Exposure to Repetitive Head Impacts Is Associated With Corpus Callosum Microstructure and Plasma Total Tau in Former Professional American Football Players

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Background: Exposure to repetitive head impacts (RHI) is associated with an increased risk of later-life neurobehavioral dysregulation and neurodegenerative disease. The underlying pathomechanisms are largely unknown.
Purpose: To investigate whether RHI exposure is associated with later-life corpus callosum (CC) microstructure and whether CC microstructure is associated with plasma total tau and neuropsychological/neuropsychiatric functioning.
Study Type: Retrospective cohort study.
Population: Seventy-five former professional American football players (age 55.2 ± 8.0 years) with cognitive, behavioral, and mood symptoms.
Field Strength/Sequence: Diffusion-weighted echo-planar MRI at 3 T.

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Assessment: Subjects underwent diffusion MRI, venous puncture, neuropsychological testing, and completed self-report measures of neurobehavioral dysregulation. RHI exposure was assessed using the Cumulative Head Impact Index (CHII). Diffusion MRI measures of CC microstructure (i.e., free-water corrected fractional anisotropy (FA), trace, radial diffusivity (RD), and axial diffusivity (AD)) were extracted from seven segments of the CC (CC1-7), using a tractography clustering algorithm. Neuropsychological tests were selected: Trail Making Test Part A (TMT-A) and Part B (TMT-B), Controlled Oral Word Association Test (COWAT), Stroop Interference Test, and the Behavioral Regulation Index (BRI) from the Behavior Rating Inventory of Executive Function, Adult version (BRIEF-A).
Statistical Tests: Diffusion MRI metrics were tested for associations with RHI exposure, plasma total tau, neuropsychological performance, and neurobehavioral dysregulation using generalized linear models for repeated measures.
Results: RHI exposure was associated with increased AD of CC1 (correlation coefficient (r) = 0.32, $P < 0.05$) and with increased plasma total tau ($r = 0.34$, $P < 0.05$). AD of the anterior CC1 was associated with increased plasma total tau (CC1: $r = 0.30$, $P < 0.05$; CC2: $r = 0.29$, $P < 0.05$). Higher trace, AD, and RD of CC1 were associated with better performance ($P < 0.05$) in TMT-A (trace, $r = 0.33$; AD, $r = 0.31$; and RD, $r = 0.28$) and TMT-B (trace, $r = 0.31$; RD, $r = 0.34$). Higher FA and AD of CC2 were associated with better performance ($P < 0.05$) in TMT-A (FA, $r = 0.36$; AD, $r = 0.28$), TMT-B (FA, $r = 0.36$; AD, $r = 0.27$), COWAT (FA, $r = 0.36$; AD, $r = 0.32$), and BRI (AD, $r = 0.29$).
Data Conclusion: These results suggest an association among RHI exposure, CC microstructure, plasma total tau, and clinical functioning in former professional American football players.
Level of Evidence: 3
Technical Efficacy Stage: 1

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Exposure to repetitive head impacts (RHI) is commonly observed in contact sports such as American football. Exposure to RHI may lead to symptomatic brain injury such as concussion, as well as the more common asymptomatic subconcussive injuries.[1] RHI exposure sustained over longer periods of time is associated with an increased risk of later-life cognitive impairment, neurobehavioral dysregulation, and neurodegenerative disease.[2] The underlying pathomechanisms of potential cumulative effects are, however, not fully understood.

RHI exposure has previously been linked to brain alterations.[3] Structural alterations of the brain can be noninvasively characterized using advanced neuroimaging. Diffusion MRI (dMRI) is particularly sensitive to subtle microstructural alterations of brain tissue following RHI.[3] More specifically, studies have revealed decreased fractional anisotropy (FA) and increased radial diffusivity (RD), axial diffusivity (AD), and mean diffusivity (MD) following RHI.[4,5] Of note, the corpus callosum (CC) is predominantly affected, likely due to its central location and increased vulnerability to shear strain.[6] Importantly, the CC is involved in brain functions that are often impaired following brain injury, such as cognition, mood, and behavior.[7] A previous study found an association between younger age at first exposure to American football and later-life microstructural alterations of the anterior CC.[5] However, whether exposure to RHI while participating in professional American football leads to later-life alterations in CC microstructure is not known. Moreover, the pathomechanism underlying microstructural alterations following exposure to RHI is not fully understood.

