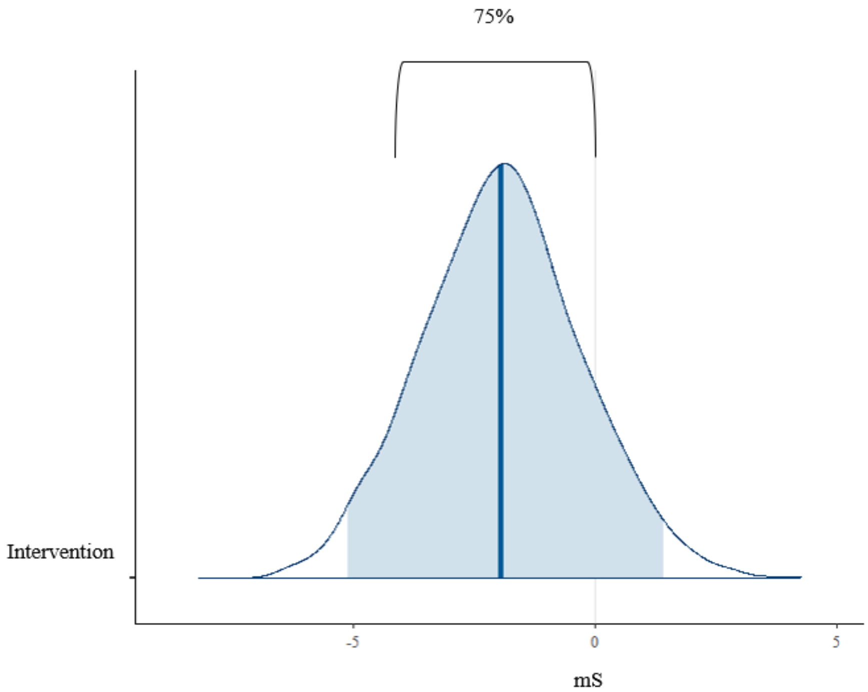

Figure 9.5. Posterior distribution of the Bayesian model showing the probability of an effect of intervention. Shaded blue area represents the 95% credibility interval and the blue line the coefficient of the effect. Values towards the left indicate greater chance for an intervention effect caused by increased CSp in the kicking condition and values towards the right indicate greater chance for an effect caused by increased CSp in the heading condition.

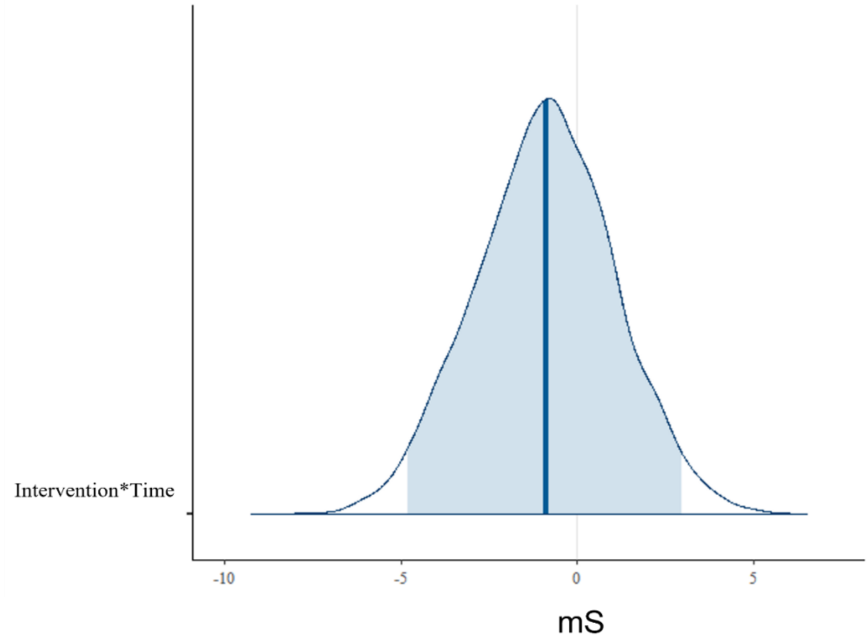

Figure 9.6. Posterior distribution of the Bayesian model showing the probability of an intervention*time interaction. Shaded blue area represents the 95% credibility interval and the blue line the coefficient of the interaction. Values towards the left indicate greater chance for an interaction caused by increased CSp in the kicking condition and values towards the right indicate greater chance for an interaction caused by increased CSp in the heading condition.

Lastly, the Bayesian repeated measures ANOVA in JASP revealed that there is not enough (anecdotal) evidence of an effect of intervention; Bayes factor of 1.2, moderate evidence for no effect of time, Bayes factor of 5 and moderate evidence for no effect of intervention*time interaction, Bayes factor of 7.21.

SRTT. Data from one athlete was lost and one athlete did not return for the 8-hour follow up, leaving a sample size of n = 17 athletes for the SRTT analysis. Two more athletes were identified as outliers and excluded from the analysis. The repeated measures ANOVA revealed no main effect of intervention (*p* = .41) or time (*p* = .56). There was no effect of intervention*time interaction, F(2,28) = 1.6, *p* = .22 (Figure 9.7).

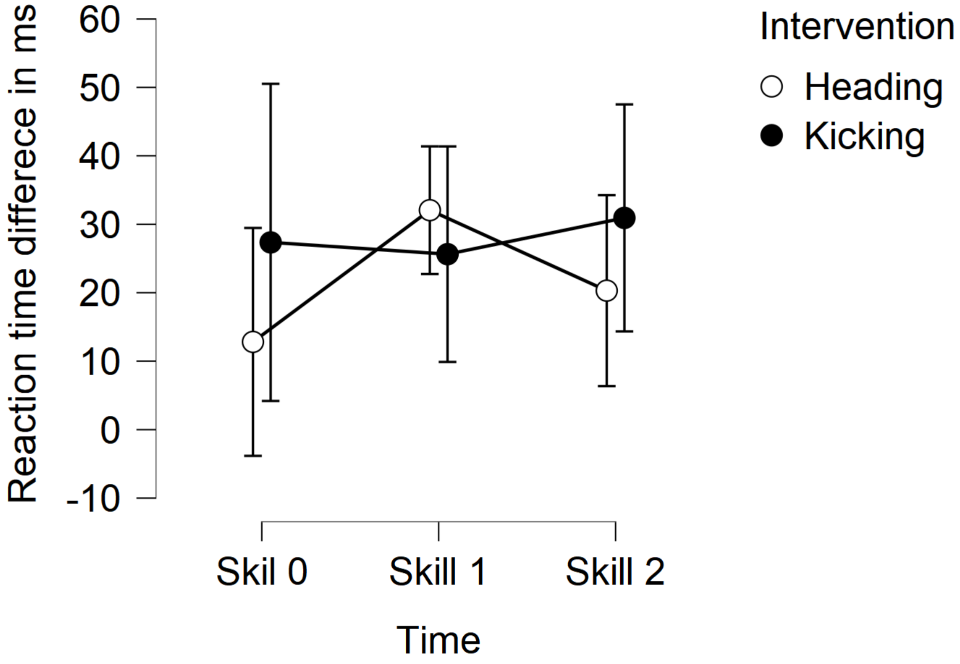

Figure 9.7. Mean reaction time difference per skill and intervention. Error bars denote standard error of the mean.

DISCUSSION

The hypothesis that heading will increase cortical inhibition over the motor cortex, as indicated by prolongation of CSp was not supported by the data. Notably, data provides moderate evidence for no effect of heading on CSp. Specifically, the Bayes factor of 7, indicates that the null hypothesis that there is no intervention*time interaction is 7 times more likely that the alternative hypothesis. The null finding is strengthened by the fact that, after inspecting the posterior distribution of the Bayesian model, it can be seen that this very small likelihood of an interaction effect is driven by a slight pre-post CSp prolongation during the kicking session, which is an interaction in the opposite direction than expected.

This finding raises questions about the replicability of the findings reported by Di Virgilio et al. (2016). Their study reports a 4.33% (5.2ms) prolongation of CSp, remarkably lower than CSp increases found after rugby tackles (27% increase in McNabb et al., 2022) and following sport related concussion (~16% increase in Pearce et al., 2015). Considering the low power of that study (~.50) and the lack of correction for multiple comparisons of TMS measures, concerns are raised about the validity of the findings reported by Di Virgilio et al. (2016).

