Grey matter adaptations to chronic pain in people with whiplash-associated disorders are partially reversed after treatment: A voxel-based morphometry study

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Abstract

Grey matter (GM) changes are often observed in people with chronic spinal pain, including those with chronic whiplash-associated disorders (CWAD). These GM adaptations may be reversed with treatment, at least partially. Pain neuroscience education combined with exercise (PNE+Exercise) is an effective treatment, but its neural underlying mechanisms still remain unexplored in CWAD. Here, we performed both cross-sectional and longitudinal voxel-based morphometry to i) identify potential GM alterations in people with CWAD (n=63) compared to age- and sex-matched pain-free controls (n=32) and ii) determine whether these GM alterations might be reversed following PNE+Exercise (compared to conventional physiotherapy). The cross-sectional whole-brain analysis revealed that individuals with CWAD had less GM volume in right and left dorsolateral prefrontal cortex (dlPFC) and left inferior temporal gyrus which was, in turn, associated with higher pain vigilance. Fifty individuals with CWAD and 29 pain-free controls were retained in the longitudinal analysis. GM in the right dlPFC increased after treatment in people with CWAD. Moreover, the longitudinal whole-brain analysis revealed that individuals with CWAD had decreases in GM volumes of the left and right central operculum and supramarginal after treatment. These changes were not specific to treatment modality and some were not observed in pain-free controls over time. Herewith, we provide the first evidence on how GM adaptations to CWAD respond to treatment.

Keywords: Chronic whiplash-associated disorders, voxel-based morphometry, grey matter, Pain Neuroscience Education

1. Introduction

Approximately half of the people who suffer a whiplash injury will develop chronic whiplash-associated disorders (CWAD).[44; 83] CWAD is a complex disorder, hallmarked by neck pain, often associated with pain-related distress (e.g., pain-related fear, hypervigilance, catastrophizing), and centrally mediated pain processing alterations.[19; 26] Chronic pain, more generally, is characterized by a functional and structural reorganization of the brain, which may partly reflect adaptations to persistent pain and its associated psychological and behavioral responses.[1; 27] Morphological alterations, predominantly lower grey matter (GM), have been consistently observed in cortical and subcortical areas implicated in sensory, interoceptive, cognitive and affective processing (e.g., prefrontal and cingulate cortex, amygdala, insula and hippocampus).[20; 32; 42] GM alterations seem to largely overlap among chronic pain disorders. While the attentional (i.e., frontoparietal, dorsal and ventral-salient) and default mode networks seem to show similar GM changes regardless of the chronic pain-related disorder, changes in other networks, like the somatosensory, appear to distinctly vary across disorders.[10; 20; 91] In CWAD specifically, lower GM in precentral and superior temporal gyri has been demonstrated.[29]

Some evidence suggests that the GM alterations observed in people with chronic pain are associated with pain features such as intensity and duration as well as its associated psychological responses.[21; 22; 41; 59] Thus, it is proposed that a better understanding of mechanisms underlying GM alterations in chronic pain as well as their response to treatment is needed to provide targets for optimizing interventions for chronic pain.[69; 99] While studies have begun to unravel the functional network changes following psychologically-based interventions such as cognitive behavioral therapy[13; 84] or mindfulness-based stress reduction[79], research on GM changes following treatment is still scarce.[79; 81] Conventional physiotherapy (advice and exercise) is considered the preferred treatment after a whiplash injury in clinical guidelines,[14; 24] yet its short-term benefits are rather small.[87; 106] Modern education-based interventions, where exercise is combined with pain neuroscience education (PNE+Exercise), have recently shown some promising results in people with chronic spinal pain when compared to conventional physiotherapy.[85] However, no such evidence is available in patients with CWAD and little is still known about its underlying therapeutic mechanisms.

In the present study, we aim to examine the effects of PNE+Exercise on GM in patients with CWAD in comparison to conventional physiotherapy. We conducted a longitudinal MRI study with CWAD patients and pain-free controls. Both cross-sectional and longitudinal voxel-based morphometry (VBM) analyses were applied to (1) identify potential baseline GM alterations in people with CWAD compared to controls, (2) determine whether these GM alterations might be reversed following treatment; and (3) examine whether there are any

other potential neuroplastic effects on GM following treatment. We hypothesized that GM alterations would be identified in attentional networks, similar to previous research,[10; 20; 91] and that those alterations would be partially reversed following treatment. In addition, given that GM alterations within the attentional networks have been found to relate to maladaptive pain cognitions and PNE+Exercise is superior to conventional physiotherapy targeting those,[62; 85] we expect a greater magnitude of change in the PNE+Exercise group.

2. Methods

2.1 Study design and participants

This is an MRI sub-study (registration number NCT04077619) of a randomized controlled trial (RCT)[23] conducted in Belgium, in which PNE+Exercise is being compared to physiotherapy in CWAD patients with moderate/severe pain-related disability (i.e., ≥15/50 in the Neck Disability Index [NDI][102]). Fifty-nine CWAD patients enrolled in the RCT took part in the MRI sub-study. Four additional CWAD participants who were not enrolled in the RCT were included in the baseline (cross-sectional) comparison. Furthermore, 32 age- and sex-matched pain-free controls with no history of neck pain were specifically recruited for the sub-study to serve as an additional comparator group. Further details on the eligibility criteria can be found in **Table S1** of the supplementary materials. Information on the randomization process can be found in the published protocol of the large RCT.[23] Baseline functional MRI data have been described already,[61] but the VBM analyses and the longitudinal aspect have not been reported yet.