Exposure to RHI has been associated with increased levels of total tau in former National Football League (NFL) players.[8] Total tau is a nonspecific marker of general neurodegeneration and axonal damage,[9] mainly expressed in neuronal axons where it regulates the stability of microtubules and supports axonal transportation.[10] Additionally, tau crosses the blood–brain barrier (BBB) and, thus, can be detected and measured in blood plasma.[11] There are few studies that have investigated the association between brain structure and total tau in former contact-sport athletes. One such study reported higher cerebrospinal fluid (CSF) total tau in a group of 22 former professional contact-sport athletes (mean age: 55.9 ± 12.2 years; American football ($n = 12$), ice hockey ($n = 9$), and snowboarding ($n = 1$)) compared with five community control participants.[9] Athletes with higher CSF total tau showed lower FA, as well as higher RD and MD across several white matter (WM) tracts and impaired neuropsychological performance on the Trail Making Test Part B (TMT-B).[9]

RHI has also been linked to impaired neuropsychological and neuropsychiatric functioning in former professional American football players.[2] Progressive cognitive impairment (specifically episodic memory deficits and/or executive dysfunction) and neurobehavioral dysregulation (e.g., explosiveness, impulsivity, rage, "short fuse") are the core clinical features of the NINDS Consensus Diagnostic Criteria for Traumatic Encephalopathy Syndrome (TES), which is the clinical syndrome associated with underlying pathology of the neurodegenerative disease, chronic traumatic encephalopathy (CTE).[12] However, it is not clear whether or not RHI exposure is associated with CC microstructure in former professional American football players. It also remains to be elucidated whether or not later-life CC microstructure is associated with increased later-life plasma total tau and with clinical functioning following exposure to RHI.

Thus, the aim of this study was (1) to investigate whether or not RHI exposure is associated with alterations in CC microstructure, (2) to determine whether or not CC microstructure is associated with plasma total tau levels, and (3) to assess the association between CC microstructure and neuropsychological functioning and neurobehavioral dysregulation in former professional American football players.

Methods

Study Design

This study was part of the Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests (DETECT) project. The aim of this project was to develop *in-vivo* biomarkers to diagnose CTE during life. Between 2011 and 2015, participants were tested in a single 2- to 3-day visit. The test protocol included an interview to quantify previous exposure to RHI from football, as well as neuropsychological testing, completion of self-report measures of neuropsychiatric functioning, collection of blood, and MRI acquisition. More details regarding the study design can be found elsewhere.[13] The study was approved by the respective Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

Study Participants

The DETECT sample includes 96 symptomatic former NFL players. Inclusion criteria were as follows: (1) male sex, (2) age between 40 and 69 years, (3) minimum of 12 years of participation in organized American football, (4) playing at least 2 years in the NFL, and (5) self-reported (at time of telephone screening) cognitive, behavioral, and mood symptoms for a minimum of 6 months prior to study enrollment. Exclusion criteria were as follows: (1) having sustained a concussion or other traumatic brain injury (TBI) within 1 year prior to study enrollment, (2) contraindications for MRI, (3) presence of another disease affecting the central nervous system, and (4) a primary language other than English.

For the present study, 15 of the 96 former NFL players were excluded because of missing dMRI data. Another six participants were excluded due to artifacts in imaging data (e.g., motion artifacts). Thus, the final sample consisted of 75 former NFL players. Cohort characteristics and demographics of these participants are shown in Table 1.

Exposure to RHI

The Cumulative Head Impact Index (CHII) was used to quantify the players' estimated exposure to RHI.[2] The CHII is based on information about the individual's football history, including the number of seasons played, position(s) played, and levels played (youth, high school, college), as well as on an estimation of head impact frequencies established based on helmet accelerometer studies.[2] Because helmet accelerometer data at the professional level have not been published or made available, head impact frequencies from college level studies were extrapolated to estimate participants' post-college head impact frequencies.

Assessment of Plasma Total Tau

Blood was drawn by conventional venous puncture. Plastic dipotassium ethylenediamine-tetraacetic acid (EDTA) tubes were used for the collection. Plasma was centrifuged, aliquoted, and stored at a temperature of −80 °C. The frozen plasma tubes were sent out for analysis. Tests were performed in duplicate from each sample using the Simoa HD-1 analyzer (Quanterix, Lexington, MA, USA). For the procedure, two monoclonal antibodies were used for binding specific parts of human tau. The detection antibody recognizes the N-terminus, while the capture antibody connects to the mid-domain. Samples were tested in triplicate calculating standard curves and individual sample measurements. On average, a coefficient of variation of 4% across all samples was detected. More details concerning the procedure can be found elsewhere.[14]

