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Research Submission

# Altered Functional Connectivity Between the Insula and the Cingulate Cortex in Patients With Temporomandibular Disorder: A Pilot Study

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**Background.—Among the most common chronic pain conditions, yet poorly understood, are temporomandibular disorders (TMDs), with a prevalence estimate of 3-15% for Western populations. Although it is increasingly acknowledged that central nervous system mechanisms contribute to pain amplification and chronicity in TMDs, further research is needed to unravel neural correlates that might abet the development of chronic pain.**

**Objective.—The insular cortex (IC) and cingulate cortex (CC) are both critically involved in the experience of pain. The current study sought specifically to investigate IC–CC functional connectivity in TMD patients and healthy controls (HCs), both during resting state and during the application of a painful stimulus.**

**Methods.—Eight patients with TMD, and 8 age- and sex-matched HCs were enrolled in the present study. Functional magnetic resonance imaging data during resting state and during the performance of a pressure pain stimulus to the temple were acquired. Predefined seed regions were placed in the IC (anterior and posterior insular cortices) and the extracted signal was correlated with brain activity throughout the whole brain. Specifically, we were interested whether TMD patients and HCs would show differences in IC–CC connectivity, both during resting state and during the application of a painful stimulus to the face.**

**Results.—As a main finding, functional connectivity analyses revealed an increased functional connectivity between the left anterior IC and pregenual anterior cingulate cortex (ACC) in TMD patients, during both resting state and applied pressure pain. Within the patient group, there was a negative correlation between the anterior IC–ACC connectivity and clinical pain intensity as measured by a visual analog scale.**

**Conclusions.—Since the pregenual region of the ACC is critically involved in antinociception, we hypothesize that an increase in anterior IC–ACC connectivity is indicative of an adaptation of the pain modulatory system early in the chronification process.**

**Key words: chronic pain, temporomandibular disorder, functional connectivity, insular cortex, cingulate cortex**

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*Conflict of Interest:* None

**Abbreviations: ACC anterior cingulate cortex, CNS central nervous system, GBS Gracely Box Scale, IC insular cortex, MCC mid cingulate cortex, NIH National Institutes of Health, PCC posterior cingulate cortex, SF-MPQ short form of McGill Pain Questionnaire, SMA supplementary motor area, STPI State-Trait Personality Inventory, TMD temporomandibular disorder**

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Chronic non-malignant pain is a significant public health problem, thought to affect up to 40% of the general population at any single point in time.[1] Among the most common chronic pain conditions are temporomandibular disorders (TMDs), with a prevalence estimate of 3-15% for Western populations.[2] TMDs are partly defined on the basis of clinical signs such as temporomandibular joint sounds, impaired mandibular movement, or limitation of mouth opening. However, pain is in most cases the presenting and most problematic symptom and can affect various parts of the face and the head, such as preauricular, facial, and masticatory muscle regions.[3] Historically, pain in TMD was believed to be caused by peripheral mechanisms, such as acute or chronic inflammation of the joint, tenderness of the masticatory musculature resulting from microtrauma, oromotor dysfunction, or "imbalance" of the dentoskeletal and neuromuscular systems. However, in many TMD patients, no peripheral pain generator can be identified, which is especially true for the myofascial pain subgroup. On the other hand, the first brain imaging studies have begun to shed light on altered brain function and morphology in TMD patients,[4-7] giving evidence that in TMD, like in other chronic pain conditions, central nervous system mechanisms contribute to the process of pain amplification and chronification.

