Associations between

Heading Exposure and Brain Changes

in High-Level Football Players

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Declaration of Authorship

I hereby declare that the presented work is, to the best of my knowledge and belief, the result of my own research. All co-author contributions are presented for each publication. Ideas and formulations from other sources are - to the best of my knowledge and belief - cited correspondingly. The work has not been submitted, either partly or completely, for a degree at this or another university.

I have read, understood, and accepted the PhD regulations (“Promotionsordnung NW”) in its version of 31st of March 2021 (AM.UNI.PB 10.21).

Paderborn, November 26th, 2024

Franziska Katharina Mund, geb. Haase (M.A.)

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Abstract

The aim of this dissertation was to investigate the relationship between heading in football, neurocognitive performance, and structural and functional magnetic resonance imaging (MRI) parameters and to contribute to the understanding of potential effects of heading.

In a cross-sectional design, former female football players with self-reported moderate heading exposure (n=4) exhibited a worse performance in verbal memory than non-contact sport athletes (n=16) and showed higher cortical thickness in an inferior parietal region compared to players with rare exposure (n=3).

A longitudinal study in active male football players showed no differences in neurocognitive (n=22) and most structural and functional MRI parameters (n=14) over 1.5 years. Although minimal cortical thinning in a precentral cluster and increased functional connectivity within the salience network was present, neither result correlated with objectively recorded heading frequency or header characteristics. In the splenium of the corpus callosum, the microstructural parameter fractional anisotropy correlated with the number of headers from more than 20 meters flight distance. Such long-distance headers exhibit higher forces, thus indicating a potential (detrimental) effect of specific headers. This needs to be investigated in future longitudinal studies.

Although the results are limited by the small sample sizes, they do not show a clear relationship between (neurodegenerative) alterations and heading. However, they indicate an effect of long-distance headers, which should be analyzed in further studies by considering modifying factors to potentially derive heading recommendations and guidelines.

Zusammenfassung

Ziel dieser Arbeit war es, Zusammenhänge zwischen dem Kopfballspiel im Fußball, neurokognitiver Leistung sowie strukturellen und funktionellen Magnetresonanztomographie (MRT)-Parametern zu untersuchen, um zum Verständnis potenzieller Effekte von Kopfbällen beizutragen.

Ehemalige Fußballerinnen mit selbst eingeschätzter moderater Kopfballbelastung (n=4) zeigten eine schlechtere Leistung im verbalen Gedächtnis als Nicht-Kontaktsportlerinnen (n=16) und eine höhere kortikale Dicke in einer inferior parietalen Region im Vergleich zu Spielerinnen mit einer seltenen Kopfballbelastung (n=3).

In einer Längsschnittstudie zeigten aktive Spieler nach 1,5 Jahren keine Veränderungen in neurokognitiven (n=22) und den meisten strukturellen und funktionellen MRT-Parametern (n=14). Trotz einer minimalen Abnahme der kortikalen Dicke in einem präzentralen Cluster und einer erhöhten funktionellen Konnektivität innerhalb des Salience Netzwerkes korrelierten beide Ergebnisse nicht mit objektiv erfassten Kopfballvariablen. Im Splenium des Corpus Callosum korrelierte der mikrostrukturelle Parameter fraktionelle Anisotropie mit der Anzahl der Kopfbälle aus mehr als 20 Metern Distanz. Solche Langdistanz-Kopfbälle weisen höhere Kräfte auf, was auf einen Effekt von spezifischen Kopfbällen hinweisen könnte. Dies gilt es in weiteren Studien zu untersuchen.

Obwohl die Ergebnisse durch kleine Gruppengrößen limitiert sind, zeigen sie keinen klaren Zusammenhang zwischen Kopfbällen und (neurodegenerativen) Veränderungen. Sie deuten jedoch auf einen Effekt von Langdistanz-Kopfbällen hin, der unter Berücksichtigung modifizierender Faktoren analysiert werden sollte, um Praxisempfehlungen ableiten zu können.

List of Publications Considered for Thesis

1) **Haase FK**[1], Prien A, Douw L, Feddermann-Demont N, Junge A, Reinsberger C. Cortical thickness and neurocognitive performance in former high-level female soccer and non-contact sport athletes. *Scand J Med Sci Sports* 2023;33(6):921-30. doi:10.1111/sms.14324.
2) **Mund FK**, Feddermann-Demont N, Welsch G, Schuenemann C, Fiehler J, Junge A, Reinsberger C. Heading during the season and its potential impact on brain structure and neurocognitive performance in high-level male football players: an observational study. *J Sci Med Sport* 2024;27(9):603-09. doi:10.1016/j.jsams.2024.05.012.
3) **Mund FK**, Feddermann-Demont N, Welsch G, Schuenemann C, Fiehler J, Thaler C, Meyer L, Reeschke R, Reinsberger C. High-magnitude headers are not associated with structural and functional brain changes in active high-level football (soccer) players. *BMJ Open Sport Exerc Med* (Submitted).

[1] Maiden name.

List of Other Publications

Peer-reviewed

Reeschke R, **Haase FK**, Dautzenberg L, Krutsch W, Reinsberger C. Training matters: Heading incidence and characteristics in children's and youth football (soccer) players. *Scand J Med Sci Sports* 2023;33(9):1821-30. doi:10.1111/sms.14408.

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Congress abstracts[2]

Haase FK, Feddermann-Demont N, Junge A, Welsch G, Schünemann C, Fiehler J, Reinsberger C. Potenzieller Einfluss von Kopfbällen auf funktionelle und strukturelle Hirnnetzwerke und die kognitive Performance. Sports Medicine and Health Summit, Hamburg, Germany, June 2023. In: *German Journal of Sports Medicine* 2023;74(4):114.

Reeschke R, Dautzenberg L, **Haase FK**, Krutsch W, Reinsberger C. Kopfbälle im Kindes- und Jugendalter – Auswirkungen auf Neurokognition und vestibulo-okuläre Funktionen. Sports Medicine and Health Summit, Hamburg, Germany, June 2023. In: *German Journal of Sports Medicine* 2023;74(4):114.

Haase FK, Reinsberger C. Projektforschung in einem interdisziplinären Themengebiet – Untersuchung repetitiver Kopftraumata und des Kopfballverhaltens im Fußball. 3. Nachwuchssymposium der Deutschen Gesellschaft für Sportmedizin und Prävention, Tübingen, Germany, September 2022.

Haase FK, Feddermann-Demont N, Junge A, Welsch G, Schuenemann C, Fiehler J, Reinsberger C. Functional connectivity of the brain in active professional football (soccer) players. 27th Annual Congress of the European College of Sport Science, Sevilla, Spain,

[2] First author was always the presenting author.

August 2022. In: *27th Annual Congress of the European College of Sport Science: Book of Abstracts* 2022; p.159.

Haase FK, Feddermann-Demont N, Junge A, Welsch G, Schuenemann C, Fiehler J, Reinsberger C. Kopfbälle im Fußball: Einfluss von Kopfbällen auf funktionelle und strukturelle Hirnnetzwerke // Kopfbälle 2.0. Leichte Schädel-Hirn-Traumata und Kopferschütterungen im Sport – Forschung und Transfer für die Praxis, Berlin, Germany, June 2022. In: *Leichte Schädel-Hirn-Traumata und Kopferschütterungen im Sport – Forschung und Transfer für die Praxis,* (Hrsg.: Bundesinstitut für Sportwissenschaft) 2022; p.15-17.

Schnitker R, **Haase FK**, Dautzenberg L, Reinsberger C. Incidence and characteristics of heading in youth football (soccer). World Congress on Science and Soccer, Coimbra, Portugal, June 2022.

Haase FK, Prien A, Douw L, Schnitker R, Feddermann-Demont N, Junge A, Reinsberger C. Kortikale Dicke des Gehirns bei ehemaligen Profi-Fußballspielerinnen im Vergleich zu Nicht-Kontaktsportlerinnen. Sports Medicine and Health Summit, Virtual Congress, April 2021. In: *German Journal of Sports Medicine* 2021;72(3):144.

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List of Abbreviations

ACC	Anterior cingulate cortex
AD	Axial diffusivity
BOLD	Blood oxygen level-dependent
CEN	Central executive network
CON	Former female non-contact sport athletes
Cnc	Concussion
Cnc_{sub}	Subconcussive blows
CSF	Cerebrospinal fluid
CTE	Chronic traumatic encephalopathy
DMN	Default mode network
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
FA	Fractional anisotropy
FC	Functional connectivity
FDR	False discovery rate
FIFA	Fédération Internationale de Football Association
fMRI	Functional magnetic resonance imaging
GM	Grey matter
JHU	John Hopkins University
MD	Mean diffusivity
MRI	Magnetic resonance imaging
mTBI	Mild traumatic brain injury
PASAT	Paced Auditory Serial Addition Test
pCTE	Probable chronic traumatic encephalopathy
PPCS	Prolonged postconcussive symptoms
RD	Radial diffusivity
RHIs	Repetitive head impacts
ROI	Region-of-interest
rPFC	Rostral prefrontal cortex
SD	Standard deviation
SMG	Supramarginal gyrus
smTBI	Single mild traumatic brain injury
SN	Salience network

SOC	Former high-level female football players
SS	Standard scores
T1w	T1 weighted
TBSS	Tract-based spatial statistics
TMT	Trail Making Test
WM	White matter

1 Introduction

Recent heading bans and heading guidelines in various football (soccer) federations have drawn immense attention to the topic of headers in football. Although these mostly focus on children and youth players [1,2], the Scottish Football Association expanded their guidelines on adult players [3]. The Football Association of England furthermore recommends playing a maximum of ten ‘higher forces’ headers per training week for professional players. These include headers occurring after a long pass of more than 35 meters or after a cross, a corner, or a free kick [4].

Heading in football has been a highly controversial topic since the first publications on potential consequences in the 1990s and early 2000s [5–7]. The study by Mackay et al. [8] contributed to and intensified this discussion. Although former professional Scottish football players lived longer and had lower all-cause mortality than the general population, a higher risk for mortality with neurodegenerative diseases was reported [8]. A subsequent analysis revealed a higher risk of neurodegenerative diseases in defenders, while goalkeepers showed the lowest risk [9]. Recent studies reporting elevated risks for Alzheimer’s disease and other forms of dementia in retired elite or professional[3] Swedish and French players emphasized the results [10,11]. In the media, in particular, the interpretation of these study results was directed towards heading, questioning whether the exposure to heading could have induced neurodegenerative diseases.

The mentioned studies raised awareness and led to increased research of heading effects, although they did not directly investigate heading and assessed mortality causes retrospectively using death certificates, diagnoses, medication, or drug prescriptions [8–11]. These results, therefore, do not yet provide sufficient evidence for a relationship between exposure to heading and adverse effects and do not establish causation. Nevertheless, they provided a foundation for further studies. To better understand potential short-term or long-term effects associated with heading exposure, further research is being conducted focusing on structural and functional brain changes as neurocognitive impairment and (structural) alterations are discussed in football

[3] Within this thesis, high-level, elite, and semi-professional players are defined as those players playing in top divisions. The term ‘professional’ is reserved for players in the highest league and playing for national teams.

players [12,13]. Although several neuroimaging and neurocognitive parameters were investigated and summarized in literature reviews [12–14], consistent evidence is still lacking, and it is unknown if and how heading might lead to potential consequences.

The aim of this thesis is consequently to investigate structural and functional brain changes and to determine a possible association between heading and alterations in adult football players. Established neuroimaging and neurocognitive measures were assessed to gain an initial understanding of potential heading effects in two cohorts. A cross-sectional case-control study was conducted to compare former high-level female players to a matched control group and to analyze differences between specific sub-groups based on the heading exposure. In a prospective and longitudinal study, active high-level male players were investigated regarding neuroimaging and neurocognitive outcomes. Potential associations with objectively evaluated heading exposure were subsequently tested.

This dissertation begins with the theoretical background by distinguishing head injuries, repetitive head impacts (RHIs), and heading before summarizing the current literature on the potential consequences of heading on neuroimaging and neurocognitive parameters. After presenting the relevant research questions of the thesis, the following chapters include summaries of the study projects and the resulting articles. Lastly, the findings of the dissertation are discussed in the context of current research evidence. The thesis ends with a conclusion and perspective for further research by pointing out relevant factors to consider in future studies on heading effects.

2 Current State of Research

Potential consequences of concussions, as well as of repetitive head impacts (RHIs), are highly debated in several collision and contact sports such as American football, rugby, or ice hockey. Neurocognitive impairment and structural or functional brain alterations are discussed [15–18]. Amongst other methods, these are investigated with advanced neuroimaging[4] methods and neurocognitive tests, which can detect subtle structural or functional brain changes [19–21].

Due to the unique nature of heading, studies on the consequences of RHIs have focused on football. The growing research interest is underlined by a constantly increasing number of published articles regarding the topic. To understand whether and how heading may induce possible effects, neurocognitive and neuroimaging measures are investigated, amongst other parameters [12,13,22]. However, evidence for a causal dose-response association is inconclusive and needs further investigation. Therefore, the goal of this thesis is to contribute to the understanding of possible effects of heading.

This chapter provides relevant background information by first distinguishing mild traumatic brain injury (mTBI), concussion, and RHIs. Head impacts and heading in football are specifically introduced afterwards. Before the current evidence on potential consequences of heading is summarized, theoretical background information on relevant neuroimaging and neurocognitive parameters is presented. This part includes definitions and descriptions of magnetic resonance imaging (MRI) sequences and neurocognitive tests.

[4] The term ‘neuroimaging’ is used in the following chapters of the thesis exclusively for different magnetic resonance imaging (MRI) modalities and sequences. Of note, several other methods, such as computer tomography or positron emission tomography, are considered neuroimaging methods.

2.1 Mild Traumatic Brain Injuries, Concussions, and Repetitive Head Impacts

Although often used interchangeably, mTBI and concussion need to be differentiated. On a continuum of severity, mTBI is the mildest form of a traumatic brain injury, and concussion is thought of as the least severe form of mTBI. Figure 1, the spectrum of mTBI presented by Mayer et al. [23], represents the difference. The acute and subacute phases describe the initial injury or impact, whereas the chronic stage includes potential consequences. The spectrum reveals that concussion (in blue) is a stage of injury occurring before the mTBI stage (in green to yellow), with a difference between single and complicated mTBI. A white bar before the concussion stage displays subconcussive blows to the head, which are commonly referred to as RHIs [23]. Subconcussion is defined as a cranial impact that does not lead to a diagnosed concussion or the development of any symptoms [24]. For this thesis, this definition is expanded to all (repetitive) impacts to the head that do not cause any symptoms or result in an injury.

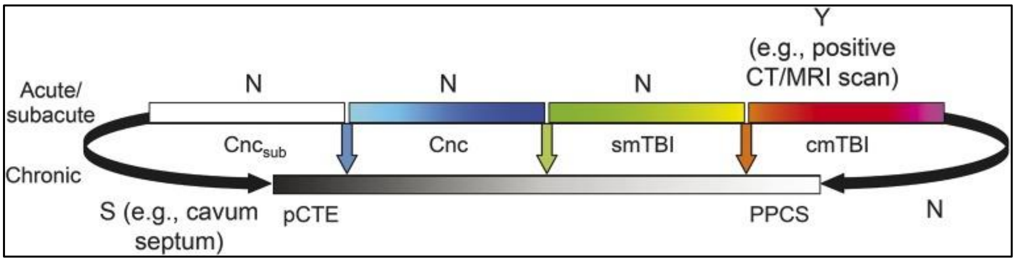

Figure 1 The spectrum of mild traumatic brain injuries. Abbreviations: cmTBI=complicated mild traumatic brain injury (mTBI), Cnc=concussion, Cnc$_{sub}$=subconcussive blows, N=no, pCTE=probable chronic traumatic encephalopathy, PPCS=prolonged postconcussive symptoms, S=some, smTBI=single mTBI, Y=yes. Source: Mayer et al. [23].

A concussion is a diffuse axonal injury that occurs after a hit or blow to the head or any part of the body. The hit results in an impulsive force that is transmitted to the brain [25]. The acceleration and deceleration lead to translational and rotational forces within the brain [26], initiating a metabolic cascade and changes in the blood flow [25,27]. Standard techniques, such as structural MRI or computer tomography, show no abnormalities after sustaining a concussion. Newer MRI techniques, such as functional or diffusion imaging, may be promising and are increasingly used, particularly in research settings [25]. However, to date, various parameters and patterns have been described and analyzed [28], and a concrete marker for concussion diagnosis is still missing [29].

A sports-related concussion is a concussion occurring during participation in sports or exercises [25]. Incidences of sports-related concussions have increased over the past years [30] and differ between sports [31,32]. Women showed a higher injury rate than men [31], even in the same sport [32,33], with female football players exhibiting a higher concussion incidence density than male players [32]. Females have weaker neck strength [34,35] and a higher likelihood of reporting incidences [36,37], which may be some of the reasons responsible for the higher concussion rate [38].

The rising incidences and awareness intensified the discussion about possible consequences or effects of (multiple) sustained concussions. The application of functional and diffusion imaging techniques revealed alterations in brain networks, specifically in the default mode network (DMN) and the salience network (SN), induced by the diffuse axonal injury [39]. Additionally, a history of concussion was found to be associated with abnormalities in the white matter (WM) and grey matter (GM) of the brain [40,41] and with long-term cognitive impairment in retired (elite or professional) athletes [16,42]. One highly debated potential consequence of (repetitive) concussions or other brain injuries is chronic traumatic encephalopathy (CTE), which is a progressive neurodegeneration characterized by the accumulation of tau proteins in the brain [43–45]. Although it can currently only be diagnosed post-mortem by brain tissue analyses [46], various symptoms or diseases can occur during life. These may include cognitive impairments up to dementia, Parkinson's disease, or amyotrophic lateral sclerosis [47].

The potential development of CTE is a topic of debate in specific cohorts, such as American football or football players, due to reported higher rates of neurodegenerative mortality among these athletes [8,48]. Although methodologically limited [25], the studies identified one common factor in these collision and contact sport cohorts, which is the exposure to (cumulative) RHIs. Therefore, it is discussed that RHIs may have an impact on the development of neurodegenerative processes and may potentially lead to CTE [49,50]. The evidence and conclusion of a causal relationship between RHI exposure and CTE development, however, is limited due to various unknown factors. These include the RHI exposure itself, socio-demographic information, genetic and environmental factors, and cardiovascular or peripheral vascular diseases [51].

Nevertheless, potential cumulative effects of RHIs on the structure and function of the brain are of great interest. As CTE seems to be associated with neurocognitive impairments during life and structural brain alterations [43,44], neurocognitive performance and neuroimaging parameters are investigated regarding the potential consequences of RHI exposure in athletes. A review on the short-term effects of RHI exposure (less than four weeks since the last impact) on cognitive function pointed out that studies reporting an effect had bigger sample sizes, included women, and primarily detected changes in verbal memory. Yet, more than 50% of the reviewed studies found no consequences of RHI exposure [52]. Youth, high school, and collegiate contact sport athletes additionally revealed unchanged or improved neurocognitive scores throughout a season [18]. Although alterations in various MRI parameters were described, no concrete pattern was detected. Even within the same MRI sequence and parameter (structural, functional, or diffusion imaging), variations in results were observed [18]. This contrasts with a conclusion stating that there was evidence across reviewed studies that RHI exposure was associated with effects on the structural integrity of the brain [17]. A more recent review underlined that conclusion. Consistent patterns were described in associations between RHI exposure and diffusion imaging parameters, which reflect the WM microstructure of the brain. The patterns were most prominent in American football and football players [53].

Currently, there is insufficient evidence in the literature to support an explicit relationship between RHI exposure and neurocognitive consequences or neuroimaging alterations [17]. This is primarily due to several limitations, such as the focus on specific cohorts and small sample sizes, different study designs and analyzed parameters, or the RHI exposure assessments (e.g., by observation, sensors, questionnaires, or interviews) [17]. Additionally, the RHI exposure itself might be of specific importance. The exposure differs between sports, age groups, or levels of play. Higher RHI exposure was described for collegiate American football players compared to high school players and compared to collegiate football and lacrosse players [54]. Consequently, more in-depth and well-designed studies focusing on sport-specific RHI exposure are needed. As football is the most popular sport in Germany [55], potential consequences of RHI exposure, and heading a ball specifically, would affect numerous players. This shows the relevance of the topic, which is therefore focused on in this thesis.

2.2 Repetitive Head Impacts, Heading, and Heading Exposure in Football

In football, repetitive impacts to the head can occur due to dives or falls (head-to-ground), unintentional contact with the ball, player-to-player contact, head-to-equipment contact (e.g., goalpost), or purposeful contact with the ball (heading) [56,57]. Although heading is the most common type of head impact [56,58], it is not the primary cause of head injuries such as contusions, fractures, or injuries to the neck or face [59] or concussions specifically [60]. Due to the higher amount of headers compared to other impacts to the head [56] and the addressed, yet not fully understood, effects due to RHI exposure in other sports, heading is of particular interest. To examine potential short- or long-term consequences of heading and concretely investigate possible associations, knowledge about the actual heading exposure of a player is essential.

Various methods are used to quantify the heading number. Sensors such as mouthguards [56], accelerometers [61], neck collars [62], or skin patches [63] can be applied, which can detect impacts to the head above a specified threshold and record the kinematics of every registered impact [56,64]. This has the advantage of providing crucial information regarding the external forces present when heading. However, when there is no verification of impacts, for example, by additional video analysis, the tools might report the number of all head impacts, including impacts with other sources, rather than just heading events [65]. Interviews or questionnaires, in which players self-categorize their heading exposure as frequent, moderate, or rare [66] or estimate their total heading number during a specific timeframe (e.g., two weeks or one year) [67–69] can also be used. Although these retrospective methods are easy to implement and of low cost [67], a limitation is the subjectivity, as players may over- or underestimate their heading frequency [70,71]. Therefore direct observation of players is needed [72], which can be achieved by persons observing training or matches and directly noting a header [58,73,74] or by analyzing video recordings of the session(s) [75–77]. The latter is considered the gold standard [72]. These approaches are time-consuming and resource-intensive, but offer the opportunity to explicitly count every header of every player. Additionally, all heading situations or concrete header characteristics can be evaluated [78]. As scenes can be reviewed when necessary, such a categorization is easier with video analysis compared to direct observation.

A systematic review of objectively and independently quantified heading frequency estimated the range between one to nine headers per player per match [79]. A recent study documented a range between 2.6 and 6.6 for mean headers per player in matches [75]. However, the heading number is influenced by several factors, including league or level of play [80,81], playing position [71,76,82], sex [58,75], and age [58,78,83]. First studies additionally revealed a higher heading number in training than in matches [70,78]. Although these evaluations mainly focused on youth cohorts [70,78,84], they assessed heading exposure with an objective method, thus establishing an important foundation for further research in other cohorts. Such heading exposure evaluations, that include matches and training sessions, are to date rare in (high-level) adult players [79].

Investigating specific header characteristics adds valuable information to the overall understanding of the heading exposure. Characteristics may include the distance the ball traveled before being headed, the purpose of the header, or whether a heading duel occurred. A header is mostly intended as a pass or as interception [61,81,85] and mostly occurs after a (high) pass [61,76,82] and from a short distance (under 5 meters) [78]. However, similar to the total heading number, header characteristics were mainly reported in matches [61,76,81,82,85], with only a few studies examining characteristics in training in youth cohorts [78,84]. These investigations report distinct differences between the sessions. For example, the flight distance of the ball is shorter in training than in matches, in which the ball more often comes from longer distances [78,84]. These results present an initial insight into the distribution and presence of different header characteristics between training and matches, on which subsequent studies can build. Header characteristics are additionally interesting regarding kinematic parameters. Head injuries, such as concussions, are induced by forces transmitted to the brain [25], and higher linear and rotational accelerations are associated with higher head injury risks [86,87]. Hence, headers with high velocities and accelerations might similarly induce different reactions or brain alterations than headers with lower accelerations. Greater mean peak linear and angular accelerations were described for balls coming from longer distances (over 10 meters) than for those coming from shorter distances before being headed [88]. Head-to-head collisions have higher accelerations than other head impacts [57,89]. These may occur in heading duels. Therefore, the flight distance of the ball and heading duels may be of specific interest. Header characteristics may consequently be an important factor to consider when investigating consequences of heading exposure. In summary, there are several possibilities to quantify the exposure to heading. As studies on high-level or professional adult players concentrated mainly on matches, little is known about the exposure in training and, hence, about the actual total heading number or possible differences in header characteristics between training and match.

2.3 Potential Consequences of Heading

Neuroimaging and neurocognitive parameters can detect subtle changes in the structure and function of the brain [19–21]. Thus, established parameters of structural, functional, and diffusion tensor MRI sequences (summarized in Figure 2, p. 13), as well as neurocognitive tests, were used in this thesis to evaluate the potential effect of heading on the brain. Before summarizing the current research on heading consequences, this part will introduce and explain different MRI sequences and the investigated neuroimaging and neurocognitive parameters.

2.3.1 Theoretical Basis for Neuroimaging and Neurocognitive Parameters

Structural MRI

MRI is a non-invasive technique to visualize (brain) tissues. The image acquisition relies on the mechanism of nuclear magnetic resonance [90]. Several sequences can be obtained, giving information about subtle brain alterations [19]. Alterations on a macrostructural level can be investigated with T1-weighted (T1w) structural sequences. These sequences are based on the magnetic characteristics of water molecules within the body (i.e., brain) that align with the magnetic field within the MRI scanner (B_0). Applying a specific radiofrequency pulse first excites the protons of the molecules that return to the alignment with B_0 when the radio frequency pulse is switched off again. While realigning with B_0, the protons emit energy and transfer it to the surroundings. The emitted signals are measured and transformed into the T1w image. As the relaxation times differ, the different tissue types appear in various shades of grey. The cerebrospinal fluid (CSF) is darker than the GM and WM. The latter appears the brightest in T1w images [91]. Structural images are used to quantify GM and WM volumes, measured in mm^3, and the cortical thickness (see Figure 2A). Cortical thickness is the distance between the GM/WM boundary and the boundary between GM and CSF [92] and is consequently measured in mm.