Neuropsychological and Neuropsychiatric Assessment

All participants were administered a neuropsychological test battery and completed self-report measures of neuropsychiatric symptoms. We chose the following tests to include in the present study because they are both sensitive to frontal system executive functioning[15] and frequently affected in people exposed to RHI[2,13]: Trail Making Test Part A and Part B (TMT-A, TMT-B), measuring visual scanning, psychomotor speed, and cognitive flexibility;[16] Controlled Oral Word Association Test (COWAT), measuring phonemic fluency;[17] the Stroop Interference Test, measuring selective attention and inhibition;[18] and the Behavioral Regulation Index (BRI) from the Behavior Rating Inventory of Executive Function, Adult version (BRIEF-A), measuring neurobehavioral dysregulation.[19] Raw scores of the tests were transformed into T scores using normative data that account for age, sex, and education.[13]

Magnetic Resonance Imaging

IMAGE ACQUISITION. All dMRI data were collected using a 3-Tesla MRI scanner (Magnetom Verio, Siemens Healthineers AG, Erlangen, Germany) with a 32-channel head coil. A diffusion-weighted echo-planar imaging (EPI) sequence was acquired with the following parameters: repetition time (TR) = 11,700 msec, echo time (TE) = 85 msec, matrix = 128 × 128, field of view = 256 × 256 mm^2, slice thickness = 2 mm, and parallel imaging using GRAPPA with an acceleration factor of 3. In total, 73 slices were acquired using 64 diffusion directions, consisting of 59 diffusion-weighted images with multiple b-values from 80 to 3000 s/mm^2 and five images with a b-value of 0 s/mm^2 for anatomical reference.

IMAGE PROCESSING. Visual inspection of the raw dMRI data (LO, FZ, JK with 20, 10, and 3 years of experience in the analysis of MRI data) was followed by generating whole-brain tractography maps using a two-tensor model. We

TABLE 1. Cohort Characteristics ($n = 75$)

	Mean	SD	Range
Sample characteristics			
Age (years)	55.16	7.98	40–69
Body mass index (kg/m^2)	33.15	5.18	25.2–52.1
Education (years)	16.49	0.98	15–20
CHII	20,352.3	7,236.02	6,860.4–48,218.3
Race	W 44; B/A 23; NA 8		
Fluid biomarkers			
Plasma total tau (pg/mL)	2.56	0.98	0.77–5.68
Neuropsychological tests			
Trail Making Test Part A	49.37	11.52	20–66
Trail Making Test Part B	45.21	15.75	20–74
Stroop Test	10.99	2.63	2–15
COWAT	49.86	11.41	24–80
BRI	63.01	12.32	37–95

Summary of demographical data, fluid biomarkers, and neuropsychological test performance represented as mean, standard deviation, range, and numeric variables.
CHII: Cumulative Head Impact Index; Race: W, White, B/A, Black or African American, NA, no data available; BRI, Behavioral Regulation Index; COWAT, Controlled Oral Word Association Test; SD, standard deviation.

applied the Unscented Kalman Filter (UKF, part of the ukftractography package (https://github.com/pnlbwh/ukftractography)) method,[20] which has previously been shown to sensitively and consistently trace fibers across various populations.[21] This two-tensor model accounts for crossing fibers,[20] which are particularly relevant in the context of tracing the CC fibers. This two-tensor model associates the first tensor with the main direction of the fiber tract that is being traced. The second tensor represents fibers crossing through the tract of interest. Whole-brain tractography was then again visually inspected and also quantitatively assessed for quality using the quality control tool in the whitematteranalysis (WMA) software (https://github.com/SlicerDMRI/whitematteranalysis).

The CC was identified in each subject by applying a fiber clustering pipeline in combination with an anatomical tract atlas.[22] This approach applies machine learning to dMRI data to identify WM tracts of each individual based on a neuroanatomist-curated WM atlas that was trained using tractography data from 100 healthy young adults (stemming from the Human Connectome Project).[23] Using the anatomical atlas, the CC was automatically divided into seven anatomical subregions[24]: CC1 = rostrum, CC2 = genu, CC3 + 4 = anterior half of the body, CC5 + 6 = posterior half of the body, and CC7 = splenium (Fig. 1). The division into seven subregions is based on a segmentation scheme by Witelson et al.[25] The CC tractography of each individual case