Two of the forebrain structures most consistently activated, when a subject experiences pain, are the insular cortex (IC) and the cingulate cortex (CC). Both structures have been reported to show structural and, in case of the IC, also neurochemical changes[8,9] in individuals with chronic pain. The structural connection between IC and CC has been extensively studied in primates, showing a connection between the anterior IC and the rostral extent of the anterior cingulate gyrus (rACC, BA 24); the mid and posterior primate IC on the other hand were shown to have connections with the dorsal CC (BA 23 and 24) and the upper banks of the cingulate sulcus and premotor cortex.[10] Only recently functional magnetic resonance imaging (fMRI) has been applied in humans to investigate functional connectivity between the IC and CC.[11] Functional connectivity has been operationally defined to refer to temporal correlations across cortical regions and can be assessed during the application of a pain stimulus, but also during resting state. The term "resting-state" functional connectivity refers to brain areas that have a strong temporally correlated activity in a non-task state. It is thought that these low-frequency (<0.1 Hz) fluctuations are functionally relevant indices of connectivity between brain regions subserving similar or related brain functions.[12]

With respect to functional connectivity, it has been suggested that the anterior and posterior IC, although part of the same anatomical structure and highly connected with each other, subserve different aspects of pain perception and are integrated into different neural networks. The anterior IC, functionally connected to the anterior cingulate cortex (ACC), has been suggested to integrate interoceptive input with its emotional salience, while the mid/posterior IC, functionally connected to the mid cingulate (MCC) and posterior cingulate cortex (PCC), is thought to be more related to environmental monitoring and response selection.[11] It is therefore not surprising that pain researchers try to explore IC connectivity, attempting to unravel neural correlates of chronic pain more thoroughly.

Given that the IC is critically involved in the experience of pain, but also in other functions, that are possibly relevant to chronic pain such as interoception and self-awareness, the current study sought specifically to investigate IC connectivity in TMD patients and healthy controls (HCs), both in the resting state and during the application of a painful stimulus. Following the approach of a recently published study by Taylor et al,[11] predefined seed regions

(SR) were placed in the IC (anterior and mid/posterior IC bilaterally totaling 4 regions overall). These predefined SR of interests' time series were used to perform a correlation with the time series of all the voxels in a whole-brain analysis. In a first step, we sought to replicate the findings of Taylor et al showing that the anterior IC is functionally connected with the posterior part of the ACC/MCC, whereas the mid/posterior IC is connected to the posterior MCC and supplementary motor area (SMA), demonstrating a segregated IC–CC connectivity along the anterior–posterior axis. We were then interested in whether TMD patients and HCs would show differences in IC–CC connectivity, both during resting state and during the application of a painful stimulus and whether IC–CC connectivity correlated with clinical pain measures and/or evoked pain ratings.

## METHODS

**Subjects and Behavioral Data.—**Originally, 10 patients with myofascial-type TMD had initially been enrolled in the study. The structural images of 9 patients and 9 HCs were analyzed within a voxel-based morphometry study, and the results reported elsewhere.[6] The fMRI data of 8 patients (8 women; aged 23 to 31 years) and 8 HCs (8 women; aged 22 to 27 years) were available for functional connectivity analysis. Groups did not differ significantly in age ($P = .49$), or ethnicity (both groups consisted of 1 African American, 3 Asian, and 5 Caucasian participants).

All subjects with TMD had been carefully examined by a dentist with experience in orofacial pain applying the research diagnostic criteria (RDC) for the diagnosis of myofascial-type TMD (Group 1a, 1b).[13] Only those subjects that fulfilled the Group I myofascial pain criteria were included. Inclusion and exclusion criteria consisted of the following: (1) presence of pain in the face, jaws, or temples greater than 1× per week; (2) presence of pain symptoms for greater than 3 months; (3) meeting the RDC criteria for myofascial pain Group 1a, 1b; (4) no comorbidities of other chronic pain disorders (eg, fibromyalgia or irritable bowel syndrome). The main inclusion criterion for HCs was absence of TMD pain, or facial pain less than 1× per week. Exclusion criteria for all subjects included physical impairment (eg, complete blindness, deafness, paraplegia), or coexisting physical injury (eg, sprained ankle, neck injury, etc.), any outstanding history of systemic or medical conditions, psychiatric illnesses, substance abuse within 2 years, and presence of head or neck pain other than masticatory myalgia. Non-steroidal anti-inflammatory drugs and other over-the-counter analgesics were allowed until 3 days before the pain and scanning trials; medication overuse had been ruled out in all patients. All subjects were right-handed. Because pain symptoms can be coupled to menstrual cycle phase in premenopausal women and women on oral contraceptives,[14] the subjects (all female) participated in pain and imaging studies within 3 days of menstrual onset. The University of Michigan Medical School Institutional Review Board for Human Subject Research determined that project title entitled, Pain Mechanisms in Chronic Multisymptom Illnesses (CMI), conforms with applicable guidelines, state and federal regulations, and the University of Michigan's Federalwide Assurance (FWA) with the Department of Health and Human Services (HHS). All participants signed an informed consent that detailed the procedures of the study.

