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Network properties and regional brain morphology of the insular cortex correlate with individual pain thresholds

Lynn Neumann[1] | Niklas Wulms[2] | Vanessa Witte[3] | Tamas Spisak[4] | Matthias Zunhammer[4] | Ulrike Bingel[4] | Tobias Schmidt-Wilcke[5,6]

[1]Medizinische Klinik I, Klinik für Innere Medizin, Nephrologie und Dialyse, Osteologie und Rheumatologie, St. Franziskus-Hospital Münster, Münster, Germany

[2]Institut für Epidemiologie und Sozialmedizin, Universitätsklinikum Münster, Münster, Germany

[3]Klinik für Dermatologie, Venerologie und Allergologie, St. Josef-Hospital Bochum, Ruhr-Universität Bochum, Bochum, Germany

[4]Klinik für Neurologie, Universitätsklinikum Essen, Essen, Germany

[5]Institut für Klinische Neurowissenschaften und Medizinische Psychologie, Heinrich Heine Universität, Düsseldorf, Germany

[6]Neurologisches Zentrum, Bezirksklinikum Mainkofen, Deggendorf, Germany

Correspondence
Tobias Schmidt-Wilcke, Neurologisches Zentrum, Bezirksklinikum Mainkofen, Mainkofen A 3, 94469 Deggendorf, Germany.
Email: tobias-schmidt-wilcke@t-online.de

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Abstract

Pain thresholds vary considerably across individuals and are influenced by a number of behavioral, genetic and neurobiological factors. However, the neurobiological underpinnings that account for individual differences remain to be fully elucidated. In this study, we used voxel-based morphometry (VBM) and graph theory, specifically the local clustering coefficient (CC) based on resting-state connectivity, to identify brain regions, where regional gray matter volume and network properties predicted individual pain thresholds. As a main finding, we identified a cluster in the left posterior insular cortex (IC) reaching into the left parietal operculum, including the secondary somatosensory cortex, where both regional gray matter volume and the local CC correlated with individual pain thresholds. We also performed a resting-state functional connectivity analysis using the left posterior IC as seed region, demonstrating that connectivity to the pre- as well as postcentral gyrus bilaterally; that is, to the motor and primary sensory cortices were correlated with individual pain thresholds. To our knowledge, this is the first study that applied VBM in combination with voxel-based graph theory in the context of pain thresholds. The co-location of the VBM and the local CC cluster provide first evidence that both structure and function map to the same brain region while being correlated with the same behavioral measure; that is, pain thresholds. The study highlights the importance of the posterior IC, not only for pain perception in general, but also for the determination of individual pain thresholds.

KEYWORDS
cluster coefficient, graph theory, pain, resting-state fMRI, voxel-based morphometry

Abbreviations: AAL, Automated Anatomical Labeling; ACC, anterior cingulate cortex; BOLD, blood oxygen level-dependent; CAT, Computational Anatomy Toolbox; CC, clustering coefficient; CPT, cold pain threshold; CSF, cerebrospinal fluid; CT-A, cortical thickness analysis; DMN, default mode network; DTI, diffusion tensor imaging; fc, functional connectivity; fMRI, functional magnetic resonance imaging; FWE, family-wise error; GABA, γ-aminobutyric acid; GM, gray matter; HPT, heat pain threshold; IC, insular cortex; MCC, mid cingulate cortex; MPT, mechanical pain threshold; MRI, magnetic resonance imaging; OP, parietal operculum; PCC, posterior cingulate cortex; QST, quantitative sensory testing; ROI, region of interest; rs-fc, resting-state functional connectivity; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; TIV, total intracranial volume; VBM, voxel-based morphometry; WM, white matter.

Lynn Neumann and Niklas Wulms contributed equally.

1 | INTRODUCTION

Pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja et al., 2020), whereby pain thresholds mark the point along a curve of increasing intensity perception of a stimulus at which pain begins to be felt. Pain thresholds vary considerably across individuals and they undergo intra-individual fluctuations across time. A number of behavioral/psychological traits (Coghill, 2010; Geva, Pruessner, & Defrin, 2014), genetic (Matic, van den Bosch, de Wildt, Tibboel, & van Schaik, 2016; Meloto et al., 2016; Vuilleumier et al., 2018) and neurobiological factors (Zunhammer et al., 2016) have been identified to influence various aspects of pain perception, for example, pain thresholds, pain tolerance and pain modulation. However, the neurobiological underpinnings that account for these differences remain to be fully elucidated.

Both structural and functional brain imaging studies have substantially advanced our understanding of functional brain anatomy underlying the percept of pain. Voxel-based morphometry (VBM), cortical thickness analyses (CT-A), as well as diffusion tensor imaging (DTI) (Wang et al., 2017) have been applied to investigate associations between pain sensitivity and regional brain morphology (Emerson et al., 2014; Ruscheweyh, Wersching, Kugel, Sundermann, & Teuber, 2018; Villemure et al., 2014), furthermore alterations in regional brain morphology in chronic pain states have been described. However, the neural mechanisms underlying these alterations, especially addressing the question whether they are a consequence of a prolonged pain experience or are preexisting, associated with an increased vulnerability to develop chronic pain, remain to be fully elucidated.

The application of experimental pain stimuli and the detection of related brain activations have been used to identify key regions involved in the processing of a nociceptive input (Apkarian, Bushnell, Treede, & Zubieta, 2005; Peyron, Laurent, & García-Larrea, 2000; Xu et al., 2020). Current research very much favors a network perspective, looking at the integration of various brain regions enabling the perception of pain with its manifold aspects. More specifically, cortical and subcortical regions like the primary and secondary somatosensory cortices (SI and SII), the insular cortices (IC), the anterior, mid and posterior cingulate cortices (ACC, MCC and PCC), the orbitofrontal cortices, as well as subcortical structures such as the basal ganglia and the thalamus have been identified to play a critical role in pain processing (Duerden & Albanese, 2013).

Besides, task and stimulus related functional magnetic resonance imaging (fMRI), resting-state fMRI and functional connectivity analyses have been used to investigate brain function and its relation to perception and behavior. While no specific stimulus is applied, the correlations of task-free BOLD fluctuations across specific brain regions serves as a marker to determine the degree of resting-state functional connectivity (rs-fc) between these regions, which in turn can then be related to behavioral measures. Importantly, resting-state activity is known to resemble task evoked activity patterns (Smith et al., 2009; Tamás Kincses et al., 2008). A number of imaging studies have used resting-state fMRI in comparing patients with chronic pain to healthy controls, demonstrating increased IC to default mode network (DMN) connectivity (Napadow, Kim, Clauw, & Harris, 2012), and IC to cingulate cortex (Ichesco et al., 2014) connectivity in chronic pain patients. However, only a few studies have performed rs-fc analyses in the context of pain thresholds (Galli et al., 2016; Spisak et al., 2020) and more research is needed to determine whether rs-fc can be used to reliably predict pain sensitivity and possibly serve as a biomarker for the vulnerability to develop chronic pain.