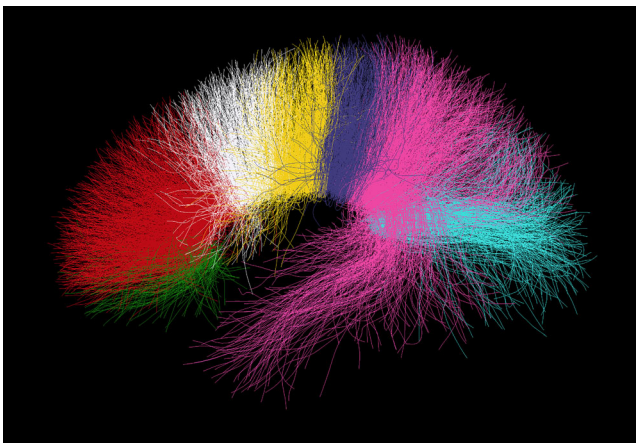

FIGURE 1: White matter (WM) fibers passing through corpus callosum (CC) subregions; green = CC1 (rostrum), red = CC2 (genu), white and yellow = CC3 + CC4 (anterior half of the body), purple and pink = CC5 + CC6 (posterior half of the body), turquoise = CC7 (splenium). WM tracts consist of grouped fiber clusters. Predefined subregions of the CC (CC1–CC7) were selected and WM fibers passing through each subregion are depicted in different colors.

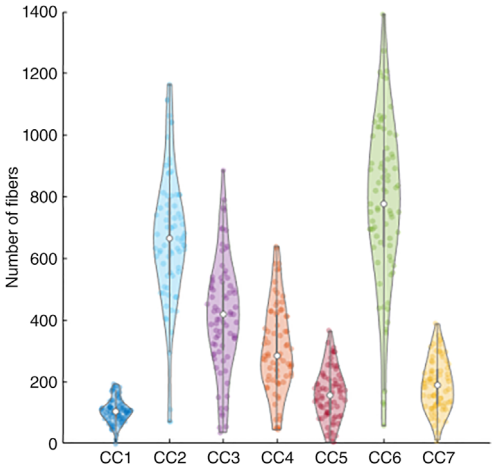

FIGURE 2: Violin plots depicting the number of fibers for each of the corpus callosum (CC) segments (CC1–CC7).

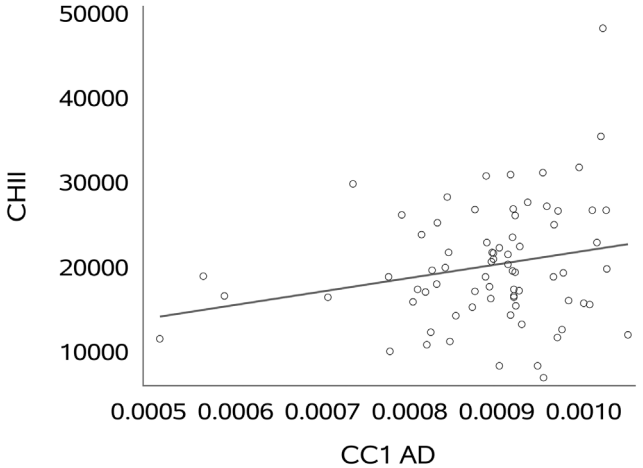

FIGURE 3: Scatter plot displaying the association between the Cumulative Head Impact Index (CHII) and axial diffusivity (AD) of the rostrum of the corpus callosum (CC1).

was visually inspected for quality and the number of fibers was quantitatively assessed (Fig. 2). 3D Slicer (The SlicerDMRI project (http://dmri.slicer.org))[26] was used to extract free-water corrected diffusion parameters (FA, trace, AD, and RD) of these seven subregions of the CC. Of note, to quantify the total amount of diffusion, one can either calculate the mean of the three eigenvalues (MD) or compute the sum (trace).

Statistical Analyses

Statistical analyses were performed using the Statistical Analysis System (SAS version 9.4; SAS Institute Inc., NC, USA). Three sets of generalized linear models (GLMs) for repeated measures with an unstructured covariance matrix were used to investigate associations among RHI exposure, CC microstructure, plasma total tau, and neuropsychological/neuropsychiatric test scores. The first set of GLM tested for associations between RHI (independent variable) and each dMRI parameter (FA, trace, AD, and RD) extracted from the seven CC subregions (dependent variables). To reduce model complexity, each dMRI parameter was tested separately. The second set of GLM investigated associations between each dMRI parameter (FA, trace, AD, and RD) from the seven CC subregions (dependent variables) with plasma total tau levels (independent variable). The third set of GLM investigated associations between neuropsychological/neuropsychiatric test scores (dependent variables) and each dMRI parameter (FA, trace, AD, and RD) from each CC subregion that revealed significant effects in previous GLM tests (independent variables). For all three sets of GLM, covariates included body mass index (BMI = body weight divided by height squared (kg/m^2)) and age (in years), in addition to years of education for the third set of GLM investigating neuropsychological/neuropsychiatric measures. BMI was included as a covariate due to evidence for a negative correlation between BMI and CC microstructure.[27] The results from the GLMs were adjusted for multiple comparisons across the number of CC subregions tested using a false discovery rate (FDR) of 5%. We set the level of statistical significance at $P < 0.05$ (FDR-corrected).