Diffusion Tensor Imaging

Diffusion-weighted imaging (DWI) informs about the diffusion of water molecules in tissue [93]. With complex mathematical calculations, DWI sequences are used to generate a tensor, which is the basis of diffusion tensor imaging (DTI) [94]. DTI can be applied to investigate the microstructure of the brain. The tensor, as a symmetric 3x3 matrix, is calculated for every voxel by applying mathematical models [95,96]. It consists of three eigenvectors and three eigenvalues (λ_1, λ_2, λ_3). The eigenvalues represent the diffusivity in the direction of each eigenvector (i.e., along the axes x, y, z). If the diffusion is similar along all axes ($\lambda_1 = \lambda_2 = \lambda_3$) and therefore unrestricted, it is called isotropic. However, diffusion of the molecules within the WM is restricted and influenced by the microstructural heterogeneity of the tissue, e.g., cellular membranes. Therefore, the diffusion is larger along one axis (e.g., $\lambda_1 > \lambda_2 > \lambda_3$) and called anisotropic [94,95,97] (see Figure 2B). This information is used in tractography approaches investigating the structural connectivity of WM fiber tracts [98,99]. '*Structural connectivity*' relies on the presence of anatomical connections between brain regions and may change over longer time periods (structural plasticity; hours to days) [100]. Instead of using tractography approaches, a tract-based spatial statistics (TBSS) approach was applied in this dissertation. This approach calculates common DTI parameters such as fractional anisotropy (FA), mean, axial, and radial diffusivity (MD, AD, RD, respectively; see Figure 2B). FA represents the direction of diffusion and has values between zero (isotropic diffusion) and one (anisotropic diffusion) without a measurement unit [94,95,97]. MD is the average magnitude of diffusion along all axes ($(\lambda_1 + \lambda_2 + \lambda_3)/3$). AD is thought to indicate axonal integrity as it is the magnitude of diffusion along the main axis (λ_1). RD is the magnitude of diffusion perpendicular to the main axis ($(\lambda_2 + \lambda_3)/2$) as an indicator of myelin integrity [94,101,102]. MD, AD, and RD are measured in mm^2/s [96].

Functional MRI

Functional MRI (fMRI) assesses the activation of brain regions in resting-state (i.e., a participant is resting with eyes open or closed) or in task-based (i.e., participant is actively engaged in a task) conditions. Functional imaging is based on the blood oxygen level-dependent (BOLD) and the oxygenation of hemoglobin [103–105]. The oxygen consumption in an active brain region, e.g., due to participating in a cognitive task, is increased. This leads to an increase in the amount of deoxygenated hemoglobin. Consequently, the regional cerebral blood flow is increased to compensate for the oxygen consumption. It ultimately leads to an overcompensation of oxygenated hemoglobin. Due to the differing magnetic characteristics of deoxygenated and oxygenated hemoglobin, fMRI sequences can detect changes in the signal [91,103–105]. The measured BOLD signal is a time series measurement used to calculate a statistical dependency between one region-of-interest (ROI) and another or between different voxels or groups of voxels. A visualization is presented in Figure 2C. The statistical dependency is referred to as '*functional connectivity*' (FC), which gives information about a statistical relationship between the regions without implying causal relationships. '*Effective connectivity*' evaluates the causality by analyzing the directed causal influences that one neural element or brain region has on the other [100]. In contrast to the above-mentioned structural connectivity, FC is time-dependent and can change rapidly (within milliseconds), for example, as a reaction to stimuli [100,106]. It can be calculated between a (pre-defined) set of voxels and all other voxels of the brain (seed-based or seed-to-voxel connectivity) or between a pair of ROIs, in which the average BOLD signal of all voxels belonging to a specific ROI is used for the calculation (ROI-to-ROI connectivity) [107]. Both can be presented as connectivity matrices (see Figure 2C).

ROI-to-ROI connectivity analyses are specifically interesting for structurally or functionally connected brain regions. A graph can be used to represent the connected regions of the brain, simplifying the complex system and displaying the regions as a network. The graph consists of nodes that are connected by edges [108]. Hence, each brain region (ROI) forms a node in a brain network, and the structural or functional connection between the regions represents the edge [109]. Graph theory approaches can be applied to evaluate the topology and organization of brain networks [108]. These approaches revealed a small-world organization of brain networks. Characteristics of small-world networks, in general, are a dense local clustering between different nodes in addition to short path lengths between all nodes [108,110]. These enable cost-efficient communication and functioning of the brain [100,111]. Graph theory approaches use complex mathematical algorithms to calculate several parameters for (brain) networks. These can be analyzed on a nodal level (measure for specific node/ROI) or a global or network level (average measure across all nodes of a specific network) [107,109]. The relevant parameters considered within this thesis are summarized in Table 1. A definition and the interpretation of the parameters (on a nodal level) are included.

Table 1 Summary of graph theory parameters (based on [106,107,109]).

Parameter	**Definition**	**Interpretation**
Degree	Number of edges to or from each node	Network centrality; measure for local connectedness of each node within the graph
Cost	Proportion of edges to or from each node	Same as for 'degree'
Average path length	Average distance (i.e., number of edges) between a node and all other connected nodes in the sub-graph	Node centrality and functional integration of a node; shorter distance = higher efficiency as fewer connections are used for information transition
Clustering coefficient	Proportion of number of edges between nearest neighbors of a node and number of all possible edges between them; ranges between 0 and 1	Estimation of graph 'cliquiness'; local integration; measure for inter-connectedness of a node within the neighboring sub-graph
Global efficiency	Inverse of the average shortest path length between the node and all other nodes of the same graph	Node's centrality within the network; degree of global connectedness of each node; measure of integration
Local efficiency	Inverse of the average shortest path length between the node and all neighboring nodes / the neighboring sub-graph	Local integration or coherence; degree of connectedness among all nodes within the node neighboring sub-graph
Betweenness centrality	Measure for how often a node is part of the shortest path between any two nodes within a graph	Role of the node to serve as "bridge" between other nodes or graphs

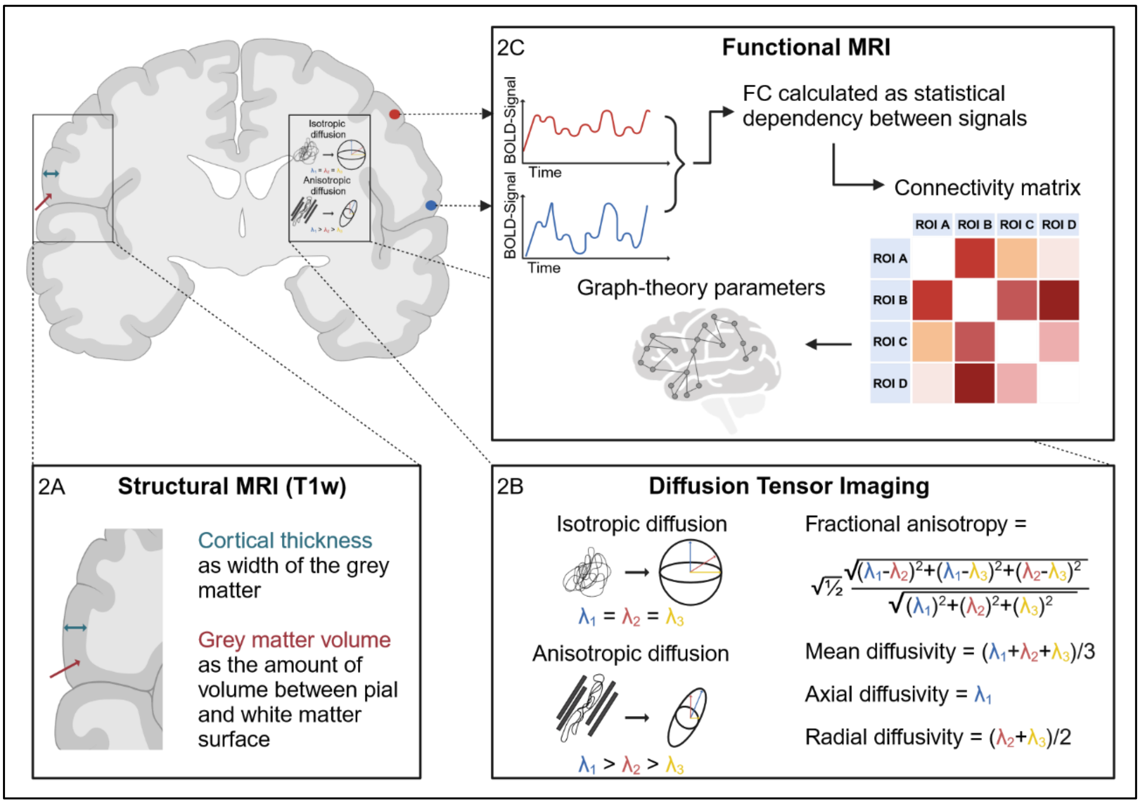

Figure 2 Visualization of investigated structural and functional neuroimaging parameters. Abbreviations: BOLD=blood oxygen level-dependent; FC=functional connectivity; ROI=region-of-interest; T1w=T1-weighted imaging. Created with BioRenders.com.

Neurocognitive tests

After the explanation of the various MRI sequences and parameters, neurocognitive tests, test administration, and outcomes are introduced in the following. Cognitive performance or tasks are planning, thinking, understanding, or learning. Neurocognition considers these tasks as a result of neuronal processes within the brain [112]. Attention, memory, executive function, or visuospatial skills build neurocognitive domains. All other abilities or neurocognitive domains that enable a person to interact with the surrounding environment are considered neurocognitive functions [21]. The evaluation and testing of these domains give information about a person's neurocognitive performance. Due to the potential effects of a (history of) concussion and inconclusive effects of RHI exposure on the neurocognitive performance of athletes [16,17,29,52], it is reasonable to test neurocognitive parameters when evaluating the potential effects of heading. There are various methods to test neurocognitive performance. However, it is advisable to select those that were shown to be sensitive to subtle cognitive changes and impairments, including those resulting from concussion. Within this dissertation, the Trail Making Test (TMT) A and B, the Paced Auditory Serial Addition Test (PASAT) 1 and 2, and the computerized test battery CNS vital signs were considered. These were shown to reveal subtle neurocognitive impairments, including changes after concussion [113–116].

The TMT provides information about processing speed, mental flexibility, and visual-motor skills [117]. The TMT A is thought to reflect visuo-perceptual skills, whereas the TMT B represents working memory and task-switching skills [118]. Both parts can be executed by using a pen and paper. In the TMT A, 25 numbers must be connected in an ascending order starting with ‘1’. This task is extended in the TMT B by adding letters. Numbers and letters have to be connected in ascending numerical and alphabetical order, alternating between numbers and letters (i.e., 1-A-2-B, etc.). The outcome measure for both parts is the time required to complete the test [117,119].

The PASAT tests attentional processes, i.e., processing speed, working memory, and sustained attention [120,121]. The PASAT is executed by presenting single-digit numbers to a participant who has to sum up the two most recent digits. For example, if the numbers ‘5’, ‘1’, and ‘7’ were presented, the answers would be ‘6’ and ‘8’, respectively. The number of correct answers is the outcome measure. The various types of the PASAT differ in the time between stimuli presentation [120,122]. Two different stimuli presentation times were considered in this thesis (PASAT 1 and PASAT 2).

Although the TMT A and B and the PASAT 1 and 2 test different neurocognitive domains, there are no concrete scores or values for specific domains. Therefore, the computerized test battery CNS vital signs can be used as an additional evaluation tool. Seven different tests are incorporated in the core battery (Verbal and Visual Memory, Finger Tapping Test, Symbol Digit Coding Test, Stroop Test, Shifting Attention Test, and Continuous Performance Test), which generate eleven domain scores (verbal, visual, and composite memory; motor, psychomotor, and processing speed; reaction time; executive function; simple, and complex attention; cognitive flexibility). The core test battery can be expanded by two tests (Four-Part Continuous Performance Test, Non-Verbal Reasoning Test), which generate three additional domain scores (working memory, sustained attention, and reasoning). All domains with domain descriptions, tests, and calculation procedures for domain scores are presented in the Appendix (Table 4). The final subject-specific report includes the raw patient scores as well as standard scores (SS) for each domain. The SS are based on a normative sample, taken from the U.S.-American population, and are additionally based on data from control subjects of the same age [123].

2.3.2 Effects of Heading on Neuroimaging Parameters

The explained neuroimaging parameters are beneficial for evaluating the potential effects of heading in football on the brain as subtle alterations can be investigated [19]. To date, the evidence for concrete effects is, however, rather unclear as there is only a small number of studies on the different MRI sequences and parameters, summarized below.

Structural MRI

Macrostructural parameters have previously been investigated in retired professional male and active amateur football players. In both studies, a cross-sectional study design was used, and heading exposure was assessed retrospectively [124,125]. Retired professional male players showed greater cortical thinning with age than age-matched former non-contact sport athletes in brain clusters in the temporal, parietal, and occipital lobes bilaterally. A cluster spanning over the right parietal and occipital lobes additionally correlated with the lifetime-estimate of headers, revealing greater thinning with a higher estimate [124]. However, a more recent study found no associations between heading exposure and cortical thickness in amateur players but reported greater GM volume with increased exposure in the left inferior parietal cortex [125].

Diffusion Tensor Imaging

To date, DTI has been the most commonly used neuroimaging modality to investigate effects of heading. One of the first studies to examine the WM microstructure in football players by analyzing DTI parameters compared professional players to a cohort of swimmers and showed increased RD and AD in several brain regions in the football players. It was hypothesized that the differences might be an effect of frequently experienced head impacts [126]. However, this study did not specifically include heading exposure, which was picked up by following investigations. Cross-sectional studies assessing the exposure to heading retrospectively with questionnaires revealed lower FA with greater exposure in the preceding year in adult amateur players [69,127,128]. In addition, there were differences between female and male players, with females showing more regions with an association between lower FA and greater heading. Male players had greater volume showing an association between lower AD and greater exposure [128]. A recent analysis of the same cohort of amateur players reported a significant difference between the group with the highest heading exposure of the preceding two weeks and the non-contact/non-collision sport athletes in low RD values. This was similarly reported for the two highest exposure groups considering the exposure of the preceding year. Additionally, players with the highest level of heading exposure in the previous year showed significant associations with FA [69]. These results led to the hypothesis that a longer-term exposure to heading might be more robustly associated with microstructural changes in the brain [69]. This hypothesis may be underlined by results showing no significant differences in FA or MD between a heading drill exercise and a catching exercise in a small cohort of young adult football athletes. These results indicate no significant alterations directly after playing headers [129]. However, the hypothesis and the results on potential long-term effects after one year of exposure need to be confirmed by longitudinal studies.

Recently, two longitudinal investigations were conducted on high school female football players [130,131]. Significant decreases in MD, RD, and/or AD were found after a season, and the change from pre- to post-measurement in MD and RD correlated significantly with the total number of head impacts. A subsequent analysis of the WM tracts with significant changes revealed additional correlations for all DTI measures (FA, AD, RD, MD). These indicated increased FA and decreased MD, RD, and/or AD at post-season with higher exposure [130]. Similar results showed that increased exposure to head impacts was negatively correlated with the change in MD and RD and positively correlated with the change in FA from pre- to post-season. These results indicated a greater reduction in MD and RD and a greater increase in FA at post-season for those with greater exposure [131]. However, both studies included all head impacts without specifically focusing on heading, and it cannot be ruled out that other impacts (e.g., contact with another player) might have influenced the results.

In summarizing the DTI results, it is striking that dissimilar findings were reported, especially for FA. The cross-sectional studies that analyzed the effects of the heading exposure of the previous year revealed lower FA with greater exposure [69,127,128]. The longitudinal studies rather reported increased FA with greater exposure after one season [130,131]. These findings might indicate that FA may be affected by longer-term heading. How it is affected and in which direction the alteration may occur needs further evaluation. Although a heading drill exercise did not lead to any differences in MD [129], changes in MD seem to be associated with heading exposure when considering longer time frames [128,130,131]. Similarly, RD and AD were found to be negatively associated with heading [69,130,131], indicating decreased RD and/or AD (or greater decreases in these parameters when compared to another group/cohort) with higher exposure.

Functional MRI

Not only the structure but also the function of the brain is discussed to be affected by heading. Consequently, FC and graph theory analyses have been conducted on football players. Three resting-state analyses recently used a longitudinal, prospective study design in male collegiate [74], male semi-professional [77], and female high school football players [62]. Overall, two studies indicate functional hyperconnectivity during rest after one football season [74,77]. Analyses revealed greater FC increases in collegiate players with the highest exposure to heading [74]. Increased FC at the post-measurement was similarly found in the semi-professional players, and greater exposure to head impacts was associated with increased connectivity. Although all head impacts (i.e., headers and involuntary impacts) were included in the analysis in one study [77], both studies used an objective exposure measurement overcoming the problem of subjectivity of retrospective assessments. The comparison of two groups of female high school players (collar-wearing vs. non-collar-wearing) regarding graph theory measures revealed increased global clustering coefficient and path length in the non-collar-wearing group. However, whether the differences between groups were associated with the heading amount was not evaluated [62], and thus, a conclusion on potential heading effects on graph theoretical parameters cannot be drawn. Nevertheless, these three projects yielded interesting results that serve as a foundation for further research.

Taken together, consistent evidence for heading-induced consequences is not yet supported by neuroimaging studies. There seem to be tendencies in some parameters that reveal alterations associated with heading. Increased FC during rest seems to be apparent after a season and decreased MD, RD, and AD or changes in FA were found. Despite the limited number of studies examining the different MRI modalities, these have provided initial insights into how heading may affect the brain's structure and function. Nevertheless, regarding definite and lasting structural brain changes, methodological shortcomings, such as low-quality methods for heading exposure assessment or missing statistical correction for multiple comparisons, limit the evidence [12]. These limitations partly also apply to studies investigating FC. Despite reported differences in DTI parameters between male and female football players [128], a higher risk for concussion, greater symptom burdens, and longer recovery times in female football players [32], (high-level) female football players are only rarely investigated in heading research. Similarly, neuroimaging studies rarely include active high-level adult football players. Hence, whether there is a clear relationship between heading and brain changes remains unknown, especially in these cohorts.

2.3.3 Effects of Heading on Neurocognitive Parameters

Some of the first studies focusing on the neurocognitive performance in football players were conducted more than 20 years ago [5,7,132]. These studies described an inverse association between heading and performance in verbal and visual memory as well as in focused attention in professional players [5]. Similarly, players of several age groups and levels of play who frequently headed a ball showed a worse performance in tests on visual search, attention, and mental flexibility [132]. However, a meta-analysis concluded that evidence was lacking for a definite association between heading and worse neurocognitive outcomes [22]. This is underlined by several more recent studies showing inconclusive results. There were no significant correlations between the estimated heading number during the career and several neurocognitive test scores in active professional male players [133]. Similarly, in former professional male players, there was no association between neurocognitive parameters and the estimated number of headers during life [124]. In contrast, sub-group analyses of former high-level female players showed differences between different heading exposure categories. The players who reported to have frequently or moderately often headed a ball showed a better performance in psychomotor speed than those players who reported to have rarely headed a ball. However, this was only statistically significant for the difference between the moderate and rare heading exposure groups. In verbal memory, the players with frequent heading exposure performed significantly worse than players with rare heading exposure [66]. Although in college players heading exposure of the previous year (long-term heading) was not associated with neurocognitive performance [134], in adult amateur players long-term heading was associated with worse performance in verbal learning and memory in higher exposure groups [68]. Higher heading exposure in the previous two weeks was associated with reduced performance in psychomotor speed, attention, and working memory in the amateur players [68,135] and with reduced performance in processing speed in collegiate players [134]. Interestingly, playing 20 headers led to transient changes in short- and long-term memory, which was worse immediately after heading but normalized to baseline within 24 hours in adult amateur players [136].

As pointed out in Chapter 2.2, specific header characteristics may be of special interest due to higher accelerations when investigating consequences of heading. This is underlined by study results of female collegiate football players, as a worse performance in visual memory was reported for those who played more impacts with a greater magnitude (i.e., over 98g) [63]. Additionally, adolescent players who played more headers coming from a longer distance (over 30 meters) showed less improvement in response time, while a significant association with the total heading number was missing [73]. A recent analysis of the same data set revealed that headers from short distances rather had short-lasting, small positive effects on response time, while long-distance headers exhibited a longer-lasting and negative effect [137].

In summary, several studies reported associations between neurocognitive performance and heading. While the results are inconclusive overall, this may be due to limiting factors such as the low quality of heading assessment (mostly retrospective self-reports [12,79,138]) and the variety of neurocognitive tests and domains [79]. Nevertheless, the studies have led to an increase in awareness of the potential effects of heading and, subsequently, to an increase in research. Interestingly, to date, only a few studies have been conducted on (high-level) adult players [12,79] that investigate neurocognitive performance and apply objective heading assessments. Furthermore, neurocognitive changes may be subtle and, therefore, only detectable with certain tests [12]. This emphasizes the need for standardized methods and the combination of several tests. Consequently, these aspects need to be focused on to provide more detailed information on a potential relationship between heading and neurocognitive performance.

3 Aims and Research Questions

The overall research aim of this dissertation is to contribute to the understanding of potential consequences of heading in football on the brain by investigating established neuroimaging and neurocognitive parameters in cohorts that are currently underrepresented in heading research. As pointed out in Chapter 2.2, little is known about the actual heading exposure over a football season as training sessions often were not included in objective assessments, though this is essential information when investigating potential effects. Chapter 2.3 additionally illustrates that there are inconclusive results and limited evidence regarding the influence of heading on structural and functional MRI parameters as well as on the neurocognitive performance.

Female football players, especially former high-level players, were only rarely examined in heading research and, to date, not included in neuroimaging studies. Similarly, research on active high-level (rather young) adult male football players using longitudinal study designs and objective methods for assessing heading exposure is scarce. Consequently, it is unclear how heading might influence the structure and/or function of the brain, especially in these player cohorts.

These aspects lead to the following research questions, which were addressed in this thesis:

1) What is the heading exposure of active high-level male players, and which header characteristics are apparent? (research papers 2 and 3)
2) Are there differences between former high-level female football and non-contact sport athletes in cortical thickness and/or neurocognitive performance, and do different heading exposure groups show differing results? (research paper 1)
3) Do macrostructural, microstructural, and fMRI parameters and/or neurocognitive performance change from pre- to post-test in active high-level male football players, and is heading associated with changes over the observation period? (research papers 2 and 3)

4 Publications and Results

After introducing the research projects, the three research papers on which this dissertation is based are summarized. Table 3 at the end of chapter 4.1 (p. 26) provides an overview of the different investigated parameters, heading data, and cohorts.

4.1 Overview of the Included Studies and Analyses Approaches

4.1.1 'Head in the Game' Study

Research Paper 1 is based on data from the 'Head in the Game' study, which was conducted and executed as a multicenter study. It was a case-control study with a cross-sectional design approved by the ethical review boards of the VU University Medical Center Amsterdam, the Netherlands (2017.360) and the Westfalian Medical Board, Münster, Germany (2016-449-f-S). The Fédération Internationale de Football Association (FIFA) funded the study.

Former high-level female football and non-contact sport athletes participated in the study. In research paper 1, a subsample of a larger cohort, described by Prien et al. [66], was analyzed. All inclusion and exclusion criteria can be found in research paper 1. As not all players underwent MRI scanning, only a sub-group of the original sample could be analyzed. Research paper 1, therefore, included 15 former high-level female football players (SOC; mean age: 38.3±5.1 years), who were compared to an age- and sex-matched control group of 16 former non-contact sport athletes (CON; mean age: 36.6±5.8 years). The non-contact sports included volleyball, korfball, hockey, (wheelchair) tennis, ice skating, and athletics. An online survey was used to obtain demographic and sport-related information. All subjects participated in a semi-structured interview in which they were asked about their history of concussion. SOC were additionally asked to categorize themselves based on their heading behavior during their career. Possible answers were 'frequent', 'moderate', or 'rare' heading exposure.

Brain MRI scans were obtained with a 3T Philips Ingenia_CX scanner. T1w images were visually and automatically checked for quality (MRIqc, v. 0.15.2.rcl) [139] and preprocessed with fMRIprep (v. 20.0.0) [140]. Differences in cortical thickness between SOC and CON and between different heading exposure groups were analyzed within FreeSurfer (v. 6.0.0). Age was added as a nuisance regressor.

Neurocognitive performance was tested by the computerized test battery CNS vital signs. In accordance with Prien et al. [66], seven domain scores were evaluated, and SS were analyzed, with higher scores representing better performance.

Cortical thickness was additionally correlated with neurocognitive performance by Pearson correlation ($p<0.05$).

Information about (pre-) processing details of the T1w sequences and statistical analyses are included in detail in research paper 1.

4.1.2 'Kopfbälle im Fußball' Study

Research papers 2 and 3 focus on the 'Kopfbälle im Fußball' ('Heading in football') study, which was a longitudinal and prospective study approved by the ethical review board of the Ärztekammer Westfalen-Lippe, Münster, Germany (2017-386-f-S). The German Federal Institute of Sport Science (Bundesinstitut für Sportwissenschaft) funded the project.

Male football players from a German team were recruited for the study. Initially, 30 players who actively played football were included (mean age: 20.0±2.5 years). All players were considered high-level players as they were highly trained and participated on a national level [141]. At the initial study visit, the medical history was documented, and sport-specific information was obtained with a questionnaire. Neurological or psychiatric disorders, as well as self-reported learning disabilities, led to the exclusion of a player. Detailed exclusion criteria are listed in research papers 2 and 3. At the start of the study, the players participated in different neurocognitive tests (i.e., TMT A and B, PASAT 1 and 2, CNS vital signs). Brain MRI scans were obtained with a 3T Philips Ingenia scanner. The neurocognitive tests and brain scans were repeated at the end of the study.