An interesting advancement of rs-fc analyses is the development of network analyses within the framework of graph theory, which takes more than two regions into account and, when performed on a voxel level, can even be performed within an explorative whole brain analysis. While the regions of interest serve as nodes, rs-fc between nodes is used to determine edges (with different strength) within the network, which allows the calculation of specific global or local (nodal) network parameters. In the case of the local network parameters, these can be mapped onto the brain to indicate which regions host these properties. In this study, we applied voxel-based graph theory, based on rs-fc, to identify brain regions where local network parameters, that is, the degree and the local clustering coefficient (CC) correlate with individual pain sensitivity, the former thought to reflect the importance of a specific node within a network, the latter indicating local cohesiveness and the tendency to form groups. We had specific a priori hypotheses for the somatosensory cortices, the ACC/MCC and the IC, which are all regions known to show robust activations during nociceptive processing, but also known to serve as major hubs within resting-state networks (Bagarinao et al., 2020).

To our knowledge, there are no studies that have linked brain morphology to brain function, specifically rs-fc and network parameters, when investigating neural correlates of pain sensitivity. Against this background, we also performed VBM analyses, searching for brain areas where regional gray matter (GM) volume correlated with pain sensitivity. We explored, whether in some brain regions the network parameters, for example, the local CC, and morphology parameters, that is, regional GM volume, would coincide, suggesting that both measures, although derived from different brain imaging methods, not only relate to human perception, but also each other, suggesting an interaction between brain function and structure. Finally, we used the clusters identified by the VBM analysis and the network analyses as seed regions to perform seed to voxel rs-fc analyses.

2 | METHODS

2.1 | Participants and behavioral measures

Data collection took place in the time period from January to August 2015. The study had been approved by the ethics committee of the Ruhr-University Bochum in 2014 [Ethics Application No.:4974-14]. The data presented here had been collected within a study performing GABA spectroscopy in pain processing brain regions (Zunhammer et al., 2016).

The study cohort consisted of 39 healthy, right-handed, nonsmoking, healthy volunteers who had been acquired at the Ruhr-University Bochum. Exclusion criteria for study participation were as follows: (1) psychiatric diseases, (2) comorbidities (e.g., diabetes mellitus, polyneuropathy), (3) regular use of medication (e.g., thyroid hormones, antidepressants, analgesics) and (4) pregnancy. For their participation in the study subjects received a reimbursement of 20 € per hour.

The test persons were instructed to avoid caffeine or alcohol on the day of the measurement and the day before. All subjects gave their written consent prior to study enrollment. The testing was carried out either on two consecutive days or with an intermediate rest day. On day 1, the subjects first underwent the QST measurements, followed by the completion of two personality questionnaires. On day 2, magnetic resonance imaging (MRI) was performed, followed by another QST measurement after a 15-min break. Measurements for heat pain (HPT), cold pain (CPT), and mechanical pain (MPT) thresholds were performed by certified examiners (Geber et al., 2009) using the standardized QST protocol (Rolke et al., 2006). Warmth and cold detection thresholds were obtained as additional control measures, to ensure normal non-nociceptive somatosensory function.

Thermal perception and nociception were assessed using the MSA thermal stimulator (Somedic, Hörby, Sweden); the thermode was placed on the palmar side of the left forearm, proximal to the wrist. Rising and falling temperatures were applied to the subjects' skin as a method of limits and participants were instructed to indicate the onset of HPT or CPT by a button press. For all thermal thresholds, instead of the three repetitions for each stimulus type required by the protocol, six passes were performed to reduce the subjects' person-dependent variations. To determine MPT, a staircase method was used, in which pinpricks (MRC Systems, Heidelberg, Germany) were alternately placed on the skin (five increasing and five decreasing trains of pinprick stimuli were applied), and had to be categorized by the participants as painful or not painful.

2.2 | MRI data acquisition

MRI was performed using a Philips 3.0 T Achieva scanner (X-Series, Best, the Netherlands) with a 32-channel head coil. Study participants were instructed to keep their head still and to avoid movement during scanning. First, a structural, high-resolution T1-weighted image was obtained in a MPRAGE sequence (repetition time: 8.5 ms, echo time: 3.9 ms, flip angle: 8°, matrix size: 240 mm × 240 mm). Two-hundred and twenty layers with 1 mm layer thickness and a voxel size of 1mm^3 were applied. Afterwards, γ-Aminobutyric acid (GABA) spectroscopic measurements were performed in the right insula and the bilateral ACC and MCC (Zunhammer et al., 2016). Spectroscopy results are not subject to the current investigation and will not be further discussed in the present article. Functional resting-state measurements were acquired by using a T2-weighted EPI SENSE sequence of images with a TR of 2,500 ms, TE = 35 ms, flip angle of 90°, a voxel size of 3 mm^3 per slice with a gap of 3.3 mm between slices, 40 slices per volume (200 volumes in the whole sequence) orientated parallel to the AC-PC line.

2.3 | Data analysis

2.3.1 | Quantitative sensory testing (QST) measures

Statistical analyses were performed using MATLAB (Version R2016a, The MathWorks Inc. Natick, MA). The evaluation of the measurement results was based on the specifications for the calculation of the quantitative sensory testing (QST) protocol (Rolke et al., 2006). Of the six test runs that were performed for each thermal stimulus, the first test run in each case was considered a trial run and rejected. Thus, the calculated values were determined from the remaining five test runs. For HPT and CPT, the arithmetic mean of the five measured temperatures was calculated and then log-transformed. The values determined from five increasing and five decreasing mechanical stimuli were averaged and log-transformed to determine the MPT. Measurements from both days were averaged to yield more robust modality specific pain sensitivity scores. Finally, all pain thresholds, for HPT, CPT and MPT, were combined into one overall pain sensitivity score (Spisak et al., 2020; Zunhammer et al., 2016). The distribution of each variable was examined visually for normality using histograms. Outliers were identified using MATLAB's boxplot function at default settings, that is, values were excluded when deviating from the median by more than 2 times the interquartile range (approximately corresponding to a probability of $p < .007$ on the 2-tailed normal distribution); scores were then normalized using a z-transformation. The results for HPT and MPT were multiplied by −1, so that high z-values indicated high pain sensitivity for all modalities. Finally, the arithmetic mean was calculated to yield the overall pain sensitivity score, used as the independent variable for the brain imaging analyses.

