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Data-driven analysis of whole-brain intrinsic connectivity in patients with chronic low back pain undergoing osteopathic manipulative treatment

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ABSTRACT

Background: Chronic Low Back Pain (cLBP) poses a significant health challenge, leading to functional disability and reduced quality of life. Osteopathic Manipulative Treatment (OMT) is emerging as a therapeutic option for cLBP, but the brain mechanisms underlying its analgesic effect remain unclear.

Materials and Methods: Thirty cLBP patients were randomly exposed to either four weekly sessions of OMT (N=16) or Sham treatment (N=14). Resting-state Magnetic Resonance Imaging (rs-MRI) scans and pain perception questionnaires were collected before and after treatment. A voxel-wise, rs-fMRI data-driven analysis was conducted to identify changes in the intrinsic functional connectivity across the whole brain that were associated with the OMT. Spearman's correlations were used to test for the association between changes in intrinsic connectivity and individual reports of pain perception.

Results: Compared to the Sham group, participants who received OMT showed significant alterations in the functional connectivity of several regions belonging to the pain matrix. Specifically, OMT was associated with decreased connectivity of a parietal cluster that includes the somatosensory cortex and an increase of connectivity of the right anterior insula and ventral and dorsal anterolateral prefrontal areas. Crucially, the change in connectivity strength observed in the ventral anterolateral prefrontal cortex, a putative region of the affective-reappraisive layer of the pain matrix, correlates with the reduction in pain perception caused by the OMT.

Conclusions: This study offers insights into the brain mechanisms underlying the analgesic effect of OMT. Our findings support a link between OMT-driven functional cortical architecture alterations and improved clinical outcomes.

1. Introduction

Chronic Low Back Pain (cLBP) is characterized by muscle tension and subsequent pain that radiates from the lower back up to the gluteal folds, often accompanied by sciatica (Vlaeyen et al., 2018). It can result in functional disability and a negative impact on the quality of life (Morlion, 2013). If the pain persists for over 3 months, it transitions from symptom to disorder, potentially maintained by factors distinct

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from its initial causes (Vlaeyen et al., 2018). Management of cLBP typically involves non-pharmacological approaches recommended by international guidelines, including exercise therapy, physiotherapy, and education (Nicol et al., 2023). Recently, there has been a growing interest in the Osteopathic Manipulative Treatment (OMT) for nonspecific chronic low back pain (cLBP). A review of the existing literature demonstrates that osteopathic interventions generally improve pain levels and functional status in chronic patients (Dal Farra et al., 2021). In particular, myofascial release within OMT demonstrates better evidence for pain reduction compared to other interventions (Dal Farra et al., 2021; Licciardone et al., 2020). Recent studies suggest that interoception, mediated through touch, may play a significant role in cLBP therapy, as fluctuations in physical symptoms and emotional states correlate with interoception. Touch operates via small-diameter, low-conducting, unmyelinated C-tactile fibers, contributing to identifying body sensations and maintaining homeostasis (Ojala et al., 2023; Tsakiris & Critchley, 2016). However, the neural bases underlying the effects of OMT remain unclear.

In making an hypothesis about the possible brain regions undergoing OMT-related changes, it is useful to refer to the concept of pain matrix, the network of cortical areas through which pain is generated from nociception (Legrain et al., 2011). According to recent formulations (Garcia-Larrea & Peyron, 2013; Tobimatsu, 2021), the pain experience results from the interplay of three cortical layers, hierarchically organized. Briefly, a first-order "nociceptive" layer, composed of brain regions receiving direct spinothalamic projections, mediates the initial quality of the pain sensation. The transition from basic nociception to conscious pain perception, and its top-down modulation requires a second "attentional-perceptive" layer, which includes the anterior insula, a region also strongly associated with interoception (Quadt et al., 2018), but also the anterior cingulate cortex and lateral fronto-parietal regions. Finally, a polymodal, third-order "affective-reappraisive" layer, including the perigenual cingulate, the orbitofrontal, the anterolateral prefrontal cortex and the ventral striatum, is responsible for the modulation of pain perception as a function of the emotional context and internal states. The ability of the second and third layers to modulate activity in the first layer and downstream subcortical pain centers, producing impressive changes in the pain experience, makes them good candidates for the manifestation of OMT effects.