The clinical pain experience of patients with TMD and HCs was assessed using the visual analog scale (VAS) and the pain rating index (PRI) from the Short-Form McGill Pain Questionnaire (SF-MPQ).[15] The VAS consists of a 10-cm line anchored on the left with "No Pain" and on the right with "Worst Possible Pain." Participants in the study were asked to rate their present orofacial pain by placing a tick along this line. The PRI component of the SF-MPQ consisted of 15 word descriptors (11 sensory and 4 affective). Participants rated these descriptors as either "none," "mild," "moderate," or "severe," giving a score of 0, 1, 2, or 3, respectively, for each descriptor. The measures were added to yield sensory, affective, and total scores. Another questionnaire used to evaluate clinical pain was the Brief Pain Inventory (BPI).[16] Information from this measure was used to determine both severity of pain and the degree of pain interference. Questions for these measures were answered using a 0 to 10 numeric rating scale for each item. The State-Trait Personality Inventory (STPI) is a self-report tool designed to measure anxiety and depression. The STPI consists of eight 10-item subscales. The trait depression scale and anxiety scale were used to assess each subject's emotional disposition, and both scales were rated on a 4-point intensity scale. Furthermore, the state anxiety scale was used to assess the current emotional state of each subject and was rated in standard fashion on a 4-point frequency scale.[17]

Prior to scanning, pressure pain values eliciting low pain (0.5 on the Gracely Box Scale [GBS], see below and Fig. S1), medium pain (7.5 on the GBS), and high pain (13.5 on GBS) were determined for every subject using the multiple random staircase (MRS) method. The GBS is a numerical scale that is used to evaluate present pain intensity. This scale is comprised of 21 boxes, sequentially numbered beginning with 0 and ending with 20. It is aligned vertically, with 0 as the lowest box. Descriptive words are arranged next to the numbers corresponding with varying levels of pain.[18] The corresponding pressures were determined for the left anterior temporalis region as follows. A form-fitting mask was created for each individual subject. The mask was molded to each subject's face using radiological thermoplastic mesh. Holes were placed for the subject's eyes and nose, and the mask was held in place using 2 Velcro straps (for an example, see Fig. 1). Once fit, a plunger with an area of ~1 cm$^2$ was attached to the mask located at the subject's left anterior temporalis region.

The following analyses were performed to describe and analyze clinical/behavioral data in both cohorts:

*Analysis 1a:* We looked for differences in age, pain scores, anxiety and depression levels between groups. Due to the relatively small sample size, we applied the Mann–Whitney *U*-test to test for significant differences in behavioral scores (pain, depression, and anxiety) between groups (Table 1). Differences were deemed significant at $P < .05$ (corrected for multiple comparisons using a Bonferroni correction).

*Analysis 1b:* We performed correlation analyses (Spearman rank correlation) looking for significant correlations between pain measures (pain duration, BPI scores, MPQ scores), depression and anxiety measures. Correlations were deemed significant at $P < .05$ (corrected for multiple comparisons using a Bonferroni correction). All statistical analyses investigating demographic and behavioral measures were assessed using SPSS, version 17.