Since CSp length is mediated by the $GABA_b$ neurotransmitter (Kobayashi & Pascual-Leone, 2003) the absence of any prolongation contradicts the theory that the adverse effects of heading on football players are caused by an influx of GABA in their cerebral cortex. This chapter, in the progression of the thesis, was the third study (each involving separate samples) targeted at assessing the evidence for cortical inhibition in the motor cortex and/or cortex in general. The evidence of this third study provides converging evidence with the previous two (Chapter 6 and Chapter 7). In Chapter 6 (behavioural measures of cognitive functioning), the procedural learning acquisition, a process linked with the GABA activity in motor cortex (Kim et al., 2014; Kolasinski et al., 2019; Stagg et al., 2011), was not affected by heading. Moreover, in Chapter 7 (ERP oddball and Go/No-Go) no evidence for alteration in response inhibition of athletes was found, again a process in which any alterations of GABA activity should manifest as changes in the N2 and/or P3a ERP components (Cheng et al., 2017; Quetscher et al., 2015). Taken together, those findings refute the premise that cortical inhibition, as manifested in motor control dampening, is the brain process predominantly affected by football heading or sport related RSHI in general, the so-called RSHI GABAergic response hypothesis (Di Virgilio et al. 2019).

However, in the TMS measurements of this thesis the leg muscles were used and stimulations were performed while athletes performed a maximum voluntary contraction (MVC), this decreased the number of stimulations that could be performed. Since an MVC produces similar motor evoked potentials with a contraction of lower intensity (Temesi et al., 2014), using a lower level of contraction and performing more stimulation might be a better way to detect subtle effects. A methodology like this produced the larger CSp differences found in rugby athletes (McNabb et al., 2020). For the purposes of this thesis, and since the aim was to replicate Di Virgilio et al. (2016; 2019) it was decided for the TMS protocol to be exactly the same as in those studies. By doing so, any null findings cannot be explained as merely a result of the deviation from Di Virgilio’s methods. Future studies should aim to use methodologies more likely to produce larger effects.

Lastly, the second hypothesis of this chapter was also not supported by the data. Heading did not affect procedural memory consolidation in a different way that kicking. This finding is in accordance with all the previous findings in this thesis that examined the functioning of motor cortex. However, the findings of this task should be approach with caution, as the sample size is limited (15 athletes) and the task did not produce off-line learning as previously reported in the literature (Brown & Robertson, 2007; Robertson et al., 2004).

This chapter was the final effort of this thesis to find proof of an inhibition of motor cortex caused by GABA increases. Recent findings suggested that cortical inhibition might be one of the main mechanisms of injury caused by RSHI in sport (Di Virgilio et al., 2016, 2019; McNabb et al., 2020), however, the experimental chapters of this thesis consistently failed to find proof of such an effect. The importance of the absence of effects on procedural learning, corticospinal silent period and response inhibition are discusses further in the next chapter (Chapter 10, general discussion).

CHAPTER 10. General discussion

Summary of findings

The main aim of the present work was to comprehensively examine if RSHI in football, and specifically heading, has detrimental effects for the athletes' brain functioning by using the widest range of sensitive neuroscientific measures available to the author.

The first step was to identify the gaps in the literature and address the potential issues that cause the mixed findings presented to date. In Chapter 2 (published narrative review of the literature), a series of issues that needed to be addressed were identified. Those were, the lack of comprehensive mapping of the literature of biofluid markers, the absence of multimodal studies, the low quality of the literature caused by failure to control for specific confounding variables like concussions and exercise, and the absence of sensitive measures of cognitive functioning and the limited use of brain-based (e.g., electrophysiological) measures of cognitive functioning.

Consequently, to address the first issue mentioned above, Chapter 3 presented the first effort to map the literature on the effects of RSHI on biofluid markers. In that scoping review a surprising high number of studies was found (79). By inspecting those studies, it becomes apparent that the use biofluid markers to examine the effects of RSHI is an emerging field with the number of studies on this topic now increasing exponentially. However, the studies found are very heterogeneous, employing a wide range of biomarkers sampled at different times to examine the effects of RSHI on active and former athletes partaking in a wide range of contact sports. Notably, a series of issues on the majority of studies was found, with only a small fraction of them being of good quality and having a low risk of bias. Even though the literature was found to be very heterogenous, some useful information was extracted, with the use of biofluid markers like NfL, tau, GFAP and NSE found to be promising in detecting brain changes caused by RSHI.

Despite the fact that the use of the aforementioned biofluid markers was found to be promising, the question on when to sample after RSHI was left unanswered. It was therefore decided that further piloting is necessary before they can be included in the experimental part of this thesis. The use of biofluid markers is also more useful in providing additional information when paired together with neuroimaging techniques like fMRI, MR Spectroscopy, DTI etc. and not measures of cognition and motor control like the ones used in this thesis. Importantly, the findings of this thesis were used to inform the design of a new study that commenced late in the second half of 2022 when the work of this thesis had concluded, which will include EEG and biofluid markers in parallel with MR spectroscopy, fMRI and MR diffusion measures to examine the acute effects of heading on football players.

The final step before moving to the experimental part of the thesis included an investigation of the burden of concussion and TBI in sport.

A graphic representation of the process followed in the experimental chapters of the thesis is presented below. The graph is a flow chart of the thought process and progression of the thesis and shows how each step forward was based on an informed decision based of the findings up to that point.

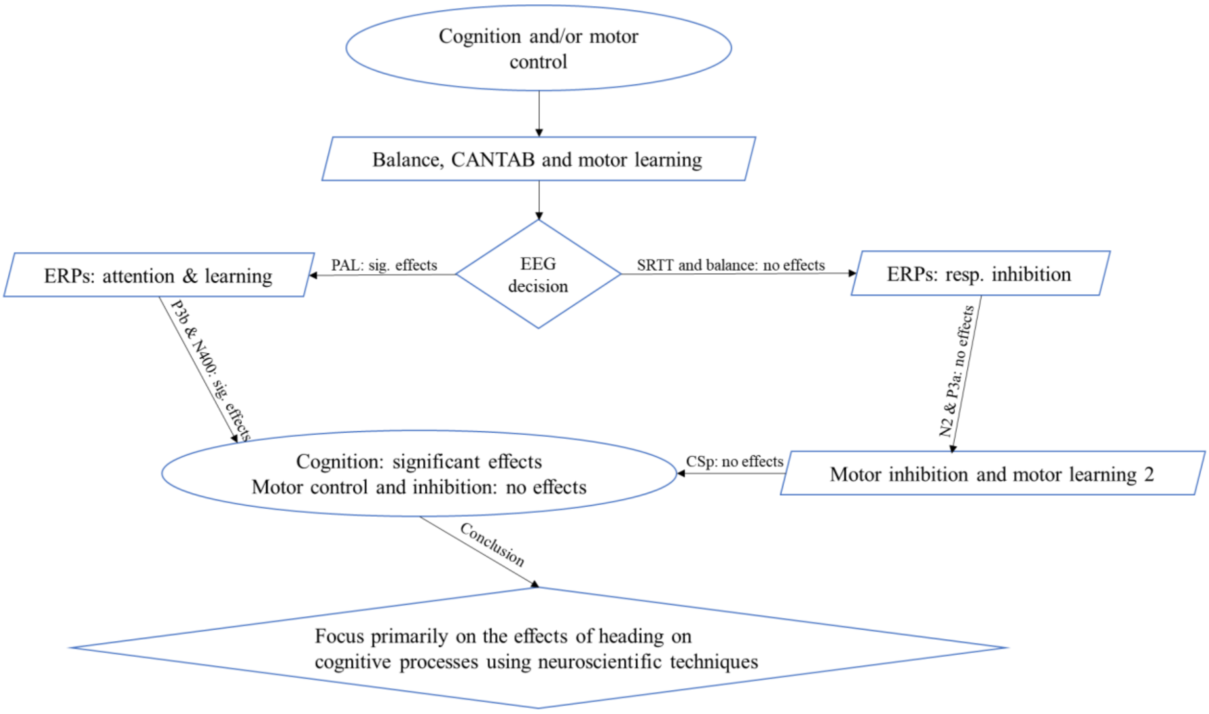

Figure 10.1. Flow chart of thesis thought process and progression.

Initially, a range of motor control and cognitive functions were assessed (balance, procedural learning, spatial working memory and paired associates learning), providing evidence only for changes on associative learning caused by heading. That task that examined athletes’ ability to form simple non-verbal paired associations. Following those findings, it was decided to focus the electrophysiological part of the experimental chapters of this study on the cognitive function of leaning.

Learning, however, is a complex process and learning disruption can be also caused by impairments on other cognitive functions, especially attention. Attention has been found to be necessary in the process of forming new paired associations (associative learning) both in animals (Mackintosh, 1975) and humans (Kruschke, 2001; Kruschke & Blair, 2000; Le Pelley et al., 2016). Therefore, an attention and working memory EEG task was included in the experimental procedure (oddball task). The electrophysiological responses of interest in the oddball task reflect attention allocation and working memory updating processes and have been linked with performance on learning tasks that do not require elaborate learning strategies (Fabiani et al., 1986, 1990; Nieuwenhuis, 2013). Thus, the use of a task that requires elaborate learning strategies like the one used in Chapter 8 (ERP verbal learning) could serve as a “differential diagnosis”, where effects on the verbal paired associations task in absence of effects in the oddball task would indicate impairments solely in the learning processes, whereas effects solely on the oddball task would reflect impairments predominantly in attention that influences some learning tasks that require attention (PAL).