Magnetic resonance imaging (MRI) offers a powerful tool to examine how osteopathy manipulative treatment (OMT) influences the brain architecture. Arterial Spin Labeling (ASL) and functional MRI (fMRI) have uncovered associations between OMT and changes in brain perfusion (Cerritelli et al., 2021) as well as task-evoked activity (Cerritelli et al., 2020a). The results of these studies highlighted the reduction of brain perfusion/activity in regions mediating nociception and the increase in regions involved in the emotional-affective components of pain (Cerritelli et al., 2020a; Cerritelli et al., 2021). Resting-state functional connectivity MRI (rs-fMRI) allows the identification of functional coupling between and within functional brain networks in absence of any explicit task (Gillebert and Mantini, 2013). Previous studies have employed various data analysis approaches (Cerritelli et al., 2017; Isenburg et al., 2021; Tramontano et al., 2020) to investigate how OMT, or manual therapy in general, alters the brain's connectome by reshaping the network topology of the pain matrix. While these studies provided the first evidence on the effect of osteopathy on brain functional connectivity, the a-priori selection of ROIs/seeds has not allowed to fully exploit the whole brain coverage of the fMRI technique. In particular, the bias towards specific seeds/ROIs is at risk of missing significant effects and requires high spatial specificity (Martuzzi et al., 2011; Scheinost et al., 2012). To overcome these methodological challenges, data-driven approaches offer an alternative by evaluating changes in brain connectivity without requiring the definition of a priori ROIs. These methods estimate changes of connectivity directly from the functional data, thereby allowing a comprehensive, whole brain assessment. ICA is one approach utilized to explore neural network dynamics (Calhoun et al., 2001; Hyvarinen, 1999) and has been previously used for the identification of the OMT effects. However, ICA is not without limitations, as it can still miss subtle functional connectivity differences that do not manifest at the level of entire networks, increasing the risk of false negatives (Martuzzi et al., 2011; McKeown, 2003). Hence, there is a need for more advanced and holistic approaches to brain connectivity data, capable of capturing the full range of changes induced by OMT avoiding potential bias associated with the a priori selection of seed/ROIs.

To that aim, in the present study, rs-MRI data from thirty patients with cLBP, randomly assigned to receive either four weekly sessions of OMT (N=16) or Sham treatment (N=14), were analyzed using a data-driven, whole-brain approach named Intrinsic Connectivity Contrast (ICC). ICC is a voxel-wise measure of degree centrality (Wang et al., 2017), indicating connectivity strength based on the root-mean-square of correlation coefficients between each voxel and all other brain voxels (Martuzzi et al., 2011). This approach identifies brain networks that show a functional connectivity change in response to OMT without prior information or operator-dependent biases. Since the ICC cannot determine with which regions a connectivity variation occurs, we further conducted a follow-up seed-to-voxel functional connectivity analysis (Zhou et al., 2023), using the regions identified in the ICC as seeds. Furthermore, the strength of intrinsic connectivity resulting from significant clusters was correlated with pain perception questionnaires to assess the clinical relevance of the OMT-induced functional reorganization.

2. Methods

2.1. Ethics statement

This study was approved by the local Ethics Committee (University of Chieti-Pescara number: 7/09–04-15), and all subjects provided informed written consent in conformity with the Declaration of Helsinki. The protocol was registered on clinicaltrial.gov (ID: NCT02464475) on 08/06/2015. All methods were carried out by relevant guidelines (CONSORT, TIDIER, and SPIRIT) and regulations.

2.2. Experimental design

The current randomized placebo-controlled study is part of a seminal project conducted by our group, from which we have already presented analyses of both task-evoked fMRI data (Cerritelli et al., 2020a) and ASL (Cerritelli et al., 2021). Detailed information on the study design, patient clinical history, and inclusion/exclusion criteria have already been reported in our previous works (Cerritelli et al., 2020a; Cerritelli et al., 2021). One subject of the Sham group was excluded from the original sample due to missing rs-fMRI data. Therefore, the OMT and sham groups consist, respectively, of 16 and 14 subjects. Clinical and pain scale data are available for 15 subjects within the OMT group.

Eligible patients were randomly split into two groups using a 1:1 ratio and allocated to either the OMT or sham group. Block randomization was employed based on a computer-generated list with a block size of 10. Patients remained unaware of the study's steps, outcomes, and group assignments. The randomization list was securely stored online, overseen by an IT consultant. Research staff was kept uninformed about the study design and outcomes, and were blind to patients' allocation, as all patients received touch from the practitioner. Only the osteopath knew the allocation. Furthermore, the practitioner delivering OMT had no role in patient care decisions.

Patients assigned to the OMT group received four osteopathic sessions including balanced-ligamentous, balanced-membranous, and fluidic techniques, following established osteopathic principles. The sham group received an osteopathic-like manual assessment and treatment, wherein the practitioner applied manual contact without specific osteopathic techniques. The operator gently placed hands on predefined

bodily areas without applying techniques, using only gentle static or dynamic touch. Areas included the low back, sacrum, pelvis, diaphragm, upper thorax, cervical spine, and cranium. The sequence was predetermined to avoid patient inference. Each session of OMT or SHAM lasted 30 min and was conducted in the same location by the same practitioner. This was done to maintain the patient-doctor relationship and prevent contamination or allocation bias. Patients in the sham group received osteopathic treatment after the trial. Throughout, patients were instructed to refrain from drug use.