This sub-study was approved by the Ethical Committee at the Ghent University Hospital (reference number 2019/1144) and all procedures were performed in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to participation. Data collection took place at Ghent Institute for Functional and Metabolic Imaging (GIfMI) from August 2019 to June 2021. Research methods and reporting were in accordance with current VBM reporting guidelines[71] and the CONSORT statement[78].

2.2 Interventions

Information on the specific content and rationale of the interventions can be found in the study protocol[23] and in **Table S2** and **S3**. In brief, the PNE+Exercise intervention combined PNE with a *time-contingent* exercise program. It consisted of 3 initial PNE sessions[18] (i.e., 1 group session and 2 one-on-one sessions, 30 min each, 1 session per week) and 15 one-on-one time-contingent exercise[63] sessions (30 min each, 1 session per week). In short, PNE sessions aimed to improve patients' knowledge of pain neurophysiology to reconceptualize maladaptive pain cognitions and affective responses (e.g., pain-related fear, hypervigilance and catastrophizing).[60; 64] The rationale is that by reducing the threat

value of pain and its associated responses, patients are able to shift away from pain control towards activity engagement in the time-contingent exercise program (i.e., “perform this exercise 12 repetitions, regardless of the pain”), breaking the fear avoidance cycle.[60; 64] In addition, stress management strategies were provided in parallel throughout the entire intervention.[88; 89] The control physiotherapy intervention combined advice with a *symptom-contingent* exercise program. It consisted of 3 advice sessions (i.e., biomedically-focused neck school education [37]), followed by 15 pain-contingent exercise sessions (i.e., biomedical approach, “stop or adjust the exercise when it hurts”). Both interventions had a duration of 4 months and were provided by physiotherapists.

2.3 Assessments

MRI data was collected at baseline before randomization (i.e., T0; within 2 weeks prior to the start of the intervention) and immediately post-intervention (i.e., T1; within 2 weeks after the end of the intervention). MRI data for pain-free controls was collected at the same timepoints with an equivalent in-between period (i.e., 4 months between T0 and T1).

In addition, pain-related questionnaires were completed online at the same timepoints and included NDI (neck pain-related disability)[102], numeric pain rating scale (NPRS; average pain intensity in the previous week), the Pain Catastrophizing Scale (PCS; catastrophic thinking)[25], the Pain Anxiety Symptoms Scale (PASS-20; pain-related fear and anxiety)[74] and the Pain Vigilance and Awareness Questionnaire (PVAQ; pain hypervigilance)[75]. Other questionnaires were administered, but are outside the scope of the current study. Assessment and data analysis were blinded to intervention allocation.

2.3.1 MRI data acquisition

MRI data were collected using a 3T MRI scanner (Siemens MAGNETOM Prisma) using a 64-channel head coil. Structural images were acquired using an MPRAGE T1-weighted protocol with 1 mm isotropic resolution, TR = 2250 ms, TE = 4.18 ms, TI = 900 ms, flip angle = 9°, FoV = 256 mm×256 mm, voxel size 1x1x1, GRAPPA acceleration factor 2.

2.3.2 Voxel-based morphometry

VBM[6] was implemented using the computational anatomy toolbox (CAT12)[35] standard pre-processing pipelines for cross-sectional (baseline sample) and longitudinal analysis (complete-case sample). In short, each participant’s T1-weighted image was corrected for bias-field inhomogeneities and later (i) segmented into GM, white matter, and cerebrospinal fluid (see Gaser et al. [35] for further details on CAT12’s tissue segmentation step). Then, individual GM segments were (ii) spatially normalized into standard MNI space using Geodesic Shooting[7] and further (iii) modulated by the Jacobian determinants from the

corresponding flow fields to restore the volumetric information lost during the high-dimensional spatial registration. This modulation step involves multiplying each voxel by the relative change in volume, which allows for a comparison of absolute GM volume (GMV) corrected for individual brain size.[109] Finally, modulated images were (iv) spatially smoothed (6 mm full width at half maximum Gaussian kernel [FWHM]). For the pre-processing of the longitudinal data, each participant's T1-weighted image from baseline and post-intervention was realigned using inverse-consistent rigid-body registrations and corrected for intra-subject bias-field inhomogeneities.[8; 70] The resulting images were then processed individually using the CAT12 longitudinal model, optimized for detecting small GMV changes over time.

Quality inspection of the MRI images was performed in stages. Raw images were visually examined for motion and scanner artifacts prior to pre-processing. After pre-processing, image quality ratings (IQR; a summary measure of noise, bias, and image resolution)[36] for each image were calculated and checked following CAT workflow.[35] Overall, the IQR scores were good (i.e., 80-90%) for all images after pre-processing (CWAD: 83.5±1.75% at T0, 85.2±1.15% at T1 and pain-free controls: 84.4±0.91% at T0, 85.6±0.53% at T1). Last, sample homogeneity was checked at the group-level adjusting for age and total intracranial volume (TIV) to identify any potential outliers. This resulted in the exclusion of no participants.

2.4 Sample size

Sample size calculation of the MRI sub-study was performed using G*Power 3.1.9.2 and based on GLM with repeated measures within-between interactions for different analyses of MRI and fMRI data. Using an alpha of .05, desired power of .80 and a drop-out rate at post-intervention of 15%[85], a minimum sample size of 40 patients with CWAD randomized to 2 treatment groups (1:1) was required to detect a moderate estimated effect size f of .25.