The data collected from both tasks provided evidence of detrimental effects of RSHI on both attentional processes and learning that involves elaborate learning strategies. Therefore, no evidence was provided that RSHI affect solely attentional or learning processes, but the findings suggest that both attentional and learning processes are affected by heading. Those findings indicate a global disruption of football players’ cognitive functioning caused by heading, which affects both their ability to focus and to learn new information. This replication of the effect on learning processes (as initially found in Chapter 5, behavioural measures of cognitive functioning) on a separate sample of athletes, strengthens the concerns that RSHI have detrimental effects on athletes cognitive functioning, and especially in learning processes. Those effects on cognitive functioning were found to be independent of level of play, position, age, years of contact sport participation and magnitude of head impact. However, grip strength (as an indicator of general body strength including neck strength) was found to be a moderator of the effects seen in the oddball task, with stronger athletes experiencing lesser decrease of the ERP component indicative of attention and working memory problems. As mentioned in Chapter 7 (ERP oddball and Go/No-Go) this finding can lead to the conclusion that stronger and better trained athletes are less affected by RSHI. However, there is an alternative explanation for this effect; stronger and better trained athletes might have sustained more RSHI throughout their career and the weeks prior to the experimental sessions, so the effect of RSHI might appear lesser compared to the rest of the athletes, primarily because their cognitive functions are already affected prior to the experimental sessions. This was assessed by using the data from the control condition. In this case grip strength and age were found to moderate attention and memory, with stronger and older athletes having smaller P3b effect amplitudes. It is hard to explain the finding that stronger and more trained athletes have reduced P3b effect amplitudes, but it can be suggested that the P3b effect amplitudes of stronger and better trained athletes are already severely affected by RSHI, due to their higher exposure to RSHI during matches and training.

This finding needs further investigation; cross sectional studies on active football players in which recent head impact exposure, as well as, cumulative head impact exposure throughout their career would be assessed as predictors of P3b effect amplitudes during an oddball task.

Along the EEG measures of attention and learning, a task assessing the response inhibition processes of the athletes was implemented. This task was chosen in order to explore any effects RSHI might have on the inhibition processes of athletes' brains, as have been previously reported (Di Virgilio et al., 2016). There is no reason to believe that increased GABAergic activity (as reported in Di Virgilio et al., 2016) is area specific[7], so if there is increased GABA concentration over cerebral cortex, a Go/No-Go task, in which performance is correlated with GABA levels in striatum and superior temporal gyrus (Cheng et al., 2017; Quetscher et al., 2015), should provide evidence of GABA level alterations. The data from this task provided no evidence of response inhibition alterations caused by heading, adding to the list of findings in this thesis that failed to make a specific link between GABA neurotransmitter with RSHI.

So far, a pattern becomes apparent, in which tasks designed to assess the effects of RSHI on cognitive functions provide evidence of effects, while tasks designed to assess motor control and specifically GABA concentrations provide no evidence of any effects. Following this, it became apparent there is a need to provide a clear answer to whether motor control problems and a GABA increase were absent because the effect was very subtle or because they are not affected by RSHI. Therefore, it was decided to examine if the findings of increased TMS-induced cortical silent period, which were the basis for the GABAergic response hypothesis and motor control problems, replicate.

This final group of measures (Chapter 9), provided evidence of no effect of heading on cortical inhibition, defined as a prolongation of the cortical silent period, and no effect of heading on the consolidation of a procedural memory, a process that again requires activation of the motor cortex. These findings complete the picture of the evidence presented, in which repetitive heading, unlike sport-related concussion, seems to primarily affect basic cognitive processes like attention and learning, while leaving postural stability and motor control processes seemingly unaffected.

Interpretation of findings & Mechanisms of "injury"

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[7] Di Virgilio et al., 2016 found increased cortical inhibition in M1, which they argue was a consequence of increased GABA in that area.

The findings presented in Chapters 5 to 9 provide very useful information with regards to potential neurological mechanisms triggered by repeated head impact exposure. The main aim of this thesis was to examine if RSHI, and specifically heading, have acute adverse effects on the brain functioning of football players. The evidence presented here shows that athletes cognitive functioning is affected immediately after 20 headers of moderate intensity. The effects on cognitive functioning were reported in separate samples of the same cohort of people and by using different methodologies, something that provides further confidence that the effects seen are not false positive or sample specific.

Although the measures used were not direct measures of pathological (degenerative) processes, they can provide valuable insights in which brain processes might be causing the attentional and learning problems found here. Importantly, the findings can provide information on the potential neurotransmitters involved following sub-concussive impacts. Although, many neurotransmitters can be potential linked with the effects of RSHI, in the findings presented in this thesis three neurotransmitters appear to be more likely to play an important role in those effects, acetylcholine, noradrenaline and GABA.

The cholinergic hypothesis

Acetylcholine appears to be the main neurotransmitter potentially affected by RSHI. To begin with, paired associative learning has been linked with the activity of the neurotransmitter acetylcholine (Hasselmo, 2006). Several studies used cholinergic antagonists and agonists to examine how acetylcholine affects performance of animals and humans on learning tasks (Tang et al., 1997; Winters & Bussey, 2005). The use of drugs that disrupts cholinergic activity impair the encoding of new information, while leaving the retrieval of previously learnt information unaffected (Hasselmo, 1995; Hasselmo et al., 1996; Tröster et al., 1989). Performance on CANTAB’s paired associatives learning (PAL) task used in Chapter 6 (Behavioural measures of cognitive function), has been found to have a dose-response relationship with the administration of a acetylcholine antagonist on healthy young adults, with higher dosages of the antagonist impairing PAL performance at a greater level (Harel et al., 2013). This impaired performance was reduced when a cholinesterase inhibitor was administered to the same individuals.

This effect of acetylcholine of associative learning is not limited to non-verbal associative learning. Verbal paired associations learning was found to be acetylcholine dependent, with acetylcholine antagonists impairing new word paired association learning by increasing proactive interference (Atri et al., 2004). In Chapter 8 (ERP verbal learning) athletes post heading were found to struggle to learn new verbal associations compared to post kicking. Moreover, cholinergic agents have been found to affect the amplitude and the latency of the P300 ERP component (Dierks et al., 1994; Hammond et al., 1987). As seen in Chapter 7 (ERP oddball and Go/No-Go) heading affected (reduced) the amplitude of the P3b effect. These findings can also be indicators of attention and learning problems caused by acetylcholine imbalances.

Interestingly, the learning impairments caused by acetylcholine imbalances are paired associative learning specific, and leave over types of learning like procedural and semantic unaffected (Broks et al., 1988; Nissen et al., 1987). Nissen et al. (1987) examined the effects of a cholinergic antagonist on different types of learning and memory. They found that reduced acetylcholine affects the memory of a list of words but does not affect procedural learning acquisition and retention as examined by the performance on a serial reaction time task (SRTT). This finding is in accordance with the findings of this thesis, in which procedural memory acquisition and consolidation, defined as the performance on a SRTT, was unaffected by heading, while the formation of words association was impaired.

The noradrenergic hypothesis

Noradrenaline is another neurotransmitter which imbalances might be responsible for the adverse effects caused by football heading.

As mentioned in Chapter 7 (ERP oddball and Go/No-Go) the parietal P300 (P3b) is associated with the noradrenaline projections of locus coeruleus (Nieuwenhuis, 2013; Nieuwenhuis et al., 2005). Those noradrenergic neurons project to frontal and parietal brain areas, and play an important role in arousal, attention and stress response (Benarroch, 2009). The relationship between P3b and noradrenergic projections was established in imaging (Soltani & Knight, 2000) and lesion studies (Pineda et al., 1989; Swick et al., 1994). So, the finding that football heading causes a decrease in the amplitude of P3b effect can be an indicator that heading affects LC functioning. This decrease of noradrenergic activity will have as a result the decreased alertness during tasks that require sustained attention, like the oddball task. Moreover, decrements in learning can be directly linked to attentional problems, thus the effects found in the learning tasks can be an outcome of decreased alertness and ability to focus. This can also explain the absence of learning decrements in procedural learning tasks like SRTT that do not require the use of attentional resources.

LC degeneration is present at the early stages of Alzheimer’s and Parkinson’s disease (Bari et al., 2020; German et al., 1992; Rommelfanger & Weinshenker, 2007; Weinshenker, 2008), so a RSHI induced LC dysfunction can provide a possible mechanism for both the acute findings found in this thesis and the long-term neurodegenerative diseases associated with RSHI.

The GABAergic hypothesis

GABA neurotransmitter was the main neurotransmitter of interest at the early stages of this thesis. This was the case because past studies had found brain alterations which could be linked with GABA and learning decrements caused by RSHI which again can be potentially linked with increased GABA activity (Di Virgilio et al., 2016; Di Virgilio et al. 2019).

