Differences in white matter structure and cortical thickness between patients with traumatic and idiopathic chronic neck pain : associations with cognition and pain modulation?

Differences in white matter structure and cortical thickness between patients with traumatic and idiopathic chronic neck pain: associations with cognition and pain modulation?

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DISCLOSURES

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LIST OF ABBREVIATIONS

CWAD= chronic whiplash associated disorders, CINP= chronic idiopathic neck pain, TBI= traumatic brain injury, CS= central sensitization, CPM= conditioned pain modulation, NRS= numeric rating scale, TMT= trail making test, PPT= pressure pain thresholds, GM= grey matter, WM= white matter, FA= fractional anisotropy, MD= mean diffusivity, AD= axial diffusivity, RD= radial diffusivity, MRI= magnetic resonance imaging, DWI= Diffusion-weighted imaging, DTI= Diffusion Tensor Imaging.

ABSTRACT

Brain alterations are hypothesized to be present in patients with chronic whiplash-associated disorders (CWAD). The aim of this case-control study was to examine alterations in cortical thickness and white matter (WM) structure, and the presence of brain microhemorrhages in a patient group encountering chronic neck pain of traumatic origin (i.e. CWAD) compared to a patient group characterized by non-traumatic chronic neck pain (i.e. chronic idiopathic neck pain (CINP)), and healthy controls. Furthermore, we aimed to investigate associations between brain structure on one hand and cognitive performance and central sensitization (CS) on the other hand. T1-weighted, diffusion-weighted and T2*-weighted Magnetic Resonance images of the brain were acquired in 105 women (31 controls, 37 CINP, 37 CWAD) to investigate regional cortical thickness, WM structure, and microhemorrhages, respectively. Next, cognitive performance, and CS encompassing distant hyperalgesia and conditioned pain modulation (CPM) efficacy were examined. Cortical thinning in the left precuneus was revealed in CWAD compared to CINP patients. Also, decreased fractional anisotropy, together with increased values of mean diffusivity and radial diffusivity could be observed in the left cingulum hippocampus and tapetum in CWAD compared to CINP, and in the left tapetum in CWAD patients compared to controls. Moreover, the extent of WM structural deficits in the left tapetum coincided with decreased CPM efficacy in the CWAD group. This yields evidence for associations between decreased endogenous pain inhibition, and the degree of regional WM deficits in CWAD. Our results emphasize the role of structural brain alterations in women with CWAD compared to CINP.

Keywords: chronic whiplash-associated disorders, chronic idiopathic neck pain, magnetic resonance imaging, white matter, cortical thickness, central sensitization, cognitive performance

INTRODUCTION

Extensive Magnetic Resonance Imaging (MRI) research has uncovered alterations in grey matter (GM) volume, cortical thickness, and white matter (WM) structure in regions and tracts involved in processing of pain and cognition in various chronic musculoskeletal pain conditions such as fibromyalgia [15, 58, 69, 73] and chronic low back pain [13, 63, 77]. In addition, these brain alterations have been reported in mild traumatic brain injury (TBI) patients [44, 57, 62, 116]. Also, in mild TBI patients evidence is available for brain microhemorrhages related to the trauma or diffuse axonal injury [60]. Yet, despite this huge body of work, there have been few attempts to investigate these findings in more detail and to examine alterations in both GM and WM, as well as associations with clinical features in patients with chronic musculoskeletal pain.

Remarkably, structural brain MRI research is limited in two prevalent musculoskeletal pain conditions namely chronic whiplash-associated disorders (CWAD), involving trauma-induced persistent neck pain [30], and chronic idiopathic neck pain (CINP), involving persistent neck pain of non-traumatic origin. However, due to the trauma inducing possible shearing forces through acceleration-deceleration of the brain [2], it could be hypothesized that subtle structural brain alterations in GM morphology and WM structure are present in CWAD, but not or to a lesser degree in CINP patients. To investigate this hypothesis and to unravel the underlying mechanisms of a wide range of possible structural brain alterations, it seemed valuable to compare patients with CINP and CWAD, who are a priori different from each other in the origin of pain. Furthermore, this research could explore pathophysiological differences between both chronic neck pain groups which is necessary to improve their treatment outcome.

Intriguingly, many chronic musculoskeletal pain disorders are characterized by central sensitization (CS) as overlapping pathophysiological mechanism, and often experience cognitive problems [82, 123]. In addition, similar to chronic pain patients, mild TBI patients often report cognitive complaints [8] and chronic pain [85]. These findings indicate that chronic pain is associated with structural brain plasticity, and suggest that cognitive deficits and CS are related to this plasticity [3, 31]. Also, it has been shown that GM and WM neuroplasticity is associated with the traumatic factor in mild TBI [44, 57, 62, 116].

Compelling evidence has shown CS in patients with CWAD [111]. In contrast, CS is not a characteristic feature in CINP patients [76, 96], and cognitive deficits are observed to a lesser extent compared to CWAD [23]. A recent systematic review concerning brain alterations in WAD and INP, only found 3 studies investigating structural brain alterations exclusively in WAD [32], and one study reported altered GM volume in CWAD. However, alterations in cortical thickness and WM structure, and their association with cognitive deficits and CS, have never been investigated in patients with CWAD compared to CINP, which is a prominent research gap.

In order to disentangle subtle structural brain alterations in patients with CWAD compared to non-traumatic CINP, innovative advanced MRI, in particular, Diffusion-Weighted Imaging (DWI) acquisition techniques, and Diffusion Tensor Imaging (DTI) analyses offer opportunities to investigate the brain's WM tissue at a (micro)structural level [6, 7, 9, 52, 64, 106]. Hence, these techniques will probably be more sensitive for detecting subtle structural brain changes in CWAD [102]. In particular, DWI examines WM structural organization by quantifying the directionality and degree of diffusion of water within tissues [6, 7, 9, 52, 64, 106]. In addition, T2*-weighted MRI can assess the presence or absence of brain microhemorrhages related to the trauma in CWAD. Furthermore, MRI techniques investigating cortical thickness could be valuable to extract additional information of the brain's GM morphology. It is suggested that cortical thickness and GM volume reflect different aspects of the neural architecture [90]. Examining cortical thickness could therefore provide additional insights into the macrostructural neural underpinnings of CWAD and CINP.

The first study objective was to investigate alterations in cortical thickness in carefully selected brain regions based on hypothesis-driven predictions regarding the localization of the expected structural brain alterations in specific regions involved in processing of cognition and/or pain in patients with CWAD compared to CINP and healthy controls. The second objective was to examine deficits in WM structure, including alterations in fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) in hypothesis-driven predefined WM regions/tracts carrying information between regions involved in processing of cognition and/or pain in patients with CWAD compared to CINP, and healthy controls. The third objective was to examine the presence or absence of microhemorrhages related to trauma in CWAD patients compared to the other study groups. The final objective was to explore in each group separately whether alterations in regional cortical thickness and WM structure were associated with cognitive performance and CS.