2.3.2 | Voxel based morphometry

Preprocessing of structural images for the VBM analyses was performed using CAT (Computational Anatomy Toolbox; www.neuro.uni-jena.de/cat/) within the SPM12 environment (Wellcome Trust Centre for Neuroimaging, University College, London, UK) running under MATLAB R2016b (Mathworks). The preprocessing steps involved spatial normalization to the same stereotactic space (using the DARTEL algorithm implemented in SPM12), segmentation and spatial smoothing (Gaussian kernel of 8 mm full-width at half maximum for GM images). Modulated images (voxel size: 1.5 mm^3) were used for statistical analyses; correspondingly, GM values are referred to as regional GM volume. Total intracranial volume (TIV) was also calculated and later added as nuisance variable to the statistical models. To avoid possible edge effects around the border between gray and white matter and to include only relatively homogenous voxel, we excluded all voxels with an absolute intensity value of <0.1 (of a maximum value of 1).

Voxel-wise statistics were performed in the framework of the general linear model implemented in SPM12. We performed regression analyses, using the multiple regression model, to identify brain regions where the independent variable, that is, the pain sensitivity score, was associated regional GM volume. Age and total intracranial

volume were added as nuisance variables to all models. Corrections for multiple comparisons were performed as follows. Statistical parametric maps were thresholded at $p < .005$ (Borsci et al., 2009). Results were corrected then for multiple comparisons throughout the whole brain ($p < .05$, cluster-level corrected, using family-wise error [FWE] correction).

2.3.3 | Resting-state functional connectivity

Resting-state functional connectivity analyses, both preprocessing as well as statistical analyses investigating seed-to-voxel connectivity were performed using the CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012) and SPM 12 (Welcome Trust Department of Cognitive Neurology, London, UK). Preprocessing steps of CONN's default MNI pipeline included slice-time correction, realignment (to the first image of the time-series), and normalizing using the structural segmented and normalized T1 weighted image that had also been warped to the Montreal Neurological Institute (MNI) template; that is, applying the warping parameters derived from the T1 warping procedure to the EPI images, generating $2 \times 2 \times 2\ mm^3$ resolution images and smoothing (FWHM: 8 mm Gaussian Kernel).

Individual GM, WM and CSF masks, derived from the normalized T1 weighted images, were used for the denoising procedure, which first applies linear regressions for the removal of physiological noise; that is, the BOLD signal from WM and CSF (5 dimensions each) and realignment parameters (6 motion parameters), and in a second step performs band-pass filtering. For bandpass filtering default settings (0.008–0.1 Hz) were applied. First level analyses comprised seed region to voxel analyses. The two clusters identified in the VBM analyses (see below), as well as the CC identified in the network analysis (see below) served as seed regions.

Correlation maps were created indicating to which degree the BOLD signal in the seed region (averaged BOLD signal) correlated with the voxels in the brain, yielding a correlation coefficient (*r*-value) per voxel. After *r* to fisher *z* transformation, the corresponding fisher *z* maps were entered into second level analyses, with the pain sensitivity score as independent variable and age serving as nuisance variable. The clusters identified were corrected for multiple comparisons throughout the whole brain ($p < .05$, cluster-level corrected, using FWE correction). Results were interpreted as follows: pain sensitivity was strongly associated with rs-fc between the clusters identified in the rs-fc analysis and the corresponding seed regions.

2.3.4 | Network analysis—local clustering coefficient

Network constructions and analyses were performed using the preprocessed resting-state time-series. For each subject, we constructed a voxel-wise functional network with a voxel size of $4 \times 4 \times 4\ mm^3$ representing a node, the inter-voxel temporal correlation of the BOLD signal, that is, the rs-fc, represented the edge weights. The BOLD signal was extracted using the REX toolbox, implemented in CONN, applying a binary GM mask (voxel size $4 \times 4 \times 4\ mm^3$) yielding a 16,931 × 16,931 adjacency matrix. In the second step the adjacency matrix was thresholded and binarized, that is, only the strongest edges (top 10%) representing the connections with the highest (positive) correlations coefficients survived (edge weight: 1), nonsurviving edges were given an edge weight of 0, yielding a weighted, undirected network. For all the nodes we calculated the degree, that is, the sum of all the remaining edges (to any other node), and the (local) cluster coefficient (CC). The degree and local CC were calculated using the brain connectivity toolbox (Rubinov & Sporns, 2010). Mathematical formula:

$$\text{Degree}: k_i = \sum_{j \in N} a_{ij}$$

$$\text{Clustering coefficient}: C_i = \frac{1}{n} \sum_{j \in N} \frac{2t_i}{k_i\,(k_i - 1)}$$

N is the set of all nodes in the network, and *n* is the number of nodes; a_{ij} is the connection status between *i* and *j*; $a_{ij} = 1$ when the link (*i*,*j*) existed; that is, in that case *i* and *j* were neighbors. In the current study the link (*i*,*j*) existed, if the correlation coefficient was among the top 10%; otherwise a_{ij} was set to zero.

While the degree of a node is considered to indicate its importance within the network (with no indication whether the neighbors are connected to each other), the local CC is a measure to quantify how close the neighbors of a given node (those nodes that are still connected to a given node after thresholding) are connected to each other (how close they are to being a complete graph). A high local CC is interpreted to indicate local cohesiveness and a high tendency to form groups.

For each subject, both parameters, degree and CC (one number per voxel) were projected back onto the MNI template, yielding one NIFTI per person. Finally smoothing was performed (Gauss filter of 4 mm FWHM), smoothed images were then submitted to further analyses; that is, a multiple regression with pain sensitivity as predictor, the network parameter as dependent variable, and age as nuisance. Corrections for multiple comparisons were performed as before. Statistical parametric maps were thresholded at $p < .005$ (voxel level). Results were then corrected for multiple comparisons throughout the whole brain ($p < .05$, cluster level corrected, using FWE correction).

3 | RESULTS

3.1 | Detection and pain thresholds

Thirty-nine healthy participants ($m = 25$, $f = 14$; mean age = 26) were enrolled in the final analyses. Mean pain scores were as follows: HPT = 43.7°C ($SD = 3.3$); CPT was at 14.1°C ($SD = 7.5$), MPT = 55.5 mN ($SD = 57.3$). There were no significant differences in pain sensitivity (QST score) or individual pain scores between male and female

study participants. There was a highly significant negative correlation between HPT and CPT ($r = -.66$; $p = .004$). No statistically significant correlations could be found between age and HPT, CPT or MPT. For details, see Table 1.