Contrary to those findings, in the chapters of this thesis no evidence for an effect of GABA was found. All GABA measures were indirect, as were the measures used by Di Virgilio et al. (2016; 2019), but they do not provide any indications of specific GABA changes. Procedural learning, a function linked directly with GABA concentrations (i.e., Stagg et al., 2011) on the motor cortex was found to be unaffected (Chapters 6 & 9). Similarly, response inhibition, a process that considerable GABA alterations should affect, was also found to be unaffected by heading (Chapter 7, ERP oddball and Go/No-Go). Finally, in order to provide a more definite answer to whether GABA is affected, in Chapter 9 (TMS and motor learning) a replication of the Di Virgilio et al. (2016) findings was performed, with the addition of a control condition (kicking). In this chapter, moderate evidence for no effect of heading on the indirect measure of GABA concentrations was found. Based on that, it can be assumed that either the Di Virgilio et al. (2016) findings were a false positive or that the effects on GABA are very subtle and athlete dependent so they cannot be easily replicated. Here it should be noted that both in Di Virgilio studies and in this thesis the methodology used (single pulse TMS, inducing a CSp) was an indirect measure of the $GABA_b$ neurotransmitter, different TMS methodologies can be used to examine changes in the concentrations of neurotransmitter $GABA_a$. Paired pulse TMS, for example, can provide information about both $GABA_a$ and $GABA_b$ neurotransmitters. As mentioned in Chapter 9, different CSp protocols that include larger number of stimulations can also be used to examine if RSHI produce subtle CSp differences.

To conclude, no direct evidence of neurotransmitters imbalances was found in this thesis, this was not possible due to the nature of the measures used, however, acetylcholine and noradrenaline were found to the neurotransmitters which should be investigated further.

Implications

The findings presented in the chapters of this thesis have very important implications about the brain health of contact sport athletes.

The finding of reduced attention and impaired verbal and associative learning in young, otherwise healthy, athletes raise concerns about the effects their contact sport participation can have in their everyday and academic life. Being able to maintain focus in class is a major factor of academic success, as is the ability to learn and comprehend new information, especially verbal information. The finding that heading impaired the ability to learn new verbal information by failing to establish a neural network that links this newly acquired knowledge with already existing knowledge (neurolexical network) should increase concerns about whether contact sport participation becomes an extra obstacle that students must surpass in order to achieve academic success.

Sports and being active are widely considered as activities that improve cognitive functioning and general health, even immediately after that exercise (Chang et al., 2015). However, in contact sports concussions and RSHI, as seen in this thesis, can not only hinder this improvement, but cause additional impairments and hinder cognitive functioning.

Contrary to the belief that RSHI might cause motor control problems (Di Virgilio et al., 2016; McNabb et al., 2020), in this thesis no evidence for such an effect was found. Such findings provide a reassurance to the rising concerns of coaches and athletes that RSHI might increase susceptibility to muscular injuries caused by problems on balance and general motor control of athletes during training and games. However, as mentioned earlier, contact sport participation should be considered an issue by teachers and schools as it can hinder learning.

In Chapter 4 (TBI prognosis) it was revealed that a considerable proportion of patients with sport-related mTBI that attend hospital have not fully recovered and report physical and mental health issues 3 and even 6 months post injury. Taken together with the finding that head impacts impair the cognitive functioning of athletes, the repeated nature of RSHI might put people recovering from mTBI at risk and prolong the recovery period.

Contribution to the field

In the 3rd chapter (Scoping review) of this thesis the first mapping of literature of biofluid markers was performed. This fills a gap in the field, since biofluid markers are the main method to assess the effects of RSHI that has not been appropriately reviewed yet. This while there is an increased interest in utilising fluid biomarkers in investigating the relationship between sport and dementia recently reported by Alzheimer research UK (Alzheimer’s research UK, 2022). The findings of the scoping review highlighted the heterogeneity of the literature, identified some promising biofluid markers and offered a set of guidelines for future studies. Those guidelines will be crucial in minimising the heterogeneity of the field and help provide a more concrete answer on which biofluid markers are more efficient in identifying the effects of RSHI and provide additional insights on the brain mechanisms behind the observed adverse effects of RSHI.

The experimental chapters of this thesis provided a much-needed comprehensive exploration of the effects of RSHI on cognition and motor control. This was the first time a sensitive and widely used neuroscientific technique (EEG) was used to assess the acute effects of football heading. The findings of chapters 7 and 8 showcase EEG as a new effective method to investigate the cognitive aspect of the RSHI issue in contact sports. There are multiple benefits in using EEG to examine RSHI effects on brain function. First, EEG is a quick and very sensitive way to assess cognitive functioning and the rise in use of modern validated quick-fit EEG systems means that sports can incorporate EEG measures in the assessments their athletes' go through routinely, such as health and fitness assessments. So far, the sensitive measures to assess the effect of RSHI involved the use of MR scanners or complex non-mobile and non-user-friendly neuroscientific equipment, however, new EEG systems can offer the benefits of a sensitive neuroscientific method paired with the quickness and the ease of use found in balance and cognitive tasks which are routinely used in concussion assessments (i.e., SCAT5). EEG measures also offer a very sensitive tool to investigate multiple cognitive and motor control functions, that can further inform the studies aiming to examine the effects of RSHI on a brain level and also offers a sensitive way to examine and assess the effects of RSHI in real time. Another method that utilises EEG is the TMS evoked EEG potential, a technique in which the effect of the TMS stimulation is assessed by changes in EEG activity, a process that is not brain area restricted and removes the confounding effects of spinal cord excitability, as is the case with conventional TMS research (Opie et al., 2017).

Furthermore, the parallel investigation of cognitive and motor control processes offers a richer interpretation of the findings of this thesis, rarely found in RSHI studies. Here, and unlike what seen in concussion, motor control was not the main function affected by RSHI (motor control was found to be unaffected by RSHI in Chapters 5, 6 & 9). Unlike motor control, cognitive processes appeared to be way more promising in the examinations of such effects and should become the main measures used in future dose-response evaluations. Identifying the right measures to examine RSHI is of huge importance for the field, as, especially in its early stages, it suffered from the use of inefficient and non-sensitive methods. Here it is argued that impairments in cognitive functioning as assessed by sensitive neuroscientific methods, paired with neuroimaging and/or certain biofluid markers where possible, are the way forward for the field.

Future steps

Repetitive subconcussive impacts in contact sports gain more and more attention the past two decades. This increase in interest is also evident in the number of efforts to address this issue. However, since RSHI are not linked with observable “concussion-like” acute effects, but with subtle acute effects and long-term neurodegenerative problems it is crucial that any attempts to examine those effects are performed with the appropriate measures.

Based on the findings presented in this thesis, more EEG studies are necessary to form a better picture of the subtle effects RSHI have on athletes’ cognition. To avoid confusion and mixed findings, neurocognitive tasks used in concussion assessment or in clinical populations should be avoided. EEG signal can be used to examine any aspect of cognition and neural network connectivity, so its use in the field of RSHI has the potential to provide evidence for multiple functions that might be affected. Future studies should employ ERPs used to examine subtle individual differences in cognitive functions like working memory, recall, decision making, visual processing, selective attention, executive functioning etc. as well as, resting-state EEG measures to examine the effect of RSHI on neural networks’ connectivity.

Moreover, NfL, tau, GFAP and NSE were found as the biomarkers most likely to be sensitive enough to identify those effects, therefore future studies should aim to examine how RSHI affect those biomarkers both acutely and chronically and link them with specific brain mechanisms (i.e., axonal damage, glial damage etc.).

Earlier in this chapter the neurotransmitters acetylcholine and noradrenaline were presented as the possible neurotransmitters which alterations might cause the adverse effects on cognition. Those hypotheses should be further examined by investigating the cholinergic and noradrenergic activity at a brain level by using neuroimaging techniques or acetylcholine and noradrenaline agonists. Studies like those have the potential to provide the link between the effects on cognition and brain alterations.

Apart from the acute effects of RSHI on athletes’ brains, it is important to examine the effects those RSHI have throughout athletes’ careers. RSHI are performed routinely in training and games. Therefore, after establishing which cognitive functions and neurological processes are acutely affected by heading, longitudinal studies will be necessary in order to examine how repetitively inducing those brain alterations affects athletes throughout their career. Longitudinal studies are not a new addition to RSHI literature, however, here it should be stressed that what the author suggests is that those longitudinal studies should employ the sensitive measures that have been reliably found to detect the subtle acute effects of RSHI in experimental lab settings.

