Spontaneous brain activity and connectivity in female patients with temporomandibular joint synovitis pain: a pilot functional magnetic resonance imaging study

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Objective. It has been proposed that mechanisms in the central nervous system contribute to the development and maintenance of pain in temporomandibular disorders. In this study, we tested whether spontaneous brain activity and functional connectivity (FC) were altered in patients with temporomandibular joint synovitis pain.

Study Design. A prospective, cross-sectional design was adopted. Each of 8 patients and 10 healthy controls (HCs) underwent 2 sessions of functional magnetic resonance imaging: mouth closed and mouth open (painful for patients). Regional homogeneity (ReHo) was used to measure spontaneous brain activity in each participant. Brain areas with altered ReHo in patients compared with HCs were identified, and their FCs with the rest of the brain was examined and compared.

Results. Compared with HCs, patients showed decreased pain-related ReHo in the right anterior insula (rAI). The rAI showed a weaker positive FC with the left middle cingulate cortex (MCC) and a weaker negative FC with the right precuneus in patients compared with HCs. Furthermore, the rAI-MCC FC showed a negative correlation with pain intensity in patients.

Conclusions. These results provide evidence supporting altered pain-related spontaneous brain activity and functional connectivity in the central nervous system in patients with temporomandibular joint synovitis pain. (Oral Surg Oral Med Oral Pathol Oral Radiol 2018;126:363–374)

Temporomandibular disorders (TMDs) are considered one of the most common causes of chronic orofacial pain,[1] and pain is the primary symptom that motivates patients to seek treatment.[2] Patients with TMD-related pain are likely to develop psychological problems, such as depression, anxiety, stress, and sleep disturbances,[3] which lead to a negative effect on long-term quality of life.[4] Despite the high prevalence rate of TMDs[5] and the damage to patients' quality of life, the etiology and pathophysiologic mechanisms underlying TMD-related pain remain poorly understood. The long-standing notion that TMDs are initiated by peripheral mechanisms, such as malocclusion,[6] has been challenged by several facts: (1) the lack of adequate evidence of peripheral tissue abnormalities or specific physical pathology in patients with TMD,[7] (2) increasing evidence of abnormalities in the central nervous system from brain imaging studies,[8,9] and (3) observations that patients with TMD pain often have pain in remote body areas[10] and enhanced sensitivity to experimentally induced pain compared with healthy controls (HCs).[11] Therefore, it has been proposed that peripheral factors, such as malocclusion and trauma, only aggravate pain in TMDs but that central mechanisms initiate or at least contribute to the development or maintenance of TMD pain.[7,12]

Functional magnetic resonance imaging (fMRI) has been extensively applied to the investigation of neural activities in a number of pain conditions,[13,14] including TMD pain.[8,9] Among functional neuroimaging studies, resting-state fMRI has become increasingly popular for examining spontaneous neural activities in a variety of clinical conditions, mainly because of its practicality in clinical populations.[15,16] It is now widely accepted that resting-state fMRI is a useful tool to study spontaneous neural activities at the baseline state; it has been proven to be physiologically and pathologically meaningful. Previous studies have reported abnormalities in resting-state,[17] task-related brain activities,[18] or brain morphology[19] in several typical pain-related brain regions, such as the insula, the anterior/middle cingulate cortex (ACC/MCC), the thalamus, the primary (S1) and secondary (S2) somatosensory cortices, the salience network (SN), and the default mode network (DMN). However, there have been inconsistencies among different studies with regard to the area in the brain where the abnormality was observed and whether the abnormality increased or decreased in patients compared with HCs.

Statement of Clinical Relevance

The findings reported in the present study provide new evidence supporting central mechanisms underlying pain in temporomandibular disorders, highlighting the importance of taking the central abnormalities into account in the treatment of orofacial pain.

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Regional homogeneity (ReHo), a measure of synchronicity of neural activities within a small local area, is widely used to investigate the state of spontaneous neural activities during rest.[20] ReHo has been adopted to study many different clinical pain conditions, such as migraine,[15] abdominal pain,[21] and idiopathic trigeminal neuralgia.[22] The results of these studies indicate that abnormal resting-state neural activities may be observed by using ReHo in brain areas associated with pain processing, such as the insula, MCC, and medial prefrontal cortex in patients with chronic pain diseases. On the basis of these findings from our previous studies, we hypothesized that altered spontaneous neural activities measured by ReHo may also exist in pain-related brain regions of patients with TMD pain.

Another way of studying spontaneous neural activities during rest is analysis of functional connectivity (FC) among different brain areas. ReHo measures the synchronicity of neural activities within a small local area, whereas FC measures the temporal correlation between distinct brain regions.[23]

As temporomandibular joint (TMJ) synovitis is one of the most common and representative subtypes of TMD in clinical practice, this study aimed to investigate altered spontaneous neural activities in the whole brain of patients with TMJ synovitis pain to further explore the underlying central neural mechanisms in the development of TMD-related pain. Considering the complex interactions among the different areas of the brain during pain and the effect of long-term painful input on patients with TMDs, we hypothesized that these patients would show altered spontaneous neural activities in pain-related brain regions and altered FCs within pain-related brain networks. To test this hypothesis, we used resting-state fMRI, including a pain session and a non-pain session, to identify pain-related brain activities and functional connectivities in patients with TMJ synovitis pain. More specifically, we first performed ReHo analysis and identified the right anterior insula (rAI) showing altered spontaneous neural activities in patients with TMJ synovitis pain compared with HCs. We then examined the changes in FCs between the rAI and the rest of the brain in these patients.

MATERIALS AND METHODS

Participants

Because several different peripheral mechanisms, such as TMJ synovitis, myogenous TMD pain, and TMJ synovial chondromatosis, may be involved in TMD pain, it is still unclear whether patients with TMD pain with different peripheral mechanisms have different brain abnormalities. To reduce possible inhomogeneity of the patient group, we only focused on patients with TMJ synovitis pain. The detailed inclusion and exclusion criteria of the patients and healthy controls are given below.

Inclusion criteria for patients. The inclusion criteria for the patient group were (1) female gender (because of higher prevalence among women[12] and to minimize gender effects on brain structure and function[24,25]); (2) right-handedness (to avoid possible confounding effects induced by handedness on brain activity[26,27]); (3) presence of typical signs of disk displacement and joint synovitis with unilateral joint pain, according to the Research Diagnostic Criteria for Temporomandibular Disorders (Group IIIa)[2]; (4) pain in the open-mouth state but no pain in the closed-mouth state; (5) open-mouth pain intensity 5 or greater, as measured by a visual analogue scale (VAS, range 0-10; 0 refers to no pain and 10 refers to the worst pain imaginable); (6) no history of trauma around the TMJ or systematic diseases, such as rheumatic arthritis and neoplasia; (7) no history of myogenous categories of TMD/myalgia/fibromyalgia; (8) pain symptoms restricted to the TMJ area and without widespread pain in other body regions; (9) pain symptoms without swelling; and (10) no history of TMD treatment, such as analgesics, physical therapy, or lidocaine injection.