3.2 | Voxel based morphometry

We found two clusters displaying a positive and two clusters displaying a negative association between the pain sensitivity score and GM volume ($p < .05$, whole brain FWE corrected). One cluster in the left parietal operculum (OP 1 and 2; whereby OP 2 comprises the secondary somatosensory cortex, SII), reaching into left posterior insula and the left superior temporal gyrus ($x = -44$, $y = -24$, $z = 0$; $k = 2{,}437\ mm^3$; $p = .015$, cluster level FWE corrected) showed a positive association between the pain sensitivity score and regional GM, such that increased pain sensitivity predicted higher GM volume. Likewise, another cluster in the left orbitofrontal/inferior frontal gyrus showed a trend towards a positive association between pain sensitivity and regional GM ($x = -50$, $y = 46$, $z = -9$; $k = 1{,}708\ mm^3$; $p = .093$, cluster level FWE corrected). Furthermore, a cluster in the left thalamus ($x = -12$, $y = -26$, $z = 0$; $k = 2{,}511\ mm^3$; $p = .012$, cluster level FWE corrected) displayed a negative association with pain sensitivity, such that increased pain sensitivity was associated with less GM volume in the left thalamus. Finally, a cluster, also with a negative association, was found in the left parahippocampus ($x = -18$, $y = -32$, $z = -15$; $k = 1{,}964\ mm^3$; $p = .048$ cluster level FWE corrected). For details, see Table 2. For display purposes, statistical maps were superimposed on SPM152 template (from the MNI152) in MRIcroGL (1.2.20201102), for exact localization the Automated Anatomical Labeling (AAL) Atlas was used (Tzourio-Mazoyer et al., 2002). For details, see Table 2 and Figure 1.

3.3 | Resting state functional connectivity (seed based)

Two seed-to-voxel rs-fc analyses were performed, with the cluster found in the left thalamus and the left posterior insula/OP 1 serving as seeds with the pain sensitivity score as independent variable). The cluster identified in the left operculo-insular region (peak coordinates: $x = -44$, $y = -24$, $z = 0$; yielded several highly significant target regions: the left pre- and postcentral gyrus (ipsilateral to the seed region; $x = -60$, $y = 8$, $z = 36$; $k = 1{,}496\ mm^3$; $x = -44$, $y = -12$, $z = 62$, $k = 13{,}488\ mm^3$), the latter cluster extended in the left superior frontal and medial frontal gyrus); as well as the right pre- and postcentral gyrus ($x = 20$, $y = -6$, $z = 64$; $k = 7{,}584\ mm^3$; $x = 44$, $y = -20$, $z = 48$; $k = 1864\ mm^3$) contralateral to the seed region (all whole brain FWE corrected. Furthermore, one cluster in the left mid IC was detected ($x = -36$, $y = -2$, $z = 6$; $k = 2{,}544\ mm^3$), and one cluster in the left inferior/middle frontal gyrus ($x = -42$, $y = 26$, $z = 16$; $k = 3{,}712\ mm^3$). For all clusters, rs-fc was positively associated with the pain sensitivity score, that is, the more pain-sensitive study participants were, the higher the rs-fc between the left operculo-insular region and the target cluster. No significant clusters were identified when using the left thalamus cluster as seed region and the pain sensitivity score as independent variable. For details, see Table 3 and Figure 2.

3.4 | Network analysis—local clustering coefficient

After correction for multiple comparisons a significant association between the pain sensitivity score and the local CC was found in the left IC ($x = -38$; $y = -24$, $z = -2$; cluster size: 111 voxels = 7,104-mm^3; $p = .041$, cluster level FWE corrected). Higher CC values were associated with higher pain sensitivity scores. No such association was found for the network parameter "degree." For details, see Table 4 and Figure 3a,b.

The VBM cluster and the CC cluster showed an overlap (64 mm^3, center: $-x = 42$, $y = -22$, $z = 2$). The distance of the two centers of gravity was 6.3 mm. The eigenvariates of the two clusters were not significantly correlated ($r = .18$; $p = .13$). In order to further investigate, whether regional GM volume was related to the local CC, we also extracted the GM values of posterior IC, where the CC cluster was located, and vice versa, we extracted he CC values applying the mask of the GM cluster in the operculo-temporo-insular cortex region. In both locations we did not find a significant correlation between GM and the local CC ($p < .2$).

TABLE 1 Behavioral data

Group		Mean	Range	*SD*
Sex ratio	25 males/14 females			
Age (in years)		26	19.6–39.1	4
Heat pain threshold (in °C)		43.7	35.9–48.3	3.3
Cold pain threshold (in °C)		14.1	4–30	7.5
Mechanical pain threshold (in mN)		55.5	12–300.4	57.3
QST-score		0		1

Note: This table describes the behavioral data. There was a highly significant negative correlation between heat and cold pain thresholds ($r = -67$; $p < .001$).
Abbreviation: QST, quantitative sensory testing.

TABLE 2 Voxel based morphometry

Localization	Side	Association	MNI coordinates (*x y z*)	Cluster size in mm^3	*z*-values (peak coordinate)
Superior temporal gyrus/operculum (subregion 1)/ posterior insular cortex	L	Positive	−44 −24 0	2,437	3.42
Orbitofrontal/inferior frontal gyrus	L	Positive	−50 46 −9	1,708	3.72
Thalamus	L	Negative	−12 −26 0	2,511	3.94
Parahippocampus	L	Negative	−18 −32 −15	1,964	3.79

Note: This table displays four clusters with significant associations between the pain sensitivity score and regional gray matter volume. All results, apart from the finding in the left orbitofrontal cortex (showing a trend, $p = .093$), were statistically significant after correcting for multiple comparison (FWE, whole brain corrected). In the left operculo-insular region and the left orbitofrontal cortex positive correlations were found, that is, increased pain sensitivity was associated with an increased local gray matter volume. In the left thalamus and parahippocampus negative correlations were found, that is, increased pain sensitivity was associated with decreased regional gray matter volume.
Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right.