Between the two testing dates (during the observation period), each training session and each match of the players was videotaped for the header documentation. The videos were analyzed by trained video raters based on a standardized protocol that included predefined categories and criteria [76]. With this approach, the individual heading number of each player was obtained for training, matches, and in total (training and matches). The monthly heading exposure was calculated by dividing the total heading number by the total number of months a player was observed. Additionally, different header characteristics were evaluated. These included the flight distance of the ball, the header intention, the head area, and whether or not it was a heading duel. Headers were categorized as 'header in training or 'header in match'.

Research paper 2 is based on 22 of the initially included 30 players who had complete neurocognitive data (pre- and post-test; mean age: 19.9 years±2.7 years). Of the 22 players, two were goalkeepers, eight defenders, seven midfielders, and five forwards. The total heading number, header characteristics, and differences between training sessions and matches are included in research paper 2 for these 22 players. Additionally, DTI parameters (FA, MD, RD, AD) were analyzed. As eight of the 22 players had aborted MRI scans or incomplete data, these analyses included 14 players (mean age: 20.4±3.3 years).

Results from the TMT A and B, PASAT 1 and 2, and the computerized test battery CNS vital signs were evaluated. From the latter, seven domains (executive function; working, verbal and visual memory; sustained, simple, and complex attention) were investigated as these domains seem to be most likely affected in football players [12]. SS were analyzed, and a better performance is reflected by higher values.

DTI parameters were calculated and extracted from DWI sequences, of which 32 images were diffusion-weighted (b=1000 s/mm²), and one was non-diffusion-weighted (b=0 s/mm²). The Functional MRI of the Brain Software Library (FSL; v. 6.0.5.1) was used for data processing. FA, MD, RD, and AD maps were calculated and analyzed regarding a difference between pre- and post-test. Age was controlled for. In an explorative approach, values for the four DTI parameters were additionally extracted for each WM tract by dividing the skeletonized masks into anatomical WM tracts based on the John Hopkins University (JHU) ICBM-DTI-81 WM atlas [142]. These values were only used for correlation analyses with heading data and not used to test a potential difference between the two measurement time points.

For the evaluation of a potential influence of heading on DTI parameters and neurocognitive variables, first, the change from pre- to post-measurement was calculated for each participant and each parameter. The post-test value was subtracted from the pre-test value. The calculated change was correlated with the individual total heading number and the number of headers that came from over 20 meters. These 'long-distance headers' were focused on due to the higher accelerations of balls coming from longer distances, as explained in Chapter 2.2. A similar categorization was implemented by Kern et al. [143], who considered passes from over 20 meters as ‘high-intensity headers’.

Details on the preprocessing and processing steps, as well as information on the statistical approaches, are included in research paper 2.

Research paper 3 focused on structural and functional neuroimaging parameters. As in research paper 2, the 14 players with complete brain MRI data were included (mean age: 20.4±3.3 years).

After visual and automatic quality check (MRIqc, v. 0.15.2rcl) [139], T1w images were processed with the longitudinal stream provided by FreeSurfer (v. 6.0.0) [144]. Cortical thickness and GM volume were analyzed to determine potential differences between pre- and post-measurement. Age was included as the nuisance regressor. In the GM volume analysis, estimated intracranial volume was additionally controlled for.

Resting-state fMRI data analysis was performed with the CONN functional connectivity toolbox (v. 20b; nitrc.org/projects/conn) [145] in conjunction with the Statistical Parametric Mapping software 12 (Wellcome Centre for Human Neuroimaging, London, United Kingdom) implemented in Matlab R2020a (MathWorks Inc., Natick, MA, USA). Data quality was visually checked. An ROI-to-ROI approach was applied to investigate FC in two brain networks. The fMRI data analysis of this thesis specifically investigated the DMN as RHI exposure was found to alter DMN connections [146] and the FC of the DMN [147,148]. The DMN is the most widely studied and hierarchically superior brain network, which is active when a person is daydreaming or not actively doing something [149,150]. The second network included in this dissertation was the SN. This large-scale intrinsic network mediates the switch between internally directed processes of the DMN and externally directed processes of the central executive network (CEN) [151–153]. Concussion and the diffuse axonal injury seem to impact the connections between DMN and SN and, hence, the organization of the networks [39]. Additionally, FC alterations within the SN were described in postconcussion syndrome patients [154,155] and after sustaining mild to severe TBI or a concussion [156,157]. These results indicate that the SN may also be of specific interest in regard to RHI exposure. To date, however, it has only been included in one investigation showing FC alterations within the network after a season of high school American football [158]. Research on heading exposure has not yet focused on the SN. The brain regions considered for the networks in the dissertation are summarized in Table 2.

Table 2 Brain regions considered for DMN and SN.

Network	**Regions**
DMN	posterior cingulate cortex, precuneus, medial prefrontal cortex, left and right lateral parietal regions [149,150,159]
SN	anterior cingulate cortex (ACC), left and right anterior insula, left and right rostral prefrontal cortex (rPFC), left and right supramarginal gyrus (SMG) [107,145,151,153,160]
Abbreviations: DMN=default mode network; SN=salience network	

To investigate the two networks, FC was analyzed for 12 ROIs (Table 2) with 66 connections and age added as a nuisance regressor. Additionally, the introduced graph theory parameters (Table 1, p. 12) were evaluated to ultimately determine whether heading may impact or influence the small-world organization of the networks and the efficient communication.

A potential association between heading and the change of significant results from the structural and functional analyses was tested afterwards. Correlation analyses included the individual total heading number, as well as the individual number of long-distance headers and the individual number of heading duels. The latter two were included due to the described greater accelerations associated with long-distance headers and head-to-head impacts (see Chapter 2.2).

The preprocessing and processing details of the MRI data and information on statistics are described in research paper 3.

Table 3 Overview of included cohorts and investigated parameters for the three research papers.

Parameter	**Research paper 1**	**Research paper 2**	**Research paper 3**
Cohort			
‘Head in the Game’/former females	✓	×	×
‘Kopfbälle im Fußball’/active males	×	✓	✓
Structural imaging			
Cortical thickness	✓	×	✓
GM volume	×	×	✓
Functional imaging			
FC	×	×	✓
Graph theory parameters	×	×	✓
Diffusion tensor imaging			
FA	×	✓	×
MD	×	✓	×
RD	×	✓	×
AD	×	✓	×
Neurocognitive performance			
CNS vital signs	✓	✓	×
PASAT 1 & 2	×	✓	×
TMT A & B	×	✓	×
Abbreviations: AD=axial diffusivity; FA=fractional anisotropy; FC=functional connectivity; GM=grey matter; MD=mean diffusivity; PASAT=paced auditory serial addition task; RD=radial diffusivity; TMT=trail making test ✓=investigated; ×=not investigated			

4.2 Research Paper 1

Haase FK, Prien A, Douw L, Feddermann-Demont N, Junge A, Reinsberger C. Cortical thickness and neurocognitive performance in former high-level female soccer and non-contact sport athletes. *Scand J Med Sci Sports* 2023;33(6):921-30. doi:10.1111/sms.14324.

Previous studies found significantly greater cortical thinning with age in retired professional male football players compared to a matched control group of non-contact sport athletes. Higher lifetime-estimates of heading were associated with greater thinning. Additionally, the former football players performed worse in memory when compared to the non-contact athletes [124]. However, little is known about retired high-level female football players. The aim of research paper 1 was to compare cortical thickness and neurocognitive parameters between former high-level female football players (SOC) and former female non-contact sport athletes (CON). An additional goal was to evaluate whether there are differences between sub-groups based on concussion history and heading exposure.

There were no significant differences between SOC and CON in demographics and sport-related data. Eight former football players reported frequent heading exposure. Four and three players categorized themselves as having had moderate and rare exposure, respectively. All CON had never headed a ball and were never diagnosed with a concussion. Eight, four, and three SOC were diagnosed with none, one, and more than one concussion, respectively.

After correction for multiple comparisons, the cortical thickness analysis revealed no significant differences between SOC and CON nor between sub-groups based on concussion history. Additionally, CON (having never headed a ball) and the group of SOC with a frequent heading exposure did not differ from all other heading exposure sub-groups. There was one significant cluster for the comparison between moderate and rare heading exposure groups. Players reporting moderate exposure had higher cortical thickness in a right inferior parietal cluster (clusterwise p=0.016, Figure 3).

In five of seven domains, SOC and CON did not differ in neurocognitive performance. However, CON performed significantly better than SOC in psychomotor speed (Bonferroni corrected p=0.018) and verbal memory (Bonferroni corrected p=0.023). In the verbal memory domain, CON performed significantly better than the sub-group with one concussion (Bonferroni corrected p=0.041) and better than the moderate heading exposure group (Bonferroni corrected p=0.043). Overall, 42 differences were analyzed for group differences in neurocognitive performance based on concussion history. Similarly, 42 differences were tested for the self-categorized heading exposure groups. All other analyses were not significant.

Visual memory performance correlated with cortical thickness values of the inferior parietal cluster of CON and of the group with frequent heading exposure. CON showed a negative (Pearson correlation coefficient=-0.523, p=0.038), whereas SOC with a frequent heading exposure showed a positive association (Pearson correlation coefficient=0.721, p=0.044). There were no other significant correlations for either heading exposure group.

In summary, SOC and CON had similar cortical thickness and neurocognitive performance, except for psychomotor speed and verbal memory, in which SOC performed worse. The clinical relevance of the significant difference between moderate and rare heading exposure groups in cortical thickness is unknown, and the relationship between the structure and function of the brain needs further investigation.

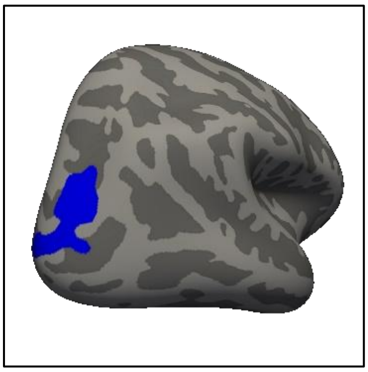

Figure 3 Significant cluster for the cortical thickness comparison between moderate (n=4) and rare (n=3) heading exposure groups (clusterwise p=0.016, cluster size=1003.34mm²; published as Supplemental Material in research paper 1).

Author contributions:

Annika Prien, Linda Douw, Nina Feddermann-Demont, Astrid Junge, and Claus Reinsberger contributed to study design. Annika Prien collected data. Franziska K. Haase, Annika Prien and Linda Douw analyzed the data. All authors contributed to data interpretation. Franziska K. Haase and Claus Reinsberger wrote the manuscript. Franziska K. Haase, Astrid Junge, and Claus Reinsberger contributed to the revision of the manuscript. All authors approved the final version for publication.

4.3 Research Paper 2

Mund FK, Feddermann-Demont N, Welsch G, Schuenemann C, Fiehler J, Junge A, Reinsberger C. Heading during the season and its potential impact on brain structure and neurocognitive performance in high-level male football players: an observational study. *J Sci Med Sport* 2024;27(9):603-09. doi:10.1016/j.jsams.2024.05.012.

A previous investigation, which was one of the first studies investigating the microstructure of the brain in football players, did not find any differences between football players and swimmers in FA and MD but in AD and RD. However, heading exposure was not taken into account [126]. Subsequent studies showed associations between heading and altered FA, AD, and/or RD [69,127,128]. The generalizability of results is limited due to cross-sectional designs or retrospective assessments of heading exposure [69,127,128]. Similarly, results on associations between heading and neurocognitive performance are limited due to low-quality assessments of heading exposure [12] or focusing on youth or student cohorts [79]. DTI measures and neurocognitive performance have rarely been investigated in active high-level football players. Therefore, in research paper 2, these players were included, and the aim was to investigate potential longitudinal changes in DTI parameters and neurocognitive performance. An additional goal was to evaluate the actual heading exposure of the players, as prospective heading documentation is scarce in this cohort. Lastly, potential associations between heading, DTI, and neurocognitive variables were tested.

22 players were observed between 9 and 17.8 months (median: 16.9 months), in which 8052 headers were documented overall. More headers were played in training sessions than in matches (6705 (83.27%) and 1347 (16.73%), respectively). The individual total heading number ranged from 57 to 943 headers. The mean heading number per month per player was 24.19 (standard deviation (SD)=12.83). The eight defenders played the most headers (3983), followed by the midfielders (2677). The forwards headed 1104 times, and the two goalkeepers played 288 headers. Regarding the mean heading number per position, a similar distribution was apparent. Headers mostly appeared after a flight distance of the ball of under 5 meters (59.62%), were played with the frontal part of the head (96.31%), and were intended as a pass (59.94%). Interestingly, header characteristics differed between training and matches, which was most prominent for the flight distance of the ball. In matches, balls more often came from longer distances before headed than in training sessions.

Neurocognitive performance (n=22) did not change significantly from pre- to post-measurement after Bonferroni correction. Similarly, the TBSS approach (n=14) did not show significant changes from pre- to post-test in any of the four DTI parameters (FA, MD, RD, AD).

The correlation analyses revealed no significant associations between heading variables and the individual change from pre- to post-test in neurocognitive parameters after correction for multiple comparisons. Likewise, the calculated change for FA, MD, RD, and AD extracted from the JHU atlas were not significantly correlated with the total heading number. The change in FA in the splenium of the corpus callosum showed a significant correlation with the number of headers over 20 meters (Pearson correlation coefficient=-0.884; p=0.000027). Due to the calculation of change, this association indicates an increase in FA from pre- to post-test with a higher number of long-distance headers. There were no significant correlations for MD, RD, or AD.

Overall, the results indicate stable neurocognitive performance and unchanged WM integrity over the observation period in the football players. Although only one significant correlation was found, the data may potentially indicate that header characteristics might be of specific interest. As long-distance headers showed higher linear and angular accelerations than headers from shorter distances [56,64,88], further investigations of header characteristics may be helpful in the evaluation of the potential effects of heading.

Author contributions:
Nina Feddermann-Demont, Götz Welsch, Carsten Schuenemann, Jens Fiehler, Astrid Junge, and Claus Reinsberger contributed to study design, conceptualization, and investigation. Franziska K. Mund analyzed the data. Franziska K. Mund, Nina Feddermann-Demont, and Astrid Junge contributed to data curation. Franziska K. Mund and Claus Reinsberger wrote the first draft of the manuscript. All authors commented on previous versions and agreed to the final version.

4.4 Research Paper 3

Mund FK, Feddermann-Demont N, Welsch G, Schuenemann C, Fiehler J, Thaler C, Meyer L, Reeschke R, Reinsberger C. High-magnitude headers are not associated with structural and functional brain changes in active high-level football (soccer) players. *BMJ Open Sport Exerc Med* (Submitted).

Previous research has shown that concussions or RHIs can cause structural and/or functional alterations in intrinsic brain networks such as the DMN and SN [39]. Concentrating on football players, results on cortical thickness are inconclusive, showing greater cortical thinning with age in former athletes [124] or rather no effect of heading on cortical thickness [125]. Additionally, greater GM volume was associated with heading [125], and there seem to be associations between FC and impact exposure in different brain networks, including the DMN [74,77]. High-level players actively playing football have only rarely been investigated, and heading exposure assessments had several limitations. Consequently, the goals of research paper 3 were to examine potential structural and/or functional changes in active high-level male football players and to evaluate whether alterations were associated with the objectively assessed heading amount. In addition, specific header characteristics were evaluated, and the number of long-distance headers and heading duels was subsequently included in the correlation analyses.

14 players were included in the analysis, and 5822 headers were documented in training sessions and matches over the observation period (median=17.2 months). 55.79% of all headers occurred after the ball traveled a short distance (under 5 meters). Additionally, headers were mostly played without a heading duel (51.65%). There were no structural differences in DMN or SN regions, but cortical thickness decreased from pre- to post-test in a left precentral cluster with a mean change of 0.048mm (SD=0.128, cluster size=64.6mm^2, clusterwise p=0.042).

FC did not change within the DMN or between the DMN and SN. Within the SN, one cluster showed significant changes (false discovery rate (FDR)-corrected p=0.026). The FC between the right SMG and left rPFC showed a mean change of -0.0563 (SD=0.297, uncorrected p=0.0004, FDR-corrected p=0.025). Similarly, a negative mean change of -0.0076 (SD=0.45) from pre- to post-measurement in FC between the right SMG and ACC was detected (uncorrected p=0.0016, FDR-corrected p=0.054). Due to the calculation of change, these two connections showed a higher FC in the post-test. The graph theory analysis did not yield any significant results.

Neither change in cortical thickness nor change in FC correlated significantly with the total heading number of the players. Similarly, the change in cortical thickness and FC was not associated with the number of long-distance headers and heading duels.

The functional and clinical relevance of the small structural change and the FC alterations within the SN is unknown and should be further investigated. However, there were no other structural changes or functional alterations within the DMN or between the DMN and SN. The calculated change of the significant results did not correlate with the total heading number or specific header characteristics. Therefore, it is possible that the observed alterations may be attributable to other underlying mechanisms rather than heading.

Author contributions:

Nina Feddermann-Demont, Götz Welsch, Carsten Schuenemann, Jens Fiehler, and Claus Reinsberger contributed to study concept and design. These authors and Christian Thaler and Lukas Meyer had a major role in data acquisition. Franziska K. Mund, Rebecca Reeschke, and Claus Reinsberger analyzed the data. Franziska K. Mund, Nina Feddermann-Demont, Rebecca Reeschke, and Claus Reinsberger performed data interpretation. Franziska K. Mund and Claus Reinsberger wrote the original draft of the manuscript, and all authors commented on previous versions. All authors agreed to and approved the final version of the manuscript.

5 Discussion

The main goal of this thesis was to contribute to understanding the potential effects of heading in football. As Beaudouin et al. [78] pointed out, the individual heading exposure itself needs to be analyzed before investigating associations between heading and consequences. Hence, the first part of the discussion focuses on the analysis of heading exposure in active high-level male players and illustrates influencing factors and aspects to consider in future studies. Afterwards, the neuroimaging and neurocognitive findings in football players and the impact of heading are discussed critically. Lastly, the limitations of both studies are presented, and consequences and recommendations for further research that emerge from the results are included.

5.1 Heading Exposure in Active High-Level Male Football Players

Although research paper 3 similarly reports the results of the heading evaluation, the discussion concentrates on the results presented in research paper 2. This is due to the fact that all players included in research paper 3 were part of the larger cohort presented in research paper 2. However, as research paper 2 did not include heading duels, the results of research paper 3 regarding this variable are briefly included.

The analysis in research paper 2 identified a total of 8052 headers in the active high-level male cohort played over a 17-month observation period, exceeding the actual length of one season. Although studies on youth cohorts or female collegiate players similarly included training sessions and matches, higher or lower total heading numbers were reported [84,88]. This might be due to different lengths of observation periods [88] or a much bigger cohort presented in [84]. As males tend to head more often than females [58], sex might be a factor contributing to the differences.
The individual total heading number with a range between 57 and 943 demonstrates a high inter-individuality, which has similarly been described in other studies [58,161]. The explorative calculation of the monthly heading exposure showed a similar distribution and range (from 3.37 to 53.01). One explanation for the differences in the total heading exposure may be the varying playing positions. Previous studies found that defenders had the highest number of headers [80,85], while goalkeepers headed the least [71,82,85]. This was corroborated in our cohort. Therefore, the range of the total header number might be explained by including all players in the analysis, regardless of their position.

Similar to previous study results in youth and university football players [64,70,88], the differentiation between training and matches revealed a higher number of headers occurring in training sessions. A recent study additionally found the greatest heading exposure in training and off-season sessions [161]. These results altogether indicate that all occurring sessions should be included in heading exposure evaluations for a realistic representation.

Regarding the distribution of all documented headers in training and match, our results are similar to those previously described. These studies showed that 80% to 90% of all headers occurred during training sessions in youth cohorts [70,84]. However, the heading exposure is influenced by the number of observed sessions. As there are presumably more training sessions than matches, this might consequently lead to a higher heading number in training. Additionally, coaching style may be a relevant and important aspect as some coaches consider more technical drills (e.g., learning a specific skill/learning a header) than others [162]. The coaching staff of the included cohort was told to proceed with training as usual. However, it cannot be excluded that participation in the study influenced the coaching styles and that more or less headers were trained than before the study's start. In addition, there were training sessions that included special (warm-up) games such as 'football tennis' or that focused on heading training. These sessions may have influenced the total heading number, while at the same time, they represent everyday training.

The number of headers played in matches over the observation period (1347) is lower than the number reported by Cassoudesalle et al. (2115 headers) [80]. This may be explained by the bigger cohort of 54 included players compared to the cohort of 22 players included in research paper 2. Several other studies analyzed matches of (semi-) professional male cohorts [75,76,81,85]. However, the total heading number cannot be compared as a varying number of matches was analyzed, and all headers played in the matches, regardless of the team, were included. This may have led to higher heading numbers in those studies.

Overall, little is known about the actual exposure in training sessions and, consequently, about the total heading number. The results of research paper 2 add valuable information by giving a realistic representation of the heading exposure in that specific cohort.

In addition to the total heading number, headers were characterized in research papers 2 and 3. In (semi-) professional adult cohorts, this was previously limited to analyses of matches [75,76,81,85]. The data presented in research paper 2 showed that most headers occurred after the ball traveled a short distance (under 5 meters). Headers were mostly intended as a pass, which is in line with the results presented by Tierney & Higgins [85]. Similar to other cohorts [75,76,82], the frontal part of the head was mostly used to head the ball, which indicates a proper heading technique in the cohort [163]. In research paper 3, we additionally found that headers mostly occurred without a heading duel, although it must be noted that information on heading duels was missing for a large number of headers.

Comparing headers in training and headers in matches revealed distinct differences in characteristics, similarly reported in youth cohorts [78,84]. In both sessions, the primary intention of a header was to play a pass. In training sessions, this was followed by a kick. In matches, the second most often intention was clearing a (dangerous) situation (e.g., goal attempt of an opponent). The latter was similarly described for professional defenders [85]. More long-distance headers (balls coming from over 20 meters) were played in matches than in training, which was found in youth cohorts as well [84]. Additionally, proportionally more headers were played with the parietal or temporal area of the head in matches than in training sessions. Parietal or temporal contact may have occurred more frequently during matches because players had to react spontaneously to a situation. Additionally, parietal or temporal contact may indicate that headers happened unintentionally, that a player was unaware of the header, or that an incorrect heading technique was used [78]. Unintentional contact or headers and headers played with the parietal part of the head had higher linear accelerations than purposeful headers [89,164]. Therefore, improper heading techniques or unintentional headers may be a cause for concern and of interest in further studies.

Most importantly, the differences between heading in training and heading in matches show that heading is variable and that headers may be similar but not identical.

Regarding research question 1 of this thesis, it is important to note that the total heading number seems to be highly individual and may be influenced by factors such as position, coaching style, or the number of analyzed sessions. More headers seem to be played in training sessions, and header characteristics differ between training and matches. As this was one of the first studies to objectively assess the heading exposure in a high-level cohort, more observational studies that include all matches and training sessions are required to confirm the results. These could span over a season or even longer to also assess potential progressions regarding the individual heading exposure over time. To date, there are rather estimations regarding the total heading number over a whole football career. Hence, longer observation periods would additionally help to evaluate the total exposure over a longer period. High-level or professional, former, active, and/or female players are still underrepresented in heading research and thus need to be focused on in future studies. Such investigations would help to gain more precise insight into heading exposure and different header characteristics. These can thus be used in the development of evidence-based heading guidelines. Additionally, the results of objective assessments, including the different header characteristics, could be used in the analyses of potential heading effects.

5.2 Influence of Heading on Brain Alterations

5.2.1 Overall Heading Exposure and Associations with Neuroimaging Parameters

Structural MRI

Relationships between macrostructural alterations and heading exposure were reported by few studies. Cortical thinning was greater for players with a higher lifetime-estimate of heading, suggesting a dose-response-relationship [124]. Additionally, GM volume was higher with greater heading exposure in amateur players [125]. However, our analyses in research papers 1 and 3 could not confirm or underline these macrostructural findings. Research paper 1 revealed greater cortical thickness in an inferior parietal cluster for players who reported to have moderately often headed a ball compared to players who reported to have only rarely headed a ball. In addition, cortical thickness change in research paper 3 was not associated with the total heading number, and we did not find any significant GM volume alterations.

Interestingly, in research paper 1, we found the greatest cortical thickness values in players who frequently headed the ball (Table 5 in the Appendix). However, this was non-significant. But together with the significant difference between the moderate and rare heading exposure groups and the absence of significant differences between SOC and CON, one possible explanation may be an adaption to football training, including heading as a complex motor skill. This is underlined by the function of the inferior cluster, which is known for higher-order motion and eye-body coordination [165]. Overall, the idea is based on research showing that aerobic exercises are associated with greater cortical thickness [166] and increased cortical thickness after learning unicycling [167]. Additionally, greater GM volume with greater heading exposure in amateur football players was interpreted as an effect of skill acquisition (i.e., heading) [125]. Similarly, greater GM volume was present after learning to juggle, and it was consequently concluded that learning seems to induce cortical plasticity [168].

In research paper 3, the calculated change from pre- to post-test for the finding of decreased cortical thickness in the active high-level male players was not significantly correlated with the total heading number. Amateur adult players similarly did not show significant associations between cortical thickness and heading, although a comparison is limited due to the retrospective assessment of heading exposure in the amateur players [125]. Although the finding of cortical thinning fits the results reported for retired professional players when compared to non-contact sport athletes [124], our finding was restricted to one small cluster in the left precentral region (64.6 mm^2). This contrasts with the results by Koerte et al. [124], who report bigger clusters in several brain areas. Reduced cortical thickness is associated with several neurodegenerative processes and diseases (e.g., Alzheimer's [169], other forms of dementia [170] or Parkinson's [171]). Therefore, although highly speculative, our finding could indicate the presence of early neurodegenerative change in the cohort. This change, however, may not be related to the total heading number, as there was no significant correlation between the two. Alternatively, it may be the result of a normal aging or developmental process [172,173], although we included age as a nuisance regressor in the analysis. A potential developmental or aging process should be tested against a control group of other contact or collision sport athletes in future investigations. This would determine whether cortical thinning is exclusively found in the football cohort or whether it is also present in other cohorts. As cortical thinning was additionally associated with a decline in processing speed in football players [124], further analyses could focus on the potential (functional) impacts of our finding. Nevertheless, we only found one small region of decreased cortical thickness in research paper 3 and the significance or clinical impact of our result remains to be determined.