Inclusion criteria for the HC group. The inclusion criteria for the control group were (1) female gender, (2) right-handedness, (3) no TMD symptoms or any other orofacial pain disorders, (4) no TMJ pain symptom in both open-mouth and closed-mouth states.

Exclusion criteria for both groups. For both groups, the exclusion criteria were (1) pregnancy, (2) history of any neurologic or psychiatric disorders, (3) history of substance abuse, (4) presence of other chronic pain disorders in areas other than the TMJ area, and (5) MRI contraindications.

The selection of patients was based on clinical examination and MRI examination of the TMJ. In the first stage of screening, patients were diagnosed by using the Research Diagnostic Criteria for Temporomandibular Disorders (Group IIIa) by a TMD specialist with experience in orofacial pain. All patients exhibited typical signs of disk displacement and pain in the TMJ area, and their pain intensity was aggravated with increased TMJ functional activities, especially pressure and palpation in the upward and backward direction. In the second stage, MRI examination was used to exclude other abnormalities in bilateral TMJ structures, such as synovial chondromatosis, calcium pyrophosphate dihydrate deposition disease, and pigmented villonodular synovitis. Two patients who were initially recruited but later found to have TMJ synovial chondromatosis were excluded from the study.

According to the above inclusion and exclusion criteria and the clinical examination and MRI examination findings, 8 female patients with right-side TMJ synovitis with unilateral open-mouth pain (5 with pain on the left side and 3 on the right side) and 10 age- and gender-matched HCs were recruited in the present study. The

sample size was relatively small because of the strict inclusion and exclusion criteria. It is worth noting that power calculations for sample size estimation are not commonly performed in the neuroimaging field because the true effect size is difficult to estimate. All participants were recruited from northern China.

This study was approved by the ethics board of the Peking University Health Science Center in compliance with the tenets of the Helsinki Declaration. All participants read and signed an informed consent form.

Brain MRI data acquisition

Brain functional and structural MRI data were collected on a 3.0-T Siemens Tim (total imaging matrix) Trio scanner (Siemens AG, Berlin, Germany). Participants lay supine, with their head fixed by a strap and foam pads to minimize head motion. Ear plugs were used to reduce the effect of noise. High-resolution T1-weighted structural images were acquired with a spoiled gradient echo recall sequence, using the following parameters: repetition time/echo time = 2300 ms/3.01 ms; flip angle = 90 degrees; 176 slices; slice thickness = 1.0 mm; intersection gap = 0.5 mm; field of view = 256 × 240 mm; and matrix = 256 × 256. Seven-minute resting-state fMRI data were obtained with a T2-weighted gradient-echo echo-planar imaging sequence, using the following parameters: repetition time/echo time = 2000 ms/30 ms; flip angle = 9 degrees; 30 axial slices; slice thickness = 4.0 mm; intersection gap = 0.8 mm; field of view = 210 × 210 mm; and matrix = 64 × 64. During the scanning period, participants were asked to relax and stay awake with their eyes closed.

Experimental paradigm

All participants underwent 2 sessions of resting-state fMRI scans**:** a "mouth-closed" session (condition C) followed by a "mouth-open" session (condition O). The "mouth-open" session, aided by a trapezoid rubber biteblock placed between the upper and lower incisors to facilitate maximum opening, was designed to create a painful session for patients, and this was confirmed by patient-reported pain intensities measured by using a VAS: mean = 6.69; standard deviation (SD) = 0.80 (0 [no pain] and 10 [the worst pain imaginable], corresponding to the 2 extremities of the VAS). The order of the 2 sessions was fixed to avoid any possible carryover effects of pain in the following sessions. There was a break of approximately 8 minutes between the 2 sessions. All participants were familiarized with the experimental procedure, especially the "mouth-open" condition, before the experiment to reduce patients' psychological load and anxiety about the procedures.

To ensure that no pain was felt in HCs during the "mouth-open" session, the researchers asked all HCs "whether any painful sensations were felt" when familiarizing them with "mouth-open" condition by placing a biteblock to facilitate maximum opening. They all reported no pain but only discomfort, so the researchers did not formally assess pain in HCs by using the VAS during either the mouth-closed condition or the mouth-open condition.

In this design, the pain induced in patients during the "mouth-open" session resembled the clinical pain in patients with TMD pain. Furthermore, the design allowed for both within-subject comparison ("mouth-open" session vs "mouth-closed" session) and between-subject comparison (TMD group vs HC group) to extract pain-related brain activities in patients and minimize the effects of possible confounding factors as much as possible (e.g., stimulation of oral receptors caused by the biteblock).

ReHo analysis

The fMRI data were preprocessed by using the Data Processing Assistant for Resting-State fMRI software package (rfmri.org)[28] in the following steps: (1) The first 10 volumes were discarded to avoid nonequilibration effects of the scanner and to allow participants to adapt to the scanning environment. (2) The remaining 200 volumes were corrected for slice timing; spatially realigned for correction of head motion; spatially normalized to the standard echo planar imaging (EPI) template in the Montreal Neurologic Institute (MNI) space; and resampled to $3 \times 3 \times 3$ mm^3 voxel size. (3) The time series of each voxel were cleaned by regressing out the 6 head motion parameters, the white matter signal, the cerebrospinal fluid signal, and the global signal; and linear detrended and band-pass filtered (0.01-0.1 Hz; to remove low-frequency physiologic noises, such as respiratory and cardiac noises, and high-frequency noises). All participants' head motions were within ±1.5 mm in each translational axis, and within ±1.5 degrees around each rotational axis.

ReHo measures the synchronicity of neural activities within a small local area (typically formed by a voxel and its neighboring voxels). A brain area with highly synchronous neural activities (i.e., high ReHo) is considered more functionally organized. ReHo value was calculated for every voxel within the brain as the Kendall's coefficient of concordance of the given voxel and its 26 neighboring voxels.[20] A ReHo map for each condition and each participant was obtained and standardized to z-score maps (i.e., zReHo maps, mean = 0 and SD = 1 across all voxels) by subtracting the mean ReHo value across all voxels and then being divided by the SD of all voxels, as follows:

$$zReHo_{voxel\ i} = \left(ReHo_{voxel\ i} - mean\left(ReHo_{all\ voxels}\right)\right) / SD\left(ReHo_{all\ voxels}\right)$$

To test the robustness of the results, an alternative standardization method was also used: The ReHo value of

each voxel was divided by the mean across all voxels within the whole brain, resulting in mean ReHo (mReHo) maps (i.e., mean = 1), as follows:

$$\mathrm{mReHo}_{\mathrm{voxel\ i}} = \mathrm{ReHo}_{\mathrm{voxel\ i}} / \mathrm{mean}\left(\mathrm{ReHo}_{\mathrm{all\ voxels}}\right)$$

The zReHo or mReHo maps were spatially smoothed with an 8-mm full-width at half-maximum Gaussian kernel to lessen error of spatial normalization and improve data normality.