Based on a-priori cortical thickness and WM anatomical hypotheses, cortical thinning, and deficits in WM structure of predefined regions/tracts were hypothesized in CWAD due to the trauma, and because of cognitive deficits [25] and CS [111], compared to CINP patients and healthy controls. Also, previous MRI studies revealed associations between altered cortical thickness and WM structure, and measures of cognition and pain in various chronic pain conditions [26, 72]. Therefore, it is hypothesized that alterations in cortical thickness and WM structure in CWAD are associated with worse cognitive performance and signs of CS in CWAD compared to CINP patients.

To guide the selection of cortical regions of interest (ROIs) from the Desikan parcellation scheme [33] and the selection of WM tracts/ROIs from the Mori atlas [81] (see Fig. 1) evidence was retrieved from our systematic review appraising the evidence for brain alterations in INP and WAD [32], based on previous studies in patients with chronic pain or mild TBI examining GM morphological alterations [15, 17, 24, 44, 63, 116], demonstrating WM structural alterations, and regarding studies exploring associations between cortical thickness or WM structure, and measures of cognition and pain [4, 10, 19, 26, 34, 37, 58, 59, 62,

[69, 70, 74, 81, 88, 94, 95, 104, 108]. In addition, only regions demonstrated to be mainly involved in processing pain or cognition or the combination thereof could be selected.

METHODS

Participants

One hundred and five female participants - 37 patients with CINP, 37 patients with CWAD, and 31 healthy pain-free controls - were enrolled. A subject sample presenting overlap with the current study sample was used in our recent study with regard to GM volume alterations **[22]**, and concerning clinical differences between women with CINP and CWAD [23]. In order to exclude the confounding factor of gender on brain structure and pain modulation, only women were included, as research has demonstrated differences between men and women regarding brain structure, pain sensitivity and processing in healthy persons and pain patients [14, 91, 110]. All participants were Dutch native speakers between 18 to 65 years old. Participants were recruited by calls on social media and through advertisements placed in health magazines and a patient information brochure, on the Ghent University website, and via local radio. Furthermore, informative flyers and posters were distributed in different medical institutes and associations in Flanders, Belgium (various hospitals, medical physician practices, and physical therapist practices). As general inclusion criteria, approximately 90 percent of all participants were right-handed and 10 percent left-handed. This is a representative sample, because approximately 10 percent of the general population is ambidextrous or left-handed [87].

Inclusion criteria for patients with CINP and CWAD were persistent neck pain lasting more than 3 months [12], with a mean pain intensity of more than 3 of 10 on a Numeric Rating Scale (NRS) during the preceding month. All chronic neck pain patients had to report mild/moderate to severe pain-related disability, established by a score of 10 or more of a maximum of 50 on the Neck Disability Index [114]. Additionally, patients with chronic neck pain had to report stability of pain medication intake for at least 4 weeks before study participation.

A specific inclusion criterion for CINP patients was persistent idiopathic neck pain. Patients with CINP were excluded if they ever experienced whiplash trauma, or any other specific cause of neck pain, e.g. cervical hernia with clinical symptoms. Patients with CWAD were included only if they had neck pain resulting from a motor vehicle crash or traumatic event and classifiable as WAD II A, B, or C on the modified Quebec Task Force Scale [101]. Patients with CWAD grades I, III (neurological signs), or IV (fracture or dislocation) on the modified Quebec Task Force Scale were excluded. Additionally, CWAD

patients who lost consciousness as a result of the traumatic event, and who had suffered distinct posttraumatic amnesia were excluded.

Healthy women could participate only if they were pain-free on each test day (NRS score of <2/10), had no history of neck-shoulder-arm pain for longer than 8 consecutive days during the preceding year, with a pain intensity of 2 or more of 10 on the NRS, no medical consultation for neck-shoulder-arm pain during the preceding year, and no history of whiplash trauma. Additionally, healthy controls were included only if they had a score of less than 8 of 50 on the Neck Disability Index.

General exclusion criteria for all study groups were the presence of major depression or psychiatric illness; neurologic, or cardiovascular disorders; inflammatory disorders; fibromyalgia; chronic fatigue syndrome; and a history of neck or shoulder girdle surgery. All participants completed the MRI safety checklist and participants who presented contra-indications for MRI were excluded. To preclude confounding factors, all participants were asked to discontinue intake of non-opioid analgesics 48 hours before study participation. In addition, participants were asked to avoid heavy physical exertion and to refrain from consuming alcohol, caffeine, and nicotine on each testing day.

Study design and procedure

This cross-sectional case-control study took place at Ghent University in cooperation with the Ghent Institute for Functional and Metabolic Imaging. The study was performed from February 2014 to September 2015 and was carried out in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of the Ghent University Hospital (EC/2013/1053) approved the research protocol. All participants were informed about the study procedures and signed an informed consent form prior to study enrolment.

First, all participants completed a survey to acquire information on demographics, and completed a series of questionnaires to obtain information on pain-related disability, pain intensity and frequency (as described below). Subsequently, experimental assessments to investigate cognitive performance and CS were performed. On a separate test day (10 +/- 7 days apart), T1-weighted MR images, diffusion-weighted MR images, and T2*-weighted gradient echo MR images of the brain were acquired.

Self-reported pain and disability measures

On each test day, participants scored current neck pain intensity on a NRS. Scores ranged from 0 to 10, with 0 reflecting ‘no pain’ and 10 reflecting ‘the worst pain imaginable’. Patients reported their neck pain frequency in number of days per week. The Dutch Neck Disability Index was used to investigate self-reported pain-related disability levels (0-50) [114]. Higher scores on the Neck Disability Index indicate higher levels of pain-related disability. The Dutch language version of the Neck Disability Index has been proven to be valid and reliable to assess self-reported disability in patients with chronic neck pain [53, 54].

Cognitive performance

In order to obtain an objective measure of cognitive performance the Trail Making Test (TMT) was administered. This test consists of two parts, trail A and trail B. TMT part A requires mainly visuo-perceptual [93] and processing speed [105] abilities, whereas TMT part B reflects primarily working memory and secondarily task-switching ability [93]. In trail A, the participant had to draw lines connecting 25 numbers in ascending order as fast as possible, without lifting the pencil from the paper. In trail B, the participant was instructed to draw lines alternating between numbers and letters in ascending order (going from 1 to A, from A to 2, etc.). The goal of the TMT was to finish part A and part B as accurate and as quickly as possible. The time taken to complete each part of the test was used as outcome measure. Both subtests necessitate graphomotor speed, visual scanning ability, sufficient attention, and numeric sequencing [80, 93, 105]. The TMT part B further requires letter sequencing, mental double tracking, and alternation (i.e. shifting between number and letter series) [80]. The TMT has been proven to be valid for assessing cognitive deficits [93], and is a quickly administered, widely used neuropsychological test that provides information on a broad range of cognitive skills [11].