At enrollment, patients were asked to provide socio-demographic data (gender, BMI, age, academic degree, smoking habits, type of work). The State-Trait Anxiety Inventory (STAI-Y1 and Y2) was utilized to assess trait anxiety (Spielberger et al., 1983), and the Edinburgh Handedness Inventory was employed to determine handedness predominance (Oldfield, 1971). The Body Awareness Questionnaire (BAQ) measured self-reported attentiveness to bodily processes (Mehling et al., 2009; Shields et al., 1989). The Temperament Evaluation of the Memphis, Pisa, Paris, and San Diego-Auto-questionnaire (TEMPS-A) evaluated the affective temperament defining the bipolar spectrum, with depressive (D), cyclothymic (C), hyperthymic (H), irritable (I), and anxious (A) subscales.

All participants underwent two experimental sessions, each involving an MRI scan and the completion of pain perception questionnaires. The first session occurred before treatment (T0), while the second took place at the end of the study period (T2, after 4 weeks), which included four treatments overall. The original protocol included MRI and pain perception assessments immediately after the first manual session (T1) to study acute treatment effects. However, this aspect is irrelevant to the current study, which focuses on the chronic treatment response.

2.3. Pain perception evaluation

Physical disability due to cLBP was assessed using the Visual Analogue Scale (VAS) for pain assessment, the Roland-Morris Disability Questionnaire, the Oswestry Low Back Disability Questionnaire (OSW), and The McGill Pain Questionnaire. The VAS represents a widely employed and validated tool for pain assessment. Utilizing a 100-mm line (0 no pain, 100 worst pain), the scale allows individuals to self-report their pain intensity by marking a single point along the line. The Roland-Morris Disability Questionnaire is a health status measure to assess physical disability in low back pain patients. It comprises 24 yes/no items and demonstrates good psychometric properties, as evidenced by internal consistency and responsiveness (Roland and Fairbank, 2000). The Roland-Morris Disability Questionnaire has four parts: Sensory Pain Rating Index (PRI-S), Affective Pain Rating Index (PRI-A), Present Pain Intensity (PPI), and Global Pain Experience Evaluation. The OSW assesses disability resulting from low back pain. It consists of 10 items addressing various aspects of functioning, such as pain intensity, physical functioning, sleep functioning, and social functioning (Fairbank et al., 1980; Roland and Fairbank, 2000). The McGill Pain Questionnaire evaluates an individual experiencing significant pain and has been used to monitor pain over time and assess the effectiveness of interventions.

2.4. MRI data analysis

MR images were acquired using a Philips Achieva 3 Tesla scanner (Philips Medical Systems, Best, Netherlands). Structural MR images, obtained using a 3D fast field echo T1-weighted sequence (sagittal, matrix 256 × 256, FOV=256 mm, slice thickness = 1 mm, no gap, in-plane voxel size = 1 × 1 mm, flip angle = 12°, TR=9.7 ms and TE=4 ms), were used in rs-fMRI preprocessing to derive the grey/white/CSF masks utilized in the confound removal process. For each session, two runs of BOLD rs-fMRI data were acquired with eyes closed using a gradient-echo T2*-weighted echo-planar (EPI) sequence (matrix 80 × 80, voxel size 3 mm × 3 mm × 3.5 mm, SENSE 1.8, TE=30 ms, TR=1.8 s, 185 volumes per run). The subjects were awake and responsive during the MRI session, as periodically verified by asking them to make a small movement with their right foot in the time intervals between the different acquisition runs.

The data were preprocessed and analyzed using the Conn toolbox (https://web.conn-toolbox.org). The preprocessing pipeline consisted of the following steps: slice-timing correction (STC) using sinc-interpolation, motion correction, ART-based identification of potential outlier scans for scrubbing, and spatial smoothing (8 mm FWHM). The scrubbing outlier covariate was set from infinite to 0.5 mm (Table 1). Subsequently, preprocessed functional images were co-registered to the corresponding anatomical data, normalized to the MNI space, linearly detrended, and band-pass filtered (0.008–0.09 Hz, after regression).

To overcome the need for a priori assumptions in defining any ROIs, the rs-fMRI data were analyzed using a fully data-driven whole-brain intrinsic voxel-wise level analysis, specifically the ICC (Delli Pizzi et al., 2023; Martuzzi et al., 2011). The primary advantage of this approach is the ability to avoid any correlation threshold requirement (it does not rely on any a priori information) while still providing high-resolution results. The ICC analysis is typically employed to generate a picture illustrating potential global voxel-wise connectivity pattern alterations resulting from experimental manipulations (e.g., before and after a given treatment). Based on this, the ICC can be associated with degree centrality, a graph theory-based network analysis where the term 'degree' refers to the number of connections to a given voxel that exceeds a specific correlation threshold (Scheinost et al., 2012) (Zuo and Xing, 2014). The major difference between these two methods lies in the absence of a correlation threshold for the ICC. Although these thresholds are usually set to be large enough to prevent false positive results but small enough to maintain the important connections, the dependence on their choice may lead to flawed results (Scheinost et al., 2012). The ICC eliminates the need for a specific threshold to be chosen. This measure employs a graph-theoretical approach where each voxel-wise connection is weighted with its average r^2, signifying that the ICC is defined as the root mean square of correlation coefficients between each individual voxel and all of the voxels in the brain (Martuzzi et al. 2011). Consequently, higher values in ICC maps represent greater connectivity strength of that voxel with the whole brain.