Results

Cohort Characteristics

Demographics, neuropsychological test scores, and total tau in blood plasma of the 75 former American football players included in this study are shown in Table 1.

Association Between RHI Exposure and Diffusion Measures

The average CHII was 20,352.3 ± 7,236.02 (range 6,860.4–48,218.3). Among the seven subregions of the CC, AD of CC1 was associated with RHI exposure ($r = 0.32$, $P < 0.05$; Fig. 3). Thus, the higher the CHII (indicating greater exposure to RHI), the higher the AD in CC1. No other subregional dMRI parameters were significantly associated with CHII ($p \geq 0.05$) (FA: CC1 $P = 0.2831$, CC2 $P = 0.2831$, CC3 $P = 0.3198$, CC4 $P = 0.5820$, CC5 $P = 0.4396$, CC6 $P = 0.3198$, CC7 $P = 0.5220$; Trace: CC1 $P = 0.2331$, CC2 $P = 0.7622$, CC3 $P = 0.7622$, CC4 $P = 0.2331$, CC5 $P = 0.7688$, CC6 $P = 0.6465$, CC7 $P = 0.7622$; AD: CC2 $P = 0.2402$, CC3 $P = 0.9462$, CC4 $P = 0.2402$, CC5 $P = 0.9462$, CC6 $P = 0.8669$, CC7 $P = 0.9462$; RD: CC1 $P = 0.8361$, CC2 $P = 0.5558$, CC3 $P = 0.5558$, CC4 $P = 0.5558$, CC5 $P = 0.5558$, CC6 $P = 0.8361$, CC7 $P = 0.5558$).

Association Between Diffusion Measures and Plasma Total Tau

AD of CC1 and CC2 were associated with plasma total tau (CC1: $r = 0.30$; CC2: $r = 0.29$, both $P < 0.05$; Table 2 and

TABLE 2. Corpus Callosum Diffusion MRI Measures and Plasma Total Tau

	Region	Mean	SD	Partial *r*	*P* value
FA	CC1	0.6096	0.0406	0.1584	0.4374
	CC2	0.6738	0.0366	0.2093	0.4374
	CC3	0.7069	0.0367	0.1528	0.4374
	CC4	0.7306	0.0323	0.0249	0.8705
	CC5	0.7346	0.0373	0.0733	0.8705
	CC6	0.7281	0.0242	0.0190	0.8705
	CC7	0.7391	0.0238	−0.0557	0.8705
Trace (mm^2/s)	CC1	0.0014	0.0001	0.2424	0.2439
	CC2	0.0016	0.0001	0.1387	0.4064
	CC3	0.0015	0.0001	−0.0535	0.7536
	CC4	0.0015	0.0001	−0.1565	0.4064
	CC5	0.0016	0.0001	−0.0121	0.9171
	CC6	0.0016	0.0001	−0.1412	0.4064
	CC7	0.0016	0.0001	−0.0979	0.5602
AD (mm^2/s)	CC1	0.0009	0.0001	**0.2996**	**0.0440**
	CC2	0.0011	0.0001	**0.2850**	**0.0440**
	CC3	0.0011	0.0001	0.0499	0.7797
	CC4	0.0011	0.0001	−0.1268	0.5544
	CC5	0.0012	0.0001	0.0129	0.9119
	CC6	0.0012	0.0001	−0.1164	0.5544
	CC7	0.0012	0.0001	−0.0890	0.6227
RD (mm^2/s)	CC1	0.0003	<0.0001	0.0970	0.7583
	CC2	0.0003	<0.0001	−0.1013	0.7583
	CC3	0.0002	<0.0001	−0.1340	0.7583
	CC4	0.0002	<0.0001	−0.0711	0.7583
	CC5	0.0002	<0.0001	−0.0142	0.9034
	CC6	0.0003	<0.0001	0.0804	0.7583
	CC7	0.0002	<0.0001	−0.0342	0.8973

Associations of plasma total tau with diffusion parameters of corpus callosum (CC) subregions (CC1–CC7). Mean, standard deviation, partial *r,* and *P* values are presented. Significant results are marked in bold.
For CC1 there were only 74 subjects included since one data point was missing after clustering.
FA, fractional anisotropy; AD, axial diffusivity; RD, radial diffusivity; SD, standard deviation.