**Fig 1.—Mask used for the application of pressure pain. An example of the mask used to deliver pressure pain stimuli to each subject.**

**Neuroimaging – Data Acquisition.—***Resting State.*—Magnetic resonance imaging was performed on a 3.0 Tesla GE Signa scanner (LX [VH3] release, Neuro-optimized gradients). Resting-state fMRI data were acquired using a T2*-weighted spiral sequence (repetition time [TR] = 2.0 s, echo time [TE] = 30 ms, flip angle [FA] = 90°, matrix size 64 × 64 with 43 slices, field of view [FOV] = 20 cm and 3.12 × 3.12 × 3 mm voxels), using a General Electric Signa scanner 9.0, VH3 with 16 rod birdcage transmit-receive radio frequency coil. During the ~6-minute resting-state fMRI acquisition period (179 scans), the subjects were asked to remain awake with their eyes open. A motionless cross was presented on the screen. Minimal cognitive tasks such as staring at a cross are thought not to disrupt resting-state networks.[19] A T1-weighted gradient echo data set (TR 1400 ms, TE 1.8 ms, FA 15°, FOV 256 × 256, yielding 124 sagittal slices with a defined voxel size of 1 × 1 × 1.2 mm) was also acquired for each subject.

**Table 1.—Behavioral Data**

|  | TMD (Mean ± SD) | HC (Mean ± SD) | *P* Value |
|---|---|---|---|
| Age | 25.4 ± 2.5 | 24.8 ± 1.4 | .796 |
| Pain duration | 2.5 ± 2.1 | – | NA |
| BPI Sev | 2.0 ± 1.3 | 0.6 ± 0.7 | .136 |
| BPI Int | 2.0 ± 3.2 | 0.1 ± 0.3 | .190 |
| MPQ Tot | 6.1 ± 5.2 | 0.4 ± 1.3 | **.001** |
| MPQ Sens | 5.6 ± 4.4 | 0.4 ± 1.3 | **.001** |
| MPQ Aff | 0.6 ± 0.9 | 0 ± 0 | .258 |
| MPQ VAS | 2.2 ± 1.4 | 0.2 ± 0.6 | **<.001** |
| STPIA Ax | 19.0 ± 6.9 | 13.1 ± 3.9 | .031 |
| STPIDA Ax | 17.2 ± 6.0 | 12.9 ± 2.7 | .136 |
| STPIDA D | 16.1 ± 6.4 | 11.1 ± 2.0 | .050 |
| Medium pressure—temple (kg/cm$^2$) | 1.2 ± 0.7 | 1.0 ± 0.8 | .654 |
| High pressure—temple (kg/cm$^2$) | 2.6 ± 1.5 | 2.4 ± 1.2 | .840 |

Mann–Whitney *U*-test was used for group comparison. *P* values were deemed significant at $P < .05$ after correction for multiple comparisons (significant differences are indicated in bold type).

BPI Int = Brief Pain Inventory pain interference; BPI Sev = Brief Pain Inventory pain severity; HC = healthy control; MPQ Aff = Short-Form McGill Pain Questionnaire Pain Rating Index—Affective Score; MPQ Sens = Short-Form McGill Pain Questionnaire Pain Rating Index—Sensory Score; MPQ Tot = Short-Form McGill Pain Questionnaire Pain Rating Index—Total Score; MPQ VAS = Short-Form McGill Pain Questionnaire Visual Analog Scale; NA = not available, missing data; STPIA Ax = State-Trait Personality Inventory state anxiety; STPIDA Ax = State-Trait Personality Inventory trait anxiety; STPIDA D = State-Trait Personality Inventory trait depression; TMD = temporomandibular disorder; – = inconclusive results.