2.5 Statistical analyses

We performed (i) cross-sectional (aim 1) and (ii) longitudinal analyses following a complete-case analysis approach (aims 2 and 3). See further details on the statistical plan in relation to the hypotheses in **Figure S1**.

2.5.1 Cross-sectional analysis

To investigate potential regional GMV alterations in people with CWAD compared to pain-free controls at baseline, we estimated a t-test model in SPM12 adjusted for TIV[57] and age. All voxels with a GMV value of <0.2 were excluded to prevent possible edge effects.[72] Threshold-free cluster enhancement (TFCE) cluster-wise correction was performed (5000 permutations, H = 2.0, E = 0.5; SPM toolbox TFCE version r256) with family-wise error

corrected p-value (*pFWEc* < 0.05).[86] TFCE correction is recommended for VBM analyses for being more sensitive to detect subtle differences in GM and more robust to non-stationary data.[52; 76]

2.5.2 Longitudinal analysis

Group-difference region of interest analyses (primary outcomes; aim 2): The regions (clusters) showing group-related GMV differences at baseline (i.e., CWAD vs pain-free in the cross-sectional analyses) were considered the primary outcomes for the longitudinal analysis. Summarized parameter estimates (Beta coefficients) for GMV were extracted for each participant and time point from these group-difference regions of interest (ROIs) using *marsbar*[16].

First, Mixed ANOVA models with Intervention (PNE+Exercise, PT) and Timepoint (T0, T1) as fixed effects and subject as random effects were fitted in R (*lmer*[11] and *car*[33] packages) for each group-difference ROI. The following hypotheses were tested: *Do effects over time on GMV differ between PNE+Exercise and Physiotherapy?* (Hypothesis 1.A; Intervention by Time interaction). If not, *is there an effect over time on GMV in people with CWAD across interventions?* (H1.B; main effect of Time). Then, in case there was no Intervention by Time interaction, similar Mixed ANOVA models were fitted with CWAD (i.e., taking PNE+Exercise and Physiotherapy interventions together) and pain-free as Group levels to test whether *effects over time on GMV differ between people with CWAD and pain-free controls* (H2; Group by Time interaction).

Whole-brain analysis (secondary outcomes; aim 3): We followed the same hypothesis testing procedure as for primary outcomes. A first model (model 1) was fitted using the Sandwich Estimator (SwE) toolbox[38] with Intervention (PNE+Exercise, PT) and Timepoint (T0, T1) as fixed effects to characterize the neuroplastic GMV changes in patients with CWAD after intervention in an undirected F-contrast (H 1.A; Group by Time interaction). If no clusters survived, a second undirected F-contrast was tested (H1.B; main effect of Time). Finally, in a similar manner as primary outcomes, both interventions were gathered into a second mixed ANOVA model (model 2) and compared with pain-free (i.e., CWAD and pain-free as Group levels). An undirected F-contrast was tested in the second model (Group by Time interaction). We constructed our model with the “classic” SwE type, which estimates the covariance matrix for each subject and session separately, using small sample adjustment type C2. Non-parametric wild bootstrappingwith 5000 permutations was used.[39] TFCE cluster-wise correction with *pFWEc* < 0.05 was performed.

Analyses in pain-free controls: Additionally, we performed post-hoc ROI analyses (summarized parameter estimates; mixed ANOVA in R) to further test whether the clusters

that were observed to change over time in CWAD (model 1) behave differently in pain-free controls.

2.5.3 Additional analyses for interpretation purposes

Neurosynth meta-analytic decoding: To further interpret the functional role of the structural regions differing in GMV between CWAD patients and controls, as well as the changes observed over time after treatment, meta-analytic decoding[108] was performed.[90; 113] This meta-analytic strategy provides the power of large datasets to compute whole-brain distributions for psychological terms improving the functional characterization of the structural findings.[108] The unthresholded T- and F-maps obtained in the cross-sectional and longitudinal whole-brain analyses, respectively, were uploaded onto the Neurosynth database (neurosynth.org) and decoded. This returned psychological and neuroanatomic terms associated with the spatial pattern of each analysis.

Baseline GMV associations with pain-related questionnaires: Post-hoc Spearman rank partial correlations were computed in the CWAD group between the beta coefficients of clusters showing significant group differences at baseline and pain-related questionnaires (i.e., NDI, PASS, PCS, PVAQ). Correlations were adjusted for the potential confounding effects of age, sex and pain duration and p-values were FDR-corrected for multiple testing across questionnaires. Potential confounders were selected based on background knowledge[2; 40; 101]

Treatment effects on pain-related questionnaires: The effect of PNE+Exercise vs Physiotherapy on the pain-related questionnaires is examined in the larger trial.[23] Results for the MRI sub-sample though are reported for completeness. In order to provide a framework for the interpretation of results, we compute the proportion of CWAD patients who exceed the minimal (clinically) important change (MIC) (i.e., percentage of patients who improved). The MIC was defined as a decrease of ≥ 5 points in NDI from pre- to post-treatment.[31; 102]

3. Results

3.1. Participants and descriptive data

The sample included in the cross-sectional analysis consisted of 63 participants with CWAD (age 42.6±10.2 years, 45 women) and 32 pain-free participants (age 41.0±10.6 years, 22 women), of which 50 CWAD (23 in the PNE+Exercise group and 27 in the physiotherapy group) and 29 pain-free participants respectively were retained in the complete-case analysis of the longitudinal phase. Participants' descriptive data per group and randomized treatment arm can be found in **Table 1** and the study's flow diagram is illustrated in **Figure 1**.