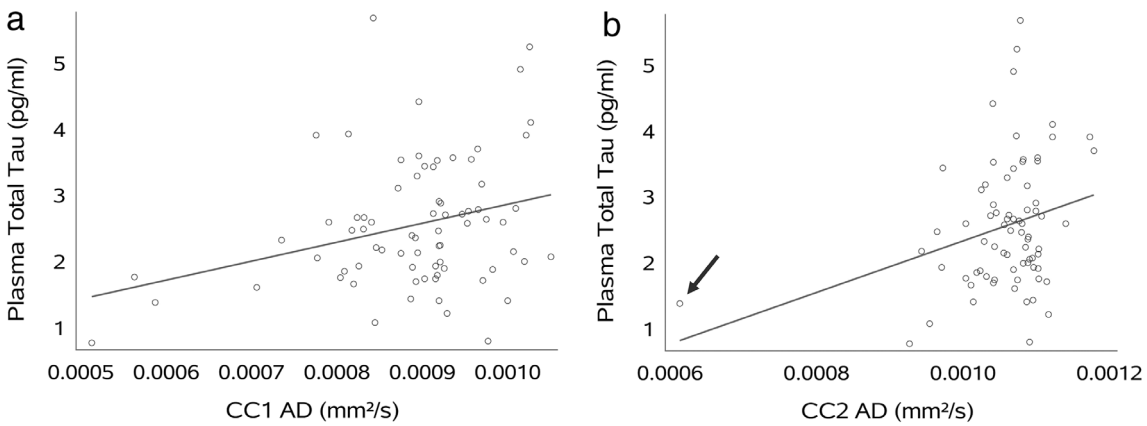

FIGURE 4: Scatter plots displaying the association between plasma total tau and axial diffusivity (AD) of rostrum (CC1) and genu (CC2) of the corpus callosum (CC). Arrow indicates outlier.

TABLE 3. Results Neuropsychological Tests

Region	DTI	Test	Estimate	SE	Partial *r*	*P* value
CC1	FA	TMT-A	63.40	25.92	0.2752	0.0657
		TMT-B	64.25	36.96	0.1993	0.1440
		STROOP	9.09	7.76	0.1358	0.2452
		COWAT	60.75	26.78	0.2566	0.0657
		BRI	36.88	27.61	0.1544	0.2324
	Trace (mm^2/s)	TMT-A	25,950.00	8,649.23	**0.3313**	**0.0184**
		TMT-B	34,753.00	12,675.00	**0.3056**	**0.0192**
		STROOP	3,472.81	2,469.56	0.1624	0.2049
		COWAT	10,628.00	9,368.05	0.1316	0.2603
		BRI	15,057.00	9,696.93	0.1788	0.2049
	AD (mm^2/s)	TMT-A	33,384.00	12,142.00	**0.3063**	**0.0376**
		TMT-B	37,878.00	17,933.00	0.2400	0.0952
		STROOP	4,289.61	3,318.86	0.1496	0.2003
		COWAT	17,530.00	12,985.00	0.1561	0.2003
		BRI	24,962.00	13,331.00	0.2141	0.1086
	RD (mm^2/s)	TMT-A	116,384.00	45,840.00	**0.2848**	**0.0331**
		TMT-B	199,840.00	64,483.00	**0.3410**	**0.0138**
		STROOP	14,044.00	12,597.00	0.1294	0.4476
		COWAT	18,110.00	49,205.00	0.0430	0.7139
		BRI	25,984.00	50,898.00	0.0596	0.7139
CC2	FA	TMT-A	87.56	26.27	**0.3613**	**0.0024**
		TMT-B	121.35	36.67	**0.3590**	**0.0024**
		STROOP	10.77	8.61	0.1439	0.2150
		COWAT	88.37	26.63	**0.3599**	**0.0024**
		BRI	39.41	28.86	0.1568	0.2150
	Trace (mm^2/s)	TMT-A	19,528.00	11,525.00	0.1933	0.1246
		TMT-B	29,599.00	17,755.00	0.1903	0.1246
		STROOP	−2,438.43	4,546.17	−0.0622	0.5933
		COWAT	21,430.00	11,654.00	0.2090	0.1246
		BRI	21,122.00	12,289.00	0.1959	0.1246
	AD (mm^2/s)	TMT-A	39,231.00	15,393.00	**0.2841**	**0.0215**
		TMT-B	59,037.00	24,880.00	**0.2659**	**0.0253**
		STROOP	−1,143.55	6,289.49	−0.0211	0.8562
		COWAT	44,808.00	15,418.00	**0.3201**	**0.0215**
		BRI	42,016.00	16,377.00	**0.2858**	**0.0215**

TABLE 3. Continued

Region	DTI	Test	Estimate	SE	Partial *r*	*P* value
	RD (mm^2/s)	TMT-A	3,135.01	48,670.00	0.0075	
		TMT-B	5,916.46	68,358.00	0.0101	0.9846
		STROOP	−7,448.39	12,534.00	−0.0689	0.9846
		COWAT	−20,847.00	49,322.00	−0.0491	0.9846
		BRI	−1,004.26	51,702.00	−0.0023	0.9846