FC analysis

The same preprocessing steps were performed as in the ReHo analysis except that the spatial smoothing step was performed immediately after spatial normalization. A cluster of voxels in the rAI showing significantly different zReHo/mReHo in the TMD group compared with the HC group were selected as the seed region of interest (ROI). For each condition and participant, the average time series across all voxels within the seed ROI were obtained. FC of the seed ROI with each voxel in the brain was calculated as the Pearson's correlation coefficient between the time series of the given voxel and the time series of the seed ROI. The correlation coefficients were then converted to z-values using Fisher's r-to-z transformation to improve the normality of the data for further statistical analyses. This FC analysis was performed by using the software package REST.[29]

Statistical analyses for ReHo and FC maps

To identify the brain areas with high zReHo/mReHo values (i.e., significantly >0 for zReHo or significantly >1 for mReHo), a random-effect 1-sample *t* test of the zReHo/mReHo maps was performed for each condition and each group. The resultant 4 statistical maps (2 conditions × 2 groups) were thresholded by using $P < .05$, corrected for multiple comparisons at cluster level by using a nonparametric Monte Carlo test with 1000 Monte Carlo simulations and the whole brain mask (using AlphaSim implemented in the REST software). A "combined ReHo mask" was then created by taking the union of the 4 thresholded maps. To identify the brain areas with significant changes in zReHo/mReHo during the pain state ("mouth-open" session; condition O) compared with the non-pain state ("mouth-closed" session; condition C) in the TMD group while controlling for any other changes unrelated to pain, zReHo/mReHo maps of "mouth-closed" condition were subtracted from those of "mouth-open" condition (O – C) in each participant. The resultant difference maps were then compared between patients and HCs with a 2-sample *t* test, performed by using the Statistical Parametric Mapping software package (http://www.fil.ion.ucl.ac.uk/spm). The statistical map of the 2-sample *t* test was thresholded similarly using $P < .05$, corrected for multiple comparisons at cluster level by using a nonparametric Monte Carlo test with 1000 Monte Carlo simulations and the "combined ReHo mask." To examine the relationship between pain intensity and spontaneous brain activities, a correlation analysis between the averaged zReHo/mReHo values of the brain areas showing altered ReHo and the VAS scores was performed in patients by using the SPSS software, version 17.0 (SPSS Inc., Chicago, IL).

The same statistical analyses described above were also performed for FC maps to identify the brain areas with significant changes in FC with the seed ROI in patients with TMD. Briefly, a random-effect 1-sample *t* test was performed to identify the brain areas showing significant FC with the seed ROI for each condition and group (thresholded using $P < .05$, corrected), and a "combined FC mask" was generated. The FC difference maps (condition O – condition C) were compared between patients and HCs by using a 2-sample *t* test (thresholded using $P < .05$, corrected at the cluster level with 1000 Monte Carlo simulations within the "combined FC mask"). A correlation analysis between the VAS scores and the averaged FC values of the brain areas showing altered FC with the seed ROI was performed in patients to examine the relationship between pain intensity and FCs.

The masks used in the above statistical analyses and the resultant cluster sizes after correction are provided in Table I.

The above ReHo and FC analyses were performed by 3 observers with experience in resting-state fMRI data analysis. The other 2 observers were responsible for data acquisition and study design.

Table I. The mask used in each statistical analysis, cluster-defining threshold, and resultant cluster size in the ReHo and FC analyses

Items	ReHo maps of each condition in each group	Pain-related ReHo changes in patients with TMDs relative to HCs	FC maps of each condition in each group	Pain-related FC changes in patients with TMDs relative to HCs
Mask	Whole-brain mask	"Combined ReHo mask"	Whole-brain mask	"Combined FC mask"
Cluster-defining threshold	$P < .001$	$P < .001$	$P < .001$	$P < .001$
Cluster size (voxels)	≥23	≥10	≥23	≥14

FC, functional connectivity; *HC,* healthy controls; *ReHo,* regional homogeneity; *TMDs,* temporomandibular disorders.

RESULTS

Average ages (with SD) of 10 HCs and 8 patients were 33.9 ± 7.3 years and 33.5 ± 8.7 years, respectively. There was no significant difference in age between the 2 groups ($P = .916$). Because the results from zReHo and mReHo were similar, only the results obtained based on zReHo are reported here.

ReHo

ReHo maps of each condition in each group. The zReHo maps of each condition in each group are displayed in Figure 1. The zReHo maps identified similar spatial distribution of spontaneous brain activities across the brain in the "mouth-closed" and "mouth-open" conditions and in both the TMD group and the HC group. The typical default mode network (including the posterior cingulate cortex (PCC)/precuneus, the medial prefrontal cortex (MPFC), the bilateral inferior parietal lobule and the lateral temporal cortex)[30] and the task-positive network (including anterior insula, ACC/MCC and dorsolateral prefrontal cortex) were clearly evident.

Pain-related ReHo changes in patients with TMDs relative to HCs. The 2-sample *t* test of the zReHo difference maps showed significantly decreased zReHo in the rAI in patients with TMDs compared with HCs (Figure 2A). No other brain areas were found. The averaged zReHo values of this rAI cluster were shown for each condition and each group in Figure 2B. The results of further 2-sample *t* tests of the averaged zReHo between different conditions and groups are given in Table II. The study did not observe a significant correlation between the zReHo of the rAI and the reported pain intensity in patients (Figure 2C).

Functional connectivity of the rAI

FC maps of each condition in each group. Two clear patterns of the FC maps were revealed in both conditions and both groups (Figure 3): The rAI was (1) positively correlated with the ACC/MCC, the other key areas of the SN, and (2) negatively correlated with the PCC and the MPFC, the key areas of the DMN.

Pain-related FC changes in patients with TMDs relative to HCs. The 2-sample *t* test of the FC difference maps showed that the rAI had significantly decreased positive FC with the left MCC (Figures 4A and 4B) and decreased negative FC with the right precuneus in patients with TMDs compared with HCs (Figures 4D and 4E). The results of further 2-sample *t* tests of the averaged FCs between different conditions and groups are given in Table II. Interestingly, pain intensity was found to be strongly correlated with the rAI-MCC FC but not with the rAI-precuneus FC: The higher the VAS score, the weaker was the FC between the rAI and the MCC in patients (Figure 4C).