As stated, the finding of unchanged GM volume is in contrast with previous literature on amateur football players, revealing greater volume with heading [125], and with literature on RHI exposure in general, revealing associations between exposure and lower volumes [174]. This suggests that the effects of RHI exposure should not be generalized across different levels of play or sports. Therefore, it may be necessary to conduct more differentiated analyses.

<u>Diffusion Tensor Imaging</u>

Similar to macrostructural MRI parameters, microstructural parameters are of interest and several studies reported WM alterations in different football cohorts. These studies presented approaches that served as a foundation for our analyses. A link between increased heading exposure and alterations in FA, MD, RD, and/or AD was reported and interpreted as a hint for an acute brain injury and for WM alterations induced by heading [69,127,128,130,131]. In research paper 2, however, we did not find any association between the total heading number and change in four DTI parameters extracted with the JHU atlas over the observation period of 17 months. Hence, our results do not support or confirm the posed hypothesis claiming that longer-term heading exposure is more robustly associated with microstructural alterations [69]. Although our findings are in line with results presented for young adult players, that study investigated the acute effect of playing ten headers [129], limiting the comparison.

The evaluation of microstructural parameters with the TBSS approach revealed no significant differences from pre- to post-test in the male players. Although a similar cohort of professional male football players exhibited significant differences in RD and AD in comparison to swimmers [126], it is unknown whether our cohort would have shown differences from a cohort of other sports athletes. The results are furthermore in contrast to a study reporting altered MD and RD after a season of high school female football [130] but similar to high-level collegiate ice hockey players not exhibiting any changes after one season [175]. This might point to the evaluated cohort. Study results may have been influenced by age, sex, or even the level of play.

Overall, the unchanged DTI parameters might indicate stable WM integrity over the observation period.

Functional MRI

Lastly, functional parameters were investigated in research paper 3. The FC analyses revealed increased FC within the SN over the observation period. Greater FC was similarly found in other football cohorts, and significant correlations between heading and FC were described. Semi-professional players had increased FC between DMN and frontal medial and temporal gyri with increased heading exposure after one season [77]. Another study revealed strong associations between FC within the central autonomic network and heading [74]. Although the SN was not yet included in analyses of football cohorts, a recent study reported altered within-network connectivity after a season of high school American football [158]. FC alterations in the SN were additionally described after concussion and mild to severe TBI [39,157]. Similar to our findings, increased FC within the SN was found four weeks after injury in concussed patients [156]. There may be multiple causes or a combination of causes, including alterations in neurochemistry, metabolism, cerebral blood flow [176], or the metabolic cascade occurring after a concussion [27] that might potentially lead to changes in brain activity after a brain injury. Generally, increased FC, or hyperconnectivity, was described as a common response to neurological disruptions or neural injury [177,178]. Previously, it was interpreted as a compensatory mechanism to maintain functioning and overcome structural or functional alterations [177,179,180] or as adaptation and plasticity resulting from brain injuries [181]. Consequently, studies revealing correlations between hyperconnectivity and heading exposure argued that heading induces similar reactions [74,77]. Our results in research paper 3, however, do not support this hypothesis as we did not find any significant associations between FC and heading. The calculated change from pre- to post-test for both connections was not correlated with the total number of headers. This indicates that potentially other factors induced the alteration and heading did not lead to a similar reaction as head injuries in our cohort. As the underlying cause and potential (behavioral, neurocognitive, functional) consequences of the increased FC within the SN are largely unknown, further investigations need to be conducted.

Finding no FC changes within the DMN or between the DMN and the SN may indicate stable functioning and communication of the DMN and between both networks. This is underlined by the graph theoretical analysis that did not show any significant changes in the topology and organization of the networks in research paper 3. These results contrast with findings in high school female football players showing alterations in global clustering coefficient and path length after one season. The authors interpreted these findings as an indication of a more segregated brain network organization [62]. Similarly, changes in graph theory parameters associated with RHI exposure or different stages of brain injuries were interpreted as altered network topology and altered functional, small-world organization after injury [182,183]. Although the results are difficult to compare as brain injuries or different RHI exposure were evaluated and the cohort's characteristics differ, finding no graph theory alterations may indicate stable topology and small-world organization of the DMN and SN, even if an increased FC within the SN was found. Due to the significant finding within the SN, a network to include in future studies could be the CEN, as the SN mediates the switch between the DMN and CEN [151,152]. Further evaluations would help to elucidate and comprehend the role and function of the SN and any potential consequences for other networks.

5.2.2 Overall Heading Exposure and Associations with Neurocognitive Parameters

Previous studies reported a link between heading and neurocognitive outcomes [5,66,68,132]. The findings served as a basis for subsequent research and for the projects presented within this thesis. However, the evidence for a definite correlation between exposure and impairments is rather weak [12,13,79]. In research paper 1, the group reporting a moderate heading exposure performed worse in verbal memory than the control group who never headed a ball. However, the actual impact of this finding is unclear, as there were no significant differences between heading exposure groups for all other neurocognitive domains. In research paper 2, we additionally did not find significant correlations between the total heading number and change in any of the evaluated neurocognitive tests. By using an objective method to evaluate heading exposure, our longitudinal study addressed one methodological limitation of previous studies, which mostly used retrospective methods [12]. Interestingly, a recent review of studies using independent observation methods summarized that two-thirds of the reviewed articles similarly did not find a negative effect of heading on neurocognitive parameters [79]. Overall, our data do not support a clear relationship between heading and neurocognitive impairments.

Compared to the controls, former high-level female football players performed worse in psychomotor speed and verbal memory in research paper 1. This was similarly found in the bigger cohort described by Prien et al. [66]. A worse performance in visual memory in former professional male players compared to a control group was interpreted as an early indication of cognitive deficits [124]. Although this interpretation may also apply to our cohort, it should be noted that our cohort was younger and that the scores remained within the normal or above-normal range. The (clinical) significance of the detected worse performance is unknown, as the two groups furthermore did not differ in the other five domains (i.e., reaction time, complex attention, cognitive flexibility, processing speed, and visual memory) in research paper 1. The analysis of several neurocognitive tests in research paper 2 showed no significant differences from pre- to post-test in the active high-level male cohort. This is in line with results on high-level collegiate and professional football players who did not differ from other contact sport athletes or non-athletes [133,184]. Altogether, these results might indicate no neurocognitive impairments in high-level football players or only very subtle deficits in the female players. An overall conclusion regarding neurocognitive performance in football players is limited by the heterogeneity of utilized neurocognitive tests [12,79], rather subtle impairments in football players and potential influences of the cohort’s characteristics [12].

In summary, regarding the posed research question 2, we report only small differences between the two female groups in neurocognitive performance with unknown (clinical) significance and no differences in cortical thickness. The sub-group analyses based on heading exposure revealed one significant difference in cortical thickness, showing greater values for the group with moderate exposure when compared to the rare exposure group. The control group performed better than the moderate heading exposure group in one neurocognitive domain. Due to the absence of any other significant differences between the heading exposure groups, the small (sub-)groups, and the self-categorized heading exposure, these findings must be interpreted cautiously.

Research question 3 of this thesis aimed to investigate whether neuroimaging and/or neurocognitive parameters change in active high-level male football players and whether there are associations with heading. WM microstructural and neurocognitive parameters remained unchanged over the observation period (research paper 2). Cortical thickness decreased in a small precentral cluster, and FC increased within the SN over the observation period, as reported in research paper 3. However, there were no other macrostructural differences on a whole-brain level or FC changes in the DMN or between the DMN and SN. The calculated change of significant findings in research paper 3 did not correlate with the total heading number. Similarly, there were no significant correlations between the total heading number and the calculated change in DTI and neurocognitive measures in research paper 2. Overall, although there are subtle structural and functional alterations in active football players, it should be noted that the majority of the parameters remained stable. Given that the study included healthy and uninjured young adults, this finding may be unsurprising. Importantly, the correlation analyses revealed no significant associations between the total heading number and any changes over the observation period. This suggests that alternative mechanisms or modifying factors may have contributed to the results rather than the total heading number.

5.2.3 Header Characteristics and Associations with Neuroimaging and Neurocognitive Parameters

While there were no significant associations between response time and total heading number, Koerte et al. [73] reported less improvement in response time with a higher number of long-distance headers. They consequently hypothesized that header characteristics may be important when investigating potential effects of heading. This idea seems reasonable as angular and linear accelerations are higher for specific headers, including those delivered via a long-kick [88]. Similarly, head-to-head impacts showed higher accelerations than other head impacts, including purposeful headers [89]. These head-to-head impacts can occur in heading duels. Higher accelerations and head-to-head impacts have greater risks of causing head injuries [14,59,86,87,185]. Consequently, in research papers 2 and 3, the differentiation between header characteristics was used in correlation analyses to investigate the potential association between brain alterations and heading. Research paper 2 focused on the number of long-distance headers, and research paper 3 additionally included the number of heading duels. There were no significant associations between the number of long-distance headers and change in neurocognitive performance or the DTI parameters MD, RD, and AD in research paper 2. Similarly, in research paper 3, no significant correlations were found between change in cortical thickness and change in FC with the number of long-distance headers or heading duels. Nevertheless, we found one significant association between FA and the number of long-distance headers.

Finding no association between change in neurocognitive parameters and number of long-distance headers is in contrast to the results by Koerte et al. [73] presented at the beginning of this chapter. However, the domain response time was not investigated in our analysis, and the difference in age between the cohorts may have contributed to the differing findings. Regarding neuroimaging parameters, to date, there are no other studies on football players using a differentiation of headers in correlation analyses. However, head impacts were differentiated in other collision or contact sports. Sensor data was used in collegiate American football players. Although the FC of the DMN did not significantly differ from pre- to post-season, DMN connectivity was associated with impact measures. Players who experienced a greater distribution of high-force impacts (i.e., impacts exceeding 80G) had greater FC increases in the DMN throughout the season [186]. Another study investigated potential differences between non-contact, contact, and collision sports. Interestingly, global FC, as the average FC over brain regions showing significant differences, decreased gradually with the level of contact exposure. More specifically, non-contact sport athletes had significantly greater global FC than collision sport athletes. Contact sport athletes showed overlapping results with the two other groups [187]. Although these results contrast our results, showing no associations between the number of long-distance headers and heading duels with the calculated change in most of the neuroimaging parameters, they illustrate that a differentiation may be meaningful. This idea is underlined by results showing longer-lasting effects of long-distance headers compared to short-distance headers on response time [137], by findings suggesting that the impact distribution is more predictive of fMRI changes than the actual impact count [188], and our results of research paper 2. We found one significant association for FA in the splenium of the corpus callosum, which indicated increased FA at the post-test with a greater number of long-distance headers. Although only one significant correlation was found, several tracts showed trends towards significance, not only for FA but also for MD and RD. Numerically, there were more non-significant associations for the number of long-distance headers than for the total heading number (Supplemental Material of research paper 2). Elevated FA was similarly detected in collision sport athletes compared to contact and non-contact sport groups [187]. It was hypothesized that the significant differences between collision, contact, and non-contact sport athletes are based on the higher magnitudes of head impacts in collision sports [187]. Due to the greater linear and angular forces of long-distance headers [56,64,88], this assumption may similarly explain our finding. However, the direction is in contrast to the results of a recent review reporting lower or decreased FA with increased RHI exposure [53]. The difference might be due to the exposure itself, as several sports were summarized and included in the review without differentiating the impacts. It may be possible that heading, or specifically headers coming from more than 20 meters, have different influences than RHIs of other sports. Moreover, the interpretation of the direction of FA change is complicated due to several assumptions that potentially explain alterations. Increased and decreased FA values were described as indications of WM injuries. Decreased FA may show a direct mechanical injury (to the myelin sheath or the axon itself), while increased FA might be based on cytotoxic edema and, therefore, indicate an acute tissue injury [189]. Additionally, repetitive injuries might induce growth processes, which may explain an increase in FA [187]. Therefore, although highly speculative, increased FA might be an indicator of (WM or repetitive) injuries. This is underlined by descriptions of increased FA values in the corpus callosum in the acute phase after a concussion [15,190]. Hence, our finding in the splenium of the corpus callosum may indicate a similar reaction induced by the long-distance headers. As increased FA may additionally be correlated with clinical outcomes [189], potential (functional) effects of the finding should be further investigated.

In the recovery phase of a concussion, FA values normalize towards baseline, suggesting a transient change [15,190]. Additionally, the direction of change in DTI parameters seems to be related to the time at which participants are scanned [191,192]. Thus, regeneration time after heading or time since the last (long-distance) header might be factors to consider in further analyses. Alterations in DTI parameters that reversed towards baseline after three months without head impacts, interpreted as a repair mechanism [130], support this idea.

It is also important to consider other approaches for interpretation. Similar to the explanation of greater cortical thickness in the group with a moderate heading exposure, increased FA might resemble an effect of learning a motor task [193]. It could also represent an adaption to experience. A positive correlation between FA and sports experience was found in football players when compared to non-athletes and, consequently, interpreted as an experience-dependent alteration [194]. Nevertheless, the measured alterations may be rather unspecific as the technique does not differentiate underlying mechanisms such as axonal count, density, or fiber organization [195].

As research question 3 of this thesis targeted associations between heading and brain alterations, it is necessary to return to this question. We found one significant association between a DTI parameter and the number of long-distance headers in the male cohort. In the absence of significant associations with the total heading number, this may point to the importance of differentiating headers when investigating potential effects of heading. Therefore, our results may serve as a foundation for future projects, focusing more specifically on headers associated with higher accelerations. The results of such studies could either reinforce or challenge the existing guidelines for professional players [4] and also aid in the development of such guidelines.

Altogether, as both projects investigated uninjured, healthy participants (i.e., no acute brain injury or neurological diseases), the results of a rather stable structure and function of the brain may not be unexpected. Nevertheless, our results do not provide sufficient evidence to support a definite relationship between heading exposure and effects but highlight the need for further well-designed studies and a differentiation of headers.

5.3 Limitations and Considerations for Future Projects

The results of the middle-aged former female high-level football group and the (relatively young) adult male active high-level players cannot be generalized due to several limitations. The findings may be specific and exclusive for the cohorts due to age and level of play. Additionally, the cohorts were rather small, and sub-group analyses in research paper 1 were based on groups of three to eight players. Subsequent analyses based on player positions were not conducted due to even smaller sub-groups in both studies. The small sample sizes may have influenced the results, and it cannot completely be ruled out that the findings are rather incidental. Consequently, larger cohorts with several age groups and levels of play are urgently needed in future studies. As suggested within the discussion, RHI exposure might not be comparable between sports. Therefore, comparisons between athletes exposed to RHIs with a similar level of play and age are needed. Such an analysis was intended in the longitudinal study as a different collision sport cohort should have been included. Due to COVID-19 pandemic restrictions, this could not be achieved. In addition, groups of athletes without RHI exposure and non-athletes could be included in bigger investigations. However, including only non-athletes as a control group may potentially demonstrate exercise-induced alterations in the football cohort rather than revealing those changes induced or influenced by heading. Nevertheless, this may be a starting point for future investigations.

When investigating potential heading effects, time might be a relevant factor to consider. Heading exposure in the preceding year or over the career or lifetime was associated with macro- and microstructural neuroimaging parameters [69,124,128]. Additionally, associations between greater long-term heading exposure and worse neurocognitive performance were described [68]. The observation period of our study in active high-level male players was longer than a year, applying a prospective and objective method for the evaluation of heading exposure. With this approach, we could not confirm such correlations. However, structural brain changes may take longer to appear [100,186], and the onset of FC alterations may be delayed due to delayed responses such as neuroinflammation or edema [196]. Therefore, it is still questionable whether the observation period was long enough to reveal alterations. It may also be possible that alterations are transient, and may only be detectable at specific stages or within specific timeframes. This indicates the necessity for studies with intermediate investigations to reveal a potential trajectory. Ideally, and to fully understand potential heading effects,

players would be observed from the start of their careers (mostly at young ages). Multiple follow-up neuroimaging and neurocognitive examinations, conducted throughout and after the career, may offer insight into the changes resulting from development or expertise and those attributable to heading.
The observation period, and hence the time between scans in research papers 2 and 3, was between nine and 17 months. It is questionable whether this period already reflects long-term alterations or whether it rather resembles a current snapshot. Similarly, the cross-sectional study design in research paper 1 may only show a current condition rather than a long-term or late effect of heading in football. Furthermore, hours or several days (for the male cohort) or years (for the female cohort) might have passed since last heading a ball, and the actual time between the last header and MRI scan was unknown. Thus, time should be considered and included in further analyses.

Another limitation is the exclusion of several potentially influencing factors in the analyses. Although age was controlled for, there may have been aging or developmental processes within the observation period in research papers 2 and 3. In research paper 1, concussion history was added and investigated, but it was not controlled for in research papers 2 and 3. As Koerte et al. [126] pointed out, lifestyle, head injuries, or sudden accelerations might contribute to changes in the brain. Greater neck strength is associated with more intact WM organization in football players [197] and lower head accelerations during heading [198], indicating that neck strength seems to be an important aspect to consider. Furthermore, the genotype affected microstructural alterations from heading [199], and elevated neurotrophic factors were apparent directly after playing headers [200]. These results demonstrate that several other factors are potentially important when investigating heading consequences. However, these were not controlled for in the studies of this thesis, mainly because the data was unavailable. Nevertheless, similar to the actual effect of heading, the influencing factors and underlying mechanisms are not completely investigated and understood. It may be possible that certain factors could have a cumulative effect or that an interaction of specific factors is crucial, although this is highly speculative. Still, multiple (potentially) confounding factors need to be considered in future analyses to exclude that results are driven by factors other than heading, which was similarly suggested by others [51,201].

The use of various MRI modalities and sequences is a huge advantage and strength. However, we did not investigate sub-cortical or WM volumes. In research paper 2, the TBSS approach was based on the WM tract skeletons. Using tractography approaches

would have extended the analysis to the whole volume of WM tracts and would have added information on structural connectivity and the architecture of the WM [98,99]. The fMRI analysis in research paper 3 was restricted to the DMN and SN, based on previous research revealing FC changes during resting-state in these networks after head injuries [39]. Whole-brain and additional network analyses may be beneficial in future studies as alterations associated with heading were found from the DMN to other brain regions [77] or within the central autonomic network [74]. The various MRI sequences should additionally be used in a complementary way. Together with different tests of neurocognitive performance, such a multimodal approach would add valuable information, help interpret results, and provide information on potentially underlying mechanisms, as similarly suggested by Koerte et al. [53].
Although the latest software was used for the MRI analyses, it remains questionable whether this might have impacted the results. Differing analysis approaches (e.g., surface- vs. voxel-based, varying (pre-) processing steps, or default pipelines in different software) also complicate an overall comparison. Consequently, future research should replicate the presented studies, add newer software or versions, and compare the results. In addition, standards for neuroimaging assessments and (pre-) processing steps are needed to increase the quality of images and the comparability of study results [53]. The latter would additionally be increased by standard outcome reporting, such as percentage change rather than absolute values.

Research papers 1 and 2 included analyses of the neurocognitive performance. While all included tests or test batteries are reliable and sensitive to mild cognitive impairments or subtle alterations [113–116], there are limitations in the domain selection. Research paper 1 focused on seven domains based on and following the analysis of the bigger cohort [66]. In research paper 2, the analysis was restricted to attention, memory, and executive function domains of the CNS vital signs based on previous findings [12]. Additionally, scores of the TMT A & B and PASAT 1 & 2 were analyzed. Therefore, it remains unknown whether the performance in other domains or tests would have differed between the two female groups or whether it would have changed over the observation period in the high-level male players. As changes might be very subtle and only detected by some tests [12], adding other domains or tests in both studies might have potentially provided a greater understanding of the overall neurocognitive performance.

One other potential limitation may be the method applied to test the effect of heading on structural and functional parameters. In research paper 2, the change in DTI parameters extracted from the JHU atlas, and neurocognitive parameters was calculated by subtracting the post-test values from the pre-test values. Similarly, in research paper 3, the change from pre- to post-test for the significant structural and functional results (i.e., cortical thickness and FC within the SN) was calculated. Afterwards, the calculated delta was correlated with the different heading variables. In future studies, it may be useful to include heading exposure and header characteristics as co-variables in statistical multivariate models or regression analyses within neuroimaging analyses to reveal potential relationships. Regardless, the applied method was feasible for investigating whether there was any association between the change from pre- to post-test and the total heading number or specific header characteristics.

The methods for heading exposure assessment in both included research projects have additional limitations. In research paper 1, the self-categorization into a specific heading exposure group (‘frequent’, ‘moderate’, ‘rare’) and the players’ interpretation of these categories are highly subjective. Objective header observations are more reliable, and video analysis is considered the gold standard [72]. This method was used in research papers 2 and 3. Although this allowed for concrete documentation and characterization of all headers, linear and/or rotational accelerations could not be investigated with this method. In addition to video analysis, sensors could be applied in further studies to analyze accelerations and forces associated with specific header characteristics. This can provide crucial information and broaden the understanding of potential heading consequences. But, to date, it remains unknown how exactly external forces are transmitted to the brain and whether this might additionally be influenced by factors such as neck strength, brain, or body size [18].
Including all training sessions and matches gave a realistic impression of the actual heading exposure in active high-level male players. However, as explained, the total heading number is influenced by several factors. Hence, it may not be the ideal parameter to consider in examinations of heading consequences, and incidence rates (e.g., by 1000 player hours) should be calculated in future studies. These depend on the actual exposure times of players and are consequently more comparable between studies. As data on the number of sessions and exposure times of the players, including winter breaks or injury-related pauses, was unavailable, incidence rates could not be calculated in research papers 2 and 3.

6 Conclusion and Perspective

This dissertation investigated associations between heading and structural and functional brain alterations in former and active high-level football players. Despite finding minor alterations, the structure and function of the brain appeared to remain largely unchanged across the investigated cohorts. Given the inclusion of uninjured and healthy former and active adult athletes, this outcome may not be unexpected. Overall, the results of the neuroimaging and neurocognitive analyses contribute to the insufficient evidence to declare a definite (dose-response) relationship between heading and structural or functional effects. However, they highlight the need to differentiate between header characteristics in upcoming studies.

The cross-sectional comparison of former female football players and non-contact sport athletes as controls showed no differences in cortical thickness and similar neurocognitive performance, except for worse results for the football players in verbal memory and psychomotor speed. The players with self-reported moderate heading exposure performed worse in verbal memory when compared to the controls and had greater cortical thickness than players with self-reported rare exposure in an inferior parietal cluster of the right hemisphere. However, as there were no further differences between heading exposure groups, the results could not establish a relationship between exposure and effects.

The objective evaluation of heading exposure in the active male players showed a high inter-individuality in the total heading number and distinct differences between heading exposure in matches and training sessions. Overall, neurocognitive performance, whole-brain GM volume, DTI parameters, and FC within the DMN and between the DMN and SN remained unchanged over the observation period of 1.5 years. Minimal cortical thinning in one small precentral cluster and increased FC within the SN were found longitudinally. The calculated change of the findings was not correlated with the total heading number or the number of heading duels and long-distance headers. This may suggest that other factors contributed to the results rather than the heading exposure alone. Therefore, the evaluation of potentially influencing factors such as age, sex, lifestyle, genetics, and history of concussion or brain injuries should be a focus of further longitudinal studies. Regeneration time or time since the last header may be of further interest and importance as transient alterations were reported [79,136].

In the male players, a subsequent correlation analysis revealed one significant association between the number of long-distance headers and increased FA in the splenium of the corpus callosum. This finding indicates an effect of specific headers on the microstructure of the brain and suggests that a differentiation between headers may be particularly important when investigating potential heading effects. As long-distance headers exhibit greater velocities, the focus of further investigations should be on headers associated with greater forces. These results and further evaluations of heading exposure and effects in various football cohorts could potentially aid in the development of evidence-based heading guidelines.

Together with the individuality of the heading exposure and the differences between exposure in training and in matches, the results suggest that heading exposure and potential effects may not be generalizable. Additionally, our results were partly contradictory to previous findings on RHI exposure in other collision and contact sports. Hence, general conclusions cannot be drawn, and the differences between results suggest differentiating exposure between sports.

Although the higher risk for neurodegenerative diseases in football players mentioned at the beginning of the dissertation is often attributed to heading, the results of the thesis do not establish a concrete relationship between heading and (neurodegenerative) alterations. However, the small sample sizes limit the generalizability of the results, and replication studies are necessary to ensure the validity of the presented findings. To truly understand a potentially dynamic response to heading, more well-designed longitudinal studies applying standardized neuroimaging methods and neurocognitive tests are required. These should additionally apply objective methods to document and evaluate the heading exposure in all occurring matches and training sessions. Such multimodal approaches, that analyze and combine various parameters, are needed to gain a deeper and more detailed understanding of how heading might affect the brain.

In summary, this dissertation does not show a clear relationship between neurodegenerative alterations and heading in football players but indicates that headers with higher accelerations and forces may potentially affect the brain’s (micro-)structure. It provides a starting point and offers ideas for future studies that investigate associations between heading exposure and potential structural and functional brain alterations in football players. To date, the evidence is still insufficient to proclaim a definite association between heading and (negative) effects.

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Appendix

Table 4 CNS vital signs domains, domain descriptions, tests, and score calculations (based on and modified from [123]).