Measures of central sensitization

Distant hyperalgesia

Pressure pain thresholds (PPTs) were measured unilaterally with a digital manual algometer (Wagner Instruments, FDX, Greenwich, Connecticut) at a distant asymptomatic region (quadriceps muscle midway between the anterior superior iliac spine and the basis patellae) to evaluate secondary or distant hyperalgesia [118]. The PPTs were assessed at the most painful side [61] and in a randomized order (with research randomizer, https://www.randomizer.org). If patients experienced the same amount of neck pain at both sides, and in healthy women, PPTs were tested at the dominant handedness side. The participant was seated and pressure was gradually increased at a rate of 1 kgf/s until the participant reported the first sensation of unpleasantness. The PPT was determined as the mean of 2 consecutive (30 seconds in between) measurements. Decreased PPTs in the patient groups compared to controls at the quadriceps muscle indicate distant hyperalgesia, and are suggestive for the presence of CS. The described PPT technique has been demonstrated to be reliable [16].

Efficacy of Conditioned Pain Modulation (CPM)

Endogenous pain inhibition was investigated by applying a CPM paradigm. This paradigm relies on the “pain-inhibits-pain” mechanism in which one noxious stimulus is used as a conditioning stimulus to induce a reduction in pain perception from another test stimulus [122]. The conditioning stimulus for eliciting CPM was the cold pressor test. The PPT assessment was used as test stimulus. For the

conditioning stimulus, the contralateral hand (of the PPT side) was first immersed in water maintained at room temperature (22°C) for 1 minute to standardize hand temperature [86], before immersing this hand (up to the wrist) in a refrigerated circulating bath (Versacool, Thermo Fisher Scientific, Waltham, Massachusetts) with cold water maintained at 12±1°C [107]. Participants were asked to keep their hand in the water bath for 2 minutes [86]. Meanwhile, the PPT was re-evaluated at the quadriceps muscle, approximately 45 seconds after immersing the hand (again with an interval of 30 seconds) [68]. If the participant removed the hand from the water before the end of the 2 minutes, the measurement was registered as missing. For analysis of CPM efficacy, the mean PPT measured before the cold pressor test was subtracted from the mean PPT measured during the cold pressor test. Hence, a lower CPM value reflected less efficient endogenous pain inhibition. The cold pressor test with cold water of 12°C and immersion of the hand for 2 minutes is sufficient to induce an endogenous pain inhibitory response [56].

MRI data acquisition

MR images were acquired on a 3T Siemens Magnetom TrioTim MRI scanner (Siemens, Erlangen, Germany) equipped with a 32-channel matrix head coil, at the Ghent University Hospital. First, high-resolution T1-weighted images of the brain were acquired using a three-dimensional magnetization prepared rapid acquisition gradient echo (MP-RAGE) (repetition time [TR] = 2250 ms, echo time [TE]= 4.18 ms, voxel size= 1 x 1 x 1 mm^3, field of view (FoV)= 256 x 256 mm, flip angle= 9°, 176 slices, 1 mm slice thickness, acquisition time = 5'14''). All T1-weighted anatomical scans were visually checked for overall quality and motion artefacts.

Second, DW images of the brain were acquired using single-shot echo planar imaging with a twice-refocused spin echo sequence with FoV = 240 x 240 mm, TR = 10800 ms, TE = 83 ms, and 60 contiguous sagittal slices (slice thickness = 2.5 mm; voxel size = 2.5 x 2.5 x 2.5 mm^3), and an acquisition time of 12' 36". Diffusion sensitizing gradients were applied at a b-value of 1200 s/mm^2, along 64 noncollinear directions. Additionally, 1 set of images with no diffusion weighting (b-value= 0 s/mm^2) was acquired as a reference image.

Finally, axial T2*-weighted brain images were acquired using a T2*-weighted acquisition gradient echo with TR= 839 ms, TE= 18.60 ms, voxel size= 1 x 0.7 x 3 mm^3, FoV= 230 x 230 mm, flip angle= 20°, 3 mm slice thickness, and an acquisition time of 3' 48". All T2*-weighted images were visually inspected by 2 expert neuroradiologists (KD, EG) to evaluate possible microhemorrhages or hemorrhagic shearing lesions related to trauma or diffuse axonal injury.

T1-weighted MRI data processing

T1-weighted anatomical scans were analyzed utilizing the FreeSurfer v5.3.0 software package (http://surfer.nmr.mgh.harvard.edu). The analyses were performed using additional computing resources from the high performance computing TIER1 cluster at the University of Ghent

(http://www.ugent.be/hpc/). The FreeSurfer analysis suite was used to extract cortical thickness using an automated approach described in detail in prior publications (see Fischl 2012 [38]). Previous research has shown that this automated procedure yields accurate and reliable results [41]. Briefly, image processing included (1) removal of non-brain tissue using a hybrid watershed/surface deformation procedure (skull stripping) [97], (2) automated Talairach transformations, (3) segmentation of the subcortical WM and deep GM volumetric structures [41], (4) intensity normalization [99], (5) tessellation of the boundary between GM and WM, automated topology correction [40, 98] and (6) surface deformation along intensity gradients for optimal placement of the borders between GM, WM and cerebrospinal fluid [29, 39]. Automated parcellation of the cerebral cortex into units with respect to gyral and sulcul structures, and calculation of cortical thickness from all vertices within the cortical parcellations was performed per hemisphere using the Desikan atlas [33]. Also, estimates of total intracranial volume, and mean total thickness of the left and right hemisphere were obtained for each subject.

The T1.mgz (i.e. the FreeSurfer T1 image) and aparc+aseg.mgz (i.e. image containing ROIs constructed by the FreeSurfer pipeline) files were converted to the Neuroimaging Informatics Technology Initiative (NIfTI) format (T1.nii and aparc+aseg.nii) to be used in the further DWI analyses.

Two independent researchers (IC, RDP) visually checked the data quality of the FreeSurfer processing output including the accuracy of skull stripping, registration, segmentation, and cortical surface reconstruction. Poor data quality, such as inclusion of dura in the pial surface after skull stripping, and surface deformations, was revealed in 12 participants (controls =3, CINP =3, CWAD =6). These cortical thickness datasets were excluded from further analyses.