*Pain Run.*—Each participant was subjected to one 10-minute evoked pressure scan in the MRI scanner and images were collected using a T2*-weighted spiral sequence (TR = 2.5 s, TE = 30 ms, FA = 90°, matrix size 64 × 64 with 48 slices, FOV = 22 cm and 3.44 × 3.44 × 3 mm voxels). Pressure pain was delivered with a pneumatic system. This system was comprised of medical grade tubing, several valves, an air supply containing medical grade air, and an analog air controller (used to regulate different pressures). Agilent VEE pro and E-prime software programs were used to coordinate pressure pain administration at the correct onsets. Further details of the pressure pain equipment setup are described in Gracely et al.[20] During the pain run, pressure pain was delivered to the left anterior temporalis region by a piston with a surface area of 1 cm$^2$. Pressures eliciting high and medium pain as previously determined (see *Subjects and behavioral data*) were applied in a pseudo random fashion and interleaved with an "off" condition (no pressure applied). A run contained a total of 12 pain blocks (6 medium, 6 high; each block 25 seconds in duration) and 12 off blocks (each block 25 seconds in duration).

**Neuroimaging – Preprocessing and Statistical Analyses.—***Preprocessing and Analysis of Functional Connectivity – Resting State.*—The first 6 images were discarded from the data set and not analyzed in order to avoid equilibration effects. Data were preprocessed and analyzed using Statistical Parametric Mapping software packages (SPM, version 8; Functional Imaging Laboratories, London, UK), as well as the functional connectivity toolbox Conn (Cognitive and Affective Neuroscience Laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA) running under Matlab 7.5b (Mathworks, Sherborn, MA, USA). Preprocessing steps included motion correction (realignment to the first image of the time series), normalization to the standard SPM–EPI template (generating 2 × 2 × 2 mm resolution images) and smoothing (convolution with an 8-mm FWHM Gaussian Kernel).

Based on the approach by Taylor et al,[11] SR were defined within the anterior and posterior IC bilaterally; SR were created as spheres (6-mm diameter) using MarsBaR software (http://marsbar.sourceforget.net). For details on center coordinates, presented in Montreal Neurological Imaging (MNI) space, see Figure 2 and Table S1. SR time series were extracted; white matter and cerebrospinal fluid signal, as well as realignment parameters were entered into the analysis as covariates of no interest, using *CompCor*, a principal component-based method for noise correction/reduction in BOLD and perfusion data.[21] A band pass filter (frequency window: 0.001-0.08 Hz) was applied, thus removing linear drift artifacts and high-frequency noise. First-level analyses were performed correlating SR signal with voxel signal throughout the whole brain, thereby creating SR-to-voxel connectivity maps (4 maps for each individual). Connectivity maps were then used for second-level (random effects) analyses.