Domain	Domain description	Tests	Score calculation
Verbal memory	Recognize, retrieve, remember words	Verbal Memory	Correct hits immediate + correct passes immediate + correct hits delay + correct passes delay
Visual memory	Recognize, retrieve, remember (geometric) figures	Visual Memory	Correct hits immediate + correct passes immediate + correct hits delay + correct passes delay
Composite memory	Recognize, retrieve, remember words and (geometric) figures	Verbal and Visual Memory	Addition of verbal and visual memory correct hits immediate + correct passes immediate + correct hits delay + correct passes delay
Motor speed	Skill/ability to perform movements in order to produce and satisfy an intention to a manual action and goal	Finger Tapping Test	Right taps average + left taps average
Processing speed	Recognize and process information, i.e., responding to incoming information, motor speed, fine motor coordination	Symbol Digit Coding	Correct answers - errors
Psychomotor speed	Perceive, attend, respond to visual-perceptual information and perform motor speed and fine motor coordination	Finger Tapping Test and Symbol Digit Coding	Right taps average + left taps average + correct responses on Symbol Digit Coding
Reaction Time	Speed of reaction to simple and increasingly complex set of directions	Stroop Test	(Complex reaction time correct + reaction time correct) / 2

Domain	Domain description	Tests	Score calculation
Executive function	Recognize rules or categories and manage/navigate rapid decision making	Shifting Attention Test	Correct answers - errors
Cognitive flexibility	Skill/ability to adapt to increasingly complex and rapidly changing set of directions and/or to manipulate the information	Stroop Test and Shifting Attention Test	Shifting Attention correct responses - Shifting Attention errors - Stroop commission errors
Simple attention	Track and respond to a single stimulus over time whilst performing vigilance and response inhibition	Continuous Performance Test	Correct answers - commission errors
Complex attention	Track and respond to various stimuli over time and/or perform mental tasks that require vigilance	Stroop Test, Shifting Attention Test and Continuous Performance Test	Stroop commission errors + Shifting Attention errors + Continuous Performance commission + omission errors
Sustained attention	Direct/Focus cognitive activity on concrete stimuli	4-Part Continuous Performance Test	(Part 2 correct + part 3 correct + part 4 correct) - (part 2 incorrect + part 3 incorrect + part 4 incorrect)
Working memory	Recognize and attend to symbols by using short-term memory processes	4-Part Continuous Performance Test	(Part 4 correct) - (part 4 incorrect)
Reasoning	Recognize, reason, respond to non-verbal (visual-abstract) stimuli	Non-Verbal Reasoning Test	Correct answers – commission errors

Table 5 Cortical thickness values in the inferior parietal cluster for the different heading exposure groups (published as Supplemental Material in research paper 1).

Heading exposure group	**Mean cortical thickness (in mm (SD))**
Frequent (n=8)	2.224 (0.115)
Moderate (n=4)	2.143 (0.139)
Rare (n=3)	2.120 (0.320)
Never/CON (n=16)	2.136 (0.104)
Abbreviations: CON=former non-contact sport female athletes; SD=standard deviation	

Original Research Articles

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Mund FK, Feddermann-Demont N, Welsch G, Schuenemann C, Fiehler J, Junge A, Reinsberger C. Heading during the season and its potential impact on brain structure and neurocognitive performance in high-level male football players: an observational study. *J Sci Med Sport* 2024;27(9):603-09. doi:10.1016/j.jsams.2024.05.012.

Mund FK, Feddermann-Demont N, Welsch G, Schuenemann C, Fiehler J, Thaler C, Meyer L, Reeschke R, Reinsberger C. High-magnitude headers are not associated with structural and functional brain changes in active high-level football (soccer) players. *BMJ Open Sport Exerc Med* (Submitted).

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ORIGINAL ARTICLE

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Cortical thickness and neurocognitive performance in former high-level female soccer and non-contact sport athletes

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Background: Long-term effects of playing soccer (football) on the brain structure and function of the brain are vividly debated. While some studies showed differences in neurocognitive performance and structural brain changes in retired male players, data on female players are scarce. The present study compares cortical thickness and neurocognitive performance in former high-level female soccer (SOC) and non-contact sport athletes (CON).
Methods: 3 T T1-weighted 3D MPRAGE MRI was performed, and vertex-wise cortical thickness was analyzed using FreeSurfer (v. 6.0.0). Neurocognitive performance in seven domains of SOC and CON was assessed. A multivariate linear model was used to analyze interactions with respect to heading frequency and a history of concussion.
Results: SOC ($n = 15$, mean age 38.3 ± 5.1 years) and CON ($n = 16$, mean age 36.6 ± 5.8 years) had a similar cortical thickness and performed similarly in the neurocognitive tests except for verbal memory and psychomotor speed, where SOC performed significantly worse than CON. Moderate headers had a significantly larger cortical thickness than rare headers in the right inferior parietal region. Visual memory and cortical thickness were positively correlated in the group of frequent headers and negatively correlated in CON, but not in the other header groups.
Perspective: In contrast to previous reports in male soccer players, female players did not reveal cortical thinning in comparison with control athletes, whereas neurocognitive profiles of female soccer players might not significantly differ from male athletes. Small sample sizes, subjective header assessment, and the case–control study design require a cautious interpretation.

KEYWORDS
(repetitive) head impacts, cognition, cortical thickness, football, MRI, soccer

Section specialty area: Sports medicine & orthopaedics (Section IV).

1 | INTRODUCTION

Soccer is one of the most popular sports worldwide and is associated with various health benefits.[1,2] Besides these positive impacts, soccer players have also been reported to be at higher risk for neurodegenerative diseases, which might be affected by playing position and career length.[3] Although those studies might be interpreted with caution, because methodological aspects (e.g., disregard of modifying factors, selection biases, cross-sectional or retrospective study designs) impair generalization of study results, those results most certainly stimulate further scientific discussion. Possible negative (long-term) effects of soccer and other contact sports regarding neurodegenerative processes and their underlying mechanisms are still not fully understood. Factors that are discussed to influence long-term effects are sustained concussions and repetitive head impacts (RHIs), which are prominent in collision and contact sports, such as American football, rugby, ice hockey, or soccer.[4] Concussions seem to functionally and structurally alter the brain.[5] The latter might classically be revealed by diffusion tensor imaging analyzing the white matter (WM) integrity, which was found to be altered in concussed athletes.[6] In American football players, a history of concussion might also be associated with gray matter (GM) abnormalities like cortical thinning.[7] Moreover, volume loss in the amygdala and the right hippocampus was demonstrated in retired boxers compared to active boxers and a control group[8] and professional rugby players with a history of concussion and RHIs exhibited smaller whole brain, smaller bilateral hippocampal, and smaller left amygdala volumes.[9] A recent review summarized the effects of a concussion history in retired athletes from different sports on neurocognitive performance. Memory, executive functioning, and psychomotor function seem to be negatively associated with a concussion history.[10] Furthermore, (mild) neurocognitive deficits were found in former elite rugby players,[11] one of the sports with a high risk of concussion[4] and a high exposure to RHIs. Overall, comparison and generalization of research on concussion as well as on RHIs is limited due to heterogeneous samples with variable injury severity or definition and various RHI characteristics.[4,14]

Soccer is the only sport, in which the head is purposefully and repetitively used to play the ball. Thus, RHIs are an area of concern in soccer in addition to concussion.[12] But in contrast to concussion, results on (long-term) effects of RHIs are still heterogeneous.[13,14] Reports on potential micro- and macrostructural changes in relationship to headers have sparked a vivid discussion in the scientific community and in (professional) sports.[12] Female soccer players demonstrated greater WM alterations than male soccer players,[15] but the underlying stimuli and clinical consequences still remain to be elucidated. Retired professional male players showed a significantly greater decrease of cortical thickness (the distance between GM/WM- and GM/cerebrospinal fluid (CSF) boundary) with age than a matched control group of swimmers in brain regions including the temporal, parietal, and occipital lobes bilaterally.[16] In contrast, Oliveira et al.,[17] did not find any associations of cortical thickness or less GM volume with heading in amateur soccer players. However, the variability of study designs, utilized methods, and examined cohorts often make it difficult to pool results and eliminate nuisance factors, when causality of cerebral changes and adaptations to playing soccer or headers should be established.

Similar to structural changes, the long-term effect of RHIs (i.e., heading) on neurocognitive performance is not clear. Koerte et al.[16] reported decreased memory performance in former professional, male soccer players when compared to non-contact sport athletes. By contrast, Guskiewicz et al.[18] found no impaired neurocognitive performance in collegiate soccer athletes. Prien et al.[19] reported that retired elite female soccer players performed similar to age-matched elite female non-contact sport athletes on various neurocognitive tests, except for significantly lower scores on verbal memory and verbal fluency tests. To evaluate neurocognitive performance in relation to concussion and to RHIs a large variety of tests was used. Computerized test batteries or structured individual testing, however, may cover all domains that are possibly affected by concussion. Similar to studies investigating effects of concussion, studies on heading and neurocognitive performance are characterized by heterogeneous designs (especially concerning determination of external loads/headers). When assessing the association between heading frequency and neurocognitive performance, results are somewhat inconclusive showing either no effects of heading[20,21] or better performances in verbal and visual memory, motor processing and speed in female soccer players compared with male soccer players.[22]

Functional deficits within the spectrum of traumatic brain injury may be associated with underlying alterations of architecture of brain network. WM integrity in the corpus callosum, fornix, internal capsule, arcuate, and uncinate fasciculi, for example, has been linked to memory and/or attention deficits following traumatic brain injury, although establishing the link between structural network integrity and cognitive deficits still needs to be replicated.[23] How those changes might relate to RHIs (vs. traumatic brain injuries) or affect cortical architecture within the affected functional networks (vs. diffusion tensor imaging-based WM integrity) is largely unknown, although those relationships may not appear unlikely, when plastic changes in aging and pathological changes in neurodevelopmental disorders are considered and regarded as models.[24]

In general, many more studies on heading and RHIs investigated male soccer players, although female soccer players had a higher incidence of concussions than male players,[4] which might be due to different neck strength or females being more open on reporting such incidences.[25] Furthermore, concussed females report a higher symptom burden and longer recovery periods.[25] Therefore, females might also be more vulnerable to long-term effects of RHIs than male players[26] despite the tendency toward less heading and subjective overestimation of heading burden in females.[27] Most studies investigating potential long-term effects of soccer, however, only rarely include and focus on female soccer players.[14] Although some studies differentiate between the sexes[15,22] others do not.[17,18] Furthermore, studies focusing on female soccer cohorts are limited by only investigating a specific age range of players (e.g., high-school or college[20,21]).

These aspects and various methodological differences and inconclusive results necessitate further investigations regarding potential (long-term) effects of soccer on structural brain changes and the neurocognitive performance, specifically in high-level retired female soccer players. Therefore, the aim of this study was to compare cortical thickness and neurocognitive performance of former high-level female soccer players to a cohort of former high-level female non-contact sport athletes.

2 | MATERIALS AND METHODS

2.1 | Design

This case–control study was approved by the ethical review boards of the Westfalian Medical Board, Münster, Germany (2016-449-f-S) and the VU University Medical Center Amsterdam, the Netherlands (2017.360).

2.2 | Study procedure

A subsample of a cohort presented in detail elsewhere (see Prien et al.[19]) was analyzed in the presented study. Female soccer players (SOC) who retired at least 2 years before the study and who played in the first league and/or the national team and non-contact sports athletes (CON) who participated at national championships and/or for the national team were included. Exclusion criteria for participation were: 1. severe non-sport related head injury (diagnosed by a physician) or 2. disease affecting the central nervous system, or for CON: 3. more than one concussion or any severe head injury. SOC and CON were matched for age and level of competition on a national level.

After informed consent was obtained, subjects underwent an online survey, a semi-structured interview, and neurocognitive testing. The subsample presented in this study additionally had an MRI scan of the brain. Demographic and sport-related characteristics were obtained using an online survey. The semi-structured interview was used to gather information about the history of concussion, which was defined as proposed by the Concussion in Sports Group,[28] other injuries to the head, neck, or face, and heading frequency. SOC had to characterize themselves as a rare, moderate, or a frequent header.

2.3 | MR imaging data acquisition and image processing

Whole-brain MR imaging was performed on a 3 T Philips Ingenia_CX with a 32-channel receive-only head coil, located at the Spinoza Centre for Neuroimaging, Amsterdam, the Netherlands. For the determination of cortical thickness, T1-weighted magnetization-prepared rapid acquisition gradient-echo images were obtained (MPRAGE, repetition time 8.3 msec; echo time 3.8 msec; inversion time 929 msec; flip angle 8°; field of view 240 mm × 188 mm (AP × RL); slice thickness 1 mm). The quality of the dataset was checked visually and automatically using MRIqc (v. 0.15.2rc1).[29] No data had to be rejected because of quality issues.

Preprocessing of the T1-weighted (T1w) images was executed by use of the automated pipeline of fMRIprep (v. 20.0.0), a Nipype based tool[30] combining established software packages for neuroimaging data, including FSL, ANTs, FreeSurfer, and AFNI.[31] In summary, the following steps were carried out:

First, all T1w images were corrected for intensity non-uniformity (INU) by using N4BiasFieldCorrection (v. 2.1.0)[32] and skull-stripped using antsBrainExtraction.sh (v. 2.1.0) with the OASIS template. Recon-all from FreeSurfer (v. 6.0.1) was used for reconstruction of brain surfaces and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of cortical gray matter of Mindboogle.[33] Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c[34] was performed through nonlinear registration with the antsRegistration tool of ANTs v2.1.0,[35] using brain-extracted versions of both T1w volume and template. Using the "fast"-stream from FSL (FSL v. 5.0.9) on the brain-extracted T1w brain tissue segmentation into CSF, WM, and GM was performed.

Data analysis was based on FreeSurfer's "recon-all" processing step in the fMRIprep pipeline. Surfaces were first checked manually for quality and corrected, when necessary, using FreeSurfer's freeview (v. 6.0.0). To include the corrected surfaces, "recon-all" was carried out again with repetition of necessary steps on the corrected data. For the parcellation of the cerebral cortex the Desikan-Killiany Atlas[36] was used.

Utilizing the intensity and continuity information from the entire three-dimensional MR volume in segmentation and deformation procedures cortical thickness was calculated as the closest distance between GM/WM boundary and GM/CSF boundary at each vertex of the surface. In a next step, the data were assembled. Subjects were already resampled onto FreeSurfer's average subject ("fsaverage") and smoothed at various full width/half maximum (FWHM) values in the "recon-all" step. With FreeSurfer's function "mris_preproc" all smoothed thickness data with a 10-mm FWHM from each subject was stacked into one file and used for further group analysis.

2.4 | Neurocognitive performance

For the evaluation of the neurocognitive performance the computerized test battery CNS Vital Signs (Morrisville, NC, USA) consisting of seven tasks generating 11 domain scores was used. As in the study of Prien et al.,[19] seven domain scores were included in the analysis (see Table 1). Excluded domain scores were considered redundant. In accordance with Prien et al.[19] and for ease of interpretation, raw scores were automatically standardized to a normative dataset of subjects with an age between eight and 90 years and to a mean of 100 and a standard deviation (SD) of 15. Standardized scores were compared between the groups based on sport, concussion history, and heading frequency.

2.5 | Statistical analyses

Demographical and neurocognitive data were processed with SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). Descriptive results are presented as means with SDs or frequencies with percentages. Normal distribution of demographics and sports characteristics were tested with the Kolmogorov–Smirnov test. When variables were normally distributed, paired student *t*-tests were carried out for group comparisons. Otherwise, Mann–Whitney *U*-tests were used. Cohen's d with 95% confidence intervals (CI) were calculated.

TABLE 1 Description and calculation of CNS vital signs domain scores (mod. from[19]).

Domain	Test	Domain score calculation	Domain description	Interpretation
Psychomotor speed	FTT, SDC	FTT total taps average + SDC correct responses	Ability to perceive, attend and respond to complex visual-perceptual information and perform simple fine motor tasks	Higher scores are better
Reaction time	ST	(ST complex reaction time correct + ST reaction time correct)/2	Speed of reaction to a simple and increasingly complex set of directions	Lower scores are better
Complex attention	CPT, SAT, ST	ST errors + SAT errors + CPT errors	Ability to track and respond to a variety of stimuli over lengthy periods of time and/or perform complex mental tasks requiring vigilance quickly and accurately	Lower scores are better
Cognitive flexibility	SAT, ST	SAT correct responses—SAT errors—ST errors	Ability to adapt to a rapidly changing and increasingly complex set of directions and/or manipulate the information	Higher scores are better
Processing speed	SDC	SDC correct responses	Ability to recognize and process information, that is, perceiving/responding to incoming information, motor speed, fine motor coordination, visual-perceptual ability	Higher scores are better
Verbal memory	VBM	Correct hits and passes immediate + correct hits and passes delay	Ability to remember (recognize and retrieve) words	Higher scores are better
Visual memory	VIM	Correct hits and passes immediate + correct hits and passes delay	Ability to remember (recognize and retrieve) geometric figures	Higher scores are better

Abbreviations: CPT, continuous performance test; FTT, finger tapping test; SAT, shifting attention test; SDC, symbol digit coding test; ST, Stroop test; VBM, verbal memory test; VIM, visual memory test.

For the cortical thickness analysis between the groups, FreeSurfer's general linear modeling (GLM) function ("mri_glmfit") was used with a "different onset, different slope" scenario. GLM allows a whole brain vertex-wise surface analysis[37] so that no region-of-interest boundaries affect the results. In the GLM, the particular group (SOC or CON; concussion history; heading frequency) was the discrete variable, and age was applied as nuisance regressor in the model. Multiple comparison correction was performed with a Monte Carlo Null Z Simulation. 10 000 iterations and a clusterwise *p*-value, as the probability of seeing a maximum cluster of that size or larger during the simulation, of 0.05 were applied using the clusterwise procedure implemented in FreeSurfer ("mri_glmfit-sim"). Results were visualized by overlaying significant clusters on the inflated cortical surfaces of the "fsaverage brain", an average and standard space into which the individual subject data was registered to. Image processing and MRI data analyses were run on a high-performance computing cluster. Neurocognitive performance was compared between the groups (SOC minus CON; concussion history; heading frequency) using a multivariate linear model with age as control variable. The comparison between the main effects was adjusted by Bonferroni to test for multiple comparisons. Correlations between cortical thickness and neurocognitive performance were calculated with Pearson correlation. Alpha was set at $p<0.05$ for all analyses.

2.6 | Sample

The present study analyzed a subsample of a larger cohort study on former high-level female athletes[19] who participated in the MRI. The 15 SOC (mean age 38.3 ± 5.1 years) and 16 CON (mean age 36.6 ± 5.8 years) were similar regarding demographical information and sports career characteristics (see Table 2) and did not differ from the larger cohort. CON included different non-contact sports (volleyball (n = 7), korfball (n = 3), hockey (n = 2), (wheelchair) tennis (n = 2), ice skating (n = 1), and athletics (n = 1)).

TABLE 2 Comparison of demographic and sport-related data of soccer and non-contact sport athletes.

	SOC (n = 15)	CON (n = 16)	p-Value	Effect size (Cohen's d (95% CI))
Age in years, mean (SD)	38.3 (5.1)	36.6 (5.8)	p = 0.232	0.444 (−0.269–1.157)
Education in years, mean (SD)	17.9 (3.2)	19.3 (1.0)	p = 0.470	0.272 (−0.436–0.98)
Competitions per year during career, mean (SD)	42.6 (14.0)	42.3 (37.6)	p = 0.163	0.529 (−0.188–1.246)
Training sessions per week during career, mean (SD)	5.4 (1.8)	6.8 (3.3)	p = 0.149	0.522 (−0.194–1.238)
Career length in years, mean (SD)	11.7 (4.4)	11.9 (4.7)	p = 0.931	0.044 (−0.661–0.748)
Retired since in years, mean (SD)	9.2 (6.4)	9.1 (4.4)	p = 0.784	0.932 (0.19–1.674)
Currently physically active, n (%)				
Yes	12 (80%)	14 (87.5%)	p = 1.00	
No	3 (20%)	2 (12.5%)		
Self-reported concussions, n (%)				
None	8 (53%)	16 (100%)		
One	4 (27%)	0 (0%)		
More than one	3 (20%)	0 (0%)		
Self-reported heading frequency, n (%)				
Frequent	8 (53%)	0 (0%)		
Moderate	4 (27%)	0 (0%)		
Rare	3 (20%)	0 (0%)		
Never	0 (0%)	16 (100%)		

Abbreviations: CI, confidence interval; CON, non-contact sport athletes; SD, standard deviation; SOC, soccer players.

3 | RESULTS

3.1 | Cortical thickness

No significant differences were found between SOC and CON nor between subgroups with different concussion history after correction for multiple comparisons. There were no differences between never headers and all other heading subgroups. Frequent headers did not differ to moderate and rare headers. Greater cortical thickness was found for moderate headers compared with rare headers in one cluster (clusterwise $p = 0.016$; see Supplemental Material Appendix S1).

3.2 | Neurocognitive performance

SOC and CON performed similar in neurocognitive tests in five of seven domains (Table 3). CON revealed a significantly better performance in the domains of psychomotor speed (Bonferroni corrected $p = 0.018$; MD = −8.307; 95% CI = −15.109, −1.506) and verbal memory (Bonferroni corrected $p = 0.023$; MD = −9.404; 95% CI = −17.406, −1.402).

Investigating potential differences between heading subgroups only one out of 42 analyzed differences was significant after correction for multiple testing. In the verbal memory domain, CON performed significantly better than the moderate headers (Bonferroni corrected p = 0.043; MD = −17.165; 95% CI = −33.941, −0.389; see Supplemental Material Appendix S1).

Similarly, the analysis of concussion history showed only one significant difference in 42 analyzed differences: SOC subgroup with one concussion ($n = 4$) performed significantly worse than CON in the verbal memory domain (Bonferroni corrected $p = 0.041$; MD = −17.226; 95% CI = −33.981, −0.471; see Supplemental Material Appendix S1).

TABLE 3 Mean differences between soccer and non-contact sport athletes in the CNS Vital Signs domains.

Domain	MD	95% CI	*p*-Value
Psychomotor speed	−8.307	−15.109, −1.506	0.018[a]
Reaction time[b]	−2.786	−13.024, 7.451	0.582
Complex attention[b]	4.010	−2.296, 10.315	0.203
Cognitive flexibility	4.182	−4.988, 13.351	0.358
Processing speed	−0.496	−8.293, 7.301	0.897
Verbal memory	−9.404	−17.406, −1.402	0.023[a]
Visual memory	−5.289	−15.457, 4.880	0.296

Abbreviations: CI, confidence interval; MD, mean difference (soccer player minus non-contact sport athletes).

[a] $p < 0.05$ (Bonferroni corrected).

[b] Lower scores are better.

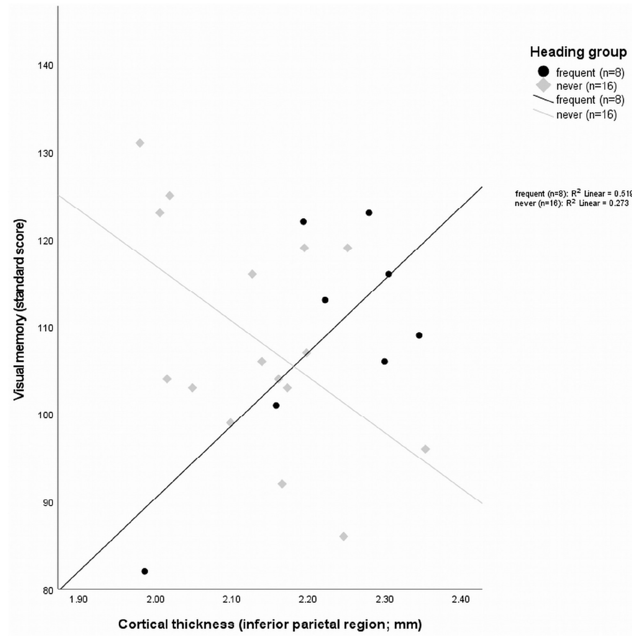

FIGURE 1 Correlation between cortical thickness and visual memory for frequent ($n = 8$) and never ($n = 16$) headers.

3.3 | Correlation cortical thickness and neurocognitive performance

The correlation of cortical thickness values of the inferior parietal region and the seven domains of neurocognitive performance did not reveal significant results, except for a negative correlation with visual performance in the CON group which is identical to the never header subgroup (Pearson correlation coefficient = −0.523, $p = 0.038$). The same correlation, with a different direction, was found in the frequent header subgroup (Pearson correlation coefficient = 0.721, $p = 0.044$), but not in the moderate or rare header subgroups (Figure 1). Further statistical investigations of this correlation in other subgroups (e.g., concussion) were not feasible due to the small sample size.

4 | DISCUSSION

This study compared cortical thickness of the brain and neurocognitive performance of 15 retired high-level female soccer players with a matched control group of 16 retired high-level female non-contact sport athletes. No cortical thinning in SOC compared with CON could be demonstrated. Self-reported heading frequency was not associated with differences in cortical thickness, except for a greater thickness in the right inferior parietal region when moderate headers ($n = 4$) were compared to rare headers ($n = 3$). SOC and CON performed similar in five neurocognitive domains, but SOC performed worse on psychomotor speed and verbal memory than CON. No relationship of neurocognitive performance and self-reported heading frequency was found except for a worse verbal memory of soccer players, who classified themselves as moderate headers compared to rare or frequent headers. Significant correlations were found between visual memory performance and cortical thickness in the frequent header group and CON, although directions were opposed with frequent headers showing a positive and CON showing a negative correlation.

4.1 | Cortical thickness

Results on cortical thickness are in contrast to previous observations in retired male soccer players that revealed cortical thinning in right inferolateral parietal, temporal, and occipital regions when compared to swimmers, which increased with age.[16] Besides the differences in sex, the male cohort was older and the technique used to calculate cortical thickness (voxel based) was different from our study, which might contribute to the different results. Recently, Oliveira et al.[17] did not find concussion or heading to be associated with reduction in gray matter volume either, but rather described an association between heading and greater volume of the left inferior parietal cortex in a large cohort of amateur male and female soccer players. Furthermore, greater cortical thickness was recently seen in American football athletes compared with volleyball players in a longitudinal approach.[38] In our study, after correction for multiple comparisons, SOC who headed the ball moderately often demonstrated a bigger cortical thickness in the right inferior parietal region than players who rarely headed the ball (see Supplemental Material Appendix S1). The absolute numbers furthermore show that frequent headers have the highest cortical thickness compared to moderate, rare and never headers (see Supplemental Material Appendix S1) but this result is not significant, and the differences were small. The small sample size and unknown clinical modifiers of this possibly neuroplastic effect (e.g., lifestyle and diet) might forbid to draw more detailed conclusions. A more in-depth investigation of sport-specific aspects (e.g., techniques or player positions) might be needed to gain a more detailed insight in the results. While for example less GM volume decrease has been shown in American football players with greater position-based head impact risk[38], correlations of structural changes with specific positions in other types of sports are still sparse.