Association between TMT-A, TMT-B, COWAT, Stroop Test, and BRI with diffusion parameters of the anterior corpus callosum (CC; rostrum (CC1) and genu (CC2)). Estimate, standard error, partial *r*, and *P* values are listed for every item. Significant results are marked with bold letters. For CC1 there were only 74 subjects included since one data point was missing after clustering.
Test scores were partly incomplete. Number of individuals with missing test scores: TMT-A, n=2; TMT-B, n=2; COWAT, n=1.
TMT-A: Trail Making Test Part A; TMT-B: Trail Making Test Part B; COWAT: Controlled Oral Word Association Test; BRI: Behavioral Regulation Index from the Behavior Rating Inventory of Executive Function, Adult version (BRIEF-A); SE: standard error.

Fig. 4). AD in CC2 from one participant represents an outlier that seems to drive the regression line (Fig. 4, indicated by arrow in the right panel) and was therefore reevaluated for data quality. Of note, data quality of this particular case is sufficiently high and therefore we did not exclude this participant from the statistical analysis. Other subregional dMRI parameters are not significantly associated with plasma total tau (see Table 2 for absolute *P* values).

Association Between Neuropsychological Function and Diffusion Measures

In light of significant associations between CHII and plasma total tau levels with WM microstructure in the anterior regions of the CC (i.e., CC1 and CC2), dMRI parameters in these specific callosal subregions were examined for associations with neuropsychological test scores and a measure of neurobehavioral dysregulation (Table 3 and Fig. S1).

More specifically, CC1 microstructure was associated with better performance ($P < 0.05$) on TMT-A (trace, $r = 0.33$; AD, $r = 0.31$; and RD, $r = 0.28$) and better performance ($P < 0.05$) on TMT-B (trace, $r = 0.31$; RD, $r = 0.34$). CC2 microstructure was associated with better performance ($P < 0.05$) on TMT-A (FA, $r = 0.36$; AD, $r = 0.28$) and on TMT-B (FA, $r = 0.36$; AD, $r = 0.27$). Higher scores on COWAT were significantly ($P < 0.05$) and positively associated with FA ($r = 0.36$) and AD ($r = 0.32$). Moreover, the BRI was associated with higher AD ($r = 0.29$, $P < 0.05$) of CC2. There were no significant associations between Stroop Test scores with dMRI parameters for CC1 or CC2 (Table 3).

Discussion

This study revealed that greater estimated RHI exposure is associated with CC microstructure in former professional American football players. CC microstructure and plasma total tau are associated, which possibly reflects the direct effects of RHI (while playing football) on WM microstructure or the secondary effects caused by later-life neurodegeneration. Measures of CC microstructure were also associated with features of neuropsychological performance, including psychomotor speed, cognitive flexibility, and phonemic fluency, as well as neurobehavioral dysregulation.

Association Between RHI Exposure, WM Microstructure, and Neuropsychological Function

Greater exposure to RHI was associated with higher AD in the rostrum of the CC. The CC has previously been shown to be particularly susceptible to RHI.[3] Findings from this study confirm previous studies that observed that within the CC, the anterior parts seem to be most often affected by exposure to RHI.[5] The anterior parts of the CC are characterized by densely packed, thin axons[28] and may therefore be especially vulnerable to shear forces.[29]

Our results indicated that greater exposure to RHI may lead to long-term microstructural alterations in the anterior CC in former professional American football players. It has previously been hypothesized that higher AD may reflect persistence of axonal injury in patients with mild TBI.[30] Such axonal damage may be expressed by axonal swelling and edema. However, whether AD also captures neuroinflammation, neurodegeneration, or axonal injury needs further investigation.

The interpretation of diffusion metrics remains challenging because a given voxel contains various structures and types of cells that influence diffusion characteristics. Additionally, animal models that use both dMRI and histology to inform the interpretation of dMRI metrics are sparse.[3] In human neuroimaging, a decrease in FA has often been interpreted as damage to axon and myelin sheath leading to less directed diffusion. An increase

in trace or MD, on the other hand, is thought to reflect an increase in diffusion and one cause for such an increase could be neuroinflammation. A decrease in AD has been associated with axonal injury and dysfunction, whereas an increase in RD has been associated with myelin injury.[31]

Our results indicated that higher diffusion measures (FA, trace, RD, and AD) were also associated with better cognitive performance as assessed using the TMT-A, TMT-B, and COWAT, and with fewer symptoms of neurobehavioral dysregulation. While the positive association between FA and better cognitive function is in line with the literature,[3] in our study, higher AD also correlated with better performance. Importantly, the cross-sectional study design of this study limits the interpretation of this finding. Accordingly, future studies need to include a longitudinal study design to capture individual changes of cognitive and neuropsychiatric functioning associated with WM microstructure.