Cortical regions of interest

Cortical thickness was extracted from 9 ROIs from the Desikan atlas [33]. The selection of these ROIs was based on our systematic review appraising the evidence for brain alterations in INP and WAD [32], on previous studies in patients with chronic pain or mild TBI examining GM morphology alterations [15, 17, 24, 44, 63, 116], and regarding studies exploring associations between cortical thickness, and measures of cognition and pain [26, 37, 88, 95]. In addition, only regions demonstrated to be mainly involved in processing pain and/or cognition were selected. Specifically, the following 9 ROIs were selected from the Desikan atlas [33]: caudal anterior cingulate cortex, posterior cingulate cortex, lateral orbitofrontal cortex, superior parietal cortex, postcentral cortex, precuneus, pars orbitalis of the inferior frontal gyrus, parahippocampal cortex and supramarginal cortex (see fig. 2 for cortical ROIs). For each ROI, cortical thickness was calculated for the right and left hemisphere separately.

DWI processing

The DWI data were analyzed and processed in ExploreDTI v4.8.6 with MATLAB [66] using the following procedure: DWI volumes were looped at a high frame rate to check for obvious artefacts in

the data, such as large signal dropouts and geometric distortions. Next, we toggled between the views of the first and last acquired DW image to observe subtle system drifts. Furthermore, we inspected the images in different orthogonal views (coronal, sagittal, axial) to check for interslice and intravolume instabilities, and visualized various image maps to check for artefacts. Then, we checked the residual map for outliers, reflecting the difference between the modelled and the measured signal [106]. Next, the DW data were corrected for distortions induced by the DW gradients, artefacts due to head motion, eddy current–induced geometric distortions, and EPI distortions [67]. EPI distortions were corrected by co-registering the DW images to the T1-weighted anatomical images, which were normalized for intensity using FreeSurfer. Moreover, we performed appropriate reorientation of the encoding vectors. Next, a tensor estimation procedure was performed on the preprocessed DW data.

Each scan was visually checked for accuracy after both the motion correction and co-registration steps by 2 independent researchers. To check the accuracy of the co-registration, the preprocessed DWI was overlaid on the normalized T1-weighted anatomical image. Poor data quality was observed in 1 healthy subject because of too much head translation due to severe head motion (exceeding the size of 1 voxel), and 2 patients with CINP, 1 due to a too high percentage of outliers in the preprocessed DW images and 1 because of a general low data quality profile (ghosting, spikes). These WM **structural** data were excluded from further analyses.

Translational motions (average of axial, coronal and sagittal) did not exceed the size of 1 voxel, i.e. 2.5 x 2.5 x 2.5 mm^3 (mean +/- SE: controls: 0.85 +/- 0.03 mm; CINP patients: 0.79 +/- 0.03 mm; 0.87 +/- 0.03 mm for CWAD patients). We re-inspected the data in 3 orthogonal planes to ensure that the motion/distortion correction was performed correctly and that no additional artefacts were introduced into the data. Finally, DWI derived metrics FA, MD, AD, and RD were extracted from the preprocessed DWI data using an automated approach based on the ICBM DTI-81 WM atlas, which will be further explained in the following paragraph [81].

White matter regions of interest

The ICBM-DTI-81 WM labels atlas, developed by Mori et al. [81], was used for automated parcellation of the ROIs. This is a stereotaxic probabilistic WM atlas that fuses DTI-based WM information with the standard MNI anatomical template (ICBM-152). The WM parcellations were applied to the preprocessed images and the DWI derived metrics were calculated in each ROI, by warping the atlas template to each individual data set.

The selection of WM ROIs was based on previous MRI research demonstrating WM structural alterations in chronic pain or mild TBI patients, or revealing associations between WM structure, and measures of cognition and pain. Based on our anatomical hypotheses, only WM atlas labels that are mainly involved in pain or cognition or the combination thereof were selected. Hereby, the following 10 WM regions/tracts were defined: projection fibers namely (1) the superior cerebellar peduncle [20, 36] (2) the anterior corona radiata, (3) the posterior corona radiate [4, 34, 59, 62, 70, 74], (4) the anterior limb

of internal capsule, (5) the posterior limb of internal capsule [69]. Association fibers, namely (6) the cingulum cingulate gyrus (i.e. subgenual and retrosplenial part of the cingulum) and (7) the cingulum hippocampus (i.e. parahippocampal part of the cingulum) [69, 81] and (8) fornix and stria terminalis [10, 19, 108]. Finally, commissural fibers i.e. (9) the tapetum of the corpus callosum [58, 78] and (10) the splenium of the corpus callosum [69]. Acceleration-deceleration of the brain is believed to affect the superior cerebellar peduncles, and periventricular WM [78], e.g. tapetum of the corpus callosum [58, 94, 104]. For visualization purposes, masks of these ROIs are displayed on MD maps of the WM Mori atlas [81] in fig. 3. To examine WM structural organization, average FA, MD, AD and RD values were computed in all WM ROI for each subject for the right and left hemisphere separately.

Statistical analyses

All statistical analyses were performed with IBM SPSS Statistics 22.0. First, normality of variables was assessed with the Shapiro-Wilk test and by visual evaluation of histograms and quantile-quantile plots. Additionally, the equality of variance was examined with the Levene's test. If the assumptions of normality and equal between-group variances were met, data were analyzed with parametric tests. Otherwise, non-parametric tests were applied.

The comparability of study groups for demographics was explored with a one-way ANOVA with post-hoc pairwise comparisons using Bonferroni correction (*Family Wise Error Rate (FWER)* < 0.05), or with the Kruskal-Wallis test with post-hoc pairwise comparisons using the Mann-Whitney U test. Differences measured with the Mann-Whitney U test were only assumed significant below the significance threshold of 0.017 (Bonferroni correction: 0.05/3) to correct for the number of groups. Categorical data were analyzed with the Fisher's exact test. Group differences for cognitive performance and CS were explored with one-way ANOVA (post-hoc pairwise comparisons using Bonferroni correction, FWER < 0.05) or the Kruskal-Wallis test (post-hoc pairwise comparisons using the Mann-Whitney U test, $p < 0.017$).

An ANCOVA model was fitted, controlling for age, to examine differences in total intracranial volume and total mean thickness of the left and right hemisphere (post-hoc pairwise comparisons using Bonferroni correction, FWER < 0.05).