Another important finding in this thesis was the indication that different athletes' might be affected at a different amount from RSHI. Chapter 7 (ERP oddball and Go/No-Go) revealed a random effect of heading on P3b effect amplitudes over Pz, which means that allowing for individual differences improved the predictive model, thus supporting the idea that different people are affected by heading at a different amount. This finding should be explored further in future studies, which should aim to identify at-risk populations (level of play, age, sex, race, history of neurological and psychiatric problems). The sample size in the experiments performed in this thesis was not large and diverse enough to allow for cross sex, race or level of play comparisons, however the fact that athletes' strength predicts the adverse effects of RSHI provides the basis for future studies to try and examine the nature of this relationship further. There is also a need to investigate and quantify if and how neck strengthening exercises mediate the effects of RSHI. For example, are professional or semi-pro athletes (who are presumably stronger and better trained) affected differently from RSHI and if so, how?

Conclusion

This thesis provides important new insights in the effects of RSHI on athletes' brains. Here we provide evidence that the cognitive functions of attention and learning, functions crucial for young athletes social, athletic and academic development and quality of life, are affected acutely post heading. These effects on attention and learning can be a signal of certain neurochemical alterations in the brain (cholinergic or noradrenergic) which when repetitively induced can cause permanent alterations to the brain and neurodegenerative diseases.

Furthermore, a methodological implication of the work is that it highlights the important role of cognitive function assessments in detecting the effects of RSHI when research is performed properly and with the use of sensitive cognitive measures like EEG. When such rigour is applied, cognitive measures can reveal the potential neurological mechanisms at play following exposure to routine impact in sport. The approach applied here also allows for differential diagnosis with regards to the problem RHSI poses, as an important distinction was found between cognitive functions and motor control, with the latter being seemingly unaffected by RSHI.

The argument that RSHI exposure should be controlled because it affects athletes' motor control, which could hinder their athletic performance or cause further musculoskeletal injuries has been widely used particularly within the world of sport. The current work, instead, highlights that the main reason to control RSHI exposure is to ensure that athletes' academic performance and cognitive abilities are not hindered and to safeguard future brain health.

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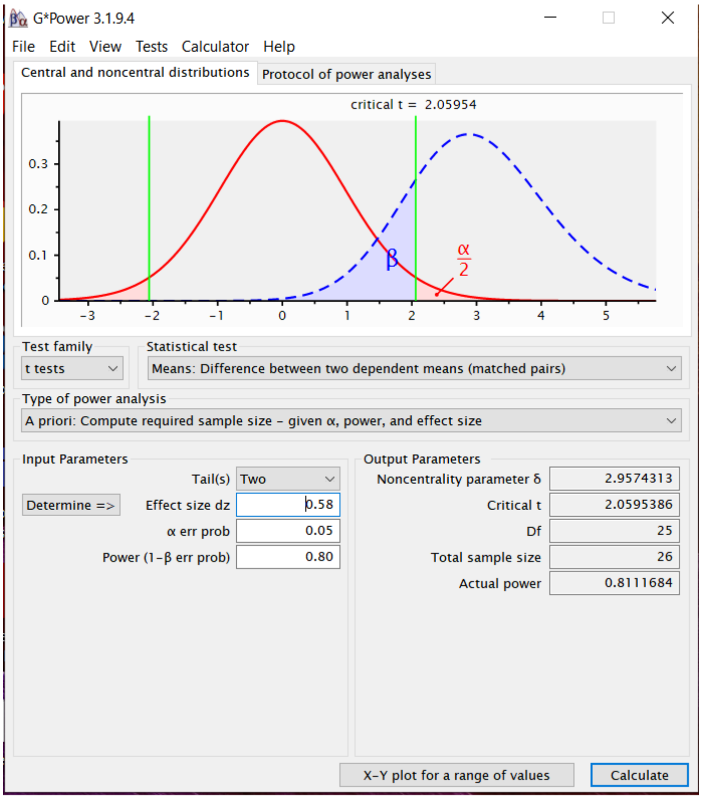
Appendix 1

1.1 Sample size calculations.

The error data on PAL recorded from Di Virgilio et al. (2016) was used to calculate the necessary sample size for a study with a power of .80. Di Virgilio et al. (2016) employed a design without control group, we used a control condition (making the presence of an effect more likely), however in order to be conservative, the power calculations were done as if our experiment had only the experimental condition. The effect size was .58, the significance value was set at .05 (two tailed test). As it can be seen in the Figure A1.1, the necessary sample size was 26 participants.



critical t = 2.05954

Test family: t tests

Statistical test: Means: Difference between two dependent means (matched pairs)

Type of power analysis: A priori: Compute required sample size – given α, power, and effect size

Input Parameters

Tail(s): Two
Effect size dz: 0.58
α err prob: 0.05
Power (1-β err prob): 0.80

Output Parameters

Noncentrality parameter δ: 2.9574313
Critical t: 2.0595386
Df: 25
Total sample size: 26
Actual power: 0.8111684

1.2 IMPACT METRICS MATLAB SCRIPT

linear acceleration:

```
function [average , linearAccel] = impacts(signal)
i = 1;
j = 1;
len(:) = length(signal);
accel = zeros(1,len);
while i <= len
   while signal(i+1,1)-signal(i,1) < 2
      SQ = sqrt(signal(i,2).^2+signal(i,3).^2+signal(i,4).^2);
      accel(i) = SQ;
      i = i + 1;
```

```
        if i == len
            break
        end
    end
linearAccel(j,1) = max(accel);
accel = 0;
j = j + 1;
i = i + 1;
end
average = mean(linearAccel);
end
```

Angular acceleration:

```
function [average , angularAccel] = angularaccel(signal)
i = 1;
j = 1;
len(:) = length(signal);
accel = zeros(1,len);
signalAcc = zeros(len);
for m = 5:7
    for n = 1:(len-1)
        signalAcc(n,m) = (signal(n+1,m)-signal(n,m))/(0.01);
        signal(n,m) = signalAcc(n,m);
    end
end
while i <= len
    while signal(i+1,1)-signal(i,1) < 2
        SQ = sqrt(signal(i,5).^2+signal(i,6).^2+signal(i,7).^2);
        accel(i) = SQ;
        i = i + 1;
        if i == len
            break
        end
    end
angularAccel(j,1) = max(accel);
accel = 0;
j = j + 1;
i = i + 1;
end
angularAccel = angularAccel.*0.0174532925;
average = mean(angularAccel);
end
```

1.3 BALANCE DATA ANALYSIS R SCRIPTS

All scripts presented below were used multiple times for each of the balance tasks and for the two planes of movement (anterior/posterior and medial/lateral).

3.3.1 Root Mean Square:

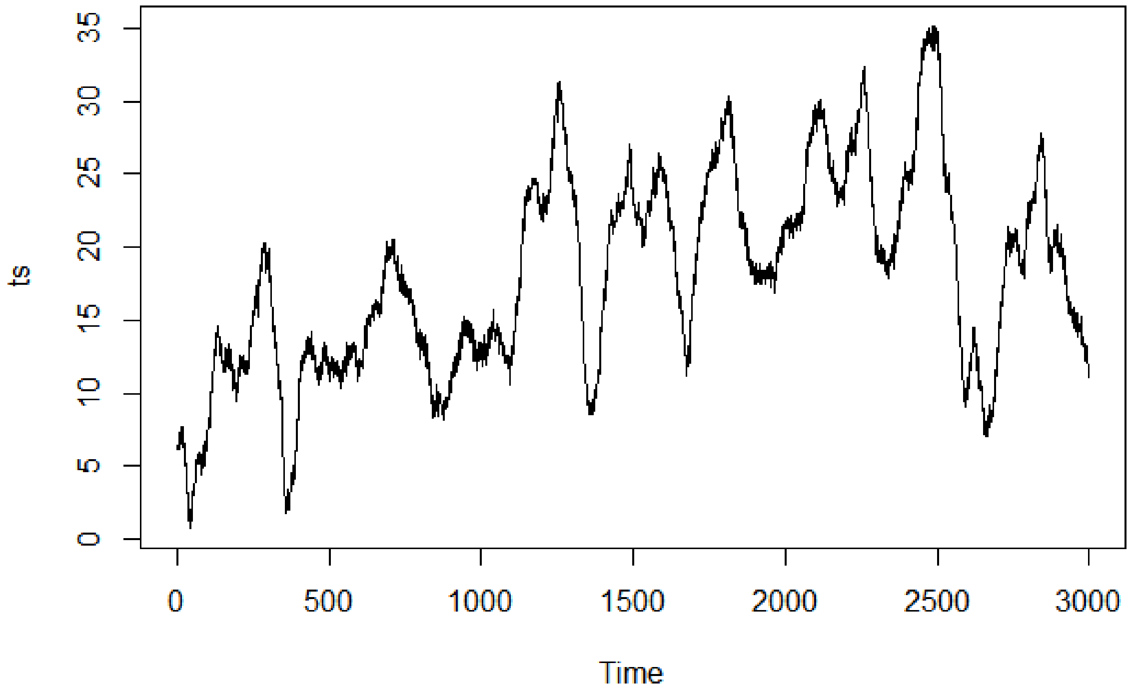
```
setwd("C:\\Users\\mixnt\\Desktop\\COP\\ECDTheading        #set path for the to-be-analysed
data
myFiles <- list.files()
vect <- 1:52                                              #set empty vector to be filled with values
for (i in 1:52) {                                 #computing RMS for each .txt file on path
indicated in line 1
  dataimport <- read.delim(myFiles[i], header = FALSE)
  mydata <- dataimport[501:3500,]
  ts <- mydata["V7"]
  ts <- unlist(ts)
  len <- length(ts)
  Mean <- mean(ts)
  Squearedsignal <- 1:len
  for (j in 1:len) {
  ts[j] <- abs(ts[j] - Mean)
  Squearedsignal[j] <- (ts[j])^2}
  MSq <- mean(Squearedsignal)
  value <- sqrt(MSq)
  vect[i] <- value}
  vect <- data.frame(vect)
  write_excel_csv(vect, file = "RMS7")                     #excel file containing the RMS values
```