Table 1. Participants' descriptive data per group at baseline.

	Cross-sectional analysis (n=95)		Longitudinal analysis (n=79)		
	pain-free (n=32)	**CWAD** (N=63)	**pain-free** (n=29)	**Physiotherapy** (N=27)	**PNE+Exercise** (N=23)
Sex					
Female, # (%)	22 (68.8)	45 (71.4)	20 (74.1)	16 (69.6)	20 (69.0)
Male, # (%)	10 (31.3)	18 (28.6)	7 (25.9)	7 (30.4)	9 (31.0)
Age (years)	41.0 (10.6)	42.60 (10.2)	43.1 (10.5)	43.1 (9.92)	40.4 (9.98)
Duration pain (months)		40.2 [3.03, 297]		23.2 [3.03, 247]	64.4 [5.87, 297]
Average pain previous week NPRS (0-10) †		5.50 [1.00, 8.00]		6.00 [1.00, 8.00]	6.00 [2.00, 8.00]
Neck-related disability NDI (0-50) †		18.00 [11.0, 35.0]		19.0 [12.0, 35.0]	18.0 [14.0, 26.0]
Pain catastrophizing PCS (0-52) †		24.00 [5.00, 49.0]		25.0 [9.00, 47.0]	29.0 [7.00, 49.0]
Pain-related fear PASS-20 (0-100) †		36.00 [4.00, 94.0]		36.0 [4.00, 78.0]	39.0 [7.00, 94.0]
Pain hypervigilance PVAQ (0-80) †		37.00 [15.0, 64.0]		37.0 [15.0, 61.0]	41.0 [23.0, 64.0]

†Median and IQR is presented instead of mean and SD.

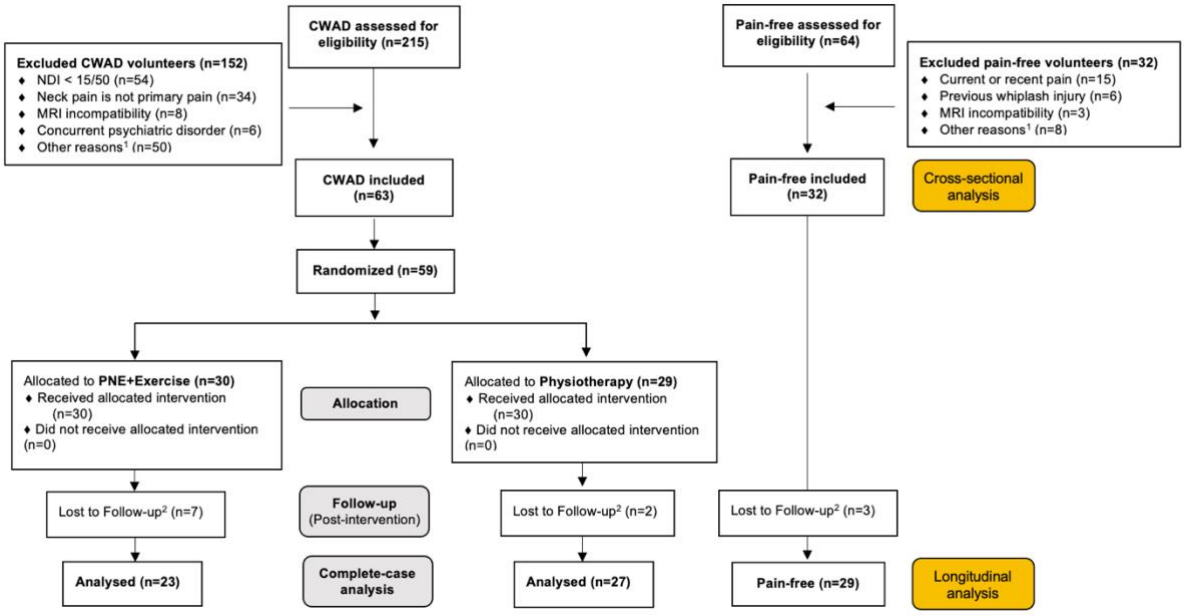

Figure 1. CONSORT Flow diagram. [1] Other reasons: fracture, surgery, non-Dutch speaking, age, (severe) cardiovascular, respiratory, neurological, rheumatic disorder, head trauma, idiopathic neck pain**[2] Reasons for lost to follow-up**: PNE+Exercise (n=7): shoulder injury/surgery (n=2), availability/working reasons (n=2), COVID (n=2), family reasons (n=1). PT (n=2): pregnancy (n=1), unknown/no response (n=1). Pain-free (n=3): moved to another country (n=1), unknown/no response (n=1), pregnancy (n=1).