A MANCOVA model controlling for age was performed to determine group differences in cortical thickness of the 9 ROIs if the assumptions for performing MANCOVA were verified (multivariate normality in the data, homogeneity of variance between groups, absence of multicollinearity). Four MANCOVA models including age as covariate were performed investigating WM **structure** with respectively FA, MD, AD and RD of 10 WM ROIs in separate models each, as dependent variables, in patients with CWAD, CINP, and controls, as independent variables. The significance threshold was Bonferroni corrected for the number of DWI derived metrics, resulting in an adjusted *p*-value of < 0.0125 (0.05/4) for the multivariate test and for the individual WM tracts. Next, post-hoc pairwise comparison using Bonferroni correction was applied for the group comparisons. Because there were

significant effects of age on various diffusion metrics in WM regions, age was included as covariate in the MANCOVA model. For each MANCOVA model, the partial eta square (η^2) was calculated. This measure shows how much variance is explained by the independent variable (study group) and is used as the effect size for the MANCOVA model.

Finally, group-specific partial correlations (controlling for age) were conducted between cognitive performance and CS measures on one hand, and cortical thickness or WM **structure** on the other hand in cortical regions or WM tracts displaying significant group differences. To correct for multiple comparisons (for the number of clinical variables), partial correlations were deemed significant only below the 0.0125 (0.05/4) level.

RESULTS

Demographic characteristics and self-reported pain and disability measures

The demographic characteristics and self-reported pain and disability measures of 102 participants (30 healthy controls, 35 CINP, 37 CWAD) are presented in table 1. No significant differences in demographic characteristics were found between all study groups ($p > 0.05$), except for age (healthy controls were younger compared to CINP; $p = 0.010$). Furthermore, both patient groups were comparable in use of medications, neck pain duration, and frequency of neck pain complaints ($p > 0.05$). In contrast, higher neck pain intensity at the MRI test day ($p < 0.001$), and higher pain-related disability were reported by patients with CWAD compared to CINP ($p = 0.001$).

Control analyses

The MANCOVA with age and handedness as covariates, revealed no significant main effect of handedness on regional cortical thickness ($p = 0.284$) or WM **structure** ($p = 0.349$ for FA; $p = 0.215$ for MD; $p = 0.170$ for AD; $p = 0.094$ for RD). Therefore, the cortical thickness and WM **structure** results of the left- and right-handed women were analyzed together. The ANCOVA controlling for age showed no significant group differences for total intracranial volume ($p = 0.137$), and total mean thickness of the left ($p = 0.563$) and right ($p = 0.404$) hemisphere.

The assumptions for all applied models were verified.

Group differences in cognitive performance and central sensitization

As shown in **table 1**, the completion time of TMT part A ($p < 0.001$) and part B ($p = 0.002$) was significantly longer in the CWAD group compared to healthy controls, denoting worse performance in CWAD patients. In addition, the time needed to perform TMT part A ($p < 0.001$) was significantly longer in patients with CWAD compared to CINP.

Decreased PPTs were demonstrated at the quadriceps muscle, reflecting distant hyperalgesia in patients with CWAD compared to healthy controls (p = 0.002). The CPM value, measured at the quadriceps muscle, was significantly lower in CWAD patients compared to controls (p = 0.005), indicating diminished endogenous pain inhibition in patients with CWAD (**table 1**).

Group differences in cortical thickness

The MANCOVA model examining cortical thickness in 9 ROIs between individuals with CWAD, CINP, and healthy controls, including age as covariate, demonstrated significant differences for cortical thickness (p = 0.015, η^2 = 0.312), based on the multivariate test (supplementary table A). Only the left precuneus showed significant group differences in cortical thickness (p = 0.037). Post-hoc pairwise comparisons between study groups using Bonferroni correction revealed significant decreased cortical thickness in the left precuneus (p = 0.032) (fig. 4, supplementary table A) in patients with CWAD compared to CINP. Hence, no significant differences in regional cortical thickness could be found between either the CWAD or CINP group and healthy controls.

Group differences in white matter structure

As can be seen in **supplementary table B**, four MANCOVA models with age as covariate investigating WM **structure** with respectively FA, MD, AD, and RD of 10 WM tracts in separate models in patients with CWAD, CINP, and controls, showed significant differences for FA (p = 0.010, η^2 = 0.293), MD (p = 0.007, η^2 = 0.274), and RD (p = 0.007, η^2 = 0.297) based on the multivariate tests. The MANCOVA of AD could not detect significant differences between study groups (p = 0.499), hence regional WM differences in AD were not further analysed.

The following WM tracts did retain significance after correcting for multiple comparisons (p < 0.0125 (0.05/4)): left cingulum hippocampus (p = 0.002 for FA, p = 0.004 for MD, p = 0.002 for RD) and left tapetum (p = 0.003 for FA, p = 0.005 for MD, p = 0.004 for RD) (**supplementary table B**). Subsequent, post-hoc pairwise comparisons between study groups, using Bonferroni correction, revealed significantly decreased FA (p = 0.007 and p = 0.013), increased MD (p = 0.010 and p = 0.025), and increased RD (p = 0.009 and p = 0.020) in the left tapetum in CWAD patients compared to controls and CINP patients respectively (**fig. 5, supplementary table B**). Also, significantly decreased FA (p = 0.002), increased MD (p = 0.004), and increased RD (p = 0.001) in the left cingulum hippocampus were found in CWAD patients compared to CINP patients (**fig. 5, supplementary table B**).

Results of T2*-weighted brain imaging analyses

Based on detailed visual inspection of the axial T2*-weighted brain images, no hemorrhagic shearing lesions or microhemorrhages related to trauma or diffuse axonal injury were detected. In one CWAD patient a small a-specific T2* hypointensity left parietal without clinical relevance was observed. Furthermore, in one CINP patient the neuroradiologists detected a small round T2*

hypointensity in the left thalamus and left globus pallidus suggesting microhemorrhages related to hypertension.

Associations between cortical thickness alterations, and cognitive performance and central sensitization

Associations (partial correlations corrected for age) between cognitive performance and CS, and cortical thickness were investigated only in ROIs showing significant group differences (**table 2**). The time to complete TMT part B was negatively correlated with cortical thickness of the left precuneus (r = - 0.520, p = 0.005) within the CINP group (n = 27). Specifically, worse performance on the TMT part B coincided with decreased cortical thickness in the left precuneus in patients with CINP, however worse TMT performance could not be revealed in CINP patients compared to healthy controls. In the CWAD (n = 26), and control group (n = 26), no significant correlations could be found (p > 0.017).

Associations between white matter structural alterations, and cognitive performance and central sensitization

As presented in **table 3**, associations (partial correlations corrected for age) between WM **structure** (only FA, MD, RD), and cognitive performance and CS measures were investigated only in WM tracts demonstrating significant group differences. In the CWAD group (n = 34), efficacy of CPM was negatively correlated with MD (r = - 0.478, p = 0.010) and RD (r = - 0.477, p = 0.010) in the left tapetum. In other words, decreased efficacy of CPM was moderately correlated with increased MD and RD in the left tapetum in CWAD patients. In the CINP (n = 33) and control group (n = 28), no significant correlations could be revealed (p-value > 0.0125 (0.05/4)).