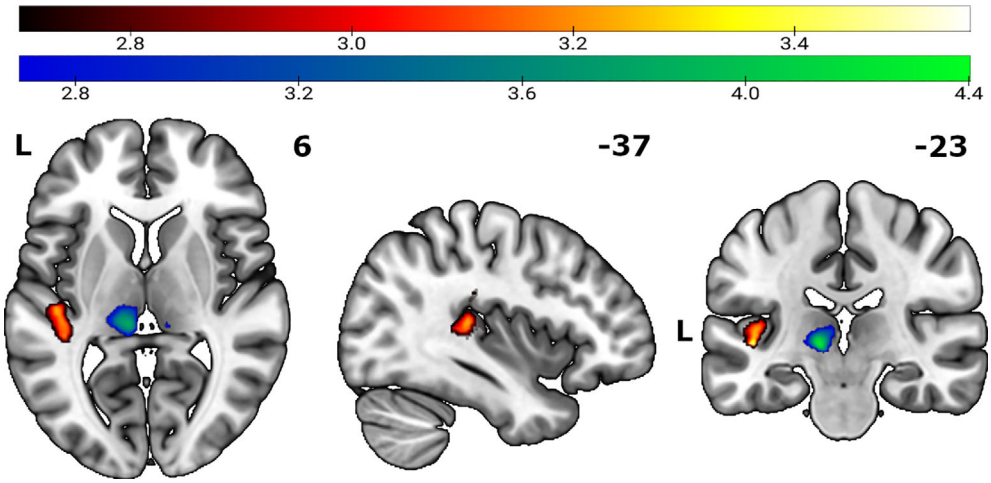

FIGURE 1 Voxel-based morphometry analysis. This figure displays the clusters identified by voxel-based morphometry analysis. Clusters in red indicate a positive association with pain sensitivity scores; that is, the higher the regional gray matter volume, the higher the pain sensitivity. Clusters in blue indicate a negative association; that is, study participants with lower local gray matter volume displayed higher pain sensitivity scores. Clusters are thresholded at $p < .005$ voxel level, with a cluster size > 500 contiguous voxels)

When using the CC cluster as seed region for seed to voxel rs-fc analyses (with the pain sensitivity score as predictor) no functionally connected regions could be identified.

4 | DISCUSSION

We used VBM and graph theory based on rs-fc to identify brain regions, where regional GM volume and the local CC correlated with individual pain thresholds in healthy volunteers. As a main finding, we identified a cluster in the left operculo-insular cortex, including the SII cortex and the posterior IC, where regional GM volume correlated with pain sensitivity. At the same time we found a cluster in the left posterior IC, located proximately anterior to the VBM cluster, where the local CC was also highly correlated with pain sensitivity. To our knowledge, this is the first study that applied graph theory in the context of pain thresholds. Although we found a co-location of the VBM and the CC cluster which might have indicated that brain structure and function relate to each other via network properties (Pur, Eagleson, de Ribaupierre, Mella, & de Ribaupierre, 2019), there were no correlations between regional GM and the local CC, neither between the clusters identified in the whole brain analyses, nor when extracting the GM values applying the CC cluster mask and vice versa. When using the VBM cluster as a seed to investigate seed to voxel rs-fc, we found very strong association between pain sensitivity and the rs-fc between the left operculo-insular cortex and the left mid insular cortex, the primary somatosensory and motor cortices bilaterally, as well as the superior and medial frontal gyrus, all key regions of the pain network.

In our study, we identified several regions where local GM volume was related to pain sensitivity, that is, in the left operculo-insular junction, the left orbitofrontal cortex, the left thalamus, and the left parahippocampus. Specifically, the operculo-insular junction and the thalamus are regions which are well known to play a key role in pain perception. Quite a few structural imaging studies have investigated the association between local GM or cortical thickness and pain

TABLE 3 Resting state functional connectivity analysis

Localization	Side	Association	MNI coordinates (*x y z*)	Cluster size in mm^3	*z*-values (peak coordinate)
Precentral gyrus/inferior frontal gyrus	L	Positive	−60 8 36	1,496	3.90
Postcental/precentral gyrus/superior frontal gyrus/ medial frontal gyrus/mid cingulum	L	Positive	−44 −12 62	13,488	4.38
Postcental/precentral gyrus/superior frontal gyrus/ medial frontal gyrus	R	Positive	20 −6 64	7,584	4.37
Postcental/precentral gyrus	R	Positive	44 −20 44 52 −8 46	1,864	3.76
Insular cortex	L	Positive	−40 −28 12 −36 −2 6	2,544	3.95
Inferior/middle frontal gyrus	L	Positive	−42 26 16	3,712	4.47

Note: This table displays multiple clusters with a significant association between the pain sensitivity score and resting state connectivity from the left operculo-insular cortex (to the identified clusters). The cluster identified in the VBM analysis in the left operculo-insular region (peak coordinates: −44, −24, 0) served as a seed region. Functional connectivity to the right and left pre- and postcentral gyrus as well as the left mid insular cortex positively correlated with the pain sensitivity score. The more pain-sensitive subjects were, the greater the resting state functional connectivity. Results are statistically significant after correcting for multiple comparison (FWE, whole brain corrected).
Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right.

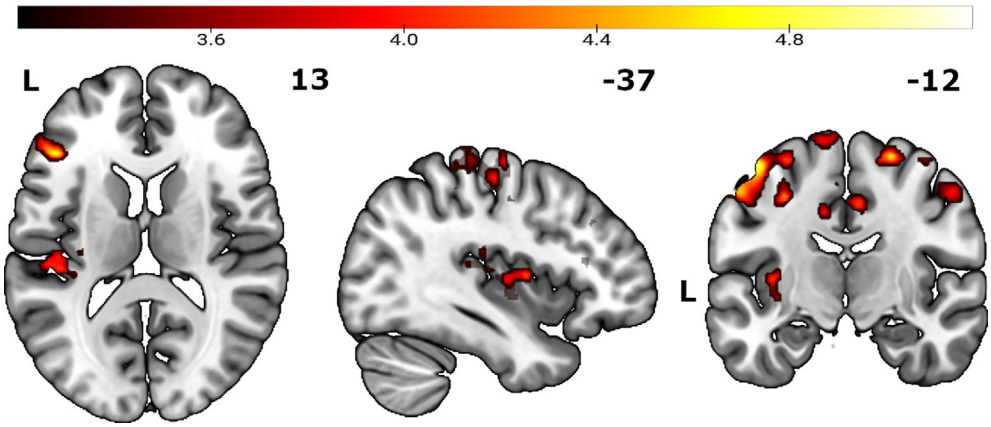

FIGURE 2 Seed to voxel resting state functional connectivity analysis. This figure shows the target clusters, derived from the resting-state functional connectivity analysis, with the cluster in the left operculo-insular cortex, identified in the voxel-based morphometry analysis (peak coordinate: $x = -44$, $y = -24$, $z = 0$), serving as seed region. Study participants with high seed to target functional connectivity displayed high pain sensitivity scores