3.2. Characterization of baseline GMV alterations in people with CWAD (cross-sectional analyses)

Whole-brain analysis: Compared to pain-free controls, people with CWAD exhibited less GMV in 3 clusters (pain-free > CWAD): the right dorsolateral prefrontal cortex (dlPFC), left dlPFC and left inferior temporal gyrus (ITG) (**Figure 2**, **Table S4** in the supplementary). No clusters were identified in the CWAD > pain-free comparison. Neurosynth meta-analytic decoding revealed that the unthresholded whole-brain t-map (pain-free > CWAD) was related to the functional terms “painful”, “reward”, “reactivity”, “fear” and “emotional value” (**Figure 2, Table S4**). Furthermore, pain hypervigilance (PVAQ) was found to negatively correlate with the beta weights of all 3 significant group-difference clusters (left dlPFC: r_s = -.38, *pFDR* = .013; right dlPFC: r_s = -.31, *pFDR* = .057 and left ITG: r_s = -.38, *pFDR* = .012). Higher levels of hypervigilance were associated with lower GMV in those 3 clusters in CWAD participants.

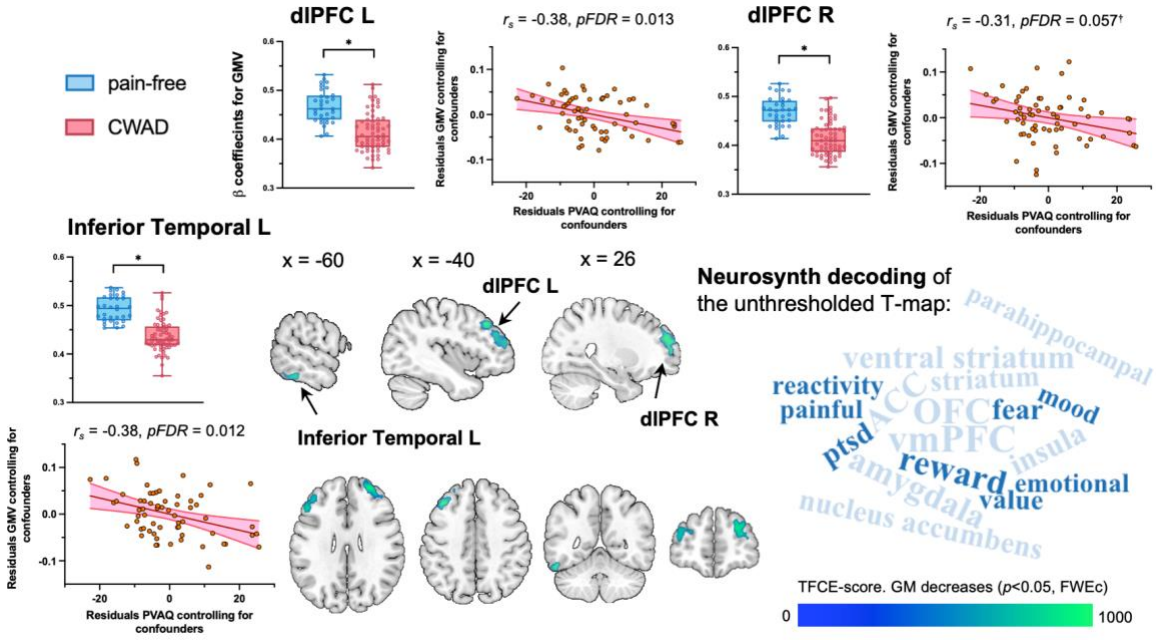

Figure 2. Results from cross-sectional analysis for baseline group differences in GMV (pain-free vs CWAD). Whole-brain analysis, partial correlations with pain-related questionnaires and Neurosynth meta-analytic decoding of the unthresholded T-map.

3.3. Effects of PNE+Exercise and conventional physiotherapy in CWAD patients over time (longitudinal analyses)

In this MRI sub-study, there was no time by intervention interaction was found for neck pain intensity (NPRS: F[1,48] = 0.16, p = 0.689) nor related disability (NDI: F[1,48] = 0.98, p = 0.326). However, a reduction in these outcomes was observed in both intervention groups (main effect for time; F[1, 48] =34.52, p < 0.001 and F[1, 48] =34.81, p < 0.001 for NPRS and NDI respectively) (**Table 2**). In terms of MIC, 54% of the people with CWAD showed a clinically relevant improvement in neck pain-related disability (52% for PNE+Exercise, and 56% for Physiotherapy).

On the other hand, PNE+Exercise was superior to physiotherapy in reducing pain-related fear and anxiety (PASS: F[1,48] = 6.34, p = 0.015) and pain hypervigilance (PVAQ: F[1,48] = 10.86, p<0.001). On average, CWAD patients in PNE+Exercise had a reduction of -11.50 points in PASS (95%CI: -20.60, -2.31) and -9.49 points in PVAQ (95%CI: -14.80, -4.20) greater than patients in the physiotherapy group. By contrast, reductions in pain catastrophizing were comparable across both groups (No time by intervention interaction in PCS; F[1,48] = 2.14, p = 0.149 but significant main effect for time; F[1, 48] = 68.44, p < 0.001).