DISCUSSION

The present study has demonstrated cortical thinning in the left precuneus, a region crucially involved in cognitive functioning, in patients with CWAD compared to CINP. Furthermore, abnormalities in WM **structure** encompassing decreased FA coinciding with increased MD and RD in the left cingulum hippocampus were revealed in patients with CWAD compared to CINP, and in the left tapetum in patients with CWAD compared to CINP and healthy controls. Interestingly, brain microhemorrhages related to trauma were not observed. This is the first study revealing alterations in GM macrostructure and WM **structure** in women with CWAD compared to CINP or healthy women, indicating a potential association between these brain alterations and the signs and symptoms induced

by the traumatic event in patients with CWAD. Moreover, decreased CPM efficacy (denoting CS) was associated with the extent of WM deficits in the left tapetum in CWAD patients. The latter yields novel evidence for underlying **structural** neural correlates of disturbed endogenous pain inhibition.

Group differences in cognitive performance and central sensitization

CWAD patients displayed worse processing speed abilities compared to CINP patients and controls based on the results of the TMT part A. Decreased working memory span was revealed in the CWAD group compared to controls, utilizing the TMT part B. Consistent with these findings, 2 recent studies revealed worse cognitive performance in CWAD patients compared to healthy volunteers, specifically on domains of sustained attention and working memory evaluated with computer-based cognitive tasks [25, 79]. Hence, our study highlights the importance of evaluating cognitive performance in patients with CWAD in clinical practice.

Furthermore, CWAD patients showed decreased PPTs at the quadriceps muscle compared to healthy controls reflecting distant hyperalgesia. This indicates significant indirect signs of CS and thus enhanced central pain sensitivity in CWAD. In accordance with our expectations, distant hyperalgesia was not demonstrated in CINP patients. Likewise, Scott and colleagues reported decreased PPTs at distant regions in CWAD but not in CINP patients [96]. Furthermore, decreased CPM efficacy was only observed in the CWAD group, implying disturbed endogenous pain inhibitory mechanisms in CWAD but not in CINP patients. Consistent with these findings, reduced endogenous pain inhibition has previously been reported in CWAD [28, 112] but could not be found in patients with CINP [21]. The lack of signs for CS in CINP patients is furthermore in line with the results of a recent systematic review concluding that CS is not a characteristic feature of CINP [76]. Our results add evidence to support that CS is present, at a group level, in CWAD and usually not in CINP patients, which further elucidates difference in underlying mechanisms.

Group differences in cortical thickness

The cortical thinning in the left precuneus in CWAD supports our hypothesis of decreased regional cortical thickness in patients with traumatic compared to non-traumatic chronic neck pain. The observed cortical thinning in the precuneus in CWAD compared to CINP patients has a valuable meaning because it demonstrates macrostructural differences between both patient groups. Moreover, this region plays a crucial role in a wide range of cognitive and mental processes, and is involved in neurodegenerative processes [18, 124]. The precuneus is part of the structural core of the brain [45], and is a core hub (i.e. highly interconnected nodes) of the default mode network [42]. The latter network is a constellation of brain regions involved in self-referential mental activity, emotional processing, and recollection of prior experiences, and is deactivated during externally focused tasks [89]. It is reported that damage to network hub regions connecting different subnetworks, such as the precuneus, causes the largest disturbances in network organization [1]. Also, functional MRI studies

have demonstrated altered resting-state functional connectivity in the precuneus in patients with chronic musculoskeletal pain [48], and in mild TBI patients [121]. Furthermore, Baliki et al. [5] have demonstrated reorganization of the default mode network dynamics in different chronic pain conditions including alterations in precuneus connectivity.

Nevertheless, opposite to our hypothesis, we could not detect significant correlations between cortical thinning in the precuneus and cognitive performance or experimental CS measures in CWAD patients. **Furthermore, no cortical thickness differences were detected in all investigated brain regions between women with CINP and healthy women.** Also, in contrast to our hypothesis and to the results of previous studies in patients with chronic musculoskeletal pain [50, 63] and mild TBI [44], differences in **regional** cortical thickness between CWAD patients and healthy controls could not be demonstrated. Yet, the cortical thickness MANCOVA model comprising all ROIs showed a partial eta squared of 0.31, which means that 31% of the variance in cortical thickness in the 9 ROIs can be explained by study group, which corresponds with a large effect size.

Possible explanations for the lack of significant cortical thickness differences between CINP or CWAD patients and controls may be that the MRI scan and analyzing procedures used in the present study were not sensitive enough to detect subtle differences or perhaps there are cortical thickness differences in other non-investigated brain regions. On the other hand, it can be that these patient conditions are just not characterized by cortical thickness changes compared to healthy controls and thus display different underlying pathophysiological mechanisms compared to for example fibromyalgia patients who present regional cortical thickness changes. Nevertheless, further research is warranted before drawing solid conclusions. Noteworthy, we recently demonstrated decreased regional GM volume in CWAD patients compared to healthy controls [24]. These contrasting findings underscore the idea that cortical thickness and GM volume reflect different aspects of the neural architecture [90].

Group differences in white matter structure

Results showed a consistent pattern of decreased FA coinciding with increased MD and RD in the left cingulum hippocampus, and in the left tapetum in patients with CWAD compared to healthy controls (tapetum) or compared to CINP patients (cingulum and tapetum). In contrast, AD values were not different between all study groups.

The observed WM **structural** differences between CWAD patients compared to healthy controls were in line with our hypothesis. In addition, differences in WM **structure** were revealed between CINP and CWAD patients in the left cingulum hippocampus and the left tapetum. The tapetum of the corpus callosum is periventricular WM, which is believed to be affected by traumatic acceleration-deceleration of the brain [94, 104]. To date, limited studies are available on WM abnormalities in the tapetum as separate region. The tapetum is the temporal component of the corpus callosum [81]

formed by decussating fibers in the splenium that arch over the atrium of the lateral ventricle and course inferiorly in the posterior and temporal horns of this ventricle [94]. In contrast, more evidence exists for WM deficits in the corpus callosum (genu, rostrum, body, splenium, tapetum) in chronic musculoskeletal pain patients [69] and mild TBI patients [120].

The revealed pattern of WM **structural** deficits encompassing decreased FA, increased MD and increased RD in CWAD is consistent with results in other chronic musculoskeletal pain conditions. For example, Lieberman et al [69] observed decreased FA and increased RD in the left cingulum hippocampus in chronic musculoskeletal pain patients compared to healthy volunteers. In patients with complex regional pain syndrome decreased FA in the left cingulum bundle has also been demonstrated [43]. Furthermore, the observed WM abnormalities are in accordance with findings of increased MD in the cingulum in mild TBI patients compared to healthy persons [113]. In literature, studies tend to show decreased FA associated with numerous neurological and neurodegenerative diseases [46, 55].