3.3.2 Approximate entropy

```
library(pracma)
setwd("C:\\Users\\mixnt\\Desktop\\COP\\ECSTkicking")  #set path for the to-be-analysed
data
myFiles <- list.files()
vect <- 1:52;
for (i in 1:52) {                           #computing appr. Entropy for each .txt file on path
dataimport <- read.delim(myFiles[i], header = FALSE)
```

```
mydata <- dataimport[501:3500,]
ts <- mydata["V6"]
ts <- unlist(ts)
plot.ts(ts)
ApEnt <- approx_entropy(ts)
vect[i] <- ApEnt}
vect <- data.frame(vect)
write_excel_csv(vect, file = "ApEnECDT")   #excel file containing appr. Entropy values
```

Example COP signal plot:



3.3.3 Sway velocity

```
setwd("C:\\Users\\mixnt\\Desktop\\COP\\ECDTkicking")  #set path for the to-be-analysed
data
myFiles <- list.files()
vect <- 1:52;                                   #set empty vector to be filled with values
for (i in 1:52) {                          #computing sway velocity for each .txt file on path
indicated in line 1
dataimport <- read.delim(myFiles[i], header = FALSE)
```

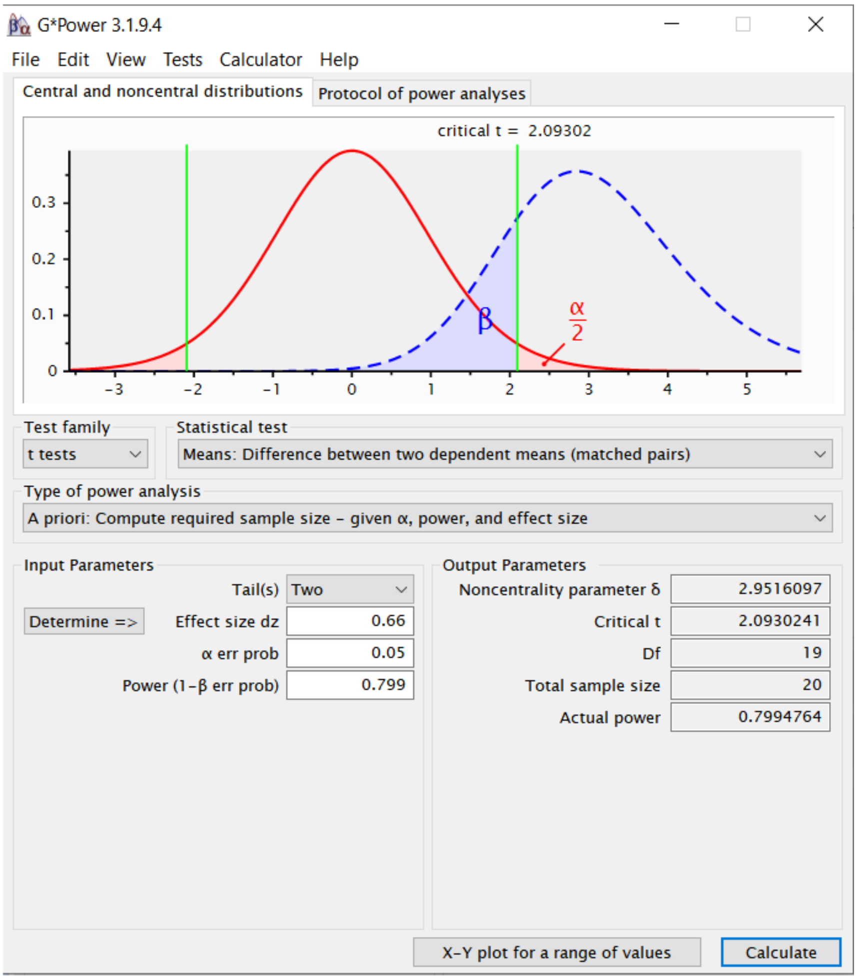
```
mydata <- dataimport[501:3500,]
ts <- mydata["V7"]
ts <- unlist(ts)
len = length(ts) -1
plot.ts(ts)
vel <- 1:len
for (j in 1:len) {
  vel[j] <- abs((ts[j+1] - ts[j]))/0.01}
Mvel<- mean(vel)
vect[i] <- Mvel}
vect <- data.frame(vect)
write_excel_csv(vect, file = "SwayVelocAP")   #excel file containing the sway velocity values
```

Appendix 2

2.1 Pilot EEG data.

EEG data from 13 athletes that took part in the studies described in Chapters 5 & 6 (Examining postural stability & examining behavioural measures of cognition) was collected in order to reveal the necessary sample size for having a study with a power of .80. Athletes performed only the oddball task, as described in chapter 7 (ERP oddball and Go/No-Go), due to time constrains. The data was analysed in the same way as reported in chapter 7, and the findings are presented below.

A paired samples t-test was performed to examine the difference of the P3b amplitude over Pz between the heading and kicking intervention. P3b was defined as the difference in amplitude caused by the rare stimulus compared to the frequent stimulus. The mean amplitude after kicking (Mean = 14.31) was significantly different compared to the amplitude after heading (Mean = 12.32); t(12) = 2.36, *p* = .36, ES = .66. The effect size of this pilot was used to determine the sample necessary for the study presented in Chapter 7 (ERP oddball and Go/No-Go). Results of the sample size calculation are presented below.



Test family: t tests

Statistical test: Means: Difference between two dependent means (matched pairs)

Type of power analysis: A priori: Compute required sample size – given α, power, and effect size

Input Parameters

Tail(s): Two
Effect size dz: 0.66
α err prob: 0.05
Power (1–β err prob): 0.799

Output Parameters

Noncentrality parameter δ: 2.9516097
Critical t: 2.0930241
Df: 19
Total sample size: 20
Actual power: 0.7994764

2.2. Scatterplot and best fit line for P3b amplitude over the Pz and N400 amplitude over the Cz. The graph includes the values of the 13 subjects that took part both in the oddball and the verbal learning task. Figure 1 represents the ERP amplitudes post heading and Figure 2 post kicking.