Finally, the cortical clusters obtained from ICC analysis were used as the seeds for standard functional connectivity analysis (Zhou et al., 2022). Follow-up analysis of the FC between seeds and voxels was conducted to clarify the specific network contributing to the differences in global brain connectivity.

2.5. Statistical analysis

OMT versus sham effects were estimated based on differences between the two conditions (T2-T0). Specifically, we calculated the 'delta' as T2 minus T0 and generated an ICC image of (T2-T0). Next, to assess the net effect of OMT, we compared the delta map of the Sham group with the delta map obtained from the OMT group. Significant results for ICC maps were adjusted by setting a cluster-wise threshold at FDR-corrected $p < 0.05$. To mitigate the limitations of cluster-wise-based thresholding, we established a voxel-wise (cluster-forming) threshold at uncorrected $p < 0.001$ (Nieto-Castanon, 2020). Significant clusters resulting from group comparison were binarized using "fslmaths"

Table 1
FD and the ratio of the scrubbed volumes for each participant at T0 and T2.

Group	FD (Mean ± SD)	Scrubbed Volumes T0 (Mean ± SD)	Scrubbed Volumes T2 (Mean ± SD)
Sham	0.13 ± 0.03	11.29 ± 12.07	21.14 ± 36.34
OMT	0.12 ± 0.12	9.93 ± 9.56	7.67 ± 13.00

(Jenkinson et al., 2012) and employed to calculate the ICC values in each study participant. Within the OMT group, correlation analysis was conducted between functional connectivity metrics and clinical outcomes to explore the clinical significance of the observed changes. Specifically, step-wise linear regressions were performed between the rs-fMRI metric obtained from ICC and seed-based analyses (i.e., independent variables) and the difference between the two time points (T2-T0) on scores of the VAS and PPI (i.e., dependent variables), serving as two measures of pain intensity. Given that two distinct models were used (one for VAS and one for PPI), Bonferroni's correction for multiple comparisons was applied, setting the significance level at 0.025.

3. Results

3.1. Study sample and clinical outcomes

Demographic and clinical outcomes remained unchanged between subjects in the OMT and Sham cohorts (Cerritelli et al., 2020a; Cerritelli et al., 2021). At baseline, the groups were comparable in pain measurements (i.e., VAS and McGill score) and disability index (i.e., the RM and OWS questionnaires).

When we compared the longitudinal variation in pain scores we observed that the OMT, as compared to controls, determines a significant reduction of pain severity (VAS, OMT group: 44.8 ± 20.9, control group: 3.8 ± 20.4, $p < 0.001$), self-rated physical disability (RMD Questionnaire, OMT group: 6.8 ± 3.3, control group: 0.4±,4.7p < 0.001), disability resulting from low back pain (OWS, OMT group: 14.1 ± 2.7, control group: 1.5 ± 4.4, $p < 0.001$), and Present Pain Intensity (PPI, OMT group: −1 (range −1,0), control group: 1 (range 0, 2), $p < 0.01$).

3.2. Whole brain intrinsic functional connectivity

By comparing the OMT and Sham groups, we identified ICC variations in four cortical clusters associated with multiple layers of the pain matrix (Fig. 1; Table 2). Specifically, the OMT groups exhibited a cluster showing decreased ICC extending across the left superior parietal lobule (SPL) and extending over the primary somatosensory cortex (S1) on the postcentral gyrus (PG). Interestingly, the portion covering the post-central gyrus spatially overlaps with the characteristic cortical representation of the low back in Penfield's homunculus. Furthermore, the OMT group showed increased ICC in the anterior insula, a key component of the "attentional-perceptive" layer but also associated with interoception (Haruki and Ogawa, 2021), and in two regions of the right anterolateral prefrontal cortex, one located more ventrally and one more dorsally, potential component of the third order "affective-reappraisive" matrix.

3.3. Follow-up seed-to-voxel functional connectivity analysis

We conducted a series of seed-based analyses to test whether the observed increases/decreases of ICC were spatially specific, denoting robust changes of ROI-to-ROI connectivity, or spatially unspecific, signaling a largely distributed effect (Table 3). Notably, both the decrease of ICC of the left L-PG/SPL region and the increase of ICC of the right anterior insula were spatially unspecific, as no region survived the significance threshold. Thus, while the parietal cluster disconnects from the rest of the brain, the anterior insula generally appears to increase its connectivity with the cortex.

In contrast, the two right anterolateral prefrontal clusters increased their connectivity in a more spatially specific manner. Specifically, the ventral anterolateral prefrontal cluster increased its connectivity with the posterior division of the right supramarginal gyrus (R-pSMG) (Fig. 2). On the other hand, the dorsal anterolateral prefrontal cluster increased its coupling with the right insular cortex-frontal operculum (R-INS/fO) and the right supplementary motor area (R-SMA) (Fig. 2).