Additionally, WM **structural** abnormalities were found in the cingulum hippocampus in CWAD compared to CINP patients but not compared to healthy persons. The cingulum bundle is a prominent WM tract extending longitudinally above the corpus callosum [51], and carrying information from the cingulate gyrus to the hippocampus. Specifically, the cingulum contains many afferent and efferent fibers associated with the cingulate cortices [83, 84, 115]. These fibers include connections with the thalamus, dorsolateral prefrontal cortex, and insula [84]. Other cingulum fibers are connected to the parahippocampal cortices, the inferior component of the hippocampal formation, and amygdala [83, 84].

Although this study has found innovative WM structural differences in the left tapetum in CWAD patients compared to healthy controls, no WM structural differences could be revealed in all other investigated WM ROIs between the CWAD or CINP group and healthy controls. Hence, the present study only found minimal and subtle differences in WM structure between women with CWAD and healthy women. The latter raises the question if investigating other WM regions/tracts or using other analyzing techniques would have revealed more differences and subsequently requires further research. It can be hypothesized that both chronic neck pain groups differ in terms of pain duration, report less maladaptive pain cognitions (e.g. pain catastrophizing), depressive and anxiety symptoms, cognitive problems, and medication use compared to other chronic pain conditions such as fibromyalgia in which more extensive WM structural alterations have been revealed compared to healthy controls [25, 26, 73]. Indeed, the role of longer pain duration, maladaptive pain cognitions, depressive and anxiety symptoms, cognitive deficits and medication use on structural brain alterations in chronic pain conditions has been reported in literature [26, 35, 47, 72, 75].

In CINP patients there is less evidence for maladaptive pain cognitions, cognitive problems and frequent medication use (e.g. narcotic analgesics) [24, 27]. It could be that the extent of debilitating associated symptoms beside the chronic pain is associated with the more extensive structural brain alterations shown in other chronic pain conditions [26, 75]. In addition, no evidence for CS or objective

cognitive deficits, and the non-traumatic origin of pain in CINP patients could play a role in the lack of GM and WM structural differences between women with CINP and controls.

We can very carefully suggest that our findings are indicative of WM structural abnormalities in the left tapetum and the left cingulum hippocampus in the CWAD group, maybe to some extent reflecting WM demyelination evidenced by the unchanged AD together with increased RD [49, 100, 103]. Interestingly, in mild TBI patients research has demonstrated that long associative WM tracts such as the cingulum bundle are frequently affected [92, 119]. As such, the underlying mechanisms of the revealed WM deficits in CWAD patients could be associated with the traumatic whiplash injury, which perhaps induced a cascade of events eventually leading to WM **(micro)structural** deficits. Nevertheless, based on visual inspection of the T2*-weighted brain images, microhemorrhages related to trauma or diffuse axonal injury were not observed in patients with CWAD. In patients with mild TBI, previous studies could detect subtle microhemorrhagic lesions suggesting differences in underlying pathophysiological mechanisms between patients with chronic WAD and mild TBI [71]. However, in future research we should include Susceptibility Weighted Imaging or Quantitative Susceptibility Mapping to detect possible microhemorrhages related to trauma in CWAD patients.

Decreased cortical thickness in the precuneus: associations with cognitive performance and central sensitization

In CINP patients, decreased precuneus thickness coincided with worse performance on the TMT part B, however decreased cortical thickness and worse cognitive performance could not be revealed in CINP patients compared to controls. The observed association between working memory capacity and cortical thickness in CINP patients is in line with studies reporting associations between regional GM morphology and working memory capacity or other features of cognition in patients with fibromyalgia [72], patients with complex regional pain syndrome [65], and in mild TBI patients [109].

Structural white matter abnormalities in the cingulum hippocampus and tapetum: associations with cognitive performance and central sensitization

Consistent with our hypothesis, deficits in WM **structure** in the left tapetum, encompassing increased MD and RD, were associated with decreased CPM efficacy (denoting CS) in the CWAD group. However, associations between cognitive performance or CS, and the observed WM alterations in the cingulum hippocampus could not be detected. Also, no associations were demonstrated between cognitive performance or distant hyperalgesia, and WM **structural** abnormalities in CWAD patients, which was in contrast with our expectations. Interestingly, we provide novel evidence for associations between **structural** WM deficits and decreased CPM efficacy in CWAD. Recently, a longitudinal MRI study uncovered **structural** WM vulnerabilities **(i.e. FA differences)** to develop chronic back pain [77]. Also, moderate evidence exists to support that higher pain intensity is associated with FA alterations in WM tracts involved in pain and cognition [26].

Clinical implications

Our findings support a role of WM **structural** abnormalities in the left tapetum in the observed dysfunctional CPM response in CWAD. This yields innovative evidence for underlying WM **structural** correlates of disturbed endogenous pain inhibition in CWAD but not in CINP patients. Accordingly, these results emphasize the role of WM abnormalities in patients with CWAD compared to CINP and maybe reflect one piece of the puzzle underlying the observed clinical differences between both neck pain conditions with possibly a role of the traumatic injury mediating the structural brain differences.

Our results furthermore indicate that chronic pain in CWAD patients should be interpreted, at least in part, as a result of structural plasticity of the central nervous system, associated with **experimental signs of CS**, alterations in cortical thickness and WM **structure** in regions involved in various aspects of pain and cognitive processing. Accordingly, it can be recommended that therapy approaches for CWAD should address the brain and take into account neuroplasticity of the central nervous system. As such, clinicians should take into account the observed **CS**, **and WM** structural differences **associated with this CS** when treating patients with CWAD.

Strengths, limitations and recommendations for further research

Several strengths can be outlined. This is the first study examining alterations in cortical thickness and WM **structure**, and their relationships with cognitive performance and CS in patients with a traumatic origin of pain (CWAD) compared to patients with non-traumatic CINP and healthy controls. This research is important because it unraveled differences between both pain conditions, and provided evidence for CS and structural brain alterations as underlying pathophysiological mechanisms in CWAD. Another strength is the evaluation of alterations in both GM macrostructure (cortical thickness) and WM **structure** (FA, MD, AD, RD).

The following limitations have to be taken into account when interpreting our results. Metrics derived from DWI data using the tensor model are indirect measures that relate to but do not directly quantify tissue features and are influenced by various methodological and biological factors [(55)]. These metrics cannot disentangle the individual microscopic contributions at the voxel level and therefore should be interpreted with caution. It remains unclear whether decreased FA is due to changes in membrane permeability, organelles, axon thickness, fiber density, or degree of myelination [(55)]. It has been suggested that alterations in RD combined with unchanged AD reflect demyelination [(100)], but the interpretation of these metrics has been a topic of controversy [(117)].