TABLE 4 Network analysis

Localization	Side	Association	MNI coordinates (*x y z*)	Cluster size in mm^3	*z*-values (peak coordinate)
Insular cortex	L	Positive	−38 −24 −2	7,104	3.92

Note: This table displays one cluster with a significant association between the pain sensitivity score and the local clustering coefficient. In the left insular cortex a positive correlation was found, that is, increased pain sensitivity was associated with an increased local clustering coefficient. The cluster reached into the left orbitofrontal cortex. Results are statistically significant after correcting for multiple comparison ($p = 0.041$; FWE whole brain corrected).
Abbreviations: L, left; MNI, Montreal Neurological Institute; R, right.

sensitivity so far (Emerson et al., 2014; Erpelding, Moayedi, & Davis, 2012; Kramer, Jutzeler, Haefeli, Curt, & Freund, 2016; Tseng et al., 2013). However, due to methodological differences in brain imaging techniques and analyses used, as well as differences in the assessment of pain sensitivity, the results are still heterogeneous. Still, the thalamic finding is in line with Elsenbruch et al. reporting that lower rectal sensory as well as pain thresholds, that is, increased sensitivity, were associated with reduced GM volume in the right thalamus. Importantly, reductions in thalamic GM have been found in various chronic pain states (Smallwood et al., 2013), such as neuropathic pain (Draganski et al., 2006; Henderson et al., 2013), chronic back pain, chronic pelvic pain (As-Sanie et al., 2012; Bhatt

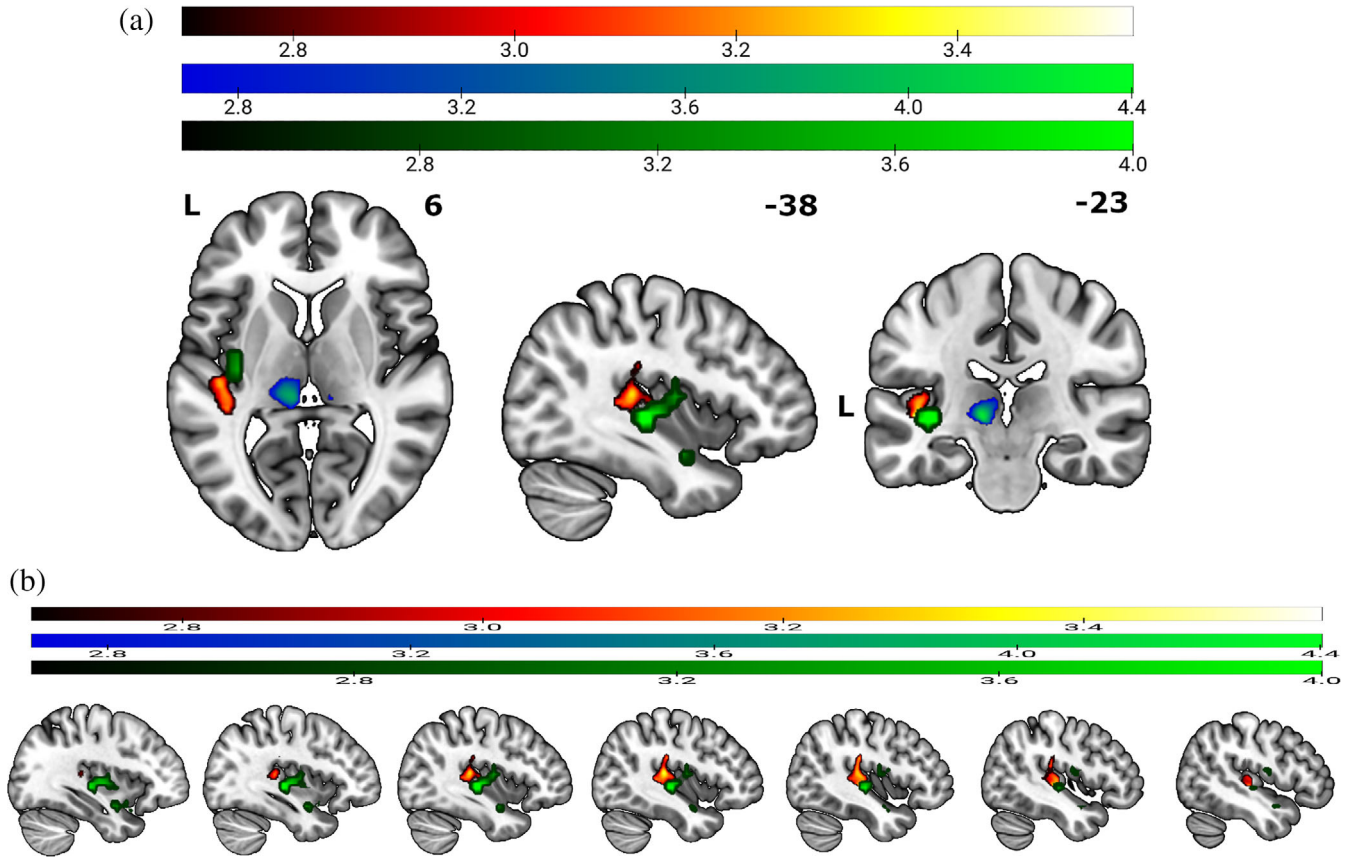

FIGURE 3 (a) Network analysis—association between local clustering coefficient and pain sensitivity. The figure displays the left insular cortex showing a positive association between the pain sensitivity score and the local clustering coefficient (in green). Study participants with a high local clustering coefficient in the left posterior insular displayed high pain sensitivity scores. The thalamic VBM cluster (blue) and the operculo-temporo-insular VBM cluster (red) are also displayed to demonstrate spatial co-localizations. L = left. (b) Network analysis—association between local clustering coefficient and pain sensitivity. The figure displays the left insular cortex showing a positive association between the pain sensitivity score and the local clustering coefficient (in green). The same analyses as in (a) are displayed. Study participants with a high local clustering coefficient in the left posterior insular displayed high pain sensitivity scores. The operculo-temporo-insular VBM cluster (red) are also displayed to demonstrate spatial co-localizations. L = left

et al., 2019), fibromyalgia (Schmidt-Wilcke et al., 2007) and furthermore in animal models of chronic pain. The thalamus is critically involved in the transmission of sensory input and not only serves as a gateway for relaying, but also filtering, incoming somatosensory and nociceptive signals to cortical regions such as the SI, SII cortices and posterior IC (Schnitzler & Ploner, 2000). Although caution is advised when making functional assumptions based on morphometric findings, it has been argued that changes in thalamic GM and neurochemistry in chronic pain might be associated with an increased transmission activity, for example, caused by a decrease in inhibitory interneurons or a change in regional blood flow (Alshelh et al., 2016; Henderson et al., 2013), leading to an increased thalamus to insular and somatosensory cortex connectivity. Accordingly, for pain perception in the healthy state, it is conceivable that a relative decrease in GM reflects an increased transmission rate of nociceptive information, leading to an increased sensory and nociceptive input to cortical regions and, and, at a behavioral level, to increased pain sensitivity.