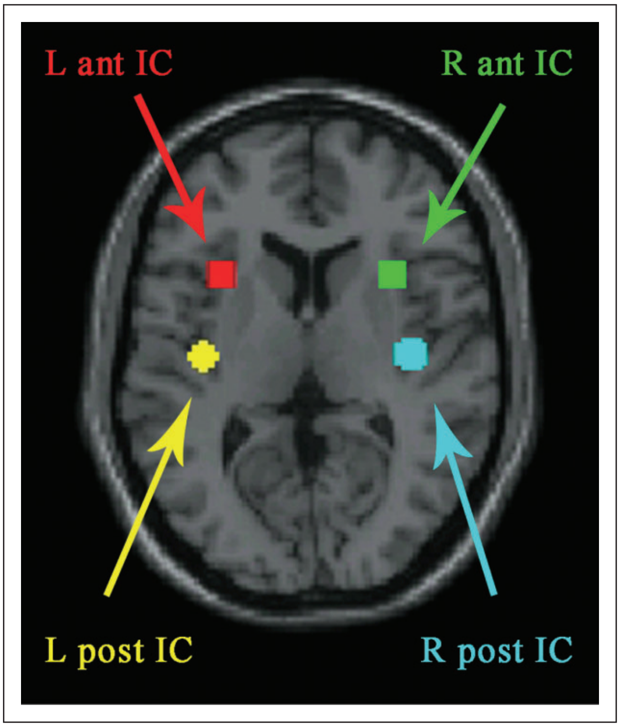

**Fig 2.—Seed regions. This figure displays the 4 seed regions used for functional connectivity analyses. Seed regions were spheres of 6 mm surrounding a peak voxel. Montreal Neurological Imaging (MNI) coordinates for each voxel include: left (L) anterior (ant) insular cortex (IC): $x = -32$, $y = 16$, $z = 6$; left posterior (post) IC: $x = -39$, $y = -15$, $z = 1$; right (R) anterior IC: $x = 32$, $y = 16$, $z = 6$; right posterior IC: $x = 39$, $y = -15$, $z = 8$.**

*Analysis 2a:* In a first step, IC connectivity was determined by performing a 1-sample *t*-test for each SR, including both TMD patients and HCs.

*Analysis 2b:* We were then interested in whether there were differences in functional connectivity between groups. To this end, 2-sample *t*-tests for each SR were performed. Age was added as nuisance variable.

*Analysis 2c:* To further evaluate behavioral/clinical relevance of the clusters found in Analysis 2b, correlations between functional connectivity and pain measures (eg, pain intensity, pain severity, pain duration) were assessed in a third step.

All statistical maps were corrected for multiple comparisons on the cluster level ($P < .05$), derived from an uncorrected $P < .001$ on the voxel level, with a cluster extent of 82 contiguous voxels, as estimated by the AlphaSim application (implemented in the Analysis of Functional NeuroImages [AFNI] software [http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim]), based on a Monte Carlo simulation (5000 simulations) applied to a whole-brain mask. For explorative reasons, as we were specifically interested in IC–CC connectivity a second mask, just covering the cingulum (anterior, medial, and posterior, bilaterally) was created using the WFU_PickAtlas (http://www.nitrc.org/projects/wfu_pickatlas). Monte Carlo simulation using that mask resulted in a lower extent threshold: 28 contiguous voxels ($P < .001$, uncorrected, on the voxel level), yielding correction for multiple comparisons on the cluster level (within that mask). As these results could be interesting for future analyses, they are reported and briefly commented on; however, as they did not survive the correction for multiple comparisons throughout the whole brain, they should be viewed with caution. These results are specifically marked in *Result* and tables.

Anatomical regions were labeled following the nomenclature of the Automated Anatomical Labeling (AAL) atlas[22] and xjView viewing program for SPM (http://www.alivelearn.net/xjview8/).

*Preprocessing and Analysis of Functional Connectivity – Pain Run.*—Preprocessing steps were performed in a similar fashion to the resting-state analysis using the same SRs. A first-level model was implemented for each subject by compiling all of the blocks for each condition respectively. Each pressure pain condition totaled six 25-s blocks and the off condition totaled twelve 25-s blocks. The block (off, medium, and high) were modeled as covariate of no interest (in addition to white matter signal, cerebrospinal fluid signal, and realignment parameters). First-level analyses were performed correlating SR signal with voxel signal throughout the whole brain for each condition, thereby creating SR-to-voxel connectivity maps (3 [conditions] × 4 [SR] maps for each individual). Connectivity maps were then used for second-level (random effects) analyses.

*Analysis 2d:* Using a flexible factorial design within the general linear model implemented in SPM, main effects across groups (medium/high pain vs off) were investigated.

*Analysis 2e:* We were then interested in whether there were differences in functional connectivity between groups (medium/high pain vs off). To this end interaction analyses were performed (group × stimulus [low pain vs off and high pain vs off]).