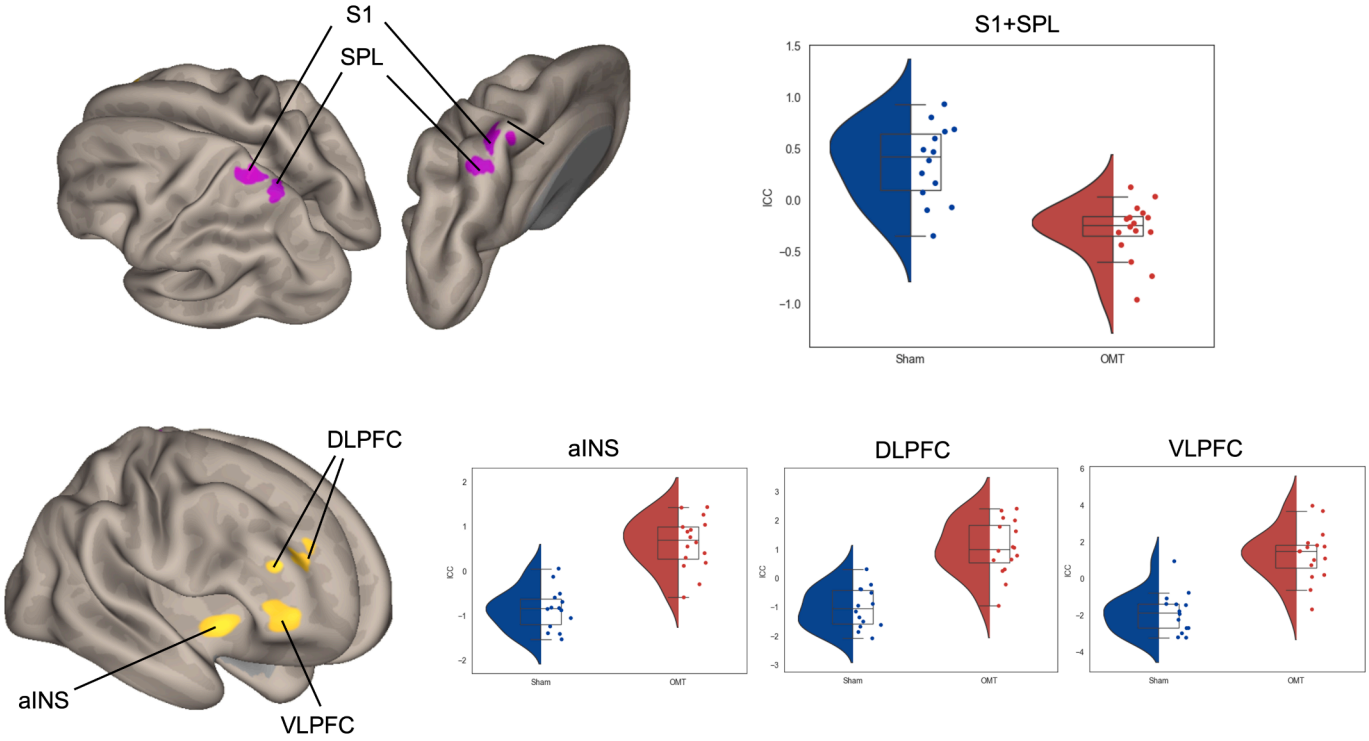

Fig. 1. Between-group differences in Intrinsic Connectivity Contrast. Maps show the clusters where intrinsic connectivity is significantly reduced (purple cluster) or increased (yellow cluster) in the OMT vs. Sham groups (T2-T0). The plots report the individual and group distribution of ICC metrics of each group and cluster. *Abbreviations:* aINS=anterior insula; DLPFC=Dorsolateral prefrontal cortex; SPL=superior parietal lobe; S1 = primary somatosensory cortex; VLPFC=Ventrolateral prefrontal cortex.

Table 2
Significant clusters obtained from ICC analysis and their characteristics/location.

Cluster	Coordinates (MNI template)			Region	size (mm^2)	size p-FWE	size p-FDR	size p-uncorr	peak p-FWE	peak p-unc
	X	Y	X							
1	−16	−40	68	L-PG/SPL	123	0.008859	0.007	0.0002	0.9862	0.000052
2	40	18	−6	R-aINS	94	0.034545	0.011	0.0009	0.1141	0.000001
3	48	48	0	R-VLPFC	92	0.038094	0.011	0.0010	0.4004	0.000004
4	32	44	16	R-DLPFC	78	0.076609	0.017	0.0021	0.8116	0.000016

Abbreviations.
aINS=anterior insula; DLPFC=dorso-lateral prefrontal cortex; PG=postcentral gyrus; SPL=superior parietal lobule; VLPFC=ventro-lateral prefrontal cortex.

Table 3
Summarized information on follow-up seed-to-voxel functional connectivity analysis.