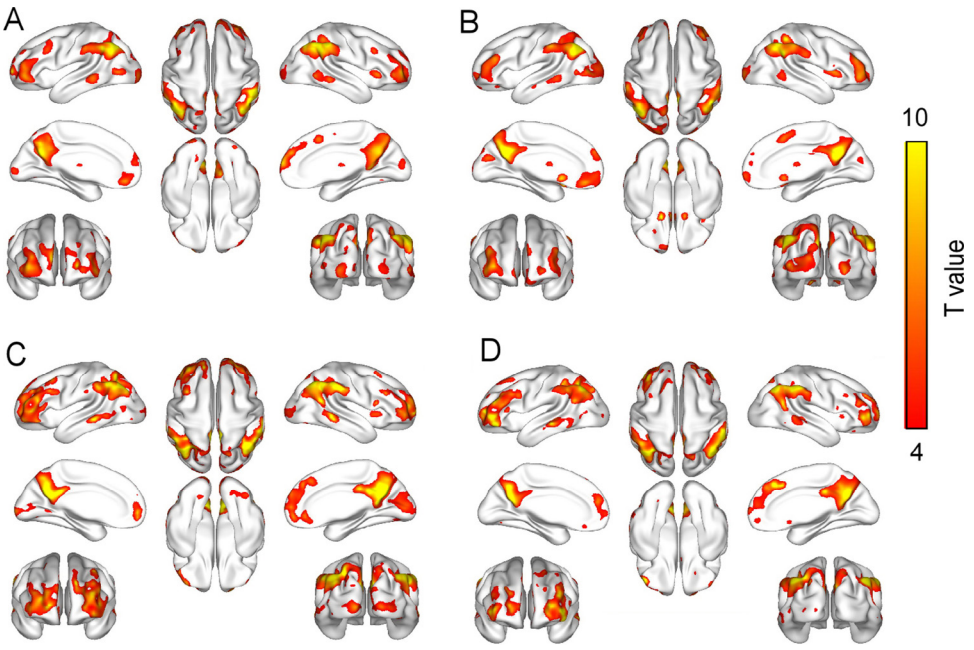

Fig. 1. Results of 1-sample t-tests of z-score regional homogeneity (zReHo) maps for different conditions in different groups. **A,** Mouth-closed condition in healthy controls (HCs). **B,** Mouth-open condition in HCs. **C,** Mouth-closed condition in patients with temporomandibular disorders (TMDs). **D,** Mouth-open condition in patients with TMDs. These maps were thresholded at $P < .05$ (corrected), and only the voxels whose zReHo were significantly greater than 0 are shown.

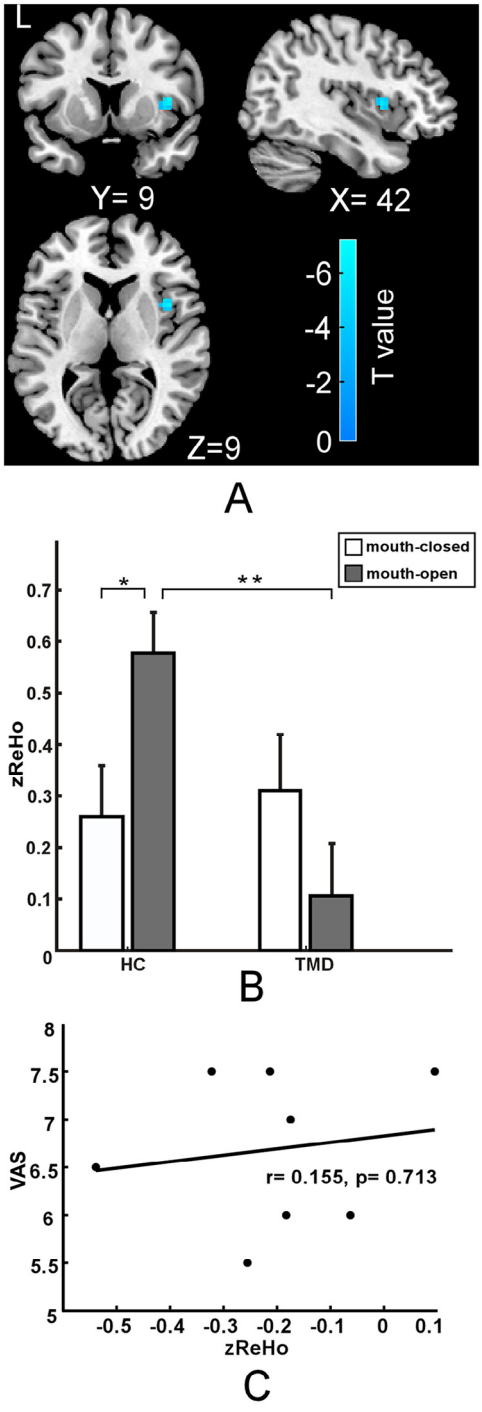

Fig. 2. The difference in z-score regional homogeneity (zReHo) between healthy controls (HCs) and the temporomandibular disorder (TMD) group. A**,** Compared with HCs, the TMD group showed decreased zReHo in the right anterior insula *(blue)*, which was used as a seed region for further functional connectivity analyses. **B,** The averaged zReHo values of the right anterior insula (rAI) cluster of each condition and each group. The error bars indicate the standard error of the mean across participants. *$P < .05$, **$P < .005$. **C,** Correlation analysis between the visual analogue scale (VAS) scores and the averaged zReHo values ("mouth-open" – "mouth-closed") of the rAI.

DISCUSSION

Decreased ReHo in the rAI in patients with TMD pain

Results of the ReHo analysis in present study showed the presence of the DMN in both HCs and patients with TMDs (see Figure 1). Numerous studies have demonstrated that the DMN, including the PCC/precuneus and the MPFC as the key components, is present in the resting brain of healthy individuals as well as patients with a number of diseases.[30,31] The DMN typically shows stronger activity and FC between its components during rest than during performance of tasks. It has been suggested to be involved in integrating cognitive and emotional processing and behavioral and self-referential processes.[32,33]

Although the DMN was evident in the TMD group, they showed decreased ReHo in the rAI compared with the HCs in the present study. The insula is one of the brain areas activated most consistently in a variety of experimental paradigms and receives inputs from and projects to a variety of brain regions.[34] It is thought that the insular cortex is an integrated area responsible for multidimensional aspects of interoceptive functions and is especially critical for pain perception.[35] Indeed, the insula, together with the thalamus, the ACC, and the primary (S1) and secondary (S2) somatosensory cortices, are the key areas composing the so-called pain matrix.[36,37] Notably, the cluster showing decreased ReHo in patients with TMDs, as observed in the present study, is located at the anterior part of the right insula. The anterior insula, together with the ACC, are the 2 key nodes in the SN, which have been hypothesized to integrate sensory input with visceral and autonomic information and to detect inputs with high emotional salience.[38] In terms of pain processing, the anterior insula is part of the medial pain system related to the affective–motivational and cognitive processing of pain, integrating interoceptive awareness with its emotional salience.[35,38] Therefore, the observation of reduced ReHo in the rAI suggests a disrupted salience processing of pain in patients with TMD pain.