Since the location and lateralization of greater cortical thickness are still not uniform, the interpretation of these findings still seems speculative. Training per se might be associated with gray matter changes, as described by a recent study revealing that aerobic exercising was associated with greater cortical thickness values in middle-aged adults (>45 years) in motor and somatosensory brain regions.[39] But underlying mechanisms are still discussed and sample sizes as well as cross-sectional study designs still forbid any further conclusions. Soccer training (including heading) resembles a complex motor skill as players need to coordinate their whole body very precisely and especially the right inferior parietal region might have a key role for higher-order motion regulation and eye-body coordination.[40] Since there was no difference in the current level of physical activity, training sessions per week and competitions per year during the respective careers of the SOC and CON in our study, the intriguing yet at this point speculative possibility of training-associated structural differences (of parietal cortical regions) should be the target of larger longitudinal studies to possibly identify features of "healthy" headers and heading training.

4.2 | Neurocognitive performance

The significantly worse performance of the SOC in the psychomotor speed domain is in concordance with study results by Prien et al.,[19] and Stewart et al.[41] In the latter study, an association between heading and psychomotor speed was also described, which was not seen in our study. Worse memory performances in verbal, but not in visual domains in soccer players, as found in our study, have also already been reported in different soccer cohorts.[16] Although a thinner cortex was associated with lower cognitive processing speed, Koerte et al.[16] did not find significant differences between soccer players and a control group, which can also be seen in our study. The similar performances of SOC and CON in the processing speed domain may be explained by the level of expertise of the study subjects since elite athletes have been found to perform better in processing speed than amateur athletes[42] or by a higher level of general motor coordination.[43] Investigating potential effects of a history of concussion or header frequencies based on subjective judgment yielded significant differences in the verbal memory, but in no other neurocognitive domain. CON performed significantly better than moderate headers or soccer players with a history of one concussion. The lack of similar differences in soccer players with a higher header frequency or more concussions leaves the interpretation of this finding debatable. Since subgroup analyses were performed with very small sample sizes, those results should be interpreted with great caution and should not be generalized. In male cohorts of retired soccer players, there was no uniform neuropsychological deficit that can be attributed to heading in general, but executive function, attention, visuospatial, or memory performance in general might be related to headers at least in some studies.[14] Overall and similar to studies on (retired) male professional soccer players, our study did not demonstrate a clear influence of heading on neurocognitive performance.

4.3 | Correlation cortical thickness and neurocognitive performance

Cortical thickness and neurocognitive performance were not correlated with only two exceptions in the subgroup analyses. Visual memory and right inferior parietal cortical thickness were positively correlated in frequent headers and negatively correlated in CON (identical to the never header subgroup), but not in the other header groups. Future studies should analyze the relation of structural and functional changes of the brain and their clinical significance.

4.4 | Limitations and future studies

The presented study has several limitations. The sample size was rather small, but the groups did not differ significantly in any demographical data and the athletic profiles were quite similar. Subgroup analysis, however, lead to even smaller samples so that player position analyses could not be conducted. Heading was subjectively assessed and not objectively counted. The necessity of direct player observation in youth soccer has already been described and pointed out.[44] This should be implemented in future studies as well, as self-report methods are not validated and it has to be assumed that not every athlete is able to accurately estimate the number of headers.[27] The presented case–control study design is useful to generate hypotheses but restricts deriving causality from the results.[45] Therefore, prospective as well as longitudinal studies are needed for the evaluation of potential long-term effects of heading.[14] All participants were middle-aged and results might be different in an older cohort. Psychomotor speed results between SOC and CON are substantially different to the larger cohort[19] which might be due to a selection bias.

5 | PERSPECTIVE

To the best of our knowledge, this is the first study that compared cortical thickness and neurocognitive performance of retired high-level female soccer players to a matched group of non-contact sport athletes. SOC and CON had a similar cortical thickness and performed similar in a well-established neurocognitive test battery, except worse results in psychomotor speed and verbal memory of SOC. No clear relationship to the subjectively perceived heading frequency or history of concussion could be established. These results need to be confirmed in future studies with larger sample sizes and ideally a prospective design and an objective documentation of concussion and heading frequency.

AUTHOR CONTRIBUTION

FKH contributed to data analysis, interpretation of the data, drafting and writing of the manuscript, revision of the manuscript, approval of the final version to be published. AP contributed to study design, data collection, data analysis, interpretation of the data, approval of the final version to be published. LD contributed to study design, data analysis, interpretation of the data, approval of the final version to be published. NF-D contributed to study design, interpretation of the data, approval of the final version to be published. AJ contributed to study design, interpretation of the data, revision of the manuscript, approval of the final version to be published. CR contributed to study design, interpretation of the data, drafting and writing of the manuscript, revision of the manuscript, approval of the final version to be published.

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CONFLICT OF INTEREST STATEMENT

CR receives grant support from the German Federal Institute of Sports Sciences and the Heinz Nixdorf Westfalian Foundation on projects not related to the presented topic. He serves as hygiene officer and as a member of the medical commission of the German Football Association (DFB) and is a medical advisor on concussion for the Union of European Football Associations (UEFA). All other authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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SUPPLEMENTAL MATERIAL

Figure S1: Significant cluster for the cortical thickness comparison between moderate (n=4) and rare (n=3) headers (clusterwise p=0.016, cluster size=1003.34mm²)

Table S1: Cortical thickness in the inferior parietal region (in mm) in different heading groups (comparison between moderate and rare headers revealed a significant difference, clusterwise p=0.016; SD=standard deviation)

Heading frequency	**Cortical thickness (in mm)** Mean (SD)
Frequent (n=8 SOC)	2.224 (0.115)
Moderate (n=4 SOC)	2.143 (0.139)
Rare (n=3 SOC)	2.120 (0.320)
Never (n=16 CON)	2.136 (0.104)
CON=non-contact sport athletes; SOC=soccer players	

Table S2: Mean differences for the CNS Vital Signs domains for the comparison between different heading groups (CI=confidence interval; MD=mean difference)

Domain	**MD**	**95% CI**	**p-value**
Psychomotor speed			
Frequent vs. moderate	-3.294	-19.414, 12.826	1.00
Frequent vs. rare	5.394	-12.824, 23.611	1.00
Frequent vs. never	-8.271	-19.703, 3.162	0.294
Moderate vs. rare	8.687	-11.959, 29.334	1.00
Moderate vs. never	-4.977	-19.669, 9.715	1.00
Rare vs. never	-13.664	-30.941, 3.612	0.195
Reaction time†			
Frequent vs. moderate	-6.157	-30.339, 18.024	1.00
Frequent vs. rare	-11.612	-38.941, 15.716	1.00
Frequent vs. never	-6.570	-23.719, 10.580	1.00

Moderate vs. rare	-5.455	-36.427, 25.517	1.00
Moderate vs. never	-0.412	-22.452, 21.628	1.00
Rare vs. never	5.043	-20.873, 30.959	1.00
Complex attention†			
Frequent vs. moderate	0.625	-14.570, 15.820	1.00
Frequent vs. rare	4.417	-12.755, 21.590	1.00
Frequent vs. never	4.969	-5.808, 15.745	1.00
Moderate vs. rare	3.792	-15.670, 23.254	1.00
Moderate vs. never	4.344	-9.506, 18.193	1.00
Rare vs. never	0.551	-15.734, 16.837	1.00
Cognitive flexibility			
Frequent vs. moderate	4.754	-17.372, 26.881	1.00
Frequent vs. rare	-1.133	-26.139, 23.873	1.00
Frequent vs. never	5.310	-10.383, 21.002	1.00
Moderate vs. rare	-5.887	-34.227, 22.453	1.00
Moderate vs. never	0.555	-19.612, 20.723	1.00
Rare vs. never	6.442	-17.272, 30.156	1.00
Processing Speed			
Frequent vs. moderate	3.467	-15.418, 22.353	1.00
Frequent vs. rare	1.515	-19.828, 22.858	1.00
Frequent vs. never	0.742	-12.651, 14.136	1.00
Moderate vs. rare	-1.952	-26.141, 22.236	1.00
Moderate vs. never	-2.725	-19.938, 14.488	1.00
Rare vs. never	-0.773	-21.013, 19.468	1.00
Verbal memory			
Frequent vs. moderate	11.368	-7.038, 29.774	0.537
Frequent vs. rare	2.415	-18.387, 23.216	1.00
Frequent vs. never	-5.797	-18.851, 7.256	1.00
Moderate vs. rare	-8.954	-32.528, 14.621	1.00
Moderate vs. never	-17.165	-33.941, -0.389	0.043*
Rare vs. never	-8.212	-27.938, 11.515	1.00
Visual memory			
Frequent vs. moderate	18.152	-4.416, 40.719	0.180
Frequent vs. rare	4.417	-21.087, 29.921	1.00
Frequent vs. never	0.571	-15.434, 16.576	1.00
Moderate vs. rare	-13.735	-42.639, 15.170	1.00
Moderate vs. never	-17.581	-38.150, 2.988	0.131
Rare vs. never	-3.846	-28.033, 20.340	1.00
*=p<0.05 (Bonferroni corrected); †= lower scores are better			

Table S3: Mean differences for the CNS Vital Signs domains for the comparison between different concussion subgroups in soccer players and non-contact sport athletes (CI=confidence interval; MD=mean difference)

Domain	MD	95% CI	p-value
Psychomotor speed			
SOC-none vs. SOC-one	-4.757	-21.723, 12.210	1.00
SOC-none vs. SOC-more	-0.301	-19.601, 18.998	1.00
SOC-none vs. CON	-9.725	-22.055, 2.605	0.198
SOC-one vs. SOC-more	4.455	-16.011, 24.921	1.00
SOC-one vs. CON	-4.968	-19.863, 9.927	1.00
SOC-more vs. CON	-9.423	-26.371, 7.524	0.747
Reaction time†			
SOC-none vs. SOC-one	-14.930	-39.398, 9.538	0.560
SOC-none vs. SOC-more	-8.553	-36.385, 19.279	1.00

SOC-none vs. CON	-8.959	-26.741, 8.8221	0.973
SOC-one vs. SOC-more	6.377	-23.137, 35.892	1.00
SOC-one vs. CON	5.971	-15.510, 27.451	1.00
SOC-more vs. CON	-0.407	-24.848, 24.034	1.00
Complex attention†			
SOC-none vs. SOC-one	-9.192	-23.831, 5.447	0.508
SOC-none vs. SOC-more	-10.763	-27.415, 5.889	0.458
SOC-none vs. CON	-1.036	-11.675, 9.602	1.00
SOC-one vs. SOC-more	-1.571	-19.229, 16.087	1.00
SOC-one vs. CON	8.156	-4.696, 21.007	0.489
SOC-more vs. CON	9.727	-4.896, 24.350	0.412
Cognitive flexibility			
SOC-none vs. SOC-one	-12.183	-34.002, 9.637	0.738
SOC-none vs. SOC-more	-12.392	-37.211, 12.428	0.995
SOC-none vs. CON	-2.082	-17.939, 13.775	1.00
SOC-one vs. SOC-more	-0.209	-26.529, 26.110	1.00
SOC-one vs. CON	10.101	-9.055, 29.256	0.865
SOC-more vs. CON	10.310	-11.485, 32.106	1.00
Processing Speed			
SOC-none vs. SOC-one	-2.616	-21.897, 16.664	1.00
SOC-none vs. SOC-more	6.155	-15.777, 28.087	1.00
SOC-none vs. CON	0.156	-13.856, 14.168	1.00
SOC-one vs. SOC-more	8.772	-14.486, 32.029	1.00
SOC-one vs. CON	2.772	-14.154, 19.699	1.00
SOC-more vs. CON	-5.999	-25.259, 13.261	1.00
Verbal memory			
SOC-none vs. SOC-one	10.085	-9.00, 29.170	0.860
SOC-none vs. SOC-more	-2.638	-24.348, 19.071	1.00
SOC-none vs. CON	-7.141	-21.011, 6.729	0.921
SOC-one vs. SOC-more	-12.723	-35.744, 10.298	0.760
SOC-one vs. CON	-17.226	-33.981, -0.471	0.041*
SOC-more vs. CON	-4.503	-23.567, 14.561	1.00
Visual memory			
SOC-none vs. SOC-one	10.019	-15.020, 35.058	1.00
SOC-none vs. SOC-more	7.254	-21.228, 35.735	1.00
SOC-none vs. CON	-0.803	-19.00, 17.393	1.00
SOC-one vs. SOC-more	-2.765	-32.968, 27.438	1.00
SOC-one vs. CON	-10.822	-32.804, 11.160	1.00
SOC-more vs. CON	-8.057	-33.068, 16.955	1.00

CON=non-contact sport athletes; SOC=soccer players ; SOC-none=soccer players without concussion; SOC-one=soccer players with one concussion; SOC-more=soccer players with more than one concussion; *=p<0.05 (Bonferroni corrected); †= lower scores are better

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Original research

Heading during the season and its potential impact on brain structure and neurocognitive performance in high-level male football players: An observational study

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ABSTRACT

Objectives: To investigate potential effects of heading on the neurocognitive performance and the white matter (WM) of the brain in high-level adult male football players.
Design: Prospective longitudinal.
Methods: Football players engaging in the highest football leagues in Germany were included. Neurocognitive performance tests and diffusion tensor imaging (DTI) were executed before and after the observation period. Video recordings of each training session and each match play during the observation period were analyzed regarding heading exposure and characteristics. Four DTI measures from tract-based spatial statistics (fractional anisotropy, mean, axial, and radial diffusivity) were investigated. Associations between heading variables and DTI and neurocognitive parameters were tested subsequently.
Results: 8052 headers of 22 players (19.9 ± 2.7 years) were documented in a median of 16.9 months. The individual total heading number ranged from 57 to 943 (median: 320.5). Header characteristics differed between training sessions and matches. Neurocognitive performance (n = 22) and DTI measures (n = 14) showed no significant differences from pre- to post-test. After correction for multiple comparisons, no significant correlations with the total heading number were found. However, the change in fractional anisotropy in the splenium of the corpus callosum correlated significantly with the total amount of long-distance headers (Pearson's $r = -0.884$; $p < 0.0001$).
Conclusions: Over the median observation period of 16.9 months, DTI measures and neurocognitive performance remained unchanged. To elucidate the meaning of the association between individual change in fractional anisotropy and long-distance headers further investigations with larger samples, longer observations, and various cohorts regarding age and level of play are required.

Practical implications

- Number and characteristics of headers vary between players and differ between training sessions and matches.
- Neurocognitive performance and WM integrity remained unchanged over the observation period and calculated pre- to post-test changes were not associated with the total number of headers.
- Header characteristics and the number of long-distance headers might be of particular interest for future studies on brain structure and/or function.
- In addition to a standardized and uniform analysis approach for headers and DTI parameters, which would increase the comparability of results, potential influencing factors, such as age or history of

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concussion, should be included in future studies to address the multifactorial causes of detected DTI changes.

1. Introduction

Potential long-term consequences of repetitive head impacts (RHIs) are an emerging research topic and a recent systematic review suggests higher risks for neurodegenerative diseases in contact sports with RHI exposure.[1] However, causality and the dose–response relationship between the actual exposure to RHIs and surrogate markers for brain injury and/or neurodegeneration have not been established. Diffusion tensor imaging (DTI) derived from diffusion-weighted magnetic resonance imaging (MRI) can be used to evaluate potential effects on white matter (WM). The integrity of the WM is assessed with DTI measures such as fractional anisotropy (FA), mean, axial, and radial diffusivity (MD, AD, RD, respectively).[2,3] The directionality of diffusion is displayed by FA. Values close to zero represent isotropic (diffusion in all directions) and values close to one represent anisotropic (diffusion along one axis) diffusion. The average diffusion along all three spatial axes, regardless of the direction, is represented by MD. The diffusion along the principal axis, parallel to fiber tracts, is displayed by AD. Finally, RD represents the degree to which the diffusion is unrestricted in the tissue perpendicular to the primary/principal diffusion direction. In athletes exposed to RHIs, lower FA compared to non-exposed controls cross-sectionally and decreased FA longitudinally over time have been described together with an increase in MD, RD, and/or AD.[2]

In football, concerns regarding potential (long-term) consequences due to RHI exposure persist.[4] Effects on neurocognitive performance are evaluated with various tests,[5] but results are inconsistent, and evidence for an influence of heading exposure on neurocognitive parameters is weak.[5,6] However, executive function, memory, and attention have been reported to be most likely affected in football players.[6] Results on WM integrity in football players have been inconclusive. One study did not find differences between professional football players and swimmers in FA and MD,[7] but one reported an association between a high amount of headers and altered FA.[8] Additionally, greater heading exposure of the previous year was associated with alterations in RD, AD, or MD.[8,9]

Several weaknesses limit the generalizability of studies reporting associations between heading exposure and neurocognitive or DTI parameters. Heading exposure was mostly analyzed with retrospective methods,[8,9] and investigated cohorts mostly consisted of children/youth, students, or amateur players.[5,9] This illustrates the need for prospective, objective header quantification and investigations on high-level, active cohorts. Therefore, the aims of this longitudinal, prospective study were to 1) objectively quantify and evaluate the heading exposure, 2) compare neurocognitive performance and DTI measures from pre- to post-measurement, and 3) assess associations of heading and header characteristics with neurocognitive performance and DTI measures in active, high-level male football players.

2. Method

The study was approved by the ethical review boards of the State Medical Board (Ärztekammer Westfalen-Lippe) in Münster, Germany (2017-386-f-S) and conducted in accordance with the Declaration of Helsinki and funded by the German Federal Institute of Sport Science. The funder did not influence data collection, analysis, interpretation, and publishing decisions.

Active, male players of a high-level German football team were included in the study. The collaboration and study settings with the club (especially with respect to video analysis) restricted the pool of potential participants. However, all participants were players of the professional, U21, or U19 team. Therefore, according to McKay et al., they were all highly trained and participated on a national level[10] and hence were considered 'high-level' players. The following criteria led to the exclusion of the player: 1) age under 18 years, 2) abnormal or pathological findings on structural imaging, 3) neurological or psychiatric disorder, 4) any self-reported learning disabilities, 5) contraindication to MRI, 6) any other native language than German, English, Spanish or French, and 7) sustaining a concussion during the study period.

The study started with obtaining informed consent, and documentation of the medical history by using a questionnaire and performing a neurological screening. Sport-specific information such as the playing position was gathered with a questionnaire. Additionally, neurocognitive tests and MRI were performed, which were repeated at the end of the study or when a player left the team. The observation period of the participants ranged from 9 to 17.8 months (median: 16.9 months). During the study period, each training and match of the team was videotaped to accurately document each player's heading exposure.

Video recording started in November/December 2017 and ended in May 2019. Recordings of training and matches were analyzed based on a standardized protocol with predefined criteria.[11] All headers were counted for an individual total heading number and additionally characterized regarding e.g., the flight distance of the ball, the area of the head, or the header intention. The latter represents the purpose of the header, i.e. why the athlete headed the ball. The different categories are briefly explained in the following. 'Pass' is defined as playing a pass to another player. 'Kick' represents (goal-directed) shots, while 'being shot' means that a player was unintentionally heading the ball and shot by another player. 'Low header' represents a header that was meant to bring the ball to the ground. When a player attempted to head the ball but instead it slipped off their head without any other intended action, this was defined as 'slip off'. 'Clearing a situation' refers to the headers played to clear for example goal attempts by the opponent. Lastly, 'presenting ball to self' is defined as headers played to retain possession of the ball. Additionally, headers were classified as 'header in training' or 'header in match'. Questionable situations regarding header characteristics were discussed within the team.

The neurocognitive tests included pen and paper tests (Trail Making Test A and B (TMT A/TMT B)[12] and Paced Auditory Serial Addition Test 1 and 2 (PASAT 1/PASAT 2)) and a standardized computerized test battery (CNS Vital Signs, Morrisville, NC, USA) consisting of nine tests, generating 14 domain scores. Seven domains (verbal, visual, and working memory; executive function; complex, sustained, and simple attention) most likely affected in football players[6] were analyzed in this study. Raw scores from all tests were standardized and inverted when necessary for ease of interpretation. This resulted in a uniform scale with a mean of 100 and a standard deviation (SD) of 15. Higher scores reflect better performance. Players with complete neurocognitive data (pre- and post-test) are abbreviated with 'NPSY' hereafter.

Whole-brain DTI was executed on a 3 T Philips Ingenia scanner with a 32-channel head coil located at the University Medical Center (Universitätsklinikum) Hamburg-Eppendorf, Germany. DTI scan parameters were: acquisition matrix $= 128 \times 128 \times 51$, voxel size $= 1.75 \times 1.75 \times 2.5$ mm^3, repetition time = 5.34 s, echo time = 0.084 s, flip angle = 90°. 33 images were acquired for each scan: 32 diffusion-weighted images (b = 1000 s/mm^2) and one non-diffusion-weighted image (b0; b = 0 s/mm^2). The Functional MRI of the Brain (FMRIB) Software Library (FSL v.6.0.5.1; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/ [13]) was used for DTI data processing and analysis on a high-performance computing cluster provided by the Paderborn Center for Parallel Computing (https://pc2.uni-paderborn.de/). The Brain Extraction Tool was used for skull removal and head motion and eddy current distortions were corrected. The individual FA maps were created with DTIfit. Tract-based spatial statistics (TBSS) applied a nonlinear registration to align all FA images to a $1 \times 1 \times 1$ mm standard space (FMRIB58_FA).[14] The mean of all FA images was created and fed into the skeletonization program resulting in a skeletonized mean FA map (threshold FA = 0.3). Similarly, MD, AD, and RD data were computed.

In addition to the voxel-based approach, the skeleton masks were divided into anatomical WM tracts based on the John Hopkins University (JHU) ICBM-DTI-81 WM atlas.[15] Pre- and post-test values for the four DTI measures for each tract were extracted. For ease of comprehension, MD, AD, and RD values were multiplied by 1000. Players with complete neurocognitive data and MRI brain scans (pre- and post-measurement data) are considered 'NPSY + MRI' hereafter.

Descriptive data of the players, total heading amount, and header characteristics as well as neurocognitive performance were evaluated with SPSS (v.29.0.0). Header characteristics are displayed as the proportion of the total number or of the number of headers played in training or matches, respectively, and as absolute number. Normal distributions were tested with Shapiro–Wilk. The difference in neurocognitive performance from pre- to post-measurement was analyzed by using a paired *t*-test for normally distributed standard scores or a Wilcoxon test for non-normally distributed variables.

The DTI parameters derived from the voxel-based TBSS approach were statistically tested within FSL. Voxelwise statistics of the skeletonized FA data were performed by using 'Randomise', FSL's nonparametric permutation inference tool with threshold-free cluster enhancement to correct for multiple comparisons (5000 permutations, $p < 0.05$). A paired *t*-test with age as nuisance regressor was applied to test the difference from pre- to post-measurement. Similarly, TBSS analyses were performed on MD, AD, and RD data.

Associations between the number of headers and changes in DTI parameters and neurocognitive performance were examined. Individual change between the measurements was calculated by subtracting the post-test-value from the pre-test-value. The changes were correlated with the number of all headers and of headers coming from over 20 m ('long-distance headers') as balls delivered via a long-kick (>10 m) have higher mean peak linear and angular acceleration.[16] These correlation analyses were performed for all neurocognitive tests and for the DTI values extracted from the JHU atlas.[15] For normally distributed variables, Pearson correlation was calculated. Spearman's rank correlation was used for non-normally distributed variables. All correlation analyses were corrected for multiple comparisons (Bonferroni, for neurocognitive data: $p < 0.0045$; for DTI data: $p < 0.001$).

3. Results

Initially, 30 players were included in the study. Seven players left the club before the study's end and did not take part in post-test measurements. Another player had no neurocognitive or MRI post-test data. Therefore, the NPSY cohort consisted of 22 players (19.9 ± 2.7 years; Table 1). Eight players were defenders, seven midfield players, five forwards, and two goalkeepers. Heading data and TMT A&B, PASAT 1&2, and the domains verbal memory, visual memory, and executive function of the CNS vital signs were analyzed for NPSY. Invalid test scores in the pre- or post-test led to the exclusion of individual player scores. Therefore, 21 NPSY were analyzed for the simple attention domain and 18 NPSY for the domains working memory, complex attention, and sustained attention. Of the 22 players, eight players had aborted or invalid MRI scans or only participated in the neurocognitive post-test. Therefore, DTI data analyses, including correlations between DTI measures and heading, are based on 14 players (NPSY + MRI; 20.4 ± 3.3 years; Table 1). NPSY + MRI did not differ significantly from players without MRI-scan regarding age, observation period, total heading number, total headers per month, total headers over 20 m, or total headers over 20 m per month (Supplements Table S1).

Heading data of NPSY and NPSY + MRI are presented in Table 2. During the observation period, 8052 headers were documented for NPSY. The total heading number per player ranged from 57 to 943 (median: 320.5). The total heading number for the different positions is included in Table 2. The large majority (83.27 %) of headers occurred in training sessions and 16.73 % in matches. Overall, headers were mostly performed after a flight distance of under 5 m, with the frontal part of the head, and played as a pass. There were distinct differences between headers in training and matches, especially regarding the flight distance of the ball and the header intention (Table 2).

There were no significant differences from pre- to post-measurement in the analyzed neurocognitive tests after Bonferroni correction (Table 3). The TBSS voxel-wise statistics approach with the included correction for multiple testing by permutation analysis did not yield significant results in any of the four DTI parameters (FA, MD, AD, RD).