Statistical maps were corrected for multiple comparisons ($P < .001$, uncorrected on the voxel level, with a cluster extent of 82 contiguous voxels, as described above). Altered functional connectivity between the SR and a target region in the TMD group, as compared to the HC group, is referred to as a hyper-connection (increased functional connectivity), respectively hypo-connection (decreased functional connectivity), between the 2 regions. To further evaluate behavioral/clinical relevance of the clusters found in Analysis 2e, correlation analyses between contrast estimates (high pain) and pressure necessary to elicit high pain (determined outside the scanner, see *Subjects and behavioral data*) were performed in a third step. Parameter estimates were extracted from group-level results (clusters defined in Analysis 2e, interaction analysis), yielding 1 parameter estimate per subject, which were then transferred to SPSS, version 17, and further analyzed (using Spearman rank correlation).

## RESULTS

**Subjects and Behavioral Data.**—*Analysis 1a:* As expected, patients with TMD displayed significantly higher clinical pain (VAS scores) than HCs (TMD: mean = 2.19, SD = 1.48; control: mean = 0.25, SD = 0.59; $P = .004$). TMD patients also showed higher scores than HCs for the MPQ Tot (TMD: mean = 6.50, SD = 5.42; control: mean = 0.50, SD = 1.41; $P = .009$) and the MPQ Sens (TMD: mean = 5.88, SD = 4.64; control: mean = 0.50, SD = 1.41; $P = .007$) measures (for details, see Table 1).

*Analysis 1b:* Within the TMD group, the STPI Trait-anxiety scores were significantly correlated with STPI Trait-depression scores ($\rho = 0.94$, $P = .001$). None of the anxiety and/or depression scores correlated significantly with BPI pain scores or MPQ scores. For details on the $\rho$ values and $P$ values, see Table S2.

**Neuroimaging – Connectivity Analyses.**—*Functional Connectivity – Resting State.*—Inspection of individual T1 MR-images revealed no gross morphological abnormalities for any participant. Functional connectivity analyses revealed functional connectivity between the chosen seeds and regions of the pain system. Results are summarized in Tables 1 and 2.

*Analysis 2a:* The anterior IC was functionally connected to the posterior ACC/MCC, and the posterior IC was functionally connected to the medial frontal gyrus/superior frontal gyrus/SMA (Fig. 3A).

*Analysis 2b:* For between-group comparisons, there were hyper-connections for the TMD patients compared to HCs. These occurred between the left anterior IC and the left rostral (pregenual) ACC (peak voxel: $x = 2$, $y = 38$, $z = 2$; Z value = 4.47), (Fig. S3) the left posterior IC and the left parahippocampal gyrus ($x = -14$, $y = -4$, $z = -26$; Z value = 5.07), and the right anterior IC with the right thalamus ($x = 8$, $y = -6$, $z = 6$; Z value = 4.35).

*Analysis 2c:* Within the TMD group, the functional connectivity of the left anterior IC and the rACC was negatively correlated with clinical pain ($\rho = -0.952$, $P < .001$, Figs. 3C and S2); that is, TMD patients with higher clinical pain had less anterior IC–rACC connectivity. The same association was found for MPQ total scores ($\rho = -0.830$, $P = .011$) in both analyses.

*Functional Connectivity – Pain Runs.—Analysis 2d:* For the main effect (high pain greater than off, across groups) an increase of functional connectivity between the left anterior IC and the left SII cortex, as well as the left cerebellum was observed (Table 2d).