When looking more closely at the ReHo values of each condition and each group, it was interesting to note that ReHo was increased in the "mouth-open" condition compared with the "mouth-closed" condition in HCs, given that both conditions were nonpainful (see Figure 2B). One possible explanation might be the increased degree of discomfort experienced by the participants while keeping the mouth open throughout the "mouth-open" session. Indeed, a trapezoid rubber biteblock was placed between the upper and lower incisors to ensure maximum mouth opening and the HCs reported nonpainful but uncomfortable sensation in this condition. Such discomfort may serve as a salient input and thus lead to a higher ReHo in the "mouth-open" condition in HCs.

Table II. The statistical results of further 2-sample *t* tests of the averaged zReHo and averaged FCs across different conditions and groups (8 patients vs 10 controls)

	Comparisons	Mean ± SD	T and P values
zReHo of the rAI	TMD(C) versus HC(C)	TMD(C): 0.309 ± 0.307	T = –0.352
		HC(C): 0.257 ± 0.315	*P* = .729
	TMD(O) versus HC(O)	TMD(O): 0.102 ± 0.293	T = 3.730
		HC(O): 0.576 ± 0.247	*P* = .002†
	HC(C) versus HC(O)	HC(C): 0.257 ± 0.315	T = –2.521
		HC(O): 0.576 ± 0.247	*P* = .021*
	TMD(C) versus TMD(O)	TMD(C): 0.309 ± 0.307	T = 1.380
		TMD(O): 0.102 ± 0.293	*P* = .189
FC: RAI-MCC	TMD(C) versus HC(C)	TMD(C): 0.261 ± 0.160	T = 0.419
		HC(C): 0.293 ± 0.166	*P* = .680
	TMD(O) versus HC(O)	TMD(O): 0.027 ± 0.179	T = 4.576
		HC(O): 0.357 ± 0.127	*P* < .001†
	HC(C) versus HC(O)	HC(C): 0.293 ± 0.166	T = –0.960
		HC(O): 0.357 ± 0.127	*P* = .350
	TMD(C) versus TMD(O)	TMD(C): 0.261 ± 0.160	T = 2.750
		TMD(O): 0.027 ± 0.179	*P* = .016*
FC: rAI-Precuneus	TMD(C) versus HC(C)	TMD(C): –0.345 ± 0.151	T = 2.487
		HC(C): –0.167 ± 0.150	*P* = .024*
	TMD(O) versus HC(O)	TMD(O): –0.212 ± 0.174	T = –2.413
		HC(O): –0.391 ± 0.142	*P* = .028*
	HC(C) versus HC(O)	HC(C): –0.167 ± 0.150	T = 3.436
		HC(O): –0.391 ± 0.142	*P* = .003†
	TMD(C) versus TMD(O)	TMD(C):–0.345 ± 0.151	T = –1.626
		TMD(O): –0.212 ± 0.174	*P* = .126

*$P < .05$.
†$P < .005$.
C, "mouth-closed" condition; *FC,* functional connectivity; *HC,* healthy controls; *O,* "mouth-open" condition; *rAI,* right anterior insula; *SD,* standard deviation; *TMDs,* temporomandibular disorders; *zReHo,* z-score regional homogeneity.

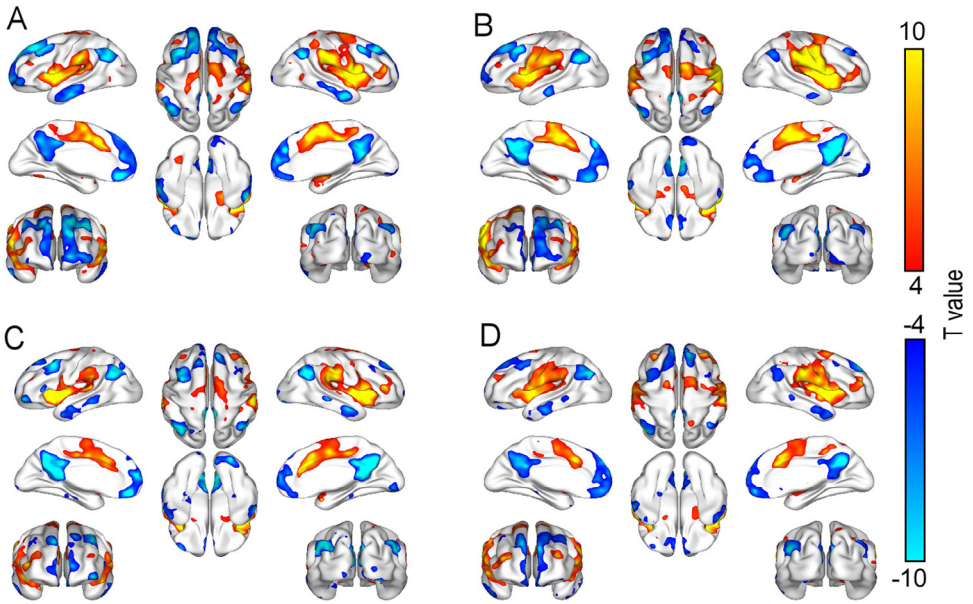

Fig. 3. Results of 1-sample *t* tests of functional connectivity (FC) maps for different conditions in different groups based on the seed region obtained from the z-score regional homogeneity (zReHo) values. **A,** Mouth-closed condition in healthy controls (HCs). **B,** Mouth-open condition in HCs. **C,** Mouth-closed condition in patients with temporomandibular disorders (TMDs). **D,** Mouth-open condition in patients with TMDs. These maps were thresholded at $P < .05$ (corrected). Warm color indicates positive FC, and cold color indicates negative FC.

In contrast, the TMD group showed a decrease in ReHo, although not significant one, in the "mouth-open" condition (i.e., during the pain state) compared with the "mouth-closed" condition (i.e., during the non-pain state). After controlling for the possible confounding effect of the discomfort caused by the biteblock, the decrease in ReHo in the TMD group compared with HCs became clearly evident (see Figure 2A). This seems to contradict the notion that pain, as a salient input, would increase the salience-related neural activities as commonly observed in acute pain studies.[38] However, brain responses to chronic pain have been shown to be different from those to acute pain.[39] The decreased ReHo of the rAI in patients with TMD pain could be a consequence of long-term, frequent pain input resulting in an overload in the SN. A previous brain morphology study in myofascial-type TMD found a decrease in gray matter density/volume in the ACC and the anterior insula,[19] reflecting a local atrophy associated with hyperactivity in pain-perceptive brain structures. This previous finding is, to some extent, consistent with the current finding; that is, atrophy of a brain area is likely to lead to decreased activity in this area.