Table 2. Effects of PNE+Exercise compared to PT on pain-related outcomes

Outcome		**PNE+Exercise** (n=23)	**Physiotherapy** (n=27)	**Intervention by Time change**
		Mean ± SE	**Mean ± SE**	
NDI (0-50)				
	Baseline	19.60 ± 0.99	19.59 ± 0.92	
	Post-intervention	14.04 ± 1.28	15.62 ± 1.17	
	Within-group change (95%CI)	**-5.56 (-7.95, -3.18)**	**-3.96 (-6.17, -1.75)**	-1.60 (-4.85, 1.64)
NPRS (0-10)				
	Baseline	5.48 ± 0.38	5.33 ± 0.35	
	Post-intervention	3.43 ± 0.36	3.55 ± 0.33	
	Within-group change (95%CI)	**-2.04 (-3.02, -1.07)**	**-1.77 (-2.68, -0.87)**	-0.27 (-1.59, 1.06)
PASS-20 (0-100)				
	Baseline	40.91 ± 4.01	37.07 ± 3.71	
	Post-intervention	21.04 ± 3.46	28.66 ± 3.22	
	Within-group change (95%CI)	**-19.87 (-27.59, -13.14)**	**-8.40 (-14.60, -2.20)**	**-11.50 (-20.60, -2.31)**
PCS (0-52)				
	Baseline	28.73 ± 2.37	25.96 ± 2.21	
	Post-intervention	15.56 ± 2.32	16.74 ± 2.15	
	Within-group change (95%CI)	**-13.17 (-17.16, -9.18)**	**-9.22 (-12.94, -5.50)**	-3.95 (-9.38, 1.48)
PVAQ (0-80)				
	Baseline	42.43 ± 2.29	37.51 ± 2.12	
	Post-intervention	33.35 ± 2.01	37.92 ± 1.87	
	Within-group change (95%CI)	**-9.09 (-12.97, -5.20)**	**-0.41 (-3.22, 4.03)**	**-9.49 (-14.80, -4.20)**

NDI, Neck Disability Index; NPRS, Numerical Pain Rating Scale; PASS, Pain Anxiety Symptoms Scale; PVAQ, Pain Vigilance and Awareness Questionnaire.

3.3.1. Do PNE+Exercise and/or conventional physiotherapy reverse GMV alterations in CWAD?

Effects over time of PNE+Exercise vs Physiotherapy: To examine whether the two CWAD intervention groups differed in their GMV changes over time, interaction effects were inspected (*Hypothesis 1.A*). No significant Intervention by Time effect was observed in any of the regions showing baseline GMV alterations in CWAD vs controls (i.e., group-difference clusters/ROIs; left dlPFC: F[1,48]=0.16, p=0.689; right dlPFC: F[1,48]=0.51, p=0.478; left ITG: F[1,48]=1.94, p=0.170).

As no interaction effect was observed, the main effect of Time was inspected. Across both interventions, CWAD participants showed increases in right dlPFC GMV over time (*Hypothesis 1.B*; main effect for time: F[1, 48]=5.49, p=0.023; see **Figure 3A**). No other main effects of time were observed in any of the other group-difference clusters. **Table S5** summarizes the results of the longitudinal ROI analysis, as well as within- and between-group changes.

Effects over time in CWAD (PNE+Exercise & Physiotherapy) compared to pain-free controls*:* As there were no differences in GMV changes over time across interventions, we examined whether GMV changes over time differed across patients with CWAD who underwent treatment and pain-free controls (*Hypothesis 2)*. Only the right dlPFC demonstrated a Group (CWAD vs pain-free) by Time interaction effect (F[1,77] = 4.53, p = 0.037) (**Figure 3A**). When compared to pain-free controls, patients with CWAD showed increases in GMV from pre- to post-intervention, while pain-free controls did not show any change in GMV over time. There was no other significant Group by Time interaction effect in any either left dlPFC (F[1,77]=0.78, p=0.780) or left ITG (F[1,77]=0.34, p=0.559) **(Table S5).**

3.3.2. Are there any additional neuroplastic effects over time following PNE+Exercise and/or Physiotherapy in CWAD?

Effects over time of PNE+Exercise vs Physiotherapy: To further examine whether the two interventions differed in their GMV changes over time, interaction effects were inspected in a whole-brain analysis as well (*Hypothesis 1.A*). No significant Intervention by Time interaction effect on GMV was found.

However, the main effects of Time (*Hypothesis 1.B*) uncovered that across the interventions, people with CWAD showed a reduction in GMV from pre- to post-intervention in 4 clusters located in left and right supramarginal gyrus and left and right central operculum (**Figure 3B**, **Table S6**). This indicated that GMV was reduced over time in these clusters irrespective of which intervention patients received. Neurosynth meta-analytic decoding revealed that the pre- vs post unthresholded F-map was related to sensory-related terms (e.g., “tactile”, “touch”), including “pain” (**Figure 3B**).

We investigated whether these changes were present for pain-free controls as well via a post-hoc region of interest analysis. This analysis showed that the changes in two of the four clusters were not observed in pain-free controls (Group by Time interaction in left supramarginal gyrus: $F[1,77] = 6.67$, $p = 0.012$ and (marginally) in right central operculum: $F[1,77] = 3.78$, $p = 0.055$) (**Table S7**).

***Effects over time in CWAD (PNE+Exercise & Physiotherapy) compared to pain-free controls**:* As there were no differences in GMV changes over time across interventions, we further examined whether GMV changes over time differed across patients with CWAD who underwent treatment and pain-free controls (*Hypothesis 2)* using a whole-brain analysis as well. No significant clusters were observed for the Group by Time interaction effect.