Our finding in the operculo-insular cortex, showing a positive association between pain sensitivity and regional GM, is in line with other brain imaging studies. Erpelding et al. reported higher pain sensitivity, derived from heat and cold pain thresholds, to be associated with higher cortical thickness in the SI cortices (Erpelding et al., 2012). Furthermore, Ruscheweyh et al., using VBM, could identify two symmetrical clusters in the temporal lobe, reaching into the putamen and IC to be positively correlated with self-rated pain sensitivity (Ruscheweyh et al., 2018). However, negative associations between pain sensitivity and GM volume and/or cortical thickness in pain processing regions, such as the, inferior parietal lobule (Emerson et al., 2014), posterior cingulate cortex/precuneus (Elsenbruch et al., 2014; Emerson et al., 2014; Zhang et al., 2020) and the IC (Elsenbruch et al., 2014) have also been found, indicating that, at the current state, results are not yet conclusive. On the other hand, it is conceivable that different brain regions contribute differentially to pain sensitivity, some displaying a positive association, others a negative. Furthermore, and even more importantly, "pain sensitivity" serves as an umbrella term covering different concepts, such as pain thresholds (with potentially different modalities), unpleasantness ratings of a given physical stimulus, pain tolerance

and self-rated pain sensitivity as determined by questionnaires (Ruscheweyh et al., 2018).

Interestingly, a study by Teutsch et al. could demonstrate that repetitive nociceptive stimulation leads to an increase in GM in pain processing areas such as the MCC and the somatosensory cortices bilaterally (Teutsch, Herken, Bingel, Schoell, & May, 2008), suggesting that a repetitive sensory input might lead to structural remodeling. Likewise, repetitive sensory (electrical) stimulation over a time period of just 40 min has been shown to induce a GM increase in the contralateral SI and SII cortex (Schmidt-Wilcke et al., 2018). Given that a decrease in thalamic gateway functioning leads to an increased nociceptive input to regions like the IC, this in turn might then cause an increase in insular GM and, at a behavioral level, to an increase in pain sensitivity.

When using the VBM cluster as a seed for rs-fc analyses, a strong association between pain sensitivity and operculo-insular to pre-and postcentral gyrus connectivity, as well as operculo-insular to mid-insular rs-fc was found, suggesting that rs-fc of these regions strongly relates to pain sensitivity. To our knowledge only a few studies using rs-fc addressing pain sensitivity have been performed (Niddam, Wang, & Tsai, 2020; Spisak et al., 2020). Our findings are in agreement with a combined spectroscopy and rs-fc study performed by Niddam et al., reporting a correlation between pain thresholds and SI to left sensory-motor network connectivity. Interestingly, specifically during nociception the posterior IC displays a tight functional connectivity to the SI and the primary motor cortex (M1; Peltz et al., 2011). Both SI and the motor cortex are key regions in pain perception mediating sensory-discriminative, but also motor aspects, in terms of withdrawal reactions. It remains to be fully elucidated, which kind of information is exchanged (during the resting-state) between brain regions displaying a high degree of rs-fc. However, given that baseline activity (Boly et al., 2007) and/or rs-fc between two or more brain regions (Keller et al., 2011) are predictive of efficient information transfer during the task situation, as seen during nociception elicited by experimental pain stimuli, it is conceivable that people with a high preexisting rs-fc are susceptible to an early generation of a pain percept requiring the integration of multiple brain regions, resulting in low pain thresholds and high pain sensitivity.

Finally, we performed network analyses within the conceptual framework of graph theory. One of the network parameters of interest was the local CC; the CC is a measure of the degree to which nodes in a graph tend to cluster together. Specifically, the local CC, which refers to a single node, and not the entire network (in our case to a $4 \times 4 \times 4\ mm^3$—voxel), indicates how close neighbors of this node are interconnected; that is, the degree to which neighbors of a specific node are also neighbors to each other. In our case, the local CC in the left posterior IC was associated with pain sensitivity, suggesting that this region serves as major integration site. The CC cluster was located in the posterior IC displaying some overlap with the VBM cluster) and extended into the mid IC. However, GM and CC values were not significantly correlated, so that at the current stage we cannot postulate an interaction between regional brain structure and local network properties.

Pain is a complex percept, based on the integration of not only discriminatory, but also emotional and motivational aspects. As such, it requires a well-functioning network where single nodes not only report to a center hub, for example, the posterior IC, but also communicate to each other, forming a functional cluster. Given that rs-fc represents some kind of information transmission between brain regions during rest, forming a network, which enables the emergence of pain from nociception, leaves a question of whether there are regions that, besides task specific activations, play an additional role in subserving the maintenance of the network (Keller et al., 2011). Given that the CC is an indicator of cohesiveness and the tendency to form groups, it is tempting to hypothesize that the posterior IC, displaying a local CC strongly related to pain sensitivity, does not only show an activation during the processing of nociceptive stimuli, but also "keeps the group together" in the offline condition, fostering its readiness for the moment the task is on.

The posterior IC, as well as the parietal operculum (OP) with its cytoarchitectonic subregions (OP 1–4) play a key role in pain perception (Segerdahl, Mezue, Okell, Farrar, & Tracey, 2015). More specifically they are thought to be part of the lateral pain system, mainly involved in decoding sensory-discriminative aspects of the nociceptive input (Segerdahl et al., 2015), while the anterior IC is believed to be involved in assessing affective-motivational aspects (Tracey, 2005), such as the significance of a stimulus, for example, the extent of potential threat (Wiech et al., 2010). Importantly, for the determination of pain thresholds, that is, the point along the curve of increasing stimulus perception, at which pain begins to be felt, the sensory-discriminative aspect is essential, while the affective-motivational aspect only plays a minor role. At the time point when the study participants first felt pain (and were instructed to press the button), the stimulus already elicited pain, but did not pose a threat, especially since study participants were in control of the situation and, furthermore, knew that the stimuli were not able to damage tissue integrity.