Decreased positive FC between the rAI and the MCC in patients with TMD pain

The present study found that the FC between the rAI and the MCC was significantly decreased in the TMD group compared with HCs (see Figure 4A). In contrast to HCs, who showed slightly greater FC during the "mouth-open" condition than during the "mouth-closed" condition, the TMD group showed clearly decreased FC during the "mouth-open" condition (i.e., the pain state) (see Figure 4B). The ACC and the middle part of the cingulate cortex are key nodes of both the SN[38,40] and the so-called pain matrix, possibly because of its role in the affective–motivational and cognitive processing of pain, automatic attention associated with pain, salience detection, and response selection.[41] The anterior insula and the ACC/MCC, the 2 key nodes of the SN, are commonly found to be coactivated in many experimental paradigms.[38,42] The decreased FC between the rAI and the MCC in patients with TMDs may reflect a disconnection within the SN, possibly because of the same mechanism underlying the decreased ReHo in the rAI observed in the present study—that is, an overload caused by frequent painful input in patients with TMD pain.[43] This is further supported by the negative correlation between pain intensity and the rAI-MCC FC: The higher the VAS score, the weaker is the FC between the rAI and the MCC in patients.

It is worth noting that a previous fMRI study using a similar design investigated the FC between the insula and the rest of the brain.[17] In this previous study, the FC maps of 4 seed regions (the left and the right anterior insula and the left and the right posterior insula) were examined. In a somewhat similar manner to the present study, each participant was scanned during a non-pain resting state and a pain task session. However, in contrast to the present study, the FC maps obtained from the resting session and the task session were not compared directly but were examined separately. The authors found increased FC between the right anterior insula and the right thalamus during the resting session and increased FC between the rAI and the ACC during the task session in patients with TMDs. In the present study, the results did not show any increased FC associated with the rAI in the TMD group compared with HCs. One possible explanation for the discrepancy in the results of the 2 studies might be the difference in the severity of pain in the patients in the 2 investigations. In the previous study, as the authors pointed out, the study patients were only mildly affected and were likely at the beginning of the chronification process and/or in a compensation stage, and thus they probably do not represent the clinical picture of "severely disabled" TMD. In the present study, all patients had severe pain (open-mouth pain ≥5 measured by the VAS). Therefore, the results of the 2 studies might represent different brain alterations at 2 different chronification stages in TMD.

Decreased negative FC between the rAI and the precuneus in patients with TMD pain

In contrast to the positive correlation between the rAI and the MCC, the correlation between the rAI and the precuneus was negative in both the HC and TMD groups. The negative correlation between these 2 areas was expected, as they belong to 2 different brain networks usually found to be anticorrelated.[32] The precuneus is a key node of the DMN and has been well documented to be negatively correlated with task-positive networks, such as the SN.[44] The SN is thought to play an important role in keeping the balance between internal-oriented state and external activity and, thus, in regulating or coordinating activity in other networks, such as promoting deactivation of the DMN in response to a salient stimulus.[45] It has been reported that damage to the integrity of SN structure leads to ineffective coordination in DMN activity, which has a negative effect on cognitive task performance.[46] The decreased anticorrelation between the SN and the DMN in the TMD group, as observed in the present study (see Figures 4 D and E), suggests disruption of balance between the 2 networks, and this might explain the cognitive deficit observed in patients with chronic pain.[47] Indeed, altered functional connectivity between the insula and the DMN have been reported in several chronic pain conditions. However, the directions of the change were inconsistent: Increased FC was reported in fibromyalgia[48] and chronic low back pain,[49] but decreased FC was reported in irritable bowel