Association Between CC Microstructure and Plasma Total Tau

In this study, we found an association between higher plasma total tau and higher AD in the anterior regions of the CC. A previous study in a cohort of 17 male high-school football players (age range: 16–17 years) has shown that higher plasma total tau was associated with both higher MD and lower fiber density in the anterior part of the CC.[32] The authors hypothesized that RHI may disrupt axons and lead to axonal degeneration and, at the same time, may release tau from its microtubule bindings.

The pathophysiological processes underlying the association of later-life plasma total tau and WM microstructure are unclear. Higher levels of t-tau have been linked to lower levels of FA, and higher levels of MD, AD, and RD across the brain. This was the case for preclinical neurodegenerative disease stages[33] as well as in cohorts of cognitive impairment.[34] Moreover, a longitudinal study on patients with mild cognitive impairment (MCI) reported a significant decrease in FA and an increase in RD in multiple brain regions in a high-tau group of MCI patients compared with the controls.[35]

In regard of RHI, possible explanations include that exposure to RHI directly leads to injury of WM microstructure. As a result, there may be neuronal death and subsequently elevated plasma total tau. However, it is unlikely that increased plasma total tau levels due to direct effects of shear injury would persist years and even decades following RHI exposure as it did in our sample of former professional American football players in their 40s, 50s, and 60s. Another pathway may be that exposure to RHI sets in motion a progressive neurodegenerative disease that results in neuronal death and elevations in plasma total tau. Microglial activation has also been discussed as a potential underlying mechanism.[36] More specifically, activated microglia release cytokines, particularly proinflammatory cytokines and excitotoxins that are purported to play a role in the development of neurodegeneration.[36]

We suggest that the underlying pathophysiology of WM alterations and increased plasma total tau is a combination of both direct injury and secondary neurodegenerative and neuroinflammatory processes. Moreover, there may be additional pathomechanisms at play. For example, in other tauopathies (e.g., Alzheimer's disease), an increase in plasma total tau has been associated with cerebral hypoperfusion resulting from cerebrovascular disease.[37] Hypoperfusion is also thought to increase perfusion of small molecules such as tau through the axonal membrane.[37] Thus, tau may accumulate in extravasal space and likely also in plasma given its ability to cross the BBB.[11] Interestingly, cerebrovascular dysfunction, including hypoperfusion, has also been described in the context of RHI exposure.[38] In Alzheimer's disease, chronic neuroinflammation and BBB dysregulation have been associated with increased plasma total tau.[39] Of further note, RHI exposure has been related to BBB dysregulation occurring predominantly in regions with high density of perivascular phosphorylated tau depositions.[40] Taken together, we hypothesize that the association between WM microstructure of the CC and plasma total tau may be due to a combination of pathomechanisms including direct injury, chronic inflammatory, and neurodegenerative processes, as well as cerebrovascular dysfunction.

Limitations

First, this is a cross-sectional study, which limits the interpretation of our results and, thus, a causal relationship cannot be determined. More specifically, the effects of acute or chronic injury cannot be differentiated from progressive neurodegeneration. Future studies using a longitudinal design are needed to further our understanding of changes in brain structure associated with exposure to RHI. Second, the results from this study cannot be generalized beyond the specific sample investigated, i.e., former professional American football players who played in the NFL during the 1970s–1990s. Third, the lack of a comparison group with asymptomatic former professional football players reflects a limitation. Fourth, our estimation of exposure to RHI approximates the real head impacts of the players. Fifth, interpretation of diffusion metrics remains challenging because a given voxel contains various structures and types of cells that influence diffusion characteristics. Moreover, to date, there are no normative values of diffusion measures in the CC. Despite these important limitations, our study contributes to understanding better the relationship between RHI exposure from American football, WM alterations, plasma total tau, and clinical measures of executive functioning and neurobehavioral dysregulation.

Conclusion

This study identified an association between RHI exposure and WM microstructure as well as an association between WM

microstructure, plasma total tau, and measures of executive functioning and neurobehavioral dysregulation in former professional American football players. WM alterations potentially reflect a combination of pathomechanisms including shear injury, chronic neuroinflammatory, and neurodegenerative processes, as well as cerebral hypoperfusion following exposure to RHI. Longitudinal studies are, however, needed to determine causal relationships between exposure to RHI and alterations in WM microstructure.

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