A second limitation of DTI analyses is the inability of the tensor model to adequately characterize diffusion in regions of complex fiber architecture. Single-shell data reconstructed with the diffusion tensor model assumes a single straight fiber orientation within each voxel and is inadequate to model more than one fiber orientation per voxel. In the brain however voxels often contain fiber populations

with more than one dominant orientation, such as crossing fibers [55]. Therefore, advanced models based on the high angular resolution DWI acquisition strategy to provide more robust estimates of the fiber orientation are recommended for further research in CINP and CWAD.

A third limitation is related to the ROI-based approach which implies a well-considered anatomical hypothesis-driven selection of investigated brain regions but on the other hand also implies that other non-selected brain regions/tracts could have revealed alterations in GM or WM structure in patients with CINP and CWAD compared to controls. Analyzing possible cortical alterations in for example the insula or the rostral anterior cingulate cortex could also have been interesting, hence the latter regions are recommended to include in future research regarding structural brain alterations in these patient conditions to increase our insight in the involved brain regions in CINP and CWAD. Furthermore, whole brain and structural network analysis could be valuable for further research in these patient conditions.

Noteworthy, the selection of CWAD and CINP patients implies that we compared two patient groups that are a priori different from each other in the origin of pain. In order to interpret the cortical thickness and WM structural results correctly it is important to emphasize that CWAD patients relate to a traumatic event in contrast to CINP patients. Interestingly, traumatic chronic neck pain patients reported higher neck pain intensity and more severe pain-related disability compared to non-traumatic CINP patients and demonstrated cognitive problems and CS compared to controls. Subsequently, the cortical thickness and WM structural differences between the study groups could also in part be interpreted in light of these more severe symptoms revealed in the traumatic CWAD group.

A final limitation pertains to the cross-sectional nature of this study implying that no conclusions can be drawn on the causality of the observed associations. To test causal inference, future longitudinal studies are necessary.

Further research investigating associations between abnormalities in WM **structure** in CWAD, and other experimental features of CS such as temporal summation of second pain could add valuable insights into the brain structural correlates of CS. Finally, it could be recommended to disentangle possible functional brain alterations using (resting-state) functional MRI techniques and perform **functional** network analyses in patients with CWAD and CINP compared to healthy controls.

CONCLUSION

In conclusion, cortical thinning in the left precuneus, a core hub of the default mode network and part of the structural brain core, was found in women with CWAD compared to CINP. Additionally, abnormalities in WM **structure** were revealed in 2 WM tracts carrying information between regions involved in affective-cognitive dimensions of pain processing and cognition in women with CWAD compared to CINP or healthy women. This study provided novel evidence for associations between

dysfunctional CPM and the degree of WM deficits in the left tapetum in patients with CWAD. This yields innovative evidence for underlying WM **structural** correlates of CS and in particular of disturbed endogenous pain inhibition in CWAD. Accordingly, these results emphasize the role of structural brain alterations in patients with CWAD compared to CINP.

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FIGURE LEGENDS

Fig 1. Schematic representation of cortical regions of interest (presented in blue) and WM regions/tracts of interest (presented in red) of cognitive and/or pain processing networks and regions, and their proposed function. Target cortical regions of the salience, central executive, default mode and somatosensory network were selected from the Desikan atlas [33]. The WM regions/tracts of interest were selected from the ICBM-DTI-81 WM labels atlas, developed by Mori et al. [81]. All regions (except for the corpus callosum) were included from both hemispheres. The careful selection of these regions of interest was based on evidence retrieved from our systematic review appraising the evidence for brain alterations in INP and WAD [32], on previous studies in patients with chronic pain or mild TBI examining GM morphological alterations [15, 17, 24, 44, 63, 116], demonstrating WM structural alterations, and regarding studies exploring associations between cortical thickness or WM structure, and measures of cognition and pain [4, 10, 19, 26, 34, 37, 58, 59, 62, 69, 70, 74, 81, 88, 94, 95, 104, 108]. In addition, only regions demonstrated to be mainly involved in processing pain or cognition or the combination thereof could be selected.

Fig. 2. Lateral (left fig.) and medial (right fig.) view of the cortical parcellation of the Desikan atlas [33] displayed on an inflated template (https://surfer.nmr.mgh.harvard.edu).
Numbered regions indicate the cortical regions of interest: 1) lateral orbitofrontal cortex; 2) pars orbitalis; 3) postcentral cortex; 4) superior parietal cortex; 5) supramarginal cortex; 6) precuneus; 7) posterior cingulate cortex; 8) caudal anterior cingulate cortex; 9) parahippocampal cortex

Fig. 3. White matter regions of interest masks.
Regions are depicted on mean diffusivity maps of the white matter atlas of Mori et al. [81]
a= Axial view of bilateral anterior limb of internal capsule (red), bilateral posterior limb of internal capsule (blue), bilateral anterior corona radiate (green), bilateral posterior corona radiate (violet); (projection fibers)
b= Axial view of bilateral tapetum (green) (commissural fibers), and bilateral cingulum cingulate gyrus (yellow)
c= Axial view of bilateral superior cerebellar peduncles (blue); (tract in the brain stem)
d= Sagittal view of the cingulum cingulate gyrus (yellow), and the cingulum hippocampus (cyan); (association fibers)

e= Sagittal view of the fornix (green) (association fibers) and superior cerebellar peduncle (blue).
f= Splenium of the corpus callosum (green) (commissural fibers).

Fig 4. Significant differences in cortical thickness of grey matter ROIs between patients with CWAD (n= 31), CINP (n= 32) and healthy controls (n= 26)

Abbreviations: HCON= healthy pain-free controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash-associated disorders, SE= standard error, ROIs= regions of interest. Data were analyzed using MANCOVA with age as covariate and post-hoc pairwise comparisons were applied using Bonferroni correction.*= p=0.032

Fig. 5. Significant differences in DTI-derived metrics of white matter ROIs between CWAD patients (n= 37), CINP patients (n= 35) and healthy women (n= 30).

Abbreviations: HCON= healthy pain-free controls, CINP= chronic idiopathic neck pain, CWAD= chronic whiplash-associated disorders, SE= standard error, DTI= diffusion tensor imaging, ROIs= regions of interest. Data were analyzed using MANCOVA with age as covariate (p<0.0125 (0.05/4)) and post-hoc pairwise comparisons were applied using Bonferroni correction.*= p<0.05, **= p<0.01