The association analyses between the number of all and of long-distance headers with the individual change from pre- to post-measurement in the neurocognitive performance revealed no significant results after correction for multiple comparisons (Table 3). Similarly, there were no significant associations between the change in FA, MD, RD, and AD extracted from the JHU-atlas with the number of total headers (Supplements Table S2). However, the change in FA in the splenium of the corpus callosum correlated significantly with the number of long-distance headers (Pearson's $r = -0.884$; $p = 0.000027$; Supplements Table S2). Due to the calculation of change (pre minus post), the negative correlation coefficient indicates increased FA at post-measurement with a higher number of long-distance headers.

Table 1
Age and observation period for players with neuro-psychological tests (NPSY) and players with neuropsychological tests and MRI (NPSY + MRI).

	NPSY (n = 22)	NPSY + MRI (n = 14)
Mean age in years (SD)	19.9 (2.7)	20.4 (3.3)
Median age in years (minimum–maximum)	19.00 (18–28)	19.00 (18–28)
Median observation period in months (minimum–maximum)	16.9 (9.1–17.8)	17.2 (9.5–17.8)
Playing position		
Defender (n)	8	8
Midfielder (n)	7	4
Forward (n)	5	2
Goalkeeper (n)	2	0

NPSY, 22 players with complete neurocognitive data; NPSY + MRI, 14 players with additional complete MRI-data; SD, standard deviation.

4. Discussion

This study aimed to objectively investigate the heading exposure as well as the neurocognitive performance and DTI parameters in active, high-level male football players. Additionally, the goal was to test associations between heading variables and the neurocognitive performance and DTI measures.

In this longitudinal study, headers played in training sessions and matches by a high-level male football team were prospectively documented over 16.9 months (median). Headers mostly occurred during training and heading characteristics differed between training sessions and matches. A higher number of headers overall, and specifically a higher number of short-distance headers were played in training. The neurocognitive performance of NPSY (n = 22) and the DTI parameters (FA, MD, RD, AD) of NPSY + MRI (n = 14) as a measure of WM integrity remained unchanged over the observation period. Explorative correlation analyses revealed no significant associations between the number of all headers with changes in neurocognitive performance or changes in FA, MD, RD, or AD after correction for multiple testing. There was one significant correlation between the change in FA in the splenium of the corpus callosum and the number of long-distance headers.

The total number of documented headers for the 22 players (NPSY) in the present study is higher than in previous studies (e.g., 2115 headers in matches over one season reported for 54 players[17] or 1307 headers in two collegiate seasons for 13 female players[16]). The difference might be explained by the inclusion of matches and training and a longer observation period compared to Cassoudesalle et al.[17] as well as a larger and male sample compared to Kenny et al., who examined female players.[16] The total number of headers is highly individual,[5,18]

Table 2
Heading information for players with neuro-psychological tests (NPSY) and players with neuropsychological tests and MRI (NPSY + MRI).

	NPSY (n = 22)			NPSY + MRI (n = 14)		
	Total	Training	Match	Total	Training	Match
Total heading number	8052	6705	1347	5822	4646	1176
Individual heading number						
Minimum–maximum	57–943	56–709	1–234	146–943	140–709	2–234
Median (25 %–75 % quartiles)	320.5 (208.5–487.25)	226 (199.5–429.75)	45 (6–116)	340 (241.75–534.5)	230 (213–461.5)	79.5 (12.75–132)
Mean heading number per month per player (SD)	24.19 (12.83)	21.77 (9.58)	4.25 (4.12)	27.34 (11.27)	21.97 (8.37)	5.37 (4.42)
Minimum–maximum	3.37–53.01	3.31–43.01	0.06–13.62	12.64–53.01	12.29–39.85	0.11–13.62
Total heading number per position (minimum–maximum)						
Defender	3983 (243–943)	2976 (213–709)	1007 (15–234)	3983 (243–943)	2976 (213–709)	1007 (15–234)
Midfielder	2677 (57–729)	2433 (56–680)	244 (1–58)	1455 (222–497)	1298 (211–441)	157 (2–58)
Forward	1104 (79–410)	1015 (69–350)	89 (6–60)	384 (146–238)	372 (140–232)	12 (6–6)
Goalkeeper	288 (120–168)	281 (116–165)	7 (3–4)	n/a	n/a	n/a
Median heading number per position (25 %–75 % quartiles)						
Defender	417.5 (317.25–667.25)	284.5 (215.75–524.5)	125.5 (104.25–148.5)	417.5 (317.25–667.25)	284.5 (215.75–524.5)	125.5 (104.25–148.5)
Midfielder	436 (222–497)	399 (211–441)	41 (2–56)	368 (229.5–493.75)	323 (213.25–437.25)	48.5 (11.75–57.5)
Forward	231 (112.5–324)	224 (104.5–291)	7 (6–35)	192 (146–n/a)	186 (140–n/a)	6 (6–6)
Goalkeeper	144 (120–n/a)	140.5 (116–n/a)	3.5 (3–n/a)	n/a	n/a	n/a
Mean heading number per position (SD)						
Defender	497.88 (239.23)	372 (190.47)	125.88 (60.51)	497.88 (239.23)	372 (190.47)	125.88 (60.51)
Midfielder	382.43 (221.77)	347.57 (203.3)	34.86 (23.98)	363.75 (146.97)	324.5 (126.07)	39.25 (25.97)
Forward	220.8 (124.4)	203 (105.85)	17.8 (23.65)	192 (65.05)	186 (65.05)	6 (0)
Goalkeeper	144 (33.94)	140.5 (34.65)	3.5 (0.71)	n/a	n/a	n/a
Distance of ball						
Under 5 m % (total)	59.62 % (4801)	69.07 % (4631)	12.62 % (170)	55.79 % (3248)	66.96 % (3111)	11.65 % (137)
5–20 m % (total)	20.46 % (1647)	17.51 % (1174)	35.12 % (473)	21.4 % (1246)	18.12 % (842)	34.35 % (404)
Over 20 m % (total)	19.91 % (1603)	13.41 % (899)	52.26 % (704)	22.81 % (1328)	14.92 % (693)	54 % (635)
No information % (total)	0.01 % (0)	0.01 % (1)	0 % (0)	0 % (0)	0 % (0)	0 % (0)
Head area						
Frontal % (total)	96.31 % (7755)	97.08 % (6509)	92.5 % (1246)	96.03 % (5591)	96.92 % (4503)	92.52 % (1088)
Temporal % (total)	1.4 % (113)	1.04 % (70)	3.19 % (43)	1.72 % (100)	1.27 % (59)	3.49 % (41)
Parietal % (total)	1.78 % (143)	1.45 % (97)	3.41 % (46)	1.84 % (107)	1.51 % (70)	3.14 % (37)
Occipital % (total)	0.37 % (30)	0.27 % (18)	0.9 % (12)	0.31 % (18)	0.17 % (8)	0.85 % (10)
Facial % (total)	0.09 % (7)	0.1 % (7)	0 % (0)	0.07 % (4)	0.09 % (4)	0 % (0)
No information % (total)	0.05 % (4)	0.06 % (4)	0 % (0)	0.03 % (2)	0.04 % (2)	0 % (0)
Header intention						
Pass % (total)	59.94 % (4826)	60.69 % (4069)	56.2 % (757)	58.96 % (3433)	59.86 % (2781)	55.44 % (652)
Kick % (total)	18.65 % (1502)	21.7 % (1455)	3.49 % (47)	16.47 % (959)	19.85 % (922)	3.15 % (37)
Low header % (total)	0.34 % (27)	0.37 % (25)	0.15 % (2)	0.36 % (21)	0.41 % (19)	0.17 % (2)
Slip off % (total)	0.94 % (76)	0.8 % (54)	1.63 % (22)	0.98 % (57)	0.84 % (39)	1.53 % (18)
Being shot % (total)	0.38 % (31)	0.39 % (26)	0.37 % (5)	0.33 % (19)	0.3 % (14)	0.43 % (5)
Clearing a situation % (total)	13.96 % (1124)	9.9 % (664)	34.15 % (460)	17.18 % (1000)	12.29 % (571)	36.48 % (429)
Presenting ball to self % (total)	2.16 % (174)	1.88 % (126)	3.56 % (48)	1.46 % (85)	1.2 % (56)	2.46 % (29)
No information % (total)	3.63 % (292)	4.27 % (286)	0.45 % (6)	4.26 % (248)	5.25 % (244)	0.34 % (4)

n/a, not applicable; NPSY, 22 players with complete neurocognitive data; NPSY + MRI, 14 players with additional complete MRI-data; SD, standard deviation.

which is also documented by the large range (from 57 to 943 headers) in our study. The individual header number might have been influenced by the different lengths of the observation period (between 9 and 17.8 months). However, examining the individual number of headers per month (total amount divided by observation period) showed a similar range (Table 2) to the overall number of headers, confirming the high interindividual variability. Of note, this might be explained by the inclusion of all playing positions and the differences regarding heading amount for the positions.[19] As displayed in Table 2, differences regarding the mean heading number per position were similarly found in our cohort.

In addition to the number of headers, different header characteristics were evaluated and compared between training sessions and matches. Long-distance headers occurred more often in matches than in training, which is in line with reports on youth players.[18] Headers were mostly played as a pass, followed by clearance in matches, which was similarly found for defenders in professional European leagues.[19] In training, the intention was a pass, followed by a (goal-directed) kick. However, other factors like style of playing, design, and contents of training might also be responsible for the difference. Objective observations and analyses of the total header number and header characteristics in training and matches were only rarely performed and mostly restricted to youth or collegiate players.[16,18] Additionally, detailed evaluations of header characteristics in high-level players mostly considered matches.[11,19] Therefore, the presented data provide a realistic representation of the complete heading exposure in a high-level male football team during the observation period. However, as a professional career can last between 8 to 11 years,[20] longer study time frames are still needed for an objective total heading number.

Neurocognitive performance did not change significantly over the observation period after correction for multiple comparisons. Similarly, no differences in verbal or visual memory over three years were observed for collegiate football players.[21] Furthermore, contact- and/or collision-sport athletes did not differ from non-contact athletes in cognitive scores.[22] Additionally, studies reporting neurocognitive impairments in football players in attention, memory, and executive function domains cross-sectionally had high rates of inappropriate control for multiple comparisons,[6] which limits the significance of those results.

None of the four DTI measures changed significantly from pre- to post-test, indicating that the WM integrity remained stable over the observation period. A similar cohort of elite football players showed increased RD and AD when compared to swimmers in a cross-sectional study.[7] The presented results are furthermore in contrast to findings reported for high-school female players, who showed decreased MD and/

Table 3
Comparison of neurocognitive pre- and post-data and correlations of change from pre- to post-measurement with the total number of headers and of headers over 20 m in NPSY.

Test	n	Pre-mean (SD)	Post-mean (SD)	Difference (pre–post)	T- or Z-value (paired *t*-test or Wilcoxon test)	p-Value	Correlation coefficient with total number of headers (95 % CI; p-value)	Correlation coefficient with number of headers over 20 m (95 % CI; p-value)
TMT A	22	96.63 (16.14)	91.62 (20.63)	5.01	T = 0.954	0.351	−0.22 (−0.596, 0.235; p = 0.33)[a]	−0.251 (−0.617, 0.203; p = 0.26)[a]
TMT B	22	93.1 (25.8)	90.7 (23.05)	2.4	Z = −0.698	0.485	0.266 (−0.175, 0.618; p = 0.23)	0.439 (0.008, 0.732; p = 0.041)[a]
PASAT 1	22	100.15 (18.64)	106.56 (11.77)	−6.41	Z = −2.283	0.022	0.174 (−0.279, 0.564; p = 0.44)[a]	0.125 (−0.325, 0.529; p = 0.58)[a]
PASAT 2	22	107.42 (10.81)	110.26 (9.25)	−2.84	Z = −1.874	0.061	0.554 (0.173, 0.791; p = 0.007)	0.385 (−0.056, 0.701; p = 0.08)[a]
CNS domain								
Working memory	18	106.06 (7.62)	109.39 (7.82)	−3.33	T = −1.21	0.243	0.13 (−0.359, 0.562; p = 0.61)	−0.09 (−0.545, 0.406; p = 0.72)[a]
Visual memory	22	97.73 (14.17)	95.82 (15.56)	1.91	T = 0.698	0.493	−0.071 (−0.478, 0.362; p = 0.76)	0.111 (−0.338, 0.519; p = 0.62)[a]
Verbal memory	22	104.09 (16.54)	101.86 (12.54)	2.23	T = 0.664	0.514	0.088 (−0.347, 0.491; p = 0.70)	−0.187 (−0.573, 0.267; p = 0.40)[a]
Executive function	22	104.64 (13.87)	105.91 (12.15)	−1.27	T = −0.66	0.517	0.311 (−0.127, 0.648; p = 0.16)	−0.147 (−0.545, 0.305; p = 0.51)[a]
Complex attention	18	105.44 (12.23)	99.11 (14.56)	6.33	Z = −2.178	0.029	0.086 (−0.397, 0.531; p = 0.90)	−0.237 (−0.643, 0.272; p = 0.34)[a]
Sustained attention	18	107.06 (4.1)	109.22 (4.2)	−2.16	T = −1.44	0.168	0.099 (−0.398, 0.552; p = 0.70)[a]	−0.16 (−0.593, 0.345; p = 0.53)[a]
Simple attention	21	105.29 (11.51)	102.52 (8.1)	2.77	Z = −1.38	0.168	0.061 (−0.381, 0.480; p = 0.79)	−0.178 (−0.575, 0.287; p = 0.44)[a]

CI, confidence interval; NPSY, 22 players with complete neurocognitive data; PASAT, Paced Auditory Serial Addition Test; SD, standard deviation; TMT, Trail Making Test.
Bonferroni corrected p = 0.0045.
[a] Spearman's rank correlation.

or RD at post-season compared to pre-season.[23] However, those studies are difficult to compare due to the cross-sectional design[7] and the investigation of a younger female cohort.[23] Of note, a study with a similar longitudinal design, a comparable cohort, and similar TBSS methods revealed similar results.[24] Professional elite ice hockey players did not show any significant changes in DTI parameters after one season either.[24] Results from a recent systematic review on effects of RHI exposure on DTI measures[2] could not be demonstrated in our cohort over the observation period of 16.9 months (median).

In summary, the analyses between pre- and post-measurement indicate unchanged neurocognitive performance and WM integrity over an observation period of 17 months in high-level male football players.

Exploratively, the total number of headers and of long-distance headers were correlated with the calculated change from pre- to post-measurement in neurocognitive and DTI parameters. These analyses did not result in any significant correlations after Bonferroni correction, which is in line with the overall weak evidence reported for (short-term) neurocognitive deficits associated with heading.[5,6] Interestingly, less improvement in response time was seen for youth football players with more headers from longer distances[25] and these effects were longer-lasting than effects of short headers.[26] However, a systematic review found that two-thirds of reviewed studies did not find any associations between heading frequency and neurocognitive performance, and those that did had a low quality of heading assessment.[6] The objective approach of the present study overcame that quality issue.

The present study did not find any significant associations between the change in FA, RD, MD, or AD and the number of total headers after correction for multiple testing. This might be in contrast to studies reporting significant correlations between changes in FA, RD, AD, and/or MD with increased heading exposure in football players,[8,9] and a recent review reporting a lower or decreased FA to be associated with greater exposure to sport-related RHIs.[2] However, parameter calculations in these studies differed from those in the present study. Previously, the amount of volume with significant alterations was reported and additionally heading exposure was retrospectively assessed.[8,9] These aspects overall limit the comparability and question the significance or clinical meaning of those results. Despite this, the dimension of the DTI values overall seems comparable and in line with reported means in retired professional male football players.[7]

In the present study, a significant correlation in the splenium of the corpus callosum indicated increased FA with more long-distance headers, which is in the contrary direction than reported for sport-related RHI exposure.[2]

To our knowledge, analyses of associations between long-distance headers or other specific header characteristics and DTI measures have not been reported yet in other studies. Cross-sectional comparisons between collision-sport athletes and contact- and non-contact-sport athletes showed increased FA values for collision-sport athletes,[22] which led to the assumption that the differences might be due to higher magnitudes of head impacts in collision-sports. As balls from longer distances (> 10 m) have higher linear and angular forces,[16] this assumption might apply to football players with a higher amount of long-distance headers and might explain the findings. The reported significant correlation together with the findings of other studies[22,25,26] might point to the relevance of header characteristics and especially to the distance of the ball. However, the direction of the association found in our study is contrary to the one previously cited for sport-related RHI exposure.[2] As different sports and RHIs were summarized in the review, it may be possible, that heading effects, or more specifically effects from long-distance headers, differ from effects of other RHIs. Interestingly, one of the brain regions described to be most frequently or most likely affected to show alterations after a concussion is the corpus callosum.[27,28] In the acute phase, increased FA values were reported, whereas in the recovery period, FA values seemed to resolve,[27,28] indicating a transient change and adaptation.[28] However, the interpretation of in- or decreased FA values might be more complex and influenced by many factors. Both, higher and lower values, were described previously as indications of WM injuries. More specifically, decreased FA might indicate a direct mechanical injury to axons or myelin sheath, whereas increased FA might indicate an acute tissue injury due to cytotoxic edema.[29] Increased FA was also interpreted as an indication of growth processes due to repetitive injuries.[22] On the other hand, increased FA was additionally found after learning a motor task (juggling)[30] and was also reported in parts of the corpus callosum in early-trained musicians when compared to late-trained persons.[31] These findings were interpreted as experience-dependent changes.

Regarding the results of the present study, increased FA with more long-distance headers in the splenium of the corpus callosum might therefore be interpreted as an acute alteration of WM tracts, a reaction to repetitive injuries, or as an indication of experience effects. It must be noted that other factors influencing FA (such as axonal count or density, fiber organization, or the degree of myelination) cannot be distinguished by DTI and therefore potential changes might be too unspecific to support cause–effect relationships.[32] Additionally, age influences FA, while influences from other parameters (i.e., education, lifestyle)[33] and the underlying mechanisms leading to FA alterations, are still unknown.

Of note, most of the correlations in the present study are not significant after applying a correction for multiple comparisons. This might be due to several factors, such as the small sample size and the observation period, which might have been too short to obtain significant results (e.g., the number of long-distance headers might have been too low to reveal results similar to some cross-sectional studies). To overcome methodological influences and potential statistical shortcomings, and to achieve more comparable results, standardized and uniform analysis approaches are needed. These should include longitudinal study designs, prospective registration and characterization of headers, conservative alpha-thresholds and/or appropriate correction for multiple comparisons. Altogether, the results highlight the need for the utilization of more specific header characteristics (i.e., distance, speed, biomechanical forces) than (estimations) of total header counts.

5. Limitations

This study has several limitations. The sample for DTI analyses was rather small ($n = 14$) and only high-level male players were included, which might limit the results to that specific group. It was not possible to calculate player heading incidences in addition to the overall number of headers as the total duration of training sessions and matches was not available. Flight distance of the ball was approximated, and velocity or force measurements were not conducted. For the video analysis, interrater reliability could not be calculated, which should be added in upcoming studies. Nevertheless, the presented data provide an objective number of headers in training sessions and matches of high-level players in the observation period, included each training and match, and did not rely on (estimated) self-reports. Another limiting factor may be the exclusion of potential co-variables (e.g., concussion history) in the analyses, which might have influenced the results. These co-variables should be addressed in future analyses.

6. Conclusion

The longitudinal analyses revealed no significant differences from pre- to post-measurement in DTI measures and neurocognitive performance of high-level male football players over an observation period of a median of 16.9 months. The total heading number was highly individual and header characteristics differed between trainings and matches. Results from correlation analyses might indicate potential influences of long-distance headers on WM integrity. However, the clinical relevance of such changes has yet to be determined as potential underlying or causative mechanisms are still unknown. To gain a more precise insight into how heading, and especially long-distance headers, might influence the structure and function of the brain, developing standardized and uniform analysis approaches might be beneficial. These should include factors like age, history of concussion, or individual exposure and regeneration times.

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Confirmation of ethical compliance

The study received ethical approval from the State Medical Board (Ärztekammer Westfalen-Lippe) in Münster, Germany (2017-386-f-S). The study was conducted in accordance with the Declaration of Helsinki.

CRediT authorship contribution statement

Franziska K. Mund: Formal analysis, Data curation, Visualization, Writing – original draft. **Nina Feddermann-Demont:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Götz Welsch:** Conceptualization, Investigation, Writing – review & editing. **Carsten Schuenemann:** Conceptualization, Investigation, Writing – review & editing. **Jens Fiehler:** Conceptualization, Investigation, Writing – review & editing. **Astrid Junge:** Conceptualization, Methodology, Writing – review & editing. **Claus Reinsberger:** Conceptualization, Methodology, Investigation, Supervision, Funding acquisition, Project administration, Writing – review & editing.

Declaration of interest statement

CR receives scientific funding for projects on traumatic brain injuries and heading from the Federal Institute of Sports Sciences (Germany) and the Heinz Nixdorf Westfalian Foundation. He is a member of the medical committee of the German Football association (DFB) and provides counseling on the management of traumatic brain injury to the Union of European Football Associations (UEFA).

All other authors declare no conflicting interests.

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Appendix A. Supplementary data

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Supplements Table S1: Comparison between players without brain MRI scans (n=8) and players with brain MRI scans (NPSY+MRI; n=14)

	Players without MRI (n=8)	NPSY+MRI (n=14)	Difference	T-value / Z-value	p-value
Mean age in years (SD)	19 (0.76)	20.4 (3.3)	-1.4	Z=-0.651	0.57
Mean observation period in months (SD)	14.96 (3.54)	15.05 (3.68)	-0.09	Z=-1.198	0.238
Mean total heading number (SD)	278.75 (230.99)	415.86 (220.93)	-137.11	T=-1.378	0.183
Mean total heading number per month per player (SD)	20 (13.49)	27.34 (11.27)	-7.34	T=-1.368	0.186
Mean headers over 20m (SD)	34.38 (22.34)	94.86 (78.62)	-60.48	Z=-1.844	0.07
Mean headers over 20m per month per player (SD)	2.4 (1.4)	6.31 (5.49)	-3.91	Z=-1.638	0.11

NPSY+MRI=14 players with complete neurocognitive and additional complete MRI-data; SD=standard deviation

Supplements Table S2: Results of the correlation analyses between change from pre- to post-measurement in DTI parameters and the total number of headers and the total number of headers over 20m in NPSY+MRI

		FA		MD		RD		AD	
JHU atlas tract		Total number of headers	Total number of headers over 20m	Total number of headers	Total number of headers over 20m	Total number of headers	Total number of headers over 20m	Total number of headers	Total number of headers over 20m
Middle cerebellar peduncle	Correlation coefficient (95% CI)	-0.43 (-0.782, 0.13)	-0.54 (-0.832, -0.013)	0.453 (-0.102, 0.793)	0.423 (-0.139, 0.779)	0.48 (-0.068, 0.805)	0.524 (-0.01, 0.825)	0.224 (-0.348, 0.675)	0.067 (-0.481, 0.577)
	r^2	0.1849	0.2916	0.205209	0.178929	0.2304	0.274576	0.050176	0.004489
	p-value	0.125	0.046	0.104	0.132	0.083	0.055	0.441	0.82
Pontine crossing tract	Correlation coefficient (95% CI)	-0.457 (-0.795, 0.097)	-0.306 (-0.72, 0.268)	0.2 (-0.385, 0.67)†	0.312 (-0.278, 0.731)†	0.183 (-0.4, 0.66)†	0.244 (-0.344, 0.695)†	0.196 (-0.389, 0.668)†	0.416 (-0.164, 0.782)†
	r^2	0.208849	0.093636	0.04	0.097344	0.033489	0.059536	0.038416	0.173056
	p-value	0.101	0.287	0.493	0.277	0.532	0.4	0.503	0.139
Genu of corpus callosum	Correlation coefficient (95% CI)	-0.535 (-0.83, -0.007)	-0.588 (-0.853, -0.083)	0.082 (-0.469, 0.587)	0.173 (-0.394, 0.645)	0.352 (-0.219, 0.744)	0.396 (-0.17, 0.766)	-0.229 (-0.677, 0.344)	-0.103 (-0.601, 0.452)
	r^2	0.286225	0.345744	0.006724	0.029929	0.123904	0.156816	0.052441	0.010609
	p-value	0.049	0.027	0.782	0.554	0.217	0.161	0.432	0.726