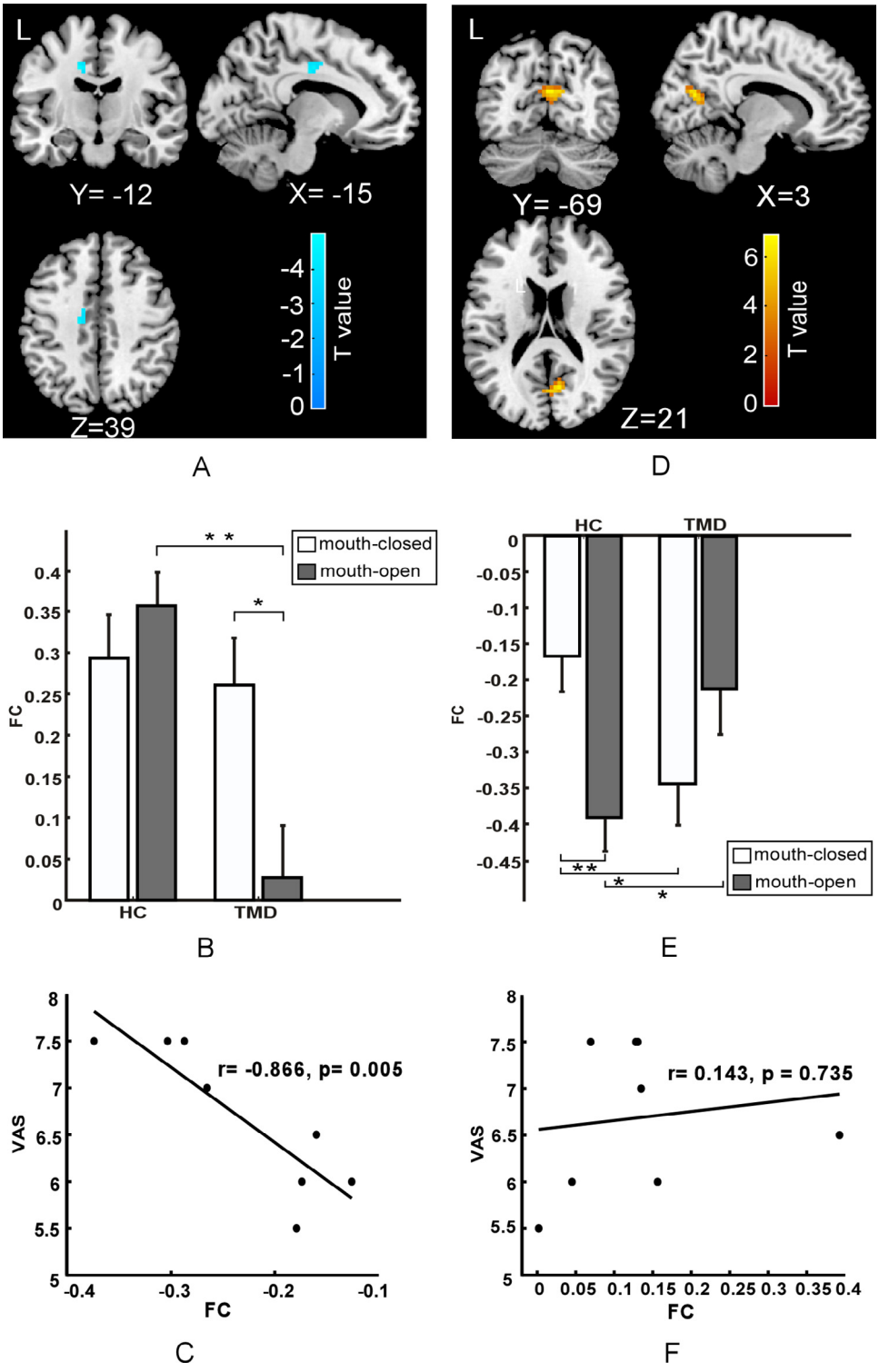

Fig. 4. The difference in functional connectivity (FC) between the healthy controls (HC) group and the temporomandibular disorders (TMD) group based on the seed region obtained from the z-score regional homogeneity (zReHo) values. **A, D,** Compared with the HC group, the TMD group showed decreased positive FC *(blue)* between the right anterior insula (rAI; seed region) and the left middle cingulate cortex (MCC; connected region, the brain area shown in blue) (**A**) and Decreased negative FC *(red-yellow)* between the rAI (seed region) and the right precuneus (connected region, the brain area shown in red-yellow) (**D**). **B, E,** The averaged rAI-MCC FC z-scores (**B**) and the averaged rAI-precuneus FC z scores (**E**) of each condition and each group. **P* < .05, ***P* < .005. **C, F,** Correlation analysis between the visual analogue scale (VAS) score and the averaged rAI-MCC FC z scores (**C**) and between the VAS score and the averaged rAI-precuneus FC z scores (**F**) ("mouth-open" – "mouth-closed"). The result showed that the higher the VAS score, the weaker was the FC between the rAI and the MCC.

syndrome.[24] One possible explanation for the inconsistency might be that different mechanisms underlie different chronic pain conditions. However, it could also result from the differences in patients' pain severity, analytical methods, or even imaging modalities used in these studies. For example, Napadow et al.[48] used independent component analysis and Hong et al.[24] used "seed regions–whole brain" connectivity analysis. Most previous studies used fMRI data, while Loggia et al.[49] used cerebral blood flow data obtained by arterial spin labeling technique.

Limitations

There were several limitations to the present study. First, the study only included typical TMJ synovitis with severe pain (VAS >5) in the patient group to make the sample as homogeneous as possible. This led to a relatively small sample size, and therefore, the present study should be considered a pilot study. Second, although pain-related brain changes in TMDs are likely to be a result of complex interactions between the general pain system and the brain areas specially related to orofacial pain, the brain areas (i.e., rAI, MCC, and precuneus) identified in the TMD group in the present study are part of the general central pain systems common to all pain conditions but not specific to orofacial pain. Two facts could have prevented the orofacial pain-specific areas from being detected in the present study: (1) The changes in these areas (e.g., the corresponding area in S1) were not significant enough to be detected with the small sample size of the present study; and (2) the side of the pain location was not consistent across patients in the present investigation. Future studies with a larger sample size and a patient group with pain on the same side are needed to confirm the results of the present research. A large sample size will also allow for investigation of differences in spontaneous brain activity and connectivity between patients with TMD pain on different sides. In addition, more advanced analytical methods, such as machine learning techniques, may be adopted to identify brain changes in TMD because these techniques have been shown to be more sensitive in detecting differences between conditions or groups.[50-53] Third, it remains undetermined whether there is a causal relationship between pain and changes in ReHo and FC in patients with TMDs. In other words, it is unclear whether chronic pain results in the abnormal spontaneous neural activities reflected by the ReHo and FC or the abnormal neural activities in these patients make them more susceptible to pain.

CONCLUSIONS

Using an experimental design with a pain state (i.e., the open-mouth state) of high clinical relevance and with the ability to perform both within-subject and between-subject comparisons to minimize possible confounding effects, the study examined the pain-related, spontaneous neural activities and connectivities in patients with TMD pain compared with HCs. The study found that TMD pain was associated with reduced synchronization of regional neural activities within the rAI and reduced FCs with the MCC and the precuneus. These results provide evidence supporting a functional maladaptation of the SN and its interaction with the DMN in patients with TMDs. These findings highlight the potential of fMRI examination in understanding and treating TMD pain in clinical practice because it can help clarify the underlying mechanisms and etiology of TMD pain and lead to the development of new medicines or treatment methods that can act on identified central neural targets.

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REFERENCES

1. Dworkin SF. Personal and societal impact of orofacial pain. In: Fricton JR, Dubner R, eds. *Orofacial Pain and Temporomandibular Disorders*. New York: Raven Press; 1995:15-32.
2. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord*. 1992;6:301-355.
3. Riley JL, Benson MB, Gremillion HA, et al. Sleep disturbance in orofacial pain patients: pain-related or emotional distress? *Cranio*. 2001;19:106-113.
4. Chisnoiu AM, Picos AM, Popa S, et al. Factors involved in the etiology of temporomandibular disorders—a literature review. *Clujul Med*. 2015;88:473-478.
5. LeResche L. Epidemiology of temporomandibular disorders: implications for the investigation of etiologic factors. *Crit Rev Oral Biol Med*. 1997;8:291-305.
6. Alanen P. Occlusion and temporomandibular disorders (TMD): still unsolved question? *J Dent Res*. 2002;81:518-519.
7. Harper DE, Schrepf A, Clauw DJ. Pain mechanisms and centralized pain in temporomandibular disorders. *J Dent Res*. 2016;95:1102-1108.
8. Moayedi M, Weissman-Fogel I, Crawley AP, et al. Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in patients with temporomandibular disorder. *Neuroimage*. 2011;55:277-286.
9. Weissman-Fogel I, Moayedi M, Tenenbaum HC, Goldberg MB, Freeman BV, Davis KD. Abnormal cortical activity in patients with temporomandibular disorder evoked by cognitive and emotional tasks. *Pain*. 2011;152:384-396.
10. Turp JC, Kowalski CJ, O'Leary N, Stohler CS. Pain maps from facial pain patients indicate a broad pain geography. *J Dent Res*. 1998;77:1465-1472.
11. Sarlani E, Greenspan JD. Evidence for generalized hyperalgesia in temporomandibular disorders patients. *Pain*. 2003;102:221-226.
12. Sarlani E, Greenspan JD. Why look in the brain for answers to temporomandibular disorder pain? *Cells Tissues Organs*. 2005;180:69-75.