ICC Cluster	Coordinates (MNI template)			SBC-derived target regions	size (mm^2)	size p-FWE	size p-FDR	size p-unc	peak p-FWE	peak p-uncorr
	X	Y	Z							
R-VLPFC	60	−40	32	R-pSMG	187	0.015641	0.011402	0.00076	0.899972	0.000052
R-DLPFC	46	12	−2	R-INS/fO	134	0.07306	0.042173	0.00383	0.429981	0.000009
	40	18	−6	R-SMA	105	0.160408	0.048595	0.00884	0.097938	0.000001

Abbreviations.
DLPFC=dorso-lateral prefrontal cortex; fO=frontal opercolum; ICC=Intrinsic Connectivity Contrast; pSMG=posterior division of the supramarginal gyrus; R=right; SMA=supplementary motor area; SFC=superior frontal cortex SBC=seed-based connectivity; VLPFC=ventro-lateral prefrontal cortex.

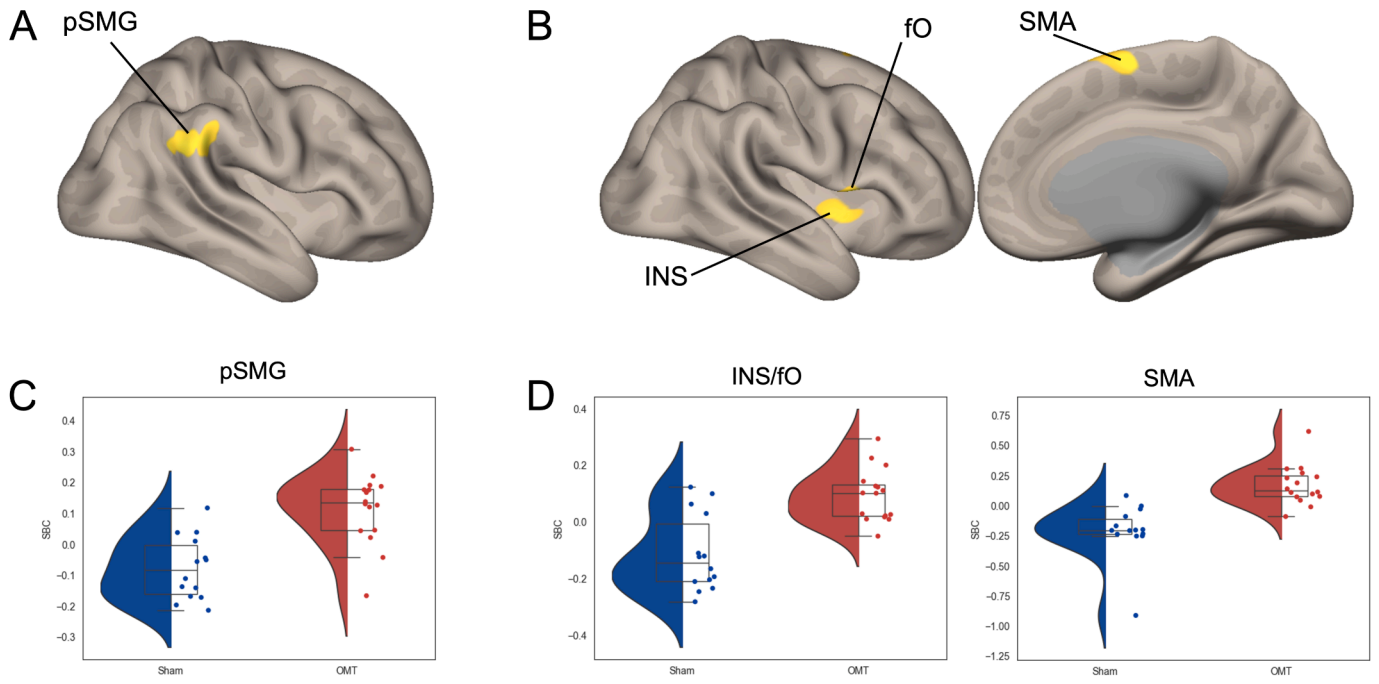

Fig. 2. Between-group differences in seed-based connectivity analysis. Maps show the clusters where functional connectivity of the VLPFC (panel A) or DLPFC (panel B) is significantly reduced (purple cluster) or increased (yellow cluster) in the OMT vs. Sham groups (T2-T0). The plots, relative to the VLPFC as the seed in panels C and the DLPFC as the seed in panel D, report the individual and group distribution of functional connectivity metrics (z-score) for each group and cluster. *Abbreviations:* INS=insula; fO=frontal opercolum; SMA=supplementary motor area; pSMG=posterior supramarginal gyrus.

3.4. Relationship of MRI measures with clinical outcome of pain perception

We tested the presence of a significant correlation between the ICC metrics and the longitudinal effect of OMT on pain perception (Fig. 3). Interestingly, we observed that only the increased ICC in the ventral anterolateral prefrontal cluster correlated with the variation of clinical measure of pain severity assessed by the VAS scale (β = -0.604, p = 0.017) as well as with self-rated physical disability, as assessed by the PPI scale (β = -0.577, p = 0.024).