Multiple studies, both in animals and humans, indicate the existence of somatotopic and modality specific representations of pain within the operculo-insular regions (Baumgartner et al., 2010; Brooks, Zambreanu, Godinez, Craig, & Tracey, 2005; Craig, 2014), forming a tight interacting network (Wu, Wang, Yang, & Chen, 2017). From a clinical perspective, it is noteworthy that cortical recordings of epilepsy patients show that electrical stimulation of this region can elicit pain sensations in the body (Mazzola, Isnard, Peyron, Guénot, & Mauguière, 2009), and regional lesions can lead to altered pain experiences (Garcia-Larrea et al., 2010). Against this background some authors have even argued that the operculo-insular region can be viewed as a primary nociceptive cortex, serving as the starting point of nociceptive-related networks (Peyron & Fauchon, 2019) .

From a rs-fc perspective, the operculo-insular region as part of the temporo-parietal junction is closely connected to the salience network (Kucyi, Hodaie, & Davis, 2012), but also plays a role in embodiment and self-awareness (Michael, Tapiero, Gálvez-García, & Jacquot, 2017). As such, it makes sense that the operculo-insular region does not only play a fundamental role in pain perception in terms of nociceptive processing, but also serves a central hub

integrating the information from various brain sites enabling the emergence of pain from nociception.

While we had a strong a priori hypothesis for the IC, serving as a network hub that integrates information to create the percept of pain, or at least significantly contributing to its evolution, we were surprised that it was the left, ipsilateral, posterior IC, rather than the right, where the local CC correlated with pain sensitivity. Also, the VBM clusters, that is, the cluster in the thalamus and parieto-insular cortex, were located on the left-hand side. Pain stimuli had been applied to the left forearm, that is, the correlations were found in the ipsilateral hemisphere. In general, nociception evokes bilateral activations, especially in the IC and SII cortex (Chen, Dillenburger, Wang, & Tang, 2012; Xu et al., 2020), while the SI cortex tends to be activated preferentially on the side contralateral to the stimulus (Timmermann et al., 2001). Some studies even provide evidence that pain is associated with a preferential left-hemispheric activation, specifically in the left IC (Brooks et al., 2005; Duerden & Albanese, 2013). Against this background, it might be either the extent of left-hemispheric or ipsilateral IC clustering ability that determines pain sensitivity, respectively the connectivity between left-hemispheric or ipsilateral operculo-insular region to the SI cortex contalateral to the stimulus presentation. Given that the percept of pain requires bilateral activation it is tempting to hypothesize that it is the ipsilateral activity/integrity that determines the transition from stimulus detection to pain perception. Importantly, rs-fc between the left operculo-insular regions with both, the left and right postcentral gyrus correlated with pain sensitivity.

4.1 | Limitations

Our study has some limitations which need to be pointed out. The pain sensitivity score used in this and previous studies comprises three pain thresholds, that is, heat pain, cold pain and pressure pain. As such it is an amalgamated metric, the idea being that certain brain areas are critically involved in the extraction of the pain aspect of a given stimulus, which usually contains several dimensions, such as stimulus detection, anticipated nociception, nociception per se, anticipated harm etc. This construct requires further validation, since it is also conceivable that each modality has its own pain system, and that the umbrella term "pain" refers to different percepts with different neurobiological underpinnings.

Another weakness is the cross-sectional design, that only allows the delineation of associations between pain sensitivity and structural and functional brain measures. Both pain sensitivity and rs-fc fluctuate and can be influenced, for example, by prior pain events. Pain sensitivity in our study was determined on the day before the MRI scan was performed and about 15 min after the second MRI scan (on day 2). As such it is conceivable that the first set of pain testing had influenced rs-fc. Future studies will need to further disentangle causes from consequences.

Finally, both structural as well as network findings were located in the left hemisphere, that is, ipsilateral to the forearm where pain stimuli had been applied. As outlined above, in fMRI studies pain is usually associated with a bilateral activation in the thalamus, the insular cortex as well as the SII, with some studies alluding to a right hemispheric, others to a left hemispheric predominance in pain processing. Our study is not capable to answer the question whether it is the left- or ipsilateral GM volume, respectively network properties, that are associated with pain sensitivity. To this end bilateral pain testing would have been required and should be performed in future studies to answer this question.

5 | CONCLUSIONS AND OUTLOOK

Our data provided evidence that regional brain morphology, in terms of local GM volume, and local network properties, in terms of the local CC in the same region, that is, the posterior IC, relate to pain sensitivity. VBM and rs-fc analyses are well established imaging methods, but the neural underpinnings of GM volume (in terms of cytoarchitecture) and correlating BOLD fluctuations in remote brain areas (in terms information transfer) remain to be fully elucidated. In our study, two completely different imaging approaches, revealed clusters in the same brain region. Although GM and CC values were not significantly correlated, and thus seem to be driven by different neural underpinnings, it will be worth investigating in future studies, in more detail whether there is a link between structural measures of a brain region and its network properties.

Graph theory is a promising advancement of connectivity analyses, which has been only recently been applied in the context of pain (Kaplan et al., 2019), where for example eigenvector centrality of the posterior IC correlated with clinical pain in fibromyalgia patients. To the best of our knowledge, this is the first study that applied rs-fc analyses and graph theory in the context of pain sensitivity. Most studies performing network analyses have done so, based on prior defined brain regions, serving as nodes. Voxel-based approaches demanding a high degree of computational power are less common, but they do have the advantage that whole brain analyses without apriori hypotheses can be performed in a rather exploratory way (Amad et al., 2017; Wang et al., 2017). In our voxel-based network analyses, we took 16,931 voxels ($4 \times 4 \times 4$ mm^3) into account, covering the cortex of both hemispheres; the cerebellum and subcortical structures were not part of the analyses. For future studies it will not only be interesting to incorporate these structures, especially the thalamus, but also to apply other network parameters such as transitivity, eigenvector centrality etc. to identify more (or the same) brain region and their specific network characteristics relating to pain sensitivity.

Finally, it remains to be fully elucidated, if and how exactly pain sensitivity and its neural underpinnings can be used to predict the evolution of chronic pain, for example, whether low pain thresholds are associated with an increased vulnerability towards the development of chronic pain, or whether pain thresholds can be used to predict a patients' response to a specific analgesic treatment. Against this background, it is of great interest, not only from a physiological, but

also from a clinical perspective to better understand the mechanisms that determine individual pain sensitivity.

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DATA AVAILABILITY STATEMENT

We hereby state that we will make the data and the code available, if requested.

ORCID

Tobias Schmidt-Wilcke https://orcid.org/0000-0002-7929-1079

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SUPPORTING INFORMATION

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

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