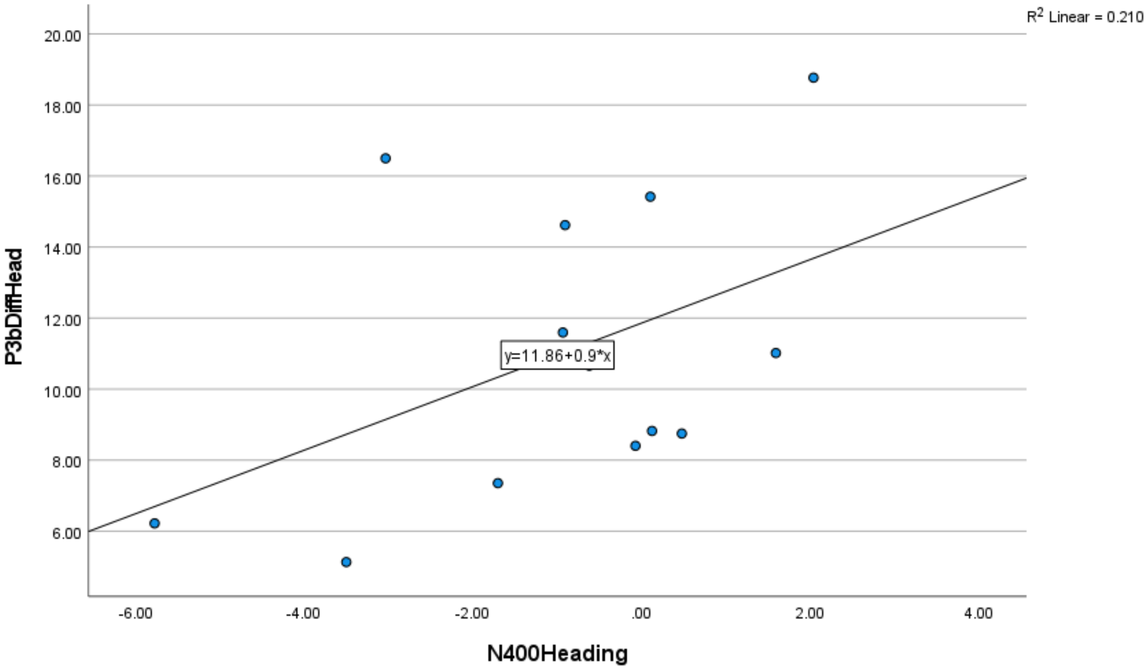


Figure 1

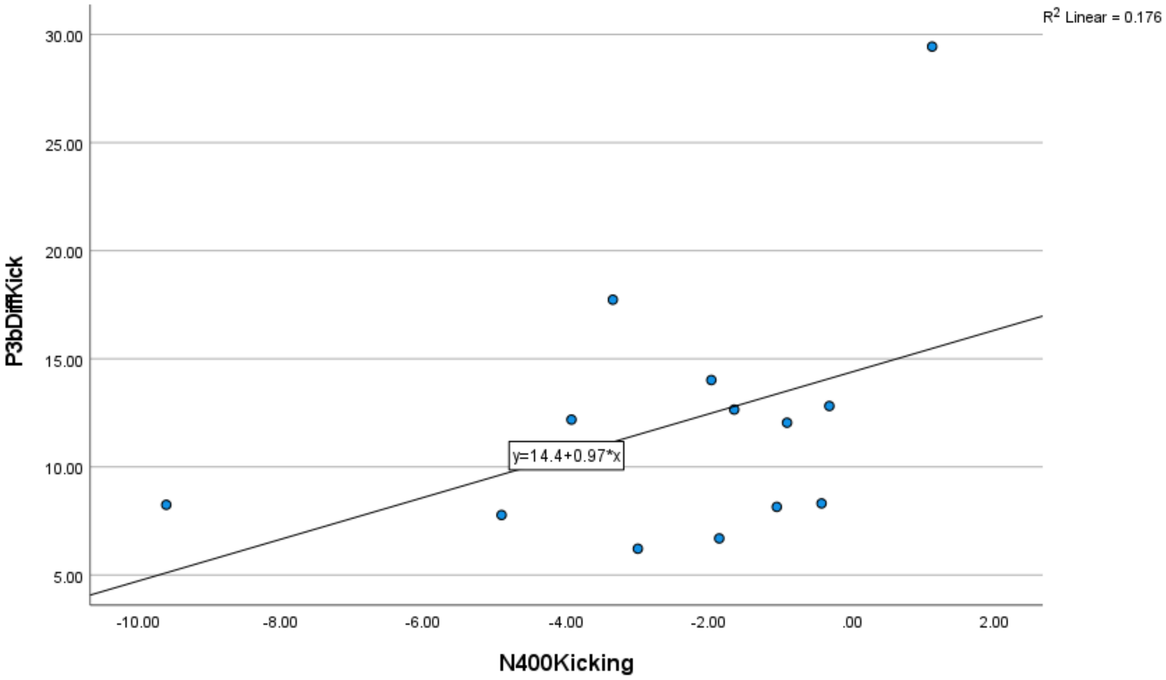


Figure 2

Appendix 3

Word	Definition	Example sentence	Matched word
Junto	A small, usually secret, number of people joining together for a common purpose	Chris joined the **junto** in hopes of meeting other Star Trek aficionados	Group
Flivver	An automobile, especially one that is small, inexpensive, and old	John decided to buy a new car after his old **flivver** broke down on the highway	Car
Zydeco	popular music of southern Louisiana played by small groups featuring the guitar, the accordion, and a washboard	They could tell the carnival celebration was about to begin when the **zydeco** melodies were heard several street blocks away	Music
Younker	a young person (especially a young man or boy)	Jan has a three-year-old **younker** and two older daughters	Child
Ultima	Most remote; furthest; last; the last syllable of a word	They're traveling across Europe by train and are planning on an **ultima** stop in Moscow.	Final
Estival	Of, relating to, or appearing June to August	Jenny's family always liked to spend some time **estival** at the Hampton's.	Summer
Cerulean	Azure; sky-blue	The **cerulean** skies were mesmerizing to Pittsburghers who see grey, cloudy skies most of the year	Blue
Gravamen	The most important/serious part	The **gravamen evidence was enough** to convict him of the murder	Significant
Napiform	Turnip-shaped; large and round in the upper part, and very slender below	The plant had a **napiform** root, and the large upper part could be eaten	Round
Xerosis	Abnormal dryness, especially of the skin and eyes	She always had horrible **xerosis** in the winter, and bought practically every bottle of lotion in the store	Dry
Heptarchy	A government by seven persons; also, a country under seven rulers	The local government rid itself of **heptarchy** because of the many disagreements between the government officials	Seven
Yttrium	a silvery metallic element	They learned in Chemistry class that **Yttrium** Oxide has industrial uses for the manufacturing of television sets	Metal
Hinny	The hybrid offspring of a male horse and female donkey	At first glance she thought the **hinny** looked like a donkey, but on closer inspection she decided the animal is more subtly like a horse	Animal

Agog	In eager desire	She explored the deck of the ship, **agog** with excitement for her first trip	Excited
Bivouac	An encampment for the night usually without tents or covering	Our troops retreated for the night and went into the **bivouac**	Camp
Chaparral	A dense thicket of shrubs and small trees	Once she lost her necklace in the **chaparral,** she knew she would never see it again because it was too dense to look through	Forest
Pluvious	Characterized by heavy rainfall	The **pluvious** weather lasted for days, causing a devastating flood throughout the small town	Rain
Schism	A separation or division into factions	The **schism** between East and West Germany ended when the Berlin Wall came down.	Split
Zucchetto	a skullcap worn by certain Roman Catholic clerics	One can tell the rank of a Roman Catholic cleric by the color of the **zucchetto** they wear on their heads	Cap
Unco	So unusual as to be surprising; uncanny; Extraordinary	He was a great traveling companion because of his **unco** sense of direction.	Strange
Pintle	A hook or bolt on the rear of a gun or trailer; the pin on which a gun carriage revolves; the pin on which a rudder turns; a pin or a bolt on which another part pivots; the one that holds a hinge together	The rusty **pintles** must be replaced soon before they a towing vehicle for attaching snap, causing the door to fall off the hinges	Pin
Grimalkin	A Felidae, especially an old female cat; an old woman considered to be ill-tempered	The irritable **grimalkin** would not play with the other cats because she was too old to run around the house	Cats
Refluent	Flowing back, ebbing	The tide is **refluent,** so we'll soon be able to walk further down the beach	Returning
Legate	An ambassador or envoy	President Bush sent a **legate** to the Middle East to represent the United States at the peace talks	Representative
Ulster	A loose, long overcoat made of heavy, rugged fabric and often belted	It is appropriate to wear a dark **ulster** over a dinner jacket or tuxedo.	Coat
Hubris	Overbearing self-confidence or presumption	With dizzying **hubris,** Shelley elevated the purpose of the poet over that of priest and statesman	Pride
Jubilee	A season or an occasion of joyful celebration	Jessica was enjoying the celebrations of the annual spring **jubilee** with her family	Anniversary

Venatic	Of or relating to hunting	John always liked **venatic** sports such as deer and duck hunting.	Hunt
Diptych	An ancient writing plaque	Ancient Greeks used **diptychs** to practice writing	Tablet
Xyloid	Resembling wood; having the nature of wood	Wormwood is neither worm-shaped or **xyloid**	Wooden
Wyvern	a two-legged mythical creature having wings and a barbed tail	The brave knight fought off the ferocious **wyvern** and saved the kingdom	Dragon
Kilderkin	A cask; an old liquid measure containing 18 English beer gallons, or nearly 22 gallons	The tavern kept its beer supply in **kilderkins** behind the bar	Barrel
Virgulate	Shaped like a small rod	The **virgulate** shape of the branch made a great makeshift stake that she substituted for the lost tent stake.	Rod
Clement	Inclined to be lenient or merciful; mild	The weather was particularly **clement,** so it was the perfect day for a walk in the park	Mild
Oblation	The act of offering something, such as worship or thanks, especially to a diety	The priest reminded everyone to offer their **oblation** throughout the week, not just on Sundays	Worship
Garboil	mix up; uproar	To avoid **garboil**, the twins never wore the same clothes	Confusion
Solfeggio	A singing exercise using the syllables: do, re, me, fa, so la, ti	Singers use the **solfeggio** exercise to warm up their vocal cords before they start singing.	Sing
Solleret	A flexible steel shoe forming part of a medieval soldier's suit.	During battle, the knights kept their feet protected with steel **sollerets.**	Armor
Glossal	Of or relating to the tongue	His **glossal** nerve had been severed, leaving him with no feeling in his tongue	Tongue
Natant	Swimming in water	She wanted to get some **natant** plants for the pond she dug in her yard	Floating
Onus	A burden; an obligation; a difficult or disagreeable necessity	The **onus** was on the prosecution to prove the man had gotten sick because of his work environment	Burden
Beleaguer	To harass; annoy persistently	The other children **beleaguered** the boy because of his lisp	Bother
Abeyant	Temporarily inactive	The plan was **abeyant** until we could get further funding	Suspended