4. Discussion

The present study investigated the brain correlates of OMT, in terms of changes of resting state functional connectivity, by employing a comprehensive data-driven approach over the entire brain. We found that OMT engenders significant modifications within the cortical functional architecture, particularly affecting four clusters belonging to different layers of the so-called pain matrix. A first effect was a decrease of connectivity in a parietal cluster that included the primary somatosensory cortex (S1), which is a basic component of the first order

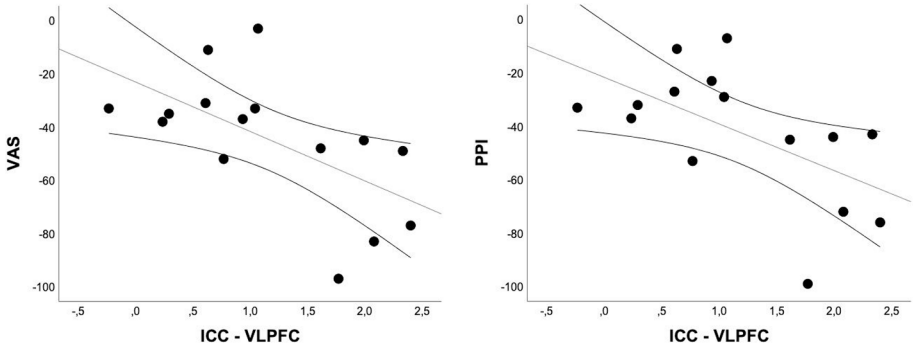

Fig. 3. Correlation between imaging and clinical outcomes. Scatterplot correlation within the OMT group between ICC values and scores measuring clinical scales of pain perception (VAS and PPI). OMT leads to a more significant increase in ICC strength, which correlates with a decreased perception of pain and vice versa.

"nociceptive" layer. Another effect was an increase of connectivity in a cluster located in the anterior insula, which is a key node of the "attentional-perceptive" layer. Notably, post-hoc analyses demonstrated that both clusters showed a diffuse effect with the rest of the cortex. Instead, two additional clusters, located in the anterolateral prefrontal cortex and putative members of the higher level "affective-reappraisive" layer, showed a more spatially specific increase in connectivity. Crucially, the change in the functional coupling of the more ventral cluster was selectively associated with the change in pain intensity, indicating a key role in the analgesic effect triggered by OMT.

Previous studies have shown that back pain is linked to diffuse increased functional connectivity of the primary somatosensory cortex (Pei et al., 2020). In this study, we found that the first effect of OMT was to produce a decrease of intrinsic functional connectivity within a portion of the primary somatosensory cortex, which allows the somatic-specific quality of the pain sensation, including localization and discrimination (Bushnell et al., 1999; Mayr et al., 2022; Pei et al., 2020). Of note, the location of the cluster that we identified closely matches the cortical representation of the low back in Penfield's homunculus (Penfield and Rasmussen, 1950) and recent studies by Gordon and colleagues (Gordon et al., 2023). Thus, this result is consistent with the idea that OMT modulate interoception in cLBP patients by directly shaping the activity of the primary somatosensory cortex. An alternative explanation considers pain perception within the active inference framework as an ongoing hierarchical process, wherein sensory inputs ascend from bottom-up sources to interact with top-down modulations and prior experiences across various cortical and subcortical regions constituting the pain matrix (Cerritelli and Esteves, 2022; Esteves et al., 2022; McParlin et al., 2022). In the specific context of our investigation, the sensory information about pain, originating from bottom-up pathways and reaching the primary sensory cortex, underwent modulation by OMT intervention. Consequently, the top-down processing of pain perception exhibited alterations, as evidenced by changes in pain scales. This suggests a potential shift in subjects' affective valence to pain stimuli (Esteves et al., 2022; Lersch et al., 2023). Consistent with the principles of active inference, it is noteworthy to recognize the roles of gain (in this case, the OMT effects) and modulation, which not only shape the present experience of pain but also contribute to the updating of prior beliefs, leading to the formation of a revised posterior estimation (Bohlen et al., 2021).