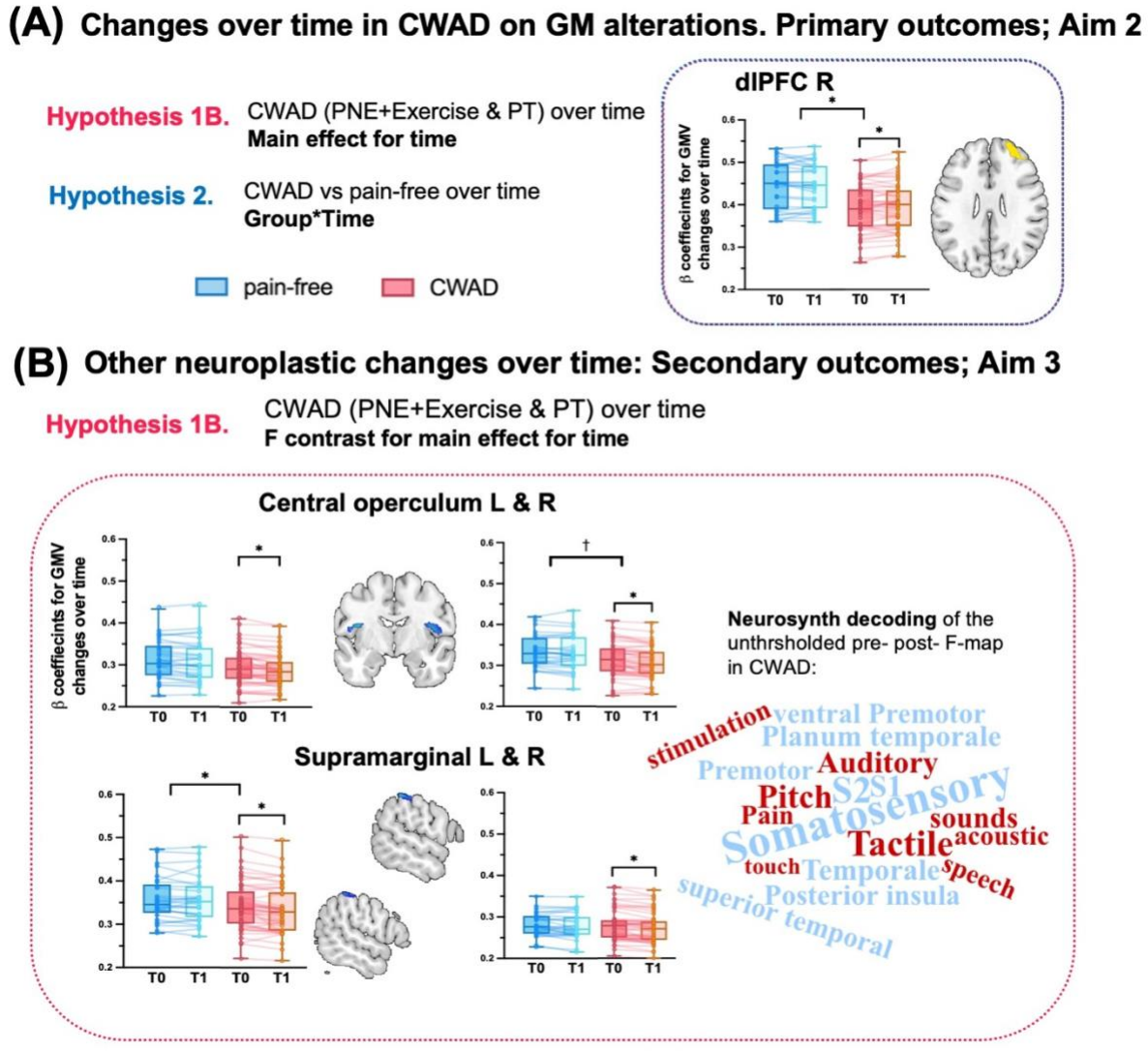

Figure 3. Results from longitudinal analysis. (A) Pre- post changes (T0, T1) in the group-difference clusters; neuroplastic changes in areas showing baseline GMV alterations in the cross-sectional analysis **(B)** Pre- post changes at the whole-brain level; additional neuroplastic changes across the whole brain. Note 1 in hypothesis 1.B. post-hoc region of interest analyses in pain-free controls for completeness purposes.

4. Discussion

Our findings indicate that people with CWAD present with GMV alterations at baseline that are, partially, reversed by intervention. Compared to pain-free controls, people with CWAD exhibited lower GMV in key regions implicated in the modulation of pain (e.g., bilateral dlPFC), which was associated with pain hypervigilance. After either PNE+Exercise or physiotherapy, the alterations in the right dlPFC were reversed to a certain degree, as people with CWAD in both treatment groups showed an increase in dlPFC GMV, which was not observed in pain-free controls. Finally, GMV decreases in areas traditionally related to nociception were additionally observed in CWAD patients after treatment, which were also not specific to the treatment modality.

The current study showed that people with CWAD have lower GMV compared to pain-free controls. These are largely located within the frontoparietal control network (i.e., dlPFC and ITG), which has a predominant role in attention to pain and its cognitive modulation.[30; 48; 66] GM decreases in dlPFC and ITG have been consistently reported in other spinal pain disorders such as chronic low back pain[2; 3; 10; 34; 77; 82] and now, our findings extend this evidence to CWAD. The dlPFC is involved in the top-down regulation of attention to nociceptive stimuli.[54; 80; 93] This region is actively implicated in the processing of sustained pain,[49] potentially modulating pain-induced activity in the somatosensory cortex.[28; 47] The ITG, on the other hand, has been recently suggested to mediate cognitive and affective responses to painful stimuli.[94; 114]