Pillory	A wooden framework on a post, with holes for the head and hands, in which offenders were formerly locked to be exposed to public scorn; to expose to ridicule and abuse	As a punishment for adultery, she was locked in a wooden **pillory** for a week at the center of town	Punishment
Ersatz	Imitation, fake, artificial	She wrote a letter full or **ersatz** sympathy to the co-worker she never liked, who had just been fired	Synthetic
Vesicate	To blister or become blistered	Her feet **vesticated** after wearing ill-fitting high-heeled shoes all day.	Bubble
Salver	A disk for serving food or drinks	She put the drinks on the **salver** and headed out of the kitchen to serve them to her guests.	Tray
Uranic	Of or relating to the heavens; celestial	It is an **uranic** principle that performing evil acts will bring punishment to the evil-doer.	Paradise
Lacrimal	Of or relating to tears	Tears are formed in the **lacrimal** gland, which is located under the upper eyelid	Crying
Yagi	A sharply directional antenna	John adjusted the **yagi** on his television because he wanted better reception of the football game	Antenna
Dandle	To move (a small child) up and down on the knees or in the arms in playful way; to pamper or pet	It is an old wives' tail that if you don't **dandle** your baby on your lap, he or she will get fat	Bounce
Maculate	To spot, blemish, or pollute	She accidentally dropped her white scarf in the muddy puddle; the **maculate** was hard to get out	Stain
Cabochon	A highly polished, rounded stone	The sapphire was cut like a **cabochon** for the ring	Gem
Temblor	The shaking of the earth	The **temblor** caused destruction throughout the city, and some could feel the tremor from miles outside of town.	Earthquake
Arcuate	Having the form of a bow; curved	The **arcuate** arteries are small curved branches of arteries supplying the brain with fresh blood	Curved
Monish	To tell the consequences of an action	His mother **monished** him of the consequences for driving without a seat belt	Warn
Girandole	An ornate candle holder; often with a mirror	She said that the antique **girandole** hanging on the wall is also known as a "mirror chandelier"	Candles

Bawdy	Humorously coarse; risqué. Vulgar, lewd	The men sat around smoking and telling **bawdy** stories about their youths	Rude
Afreet	A powerful evil spirit or gigantic monstrous devil in Arabic mythology	To protect herself from the evil **afreet**, she wore a garlic necklace around her neck	Demon
Folderol	Foolishness, not making sense	Her silly **folderol** comments discredited her ability to serve as governor	Nonsense
Ibex	Wild goat of mountain areas of Eurasia and N. Africa having large backward curving horns	The **ibex** wandered freely throughout the African mountains	Goat
Blandish	To flatter with kind words or affectionate actions	She used her ability to **blandish** to her advantage anytime she wanted a raise at her job	Compliment
Kittle	Touchy; not able to predict	Because of her **kittle** personality, her friends never knew what to expect from her when they spent time together	Unpredictable
Bibulous	Of, pertaining to, marked by, or given to the consumption of alcoholic drink	After each weekend, the **bibulous** can be found hungover, holding their heads and wearing their sunglasses	Alcoholic
Assuasive	Soothing; calming	His **assuasive** remarks really helped the family in their time of need	Soothing
Wheedle	To entice by soft words; to cajole; to flatter; to coax; to gain or get by with flattery or guile	The school was always trying to **wheedle** contributions from the parents by telling them how bright their children were	Persuade
Cygnet	A young gooselike bird	The young **cygnet** was swimming closely next to his mother	Swan
Prescient	Having foresight or knowledge of what will happen	His **prescient** that the Buccaneers would win the Superbowl was correct	Intuition
Intarsia	A decorative inlaid pattern in a surface, usually worked in wood	To construct an **instarsia,** outline drawings are used as templates for cutting the many pieces of wood	Mosaic
Leister	A three-pronged spear like weapon used in fishing	Native Americans used **leisters** to catch their fish long ago	Spear
Yashmak	Worn by Muslim women to cover their face in public.	In some middle eastern countries, women who do not wear their **yashmaks** in public can be arrested by the police	Veil
Famulus	A private secretary or waiter/ helper	In the late 1800's, it was typical for rich families to have a **famulus** working in their home	Attendant

Yenta	a person, especially a woman, who is meddlesome meddlesome or gossipy	The **yenta** is famous for knowing the latest rumors in town	Gossip
Venial	Forgivable; excusable; pardonable	Eating meat on a Friday is a **venial** sin, but murder is a mortal sin.	Forgiven
Ursine	Of or characteristic of bears or a bear	Because of its **ursine** appearance, the great panda has been identified with the bears; actually, it is closely related to the raccoon.	Panda
Ambry	In churches, a kind of closet, niche, cupboard or locker for utensils, vestments, etc.	After the church service, the priest put the silver chalice back in the **ambry**	Cupboard
Peruke	A toupe, especially one worn by men in the 17th and 18th centuries	In addition to wearing a **peruke,** Lord Wadsworth sold them to other men wanting long, flowing hair	Wig
Gloaming	Twilight; the fall of the evening	He arrived at the village station on a wintry evening, when the **gloaming** was punctuated by the cheery household lamps	Dusk
Urticant	Causing a rush or stinging	The sea anemone tentacles can be used to attack because of their **urticant** properties.	Itch
Lambaste	To give a thrashing to; to beat severely; to scold sharply; to attack verbally; to berate	The politician spent most of his campaign money **lambasting** his opponent rather than discussing the issues	Criticize
Illation	A conclusion, a deduction	Faulty deductions or unimportant **illations** form a false image of things	Inference
Ramous	Of or resembling branches	Some cancers grow in a **ramous** fashion, resembling tree limbs	Branching
Badinage	Playful teasing; banter	The playful **badinage** of her co-workers made her office a fun place to work	Teasing
Alate	Having winglike extensions or parts	The **alate** seeds of the maple floated through the forest on the wind	Winged
Myxoid	mucoid, with fluid	She had a **myxoid** cyst at the end of her finger	Mucous
Nebbish	A weak-willed, or ineffectual person	He is a **nebbish** person who might be played effectively by Woody Allen	Timid
Quisling	A man who serves as the puppet of the enemy occupying his or her country	Hitler’s plan was to use Cuesta as his Mexican **quisling**	Traitor

Quahog	An edible mollusk of the Atlantic coast of N. America, having a hard rounded shell	The restaurant used only the freshest **quahogs** to make their famous chowder	Clam
Paranymph	The bridesmaid conducting the bride to the bridegroom	The bride has one chief **paranymph** that helps her out with the wedding plans; she is also known as the maid of honor	Bridesmaid
Xanthous	Yellow; having light brown or yellow skin	The **xanthous** colouring of her skin suggested she contracted jaundice	Yellowish
Bandy	To toss or throw back and forth (especially words)	We **bandied** many words about when we tried to come up with a good title	Volley
Sibilant	Of, characterized by, or producing a hissing sound like that of (s) or (sh)	The poet used a lot of **sibilant** consonants in his poem about snakes.	Hiss
Frisson	A feeling of shaking caused by excitement	As we descended from the pinnacle of the rollercoaster track, we experienced a short **frisson** of excitement	Shudder
Roorback	A false or slanderous story used for political advantage	Politicians use **roorbacks** to defame the name of their opponents.	Slander
Piaffe	A cadenced jump executed by the horse in one spot	To win the equestrian match, John trained his horse to do a fancy **piaffe** in front of the judges	Trot
Kyphosis	Abnormal rearward curvature of the spine, resulting in protuberance of the upper back	The hunchback of Notre Dame had severe **kyphosis**	Humpback
Rivage	A coast, shore, or bank	She liked to wander along the green **ravage** in the spring, listening to the river	Seashore
Xylograph	Draw on wood	She bought some beautiful **xylographs** to hang on her office wall	Engrave
Zecchino	a spangle often sewn on cloth	Mary was wearing a beautiful dress with golden **zecchinos** that sparkled	Sequin
Vilipend	To treat something as if it has little value; to express a low opinion of	He thought I was **vilipending** his effort, but in actuality I appreciated his work very much.	Belittle
Vulpine	Of, resembling, or characteristic of a fox; cunning; clever	The sly **vulpine** lurked behind the trees preparing to attack his prey.	Foxy
Fossick	To search for gold in abandoned claims or to rummage around for anything valuable	The homeless tend to **fossick** through trash, hoping to find something they can use	Rummage

Hebetude	Mentally slow or sluggishness	Some say that too much television is leading us toward a nationwide **hebetude**	Dullness
Chafferer	A vendor who enjoys talking while making a sale	Most street vendors are **chafferers** by nature because they like to sell their merchandise at the best price	Bargainer