13. Morton DL, Sandhu JS, Jones AK. Brain imaging of pain: State of the art. *J Pain Res*. 2016;9:613-624.
14. May A. Chronic pain may change the structure of the brain. *Pain*. 2008;137:7-15.
15. Yu D, Yuan K, Zhao L, Liang F, Qin W. Regional homogeneity abnormalities affected by depressive symptoms in migraine patients without aura: a resting state study. *PLoS ONE*. 2013;8: e77933.
16. He SS, Li F, Song F, et al. Spontaneous neural activity alterations in temporomandibular disorders: a cross-sectional and longitudinal resting-state functional magnetic resonance imaging study. *Neuroscience*. 2014;278:1-10.
17. Ichesco E, Quintero A, Clauw DJ, et al. Altered functional connectivity between the insula and the cingulate cortex in patients with temporomandibular disorder: a pilot study. *Headache*. 2012; 52:441-454.
18. Youssef AM, Gustin SM, Nash PG, et al. Differential brain activity in subjects with painful trigeminal neuropathy and painful temporomandibular disorder. *Pain*. 2014;155:467-475.
19. Gerstner G, Ichesco E, Quintero A, Schmidt-Wilcke T. Changes in regional gray and white matter volume in patients with myofascial-type temporomandibular disorders: a voxel-based morphometry study. *J Orofac Pain*. 2011;25:99-106.
20. Zang Y, Jiang T, Lu Y, He Y, Tian L. Regional homogeneity approach to fMRI data analysis. *Neuroimage*. 2004;22:394-400.
21. Bao CH, Liu P, Liu HR, et al. Differences in regional homogeneity between patients with Crohn's disease with and without abdominal pain revealed by resting-state functional magnetic resonance imaging. *Pain*. 2016;157:1037-1044.
22. Wang Y, Zhang X, Guan Q, Wan L, Yi Y, Liu CF. Altered regional homogeneity of spontaneous brain activity in idiopathic trigeminal neuralgia. *Neuropsychiatr Dis Treat*. 2015;11:2659-2666.
23. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci*. 2007;8:700-711.
24. Hong JY, Kilpatrick LA, Labus JS, et al. Sex and disease-related alterations of anterior insula functional connectivity in chronic abdominal pain. *J Neurosci*. 2014;34:14252-14259.
25. Wang G, Erpelding N, Davis KD. Sex differences in connectivity of the subgenual anterior cingulate cortex. *Pain*. 2014;155: 755-763.
26. Amunts K, Schlaug G, Schleicher A, et al. Asymmetry in the human motor cortex and handedness. *Neuroimage*. 1996;4:216-222.
27. Pool EM, Rehme AK, Fink GR, Eickhoff SB, Grefkes C. Handedness and effective connectivity of the motor system. *Neuroimage*. 2014;99:451-460.
28. Yan C, Zang Y. DPARSF: a MATLAB Toolbox for "Pipeline" data analysis of resting-state fMRI. *Front Syst Neurosci*. 2010;4:13.
29. Song XW, Dong ZY, Long XY, et al. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS ONE*. 2011;6:e25031.
30. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci USA*. 2003;100:253-258.
31. Ke J, Qi R, Liu C, et al. Abnormal regional homogeneity in patients with irritable bowel syndrome: a resting-state functional MRI study. *Neurogastroenterol Motil*. 2015;27:1796-1803.
32. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci USA*. 2005;102:9673-9678.
33. Otti A, Gundel H, Wohlschlager A, Zimmer C, Sorg C, Noll-Hussong M. Default mode network of the brain. Neurobiology and clinical significance. *Nervenarzt*. 2012;83:16, 18-24 [in German].
34. Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Res Brain Res Rev*. 1996; 22:229-244.
35. Brooks JC, Tracey I. The insula: a multidimensional integration site for pain. *Pain*. 2007;128:1-2.
36. Iannetti GD, Mouraux A. From the neuromatrix to the pain matrix (and back). *Exp Brain Res*. 2010;205:1-12.
37. Garcia-Larrea L, Peyron R. Pain matrices and neuropathic pain matrices: a review. *Pain*. 2013;154(suppl 1):S29-S43.
38. Wiech K, Lin CS, Brodersen KH, Bingel U, Ploner M, Tracey I. Anterior insula integrates information about salience into perceptual decisions about pain. *J Neurosci*. 2010;30:16324-16331.
39. Henry DE, Chiodo AE, Yang W. Central nervous system reorganization in a variety of chronic pain states: a review. *PM R*. 2011; 3:1116-1125.
40. Downar J, Mikulis DJ, Davis KD. Neural correlates of the prolonged salience of painful stimulation. *Neuroimage*. 2003;20: 1540-1551.
41. Legrain V, Damme SV, Eccleston C, Davis KD, Seminowicz DA, Crombez G. A neurocognitive model of attention to pain: behavioral and neuroimaging evidence. *Pain*. 2009;144:230-232.
42. Wiech K, Jbabdi S, Lin CS, Andersson J, Tracey I. Differential structural and resting state connectivity between insular subdivisions and other pain-related brain regions. *Pain*. 2014;155:2047-2055.
43. Borsook D, Edwards R, Elman I, Becerra L, Levine J. Pain and analgesia: the value of salience circuits. *Prog Neurobiol*. 2013; 104:93-105.
44. Fransson P, Marrelec G. The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: evidence from a partial correlation network analysis. *Neuroimage*. 2008;42:1178-1184.
45. Sridharan D, Levitin DJ, Menon V. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc Natl Acad Sci USA*. 2008;105:12569-12574.
46. Bonnelle V, Ham TE, Leech R, et al. Salience network integrity predicts default mode network function after traumatic brain injury. *Proc Natl Acad Sci USA*. 2012;109:4690-4695.
47. Baliki MN, Geha PY, Apkarian AV, Chialvo DR. Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J Neurosci*. 2008;28:1398-1403.
48. Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE. Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. *Arthritis Rheum*. 2010;62:2545-2555.
49. Loggia ML, Kim J, Gollub RL, et al. Default mode network connectivity encodes clinical pain: an arterial spin labeling study. *Pain*. 2013;154:24-33.
50. Harper DE, Shah Y, Ichesco E, Gerstner GE, Peltier SJ. Multivariate classification of pain-evoked brain activity in temporomandibular disorder. *Pain Rep*. 2016;1:e572.
51. Wager TD, Atlas LY, Lindquist MA, Roy M, Woo CW, Kross E. An fMRI-based neurologic signature of physical pain. *N Engl J Med*. 2013;368:1388-1397.
52. Hazlett HC, Gu H, Munsell BC, et al. Early brain development in infants at high risk for autism spectrum disorder. *Nature*. 2017; 542:348-351.
53. Liang M, Mouraux A, Hu L, Iannetti GD. Primary sensory cortices contain distinguishable spatial patterns of activity for each sense. *Nat Commun*. 2013;4:1979.

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