People with pain after trauma may have an excessive tendency to attend to pain which can lead to an increased pain experience.[17; 68; 98] Inter-individual differences in pain hypervigilance have been shown to explain the variability in neural responses to painful stimuli in prefrontal and temporal areas.[112] In the present study, GMV reductions in dlPFC and ITG were moderately correlated with pain hypervigilance (i.e., the greater the attention to pain, the lower the GMV). This relationship was also observed in a previous study in people with CWAD.[22] This seems to agree with the notion that, in people with chronic pain, top-down pain modulation exerted within these regions is associated with hypervigilance.[50] This idea is further supported by the terms derived from decoding the statistical map, illustrating that the patterns of GMV alterations in people with CWAD are associated with terms such as “painful”, “reactivity”, “reward” and “value”. Interestingly, the pattern was also associated with the term “post-traumatic stress disorder”, which is often comorbid with CWAD and mutually dependent on hypervigilance.[17; 68] Altogether, these findings seem to indicate that impaired top-down regulation could presumably relate to GMV loss.[45; 97]

Overall, our findings indicate that GMV alterations are partially reversed following treatment, but that these changes are not specific to either PNE+Exercise or conventional physiotherapy. Modest increases in dlPFC GMV were observed in CWAD patients

immediately after treatment (main effect for time) that, by contrast, were not found in pain-free controls over the same period. This finding contradicts results from a previous PNE+Exercise trial[56] in people with chronic spinal pain, which reported no change in this region at post-intervention. On the other hand, several cohort studies have consistently reported that pain remission, either spontaneous or after treatment, is accompanied by partial normalization of the dlPFC GM.[65; 73; 81; 82] Similar to our findings, a previous mindfulness RCT conducted by Seminowicz et al. [79] found no intervention by time effect on dlPFC GM. The authors theorized that changes in this region may be dependent on treatment responses, rather than specific to the intervention.[79] However, this hypothesis seems to be challenged by our results. In the present study, a low number of patients exceeded the MIC for pain-related disability (54%; although comparable to previous trials in CWAD[55; 58]) and, therefore, it is unclear if this effect can be explained by a positive treatment response. The dlPFC is implicated in the mechanisms underlying expectations and placebo analgesia.[9; 116] Yet, these processes are somewhat independent of the modulation of bottom-up nociceptive processing and not sufficient to explain effects on pain self-reported outcomes.[53; 115] Thus, the observed increase in dlPFC GMV could reflect, to some extent, positive expectations about the consequences of engaging in treatment rather than the direct expression of treatment effects on pain. This, however, remains speculative and needs to be explored in future research as our current study is not sufficiently powered for exploring factors that could have mediated the effect or for comparing responders vs. non-responders.

In addition, the exploratory whole-brain analysis revealed other GMV changes in patients with CWAD after treatment that were also not specific to one type of treatment. Particularly, reductions in regions related to nociceptive processing (i.e., central operculum and supramarginal).[104; 107] This neuroplastic trend was further corroborated by the decoding of the pre- post-brain map, which pointed towards somatosensory-related terms in addition to “pain”. The nociceptive-related changes could potentially mirror changes in pain hypersensitivity, which is a common feature in people with CWAD.[92; 100] Importantly, exercise-induced hypoalgesia can be achieved even in the absence of substantial gains in pain intensity and related disability.[12; 43; 51; 96] The underlying (patho)physiological mechanisms behind the observed changes remain unclear. Growing evidence suggests that GMV increases/decreases are not solely dominated by neurogenesis, but also, gliogenesis (i.e., changes in the number of non-neural cells), dendritic, vascular and synaptic plasticity changes among others.[4; 45; 46; 67; 95; 111]. These local tissue and vascular adaptations are suggested to be driven by energy demands following neuronal responses and potentially induce long-term morphological changes.[92; 95]

A major strength of the longitudinal VBM analysis compared to previous similar research[79; 81] is the inclusion of a pain-free (control) group with baseline and follow-up

measurements. This allowed us to, respectively, (i) determine whether or not GM adaptations to CWAD specific to our sample were reversed by treatment and (ii) investigate whether changes in GM in CWAD patients differ from those observed in pain-free people, and hence are not solely a result of time. Although the addition of an active evidence-based comparator treatment can be considered a strength as well, it can also explain the lack of an interaction effect, and we acknowledge that the inclusion of an inert control group could have provided more specific insights into the GMV changes related to either intervention.[5] Another consideration is that the results from the longitudinal analysis could have been potentially confounded by fluctuations in pain medication intake or depressive symptoms, among others, and should therefore be interpreted with caution.[15; 105; 110] In addition, Neurosynth decoding can be subject to biases in how terms are used in different studies (and research fields),[103] and was used merely to facilitate the interpretation of the results.

Neuroimaging studies have shown GM alterations in people with chronic pain, and here we extend those findings to people with CWAD specifically. People with CWAD present with lower GMV compared to pain-free controls, mostly within areas related to pain modulation, including dlPFC and ITG. Among these areas, the right dlPFC GMV increased and hence showed reversal after treatment, yet it is difficult to determine the underlying mechanisms of this neuroplastic adaptation as it was not specific to the type of treatment. In addition, slight GMV reductions in nociceptive areas were observed in people with CWAD after treatment. Taken together, our findings provide further insights into structural brain alterations and their response to treatment in this population, pointing towards involvement of pain modulatory and sensorimotor neural circuitries that seem to adapt to chronic pain states, but also -at least partially- to its treatment.

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