Notably, the parietal cluster extended posteriorly to include a region in dorsal posterior parietal cortex. The finding is consistent with prior research on cLBP patients treated with OMT (Bohlen et al., 2021; Cerritelli et al., 2020b; Tramontano et al., 2020). The parietal cortex, recognized for its involvement in attentional processing (Behrmann et al., 2004); (Corbetta and Shulman, 2002), also plays a crucial role in pain perception (Lobanov et al., 2013; Torta et al., 2017; Zhu et al., 2024). Specifically, posterior parietal areas do not receive direct spinothalamic afferents, but elaborate information coming from the nociceptive matrix and participate to the conscious pain perception and its attentional-cognitive modulations (Garcia-Larrea and Peyron, 2013). The brain controls pain perception via placebo analgesia and attentional modulation operating via distinct pathways (Norcliffe-Kaufmann et al., 2014; Wager and Atlas, 2015). Placebo analgesia, driven by positive treatment expectations irrespective of actual analgesic effects, highlights the significant influence of psychological factors on pain (Colloca et al., 2013). Our study's design, including a sham group, allowed us to account for this effect, ensuring that observed connectivity changes result from OMT rather than placebo. Conversely, attentional modulation, characterized by the deliberate redirection of attention away from painful stimuli towards alternative stimuli or activities, emerges as a potential explanatory mechanism for the reduction in connectivity observed in the superior parietal region following OMT. This suggests that OMT alleviates pain by affecting attentional networks, reducing perceived pain intensity.

Another node of the second order attentional perceptive pain matrix that was modulated by OMT was the anterior insula. The anterior insula is particularly well-suited for integrating various types of information in sensory processing (Benarroch, 2019), playing a key role in interoception, including monitoring sensations crucial for the integrity of the body's internal state, such as arousal levels (Gray et al., 2007). Several lines of evidence suggest that anterior insula activity is associated with the magnitude of pain perception (Baliki et al., 2009), reflecting the subjective pain experience, rather than nociception (Craig, 2009). Supporting this view, prestimulus activation of the anterior insula predicts the experience of touch (Lovero et al., 2009), and activation in these regions has been associated with sensory aspects and the perceived controllability of painful stimuli (Salomons et al., 2004). Based on these findings, we hypothesize that increased connectivity of the anterior insula might be associated with its role in the management of chronic pain and the decreased likelihood of perceiving the stimulus as painful.

The other two clusters that were modulated by OMT were found in ventral and dorsal anterolateral prefrontal cortex. These high-level polymodal regions are located outside the traditional pain matrix but are nonetheless associated with the interpretation of the pain perception and its affective component, which has consequences for the formation of memories. For example, anterolateral prefrontal regions are involved in pain modulation triggered by the observation of other people's pain (the so-called "compassional hyperalgesia" effect) (Godinho et al., 2012), but also in the analgesic effect deriving from placebo (Wager et al., 2004), self-control (Wiech et al., 2006) or religious beliefs (Wiech et al., 2008). Importantly, the activity in these areas can modulate subjective perception of pain without involving low-level perceptual processes, causing a reinterpretation of the pain experience rather than a change in the sensory gain. Moreover, these regions can bias pain perception by exploiting not only their connections with lower-level layers of the pain matrix, but also with subcortical regions involved in pain modulation, such as the periacqueductal gray matter. In this

regard, the present results indicate that the anterolateral prefrontal cortex shows a preferential connectivity change with putative components of the attentional-perceptive layer, such as the posterior parietal cortex and the insula. Moreover, the presence of a significant relationship between the increase of functional connectivity induced by the OMT in the ventral anterolateral prefrontal cluster and the analgesic effect reported by the participants strongly supports the idea that the network reorganization occurring in higher-order regions of the pain matrix is the one that shows the highest association with the subjective experience.

Our study has some limitations. Firstly, care should be taken when interpreting the results in terms of ICC values. First, ICC depends on the community size (Power et al., 2013). This implies that it is not possible to disentangle whether a node with high ICC is a connector hub (connecting nodes of different modules) or a provincial one (connecting nodes within the same module). Furthermore, ICC depends on the average weight of the network and thus might be biased by an eventual large number of edges with small weights. Secondly, we recognize that the study sample is relatively small. However, we would like to emphasize that, as explained in detail in our previous study (Cerritelli et al., 2021), Cohen's apriori statistical power was adequate. Finally, the current study focused on OMT's immediate and 1-month sustained effects on chronic pain patients. Future research is necessary to investigate whether there are longer-term benefits, both clinically and in terms of brain connectivity changes. Additionally, it will be important to determine the optimal frequency and duration of OMT sessions. Previous research suggests that eight treatment sessions over six months can reduce pain, disability, and migraine attacks. Therefore, comparing immediate effects with long-term outcomes could provide insights into potential brain changes.

4.1. Conclusions

Our study supports OMT's effectiveness in chronic pain treatment and lays the groundwork for understanding its neurobiological mechanisms. Using a data-driven approach, we revealed OMT's ability to reshape brain connectivity patterns involving all the level of the pain matrix, although the analgesic effect appears to be more strongly associated with the modulation of the affective layer.

CRediT authorship contribution statement

Federica Tomaiuolo: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Francesco Cerritelli:** Conceptualization, Data curation, Formal analysis, Project administration, Resources, Writing – review & editing. **Stefano Delli Pizzi:** Conceptualization, Investigation, Supervision, Writing – original draft. **Carlo Sestieri:** Conceptualization, Writing – original draft. **Teresa Paolucci:** Writing – review & editing. **Piero Chiacchiaretta:** Writing – review & editing. **Stefano L. Sensi:** Writing – review & editing. **Antonio Ferretti:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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