Body of corpus callosum	Correlation coefficient (95% CI)	-0.349 (-0.750, 0.239)†	-0.462 (-0.803, 0.108)†	0.299 (-0.276, 0.716)	0.4 (-0.166, 0.768)	0.343 (-0.246, 0.747)†	0.434 (-0.143, 0.791)†	0.192 (-0.377, 0.656)	0.313 (-0.261, 0.723)
	r^2	0.121801	0.213444	0.089401	0.16	0.117649	0.188356	0.036864	0.097969
	p-value	0.221	0.096	0.3	0.157	0.23	0.121	0.51	0.276
Splenium of corpus callosum	Correlation coefficient (95% CI)	-0.7 (-0.897, -0.27)	-0.884 (-0.963, -0.665)	0.462 (-0.108, 0.803)†	0.573 (0.043, 0.851)†	0.51 (-0.045, 0.825)†	0.694 (0.242, 0.898)†	0.588 (0.084, 0.853)	0.698 (0.266, 0.896)
	r^2	0.49	0.781456	0.213444	0.328329	0.2601	0.481636	0.345744	0.487204
	p-value	0.005	0.000027*	0.096	0.032	0.062	0.006	0.027	0.006
Fornix	Correlation coefficient (95% CI)	-0.099 (-0.609, 0.469)†	-0.22 (-0.682, 0.367)†	0.002 (-0.541, 0.545)†	0.062 (-0.498, 0.585)†	0.037 (-0.516, 0.569)†	0.145 (-0.432, 0.638)†	-0.058 (-0.571, 0.488)	-0.119 (-0.611, 0.439)
	r^2	0.009801	0.0484	0.000004	0.003844	0.001369	0.021025	0.003364	0.014161
	P-value	0.737	0.45	0.994	0.834	0.899	0.62	0.844	0.684
Corticospinal tract (R)	Correlation coefficient (95% CI)	-0.222 (-0.683, 0.365)†	-0.139 (-0.634, 0.437)†	0.321 (-0.269, 0.736)†	0.377 (-0.209, 0.764)†	0.29 (-0.3, 0.72)†	0.256 (-0.334, 0.701)†	0.319 (-0.271, 0.735)†	0.455 (-0.116, 0.8)†
	r^2	0.049284	0.019321	0.103041	0.142129	0.0841	0.065536	0.101761	0.207025
	p-value	0.446	0.637	0.263	0.184	0.314	0.378	0.267	0.102
Corticospinal tract (L)	Correlation coefficient (95% CI)	-0.466 (-0.799, 0.086)	-0.58 (-0.849, -0.071)	0.405 (-0.177, 0.777)†	0.384 (-0.201, 0.767)†	0.35 (-0.238, 0.75)†	0.39 (-0.194, 0.77)†	0.341 (-0.248, 0.746)†	0.222 (-0.365, 0.683)†
	r^2	0.217156	0.3364	0.164025	0.147456	0.1225	0.1521	0.116281	0.049284

	p-value	0.093	0.03	0.151	0.175	0.22	0.168	0.233	0.445
Medial lemniscus (R)	Correlation coefficient (95% CI)	0.293 (-0.281, 0.713)	0.399 (-0.167, 0.767)	0.043 (-0.499, 0.561)	0.057 (-0.488, 0.57)	-0.135 (-0.621, 0.426)	-0.18 (-0.648, 0.388)	0.334 (-0.239, 0.734)	0.438 (-0.12, 0.786)
	r^2	0.085849	0.159201	0.001849	0.003249	0.018225	0.0324	0.111556	0.191844
	p-value	0.31	0.158	0.883	0.847	0.647	0.539	0.243	0.117
Medial lemniscus (L)	Correlation coefficient (95% CI)	0.204 (-0.366, 0.663)	0.202 (-0.368, 0.661)	0.174 (-0.393, 0.645)	0.16 (-0.405, 0.636)	0.004 (-0.528, 0.533)	-0.001 (-0.531, 0.53)	0.369 (-0.201, 0.752)	0.359 (-0.212, 0.747)
	r^2	0.041616	0.040804	0.030276	0.0256	0.000016	0.000001	0.136161	0.128881
	p-value	0.484	0.489	0.553	0.586	0.99	0.997	0.194	0.208
Inferior cerebellar peduncle (R)	Correlation coefficient (95% CI)	-0.504 (-0.816, 0.036)	-0.352 (-0.744, 0.22)	0.404 (-0.161, 0.77)	0.312 (-0.262, 0.723)	0.55 (0.01, 0.842)†	0.422 (-0.157, 0.785)†	0.045 (-0.497, 0.562)	0.075 (-0.474, 0.582)
	r^2	0.254016	0.123904	0.163216	0.097344	0.3025	0.178084	0.002025	0.005625
	p-value	0.066	0.217	0.152	0.277	0.042	0.133	0.877	0.799
Inferior cerebellar peduncle (L)	Correlation coefficient (95% CI)	-0.264 (-0.697, 0.31)	-0.255 (-0.692, 0.318)	0.317 (-0.256, 0.726)	0.341 (-0.232, 0.738)	0.367 (-0.203, 0.751)	0.315 (-0.259, 0.724)	0.102 (-0.453, 0.6)	0.215 (-0.356, 0.669)
	r^2	0.069696	0.065025	0.100489	0.116281	0.134689	0.099225	0.010404	0.046225
	p-value	0.362	0.378	0.269	0.233	0.197	0.273	0.729	0.461
Superior cerebellar	Correlation coefficient (95% CI)	-0.165 (-0.64, 0.401)	-0.251 (-0.69, 0.323)	0.431 (-0.129, 0.783)	0.186 (-0.382, 0.652)	0.211 (-0.36, 0.667)	0.174 (-0.393, 0.645)	0.188 (-0.38, 0.654)	-0.046 (-0.563, 0.497)

peduncle (R)	r^2	0.027225	0.063001	0.185761	0.034596	0.044521	0.030276	0.035344	0.002116
	p-value	0.573	0.387	0.123	0.524	0.468	0.552	0.519	0.877
Superior cerebellar peduncle (L)	Correlation coefficient (95% CI)	-0.131 (-0.618, 0.43)	0.087 (-0.465, 0.59)	0.275 (-0.299, 0.703)	0.185 (-0.383, 0.651)	0.269 (-0.305, 0.7)	0.041 (-0.501, 0.559)	0.213 (-0.358, 0.668)	0.292 (-0.282, 0.712)
	r^2	0.017161	0.007569	0.075625	0.034225	0.072361	0.001681	0.045369	0.085264
	p-value	0.656	0.768	0.341	0.527	0.353	0.889	0.465	0.311
Cerebral peduncle (R)	Correlation coefficient (95% CI)	-0.569 (-0.845, -0.055)	-0.737 (-0.911, -0.339)	0.41 (-0.154, 0.772)	0.573 (0.062, 0.846)	0.536 (0.007, 0.83)	0.719 (0.305, 0.905)	0.133 (-0.428, 0.62)	0.229 (-0.343, 0.677)
	r^2	0.323761	0.543169	0.1681	0.328329	0.287296	0.516961	0.017689	0.052441
	p-value	0.034	0.003	0.146	0.032	0.048	0.004	0.65	0.431
Cerebral peduncle (L)	Correlation coefficient (95% CI)	-0.61 (-0.862, -0.118)	-0.771 (-0.924, -0.408)	0.205 (-0.381, 0.673)†	0.308 (-0.282, 0.729)†	0.304 (-0.287, 0.727)†	0.533 (-0.014, 0.835)†	0.373 (-0.196, 0.754)	0.373 (-0.196, 0.754)
	r^2	0.3721	0.594441	0.042025	0.094864	0.092416	0.284089	0.139129	0.139129
	p-value	0.02	0.0012	0.483	0.283	0.291	0.05	0.189	0.189
Anterior limb of internal capsule (R)	Correlation coefficient (95% CI)	-0.594 (-0.855, -0.093)	-0.718 (-0.904, -0.303)	0.244 (-0.329, 0.686)	0.367 (-0.203, 0.751)	0.391 (-0.176, 0.763)	0.519 (-0.016, 0.823)	0.083 (-0.468, 0.588)	0.177 (-0.39, 0.647)
	r^2	0.352836	0.515524	0.059536	0.134689	0.152881	0.269361	0.006889	0.031329
	p-value	0.025	0.004	0.4	0.197	0.166	0.057	0.778	0.544

Anterior limb of internal capsule (L)	Correlation coefficient (95% CI)	-0.383 (-0.759, 0.185)	-0.554 (-0.838, -0.032)	0.235 (-0.338, 0.681)	0.295 (-0.279, 0.714)	0.386 (-0.182, 0.761)	0.52 (-0.015, 0.823)	-0.227 (-0.685, 0.361)†	-0.205 (-0.673, 0.38)†
	r^2	0.146689	0.306916	0.055225	0.087025	0.148996	0.2704	0.051529	0.042025
	p-value	0.177	0.04	0.419	0.306	0.173	0.057	0.436	0.482
Posterior limb of internal capsule (R)	Correlation coefficient (95% CI)	-0.181 (-0.649, 0.387)	-0.241 (-0.684, 0.332)	-0.032 (-0.553, 0.507)	0.115 (-0.442, 0.609)	0.165 (-0.415, 0.65)†	0.307 (0.284, 0.728)†	-0.181 (-0.649, 0.387)	-0.017 (-0.543, 0.518)
	r^2	0.032761	0.058081	0.001024	0.013225	0.027225	0.094249	0.032761	0.000289
	p-value	0.536	0.406	0.913	0.694	0.572	0.286	0.537	0.955
Posterior limb of internal capsule (L)	Correlation coefficient (95% CI)	-0.04 (-0.559, 0.501)	-0.135 (-0.621, 0.426)	0.044 (-0.498, 0.562)	0.151 (-0.412, 0.631)	0.041 (-0.501, 0.559)	0.156 (-0.409, 0.634)	0.051 (-0.493, 0.566)	0.106 (-0.45, 0.603)
	r^2	0.0016	0.018225	0.001936	0.022801	0.001681	0.024336	0.002601	0.011236
	p-value	0.892	0.646	0.881	0.606	0.89	0.595	0.862	0.718
Retro-lenticular part of internal capsule (R)	Correlation coefficient (95% CI)	-0.131 (-0.619, 0.429)	-0.4 (-0.768, 0.166)	0.228 (-0.344, 0.677)	0.319 (-0.254, 0.727)	0.248 (-0.341, 0.697)†	0.345 (-0.243, 0.748)†	0.202 (-0.368, 0.661)	0.062 (-0.485, 0.573)
	r^2	0.017161	0.16	0.051984	0.101761	0.061504	0.119025	0.040804	0.003844
	p-value	0.654	0.157	0.433	0.266	0.392	0.226	0.49	0.834
Retro-lenticular	Correlation coefficient (95% CI)	-0.321 (-0.728, 0.252)	-0.186 (-0.652, 0.383)	0.464 (-0.88, 0.798)	0.438 (-0.121, 0.786)	0.473 (-0.077, 0.802)	0.373 (-0.197, 0.754)	0.296 (-0.278, 0.715)	0.355 (-0.216, 0.745)

part of internal capsule (L)	r^2	0.103041	0.034596	0.215296	0.191844	0.223729	0.139129	0.087616	0.126025
	p-value	0.263	0.525	0.094	0.117	0.088	0.19	0.303	0.213
Anterior corona radiata (R)	Correlation coefficient (95% CI)	-0.597 (-0.856, -0.097)	-0.729 (-0.908, -0.324)	0.226 (-0.346, 0.676)	0.249 (-0.325, 0.688)	0.395 (-0.171, 0.765)	0.454 (-0.101, 0.793)	0.035 (-0.505, 0.555)	0.023 (-0.514, 0.547)
	r^2	0.356409	0.531441	0.051076	0.062001	0.156025	0.206116	0.001225	0.000529
	p-value	0.024	0.003	0.437	0.391	0.162	0.103	0.906	0.937
Anterior corona radiata (L)	Correlation coefficient (95% CI)	-0.338 (-0.736, 0.235)	-0.674 (-0.887, -0.224)	0.185 (-0.383, 0.652)	0.194 (-0.375, 0.657)	0.276 (-0.298, 0.704)	0.41 (-0.154, 0.773)	0.053 (-0.491, 0.568)	-0.086 (-0.59, 0.466)
	r^2	0.114244	0.454276	0.034225	0.037636	0.076176	0.1681	0.002809	0.007396
	p-value	0.237	0.008	0.526	0.507	0.339	0.145	0.857	0.771
Superior corona radiata (R)	Correlation coefficient (95% CI)	-0.468 (-0.8, 0.083)	-0.763 (-0.921, -0.391)	0.461 (-0.92, 0.797)	0.428 (-0.132, 0.781)	0.566 (0.05, 0.843)	0.664 (0.206, 0.883)	0.266 (-0.308, 0.698)	0.087 (-0.465, 0.59)
	r^2	0.219024	0.582169	0.212521	0.183184	0.320356	0.440896	0.070756	0.007569
	p-value	0.091	0.0015	0.097	0.127	0.035	0.01	0.357	0.768
Superior corona radiata (L)	Correlation coefficient (95% CI)	-0.551 (-0.837, -0.029)	-0.705 (-0.899, -0.278)	0.376 (-0.194, 0.756)	0.432 (-0.128, 0.783)	0.492 (-0.052, 0.811)	0.601 (0.103, 0.858)	0.194 (-0.375, 0.657)	0.171 (-0.396, 0.643)
	r^2	0.303601	0.497025	0.141376	0.186624	0.242064	0.361201	0.037636	0.029241
	p-value	0.041	0.005	0.186	0.123	0.074	0.023	0.506	0.559

Posterior corona radiata (R)	Correlation coefficient (95% CI)	-0.691 (-0.894, -0.253)	-0.765 (-0.922, -0.395)	0.627 (0.144, 0.869)	0.639 (0.165, 0.874)	0.678 (0.23, 0.889)	0.717 (0.301, 0.904)	0.463 (-0.09, 0.798)	0.432 (-0.128, 0.783)
	r^2	0.477481	0.585225	0.393129	0.408321	0.459684	0.514089	0.214369	0.186624
	p-value	0.006	0.0014	0.016	0.014	0.008	0.004	0.096	0.123
Posterior corona radiata (L)	Correlation coefficient (95% CI)	-0.651 (-0.878, -0.184)	-0.741 (-0.913, -0.347)	0.404 (-0.161, 0.77)	0.462 (-0.09, 0.797)	0.492 (-0.052, 0.811)	0.569 (0.055, 0.844)	0.258 (-0.316, 0.693)	0.279 (-0.295, 0.705)
	r^2	0.423801	0.549081	0.163216	0.213444	0.242064	0.323761	0.066564	0.077841
	p-value	0.012	0.002	0.152	0.096	0.074	0.034	0.374	0.334
Posterior thalamic radiation (R)	Correlation coefficient (95% CI)	-0.48 (-0.806, 0.067)	-0.604 (-0.859, -0.108)	0.363 (-0.224, 0.757)†	0.324 (-0.266, 0.737)†	0.545 (0.02, 0.834)	0.632 (0.152, 0.87)	0.317 (-0.256, 0.726)	0.242 (-0.331, 0.684)
	r^2	0.2304	0.364816	0.131769	0.104976	0.297025	0.399424	0.100489	0.058564
	p-value	0.082	0.022	0.202	0.259	0.044	0.015	0.269	0.405
Posterior thalamic radiation (L)	Correlation coefficient (95% CI)	-0.313 (-0.724, 0.26)	-0.591 (-0.854, -0.088)	0.392 (-0.175, 0.764)	0.578 (0.069, 0.849)	0.421 (-0.141, 0.778)	0.681 (0.235, 0.89)	0.301 (-0.274, 0.717)	0.357 (-0.214, 0.746)
	r^2	0.097969	0.349281	0.153664	0.334084	0.177241	0.463761	0.090601	0.127449
	p-value	0.275	0.026	0.166	0.03	0.134	0.007	0.296	0.21
Sagittal stratum (R)	Correlation coefficient (95% CI)	-0.574 (-0.847, -0.062)	-0.574 (-0.847, -0.062)	0.635 (0.158, 0.872)	0.444 (-0.113, 0.789)	0.765 (0.395, 0.922)	0.596 (0.096, 0.856)	0.071 (-0.477, 0.58)	-0.1 (-0.599, 0.455)
	r^2	0.329476	0.329476	0.403225	0.197136	0.585225	0.355216	0.005041	0.01

	p-value	0.032	0.032	0.015	0.112	0.0014	0.024	0.809	0.735
Sagittal stratum (L)	Correlation coefficient (95% CI)	-0.336 (-0.744, 0.253)†	-0.455 (-0.800, 0.116)†	0.209 (-0.377, 0.675)†	0.383 (-0.202, 0.767)†	0.288 (-0.302, 0.719)†	0.383 (-0.202, 0.767)†	0.13 (-0.481, 0.599)†	0.246 (-0.342, 0.696)†
	r^2	0.112896	0.207025	0.043681	0.146689	0.082944	0.146689	0.0169	0.060516
	p-value	0.24	0.102	0.474	0.177	0.318	0.177	0.659	0.396
External capsule (R)	Correlation coefficient (95% CI)	-0.1 (-0.599, 0.455)	-0.444 (-0.789, 0.113)	0.168 (-0.398, 0.642)	0.176 (-0.391, 0.646)	0.2 (-0.37, 0.66)	0.327 (-0.246, 0.731)	0.065 (-0.482, 0.576)	-0.197 (-0.658, 0.373)
	r^2	0.01	0.197136	0.028224	0.030976	0.04	0.106929	0.004225	0.038809
	p-value	0.735	0.111	0.565	0.547	0.494	0.253	0.825	0.501
External capsule (L)	Correlation coefficient (95% CI)	-0.324 (-0.729, 0.25)	-0.385 (-0.76, 0.183)	0.211 (-0.359, 0.667)	0.414 (-0.149, 0.775)	0.268 (-0.307, 0.699)	0.426 (-0.135, 0.78)	0.067 (-0.481, 0.577)	0.332 (-0.241, 0.733)
	r^2	0.104976	0.148225	0.044521	0.171396	0.071824	0.181476	0.004489	0.110224
	p-value	0.259	0.175	0.468	0.141	0.355	0.129	0.82	0.246
Cingulum cingulate gyrus (R)	Correlation coefficient (95% CI)	-0.688 (-0.893, -0.247)	-0.641 (-0.874, -0.167)	0.387 (-0.181, 0.761)	0.479 (-0.069, 0.805)	0.576 (0.065, 0.847)	0.637 (0.161, 0.873)	0.104 (-0.451, 0.601)	0.211 (-0.36, 0.667)
	r^2	0.473344	0.410881	0.149769	0.229441	0.331776	0.405769	0.010816	0.044521
	p-value	0.007	0.014	0.172	0.083	0.031	0.014	0.723	0.469
	Correlation coefficient (95% CI)	0.071 (-0.478, 0.58)	0.131 (-0.429, 0.619)	0.342 (-0.23, 0.739)	0.358 (-0.213, 0.747)	0.206 (-0.365, 0.664)	0.214 (-0.357, 0.669)	0.399 (-0.167, 0.767)	0.417 (-0.145, 0.776)

Cingulum cingulate gyrus (L)	r^2	0.005041	0.017161	0.116964	0.128164	0.042436	0.045796	0.159201	0.173889
	p-value	0.81	0.655	0.231	0.209	0.48	0.463	0.158	0.137
Cingulum hippocampus (R)	Correlation coefficient (95% CI)	-0.405 (-0.77, 0.159)	-0.081 (-0.587, 0.469)	-0.214 (-0.669, 0.357)	-0.435 (-0.784, 0.125)	0.317 (-0.257, 0.726)	-0.077 (-0.583, 0.473)	-0.47 (-0.801, 0.081)	-0.475 (-0.803, 0.074)
	r^2	0.164025	0.006561	0.045796	0.189225	0.100489	0.005929	0.2209	0.225625
	p-value	0.15	0.782	0.462	0.121	0.269	0.795	0.09	0.086
Cingulum hippocampus (L)	Correlation coefficient (95% CI)	0.072 (-0.477, 0.581)	0.251 (-0.322, 0.69)	0.077 (-0.473, 0.583)	-0.245 (-0.686, 0.328)	0.171 (-0.396, 0.643)	-0.097 (-0.597, 0.457)	-0.091 (-0.593, 0.462)	-0.339 (-0.737, 0.233)
	r^2	0.005184	0.063001	0.005929	0.060025	0.029241	0.009409	0.008281	0.114921
	p-value	0.806	0.386	0.795	0.398	0.559	0.741	0.758	0.235
Fornix Stria terminalis (R)	Correlation coefficient (95% CI)	-0.342 (-0.739, 0.23)	-0.459 (-0.796, 0.095)	0.123 (-0.436, 0.614)	0.193 (-0.376, 0.656)	0.355 (-0.217, 0.745)	0.459 (-0.095, 0.796)	-0.208 (-0.665, 0.363)	-0.213 (-0.668, 0.358)
	r^2	0.116964	0.210681	0.015129	0.037249	0.126025	0.210681	0.043264	0.045369
	p-value	0.231	0.099	0.674	0.509	0.214	0.099	0.476	0.464
Fornix Stria terminalis (L)	Correlation coefficient (95% CI)	-0.094 (-0.595, 0.459)	-0.335 (-0.735, 0.237)	-0.085 (-0.589, 0.467)	0.108 (-0.448, 0.604)	0.055 (-0.49, 0.569)	0.322 (-0.252, 0.728)	-0.201 (-0.661, 0.369)	-0.262 (-0.696, 0.312)
	r^2	0.008836	0.112225	0.007225	0.011664	0.003025	0.103684	0.040401	0.068644
	p-value	0.749	0.241	0.774	0.713	0.853	0.261	0.491	0.366

Superior longitudinal fasciculus (R)	Correlation coefficient (95% CI)	-0.362 (-0.749, 0.209)	-0.616 (-0.864, -0.127)	0.15 (-0.413, 0.631)	0.273 (-0.301, 0.702)	0.282 (-0.293, 0.707)	0.45 (-0.105, 0.792)	-0.006 (-0.535, 0.526)	0.047 (-0.496, 0.564)
	r^2	0.131044	0.379456	0.0225	0.074529	0.079524	0.2025	0.000036	0.002209
	p-value	0.204	0.019	0.608	0.344	0.329	0.106	0.983	0.872
Superior longitudinal fasciculus (L)	Correlation coefficient (95% CI)	-0.684 (-0.891, -0.241)	-0.736 (-0.911, -0.338)	0.293 (-0.281, 0.713)	0.404 (-0.161, 0.77)	0.487 (-0.059, 0.809)	0.59 (0.087, 0.853)	0.03 (-0.509, 0.552)	0.139 (-0.423, 0.624)
	r^2	0.467856	0.541696	0.085849	0.163216	0.237169	0.3481	0.0009	0.019321
	p-value	0.007	0.003	0.309	0.152	0.077	0.026	0.919	0.636
Superior fronto occipital fasciculus (R)	Correlation coefficient (95% CI)	0.115 (-0.442, 0.609)	0.014 (-0.521, 0.54)	0.245 (-0.329, 0.686)	0.288 (-0.286, 0.71)	0.21 (-0.361, 0.666)	0.28 (-0.294, 0.706)	0.232 (-0.34, 0.679)	0.253 (-0.32, 0.691)
	r^2	0.013225	0.000196	0.060025	0.082944	0.0441	0.0784	0.053824	0.064009
	p-value	0.694	0.963	0.399	0.317	0.472	0.332	0.424	0.382
Superior fronto occipital fasciculus (L)	Correlation coefficient (95% CI)	-0.478 (-0.805, 0.071)	-0.42 (-0.777, 0.142)	0.493 (-0.0051, 0.0811)	0.431 (-0.129, 0.783)	0.648 (0.18, 0.877)	0.574 (0.062, 0.847)	0.222 (-0.35, 0.673)	0.189 (-0.38, 0.654)
	r^2	0.228484	0.1764	0.243049	0.185761	0.419904	0.329476	0.049284	0.035721
	p-value	0.084	0.135	0.073	0.124	0.012	0.032	0.446	0.518

Inferior fronto occipital fasciculus (R)	Correlation coefficient (95% CI)	-0.316 (-0.725, 0.258)	-0.424 (-0.779, 0.138)	0.363 (-0.208, 0.749)	0.127 (-0.433, 0.616)	0.394 (-0.173, 0.765)	0.277 (-0.297, 0.704)	0.111 (-0.446, 0.606)	-0.201 (-0.661, 0.369)
	r^2	0.099856	0.179776	0.131769	0.016129	0.155236	0.076729	0.012321	0.040401
	p-value	0.271	0.131	0.202	0.665	0.164	0.337	0.705	0.491
Inferior fronto occipital fasciculus (L)	Correlation coefficient (95% CI)	0.141 (-0.421, 0.625)	0.059 (-0.487, 0.572)	0.148 (-0.415, 0.629)	0.166 (-0.4, 0.64)	0.044 (-0.498, 0.562)	0.108 (-0.448, 0.604)	0.238 (-0.334, 0.683)	0.19 (-0.379, 0.654)
	r^2	0.019881	0.003481	0.021904	0.027556	0.001936	0.011664	0.056644	0.0361
	p-value	0.631	0.842	0.615	0.572	0.88	0.713	0.412	0.516
Uncinate fasciculus (R)	Correlation coefficient (95% CI)	-0.383 (-0.759, 0.185)	-0.414 (-0.774, 0.149)	0.537 (0.9, 0.831)	0.404 (-0.162, 0.769)	0.485 (-0.062, 0.808)	0.386 (-0.181, 0.761)	0.562 (0.045, 0.842)	0.384 (-0.184, 0.76)
	r^2	0.146689	0.171396	0.288369	0.163216	0.235225	0.148996	0.315844	0.147456
	p-value	0.177	0.141	0.048	0.152	0.079	0.172	0.036	0.175
Uncinate fasciculus (L)	Correlation coefficient (95% CI)	0.27 (-0.304, 0.7)	0.152 (-0.412, 0.631)	0.132 (-0.429, 0.619)	0.04 (-0.501, 0.559)	-0.017 (-0.543, 0.518)	-0.03 (-0.552, 0.508)	0.242 (-0.331, 0.685)	0.087 (-0.465, 0.59)
	r^2	0.0729	0.023104	0.017424	0.0016	0.000289	0.0009	0.058564	0.007569
	p-value	0.35	0.605	0.653	0.891	0.953	0.918	0.404	0.767

Tapetum (R)	Correlation coefficient (95% CI)	0.088 (-0.464, 0.591)	0.009 (-0.524, 0.537)	0.431 (-0.129, 0.783)	0.186 (-0.382, 0.652)	0.315 (-0.259, 0.724)	0.123 (-0.436, 0.614)	0.47 (-0.081, 0.801)	0.217 (-0.354, 0.67)
	r^2	0.007744	0.000081	0.185761	0.034596	0.099225	0.015129	0.2209	0.047089
	p-value	0.764	0.976	0.123	0.524	0.273	0.675	0.09	0.456
Tapetum (L)	Correlation coefficient (95% CI)	-0.324 (-0.729, 0.25)	-0.232 (-0.679, 0.34)	0.153 (-0.411, 0.632)	-0.173 (-0.645, 0.393)	0.251 (-0.323, 0.69)	-0.06 (-0.572, 0.486)	0.028 (-0.51, 0.55)	-0.251 (-0.69, 0.322)
	r^2	0.104976	0.053824	0.023409	0.029929	0.063001	0.0036	0.000784	0.063001
	p-value	0.259	0.424	0.602	0.554	0.387	0.838	0.924	0.386

AD=axial diffusivity; CI=confidence interval; FA=fractional anisotropy; JHU= Johns Hopkins University; MD=mean diffusivity; NPSY+MRI=14 players with complete neurocognitive and additional complete MRI-data; RD=radial diffusivity; (R)=right; (L)=left; *=significant correlation

Bonferroni corrected p=0.001

†=Spearman's rank correlation