**Table 2a.—Results of 1-Sample *t*-Test – fcMRI Resting State**

| Seed Region | Connectivity Region | Brodmann Area | Cluster Size (# of Voxels) | Z Score (Peak Value) | Coordinates (MNI) | | |
|---|---|---|---|---|---|---|---|
| | | | | | *x* | *y* | *z* |
| Left anterior IC | Right anterior insula cortex | 13/47 | 2768 | 6.89 | 38 | 22 | 4 |
| | Middle cingulate cortex | 24 | 482 | 4.32 | –4 | 0 | 40 |
| | Left inferior parietal lobule* | 40 | 256 | 3.85 | –54 | –40 | 44 |
| | Right inferior parietal lobule | 40 | 126 | 3.93 | 56 | –44 | 40 |
| Left posterior IC | Right posterior insula cortex | 13 | 5007 | 6.63 | 38 | –18 | 16 |
| | Right SMA | 6 | 881 | 4.78 | 2 | –10 | 58 |
| | Right SI | 4/3 | 611 | 4.77 | 28 | –34 | 66 |
| | Left SI* | 4/3 | 150 | 4.26 | –22 | –30 | 74 |
| Right anterior IC | Left anterior insula cortex | 13 | 2353 | 6.12 | –34 | 16 | 0 |
| | Middle cingulate cortex | 32/6 | 1921 | 4.16 | 8 | 22 | 32 |
| | Left inferior parietal lobule | 40 | 312 | 4.29 | –60 | –36 | 34 |
| | Right inferior parietal lobule | 40 | 328 | 4.08 | 58 | –40 | 28 |
| | Left SI | 2/3 | 115 | 3.56 | –46 | –22 | 50 |
| Right posterior IC | Left posterior insula cortex | 13 | 5175 | 6.17 | –38 | –28 | 14 |
| | Middle cingulate cortex | 24 | 51 | 3.98 | –6 | 0 | 40 |
| | Right SI** | 3/4 | 203 | 4.78 | 42 | –28 | 58 |

Table 2a describes the resting-state connectivity regions associated with the 4 seed regions (across groups, $P < .05$ corrected). fcMRI = functional connectivity magnetic resonance imaging; IC = insula cortex; SI = primary somatosensory cortex; SMA = supplementary motor area.

*Voxel level uncorrected $P$ value = .0005 was used to separate clusters. **Voxel level uncorrected $P$ value < .0001 was used to separate clusters.

*Analysis 2e:* When groups were compared (interaction analysis), TMD patients displayed a hyper-connection between the left anterior IC and the rACC/medial frontal cortex (BA 32) compared to HCs for the high greater than off (peak voxel: $x = 4$, $y = 42$, $z = 16$; Z value = 3.92). Compared with HCs, TMD patients also displayed a hyper-connection between the right anterior IC and the ACC (peak voxel: $x = 18$,

**Table 2b.—Results of Group Analyses – Resting-State Functional Connectivity**

| Seed Region | Connectivity Region | Brodmann Area | Cluster Size (# of Voxels) | Z Score (Peak Value) | Coordinates (MNI) | | |
|---|---|---|---|---|---|---|---|
| | | | | | *x* | *y* | *z* |
| TMD > HC (2-sample *t*-test) | | | | | | | |
| Left anterior IC | Anterior cingulate cortex | 24/32 | 101 | 4.47 | 2 | 38 | 2 |
| Left posterior IC | Left parahippocampal gyrus | 34 | 176 | 5.07 | –14 | –4 | –26 |
| Right anterior IC | Right thalamus | – | 98 | 4.35 | 8 | –6 | 6 |

Table 2b describes resting-state functional connectivity. Two-sample *t*-tests with a threshold of an uncorrected voxel level $P = .001$ (cluster extent of 82 contiguous voxels) were used to determine group differences among TMD patients and HCs.

DLPFC = dorsal lateral prefrontal cortex; HC = healthy control; IC = insula cortex; MNI = Montreal Neurological Imaging; TMD = temporomandibular disorder; – = inconclusive results.

**Table 2c.—Results of TMD Behavioral Correlations With Functional Connectivity Resting State**

| | | | | | | Coordinates (MNI) | | |
|---|---|---|---|---|---|---|---|---|
| Seed Region | Connectivity Region | Behavioral Correlate | BA | Cluster Size (# of Voxels) | $\rho$ | $x$ | $y$ | $z$ |
| Left anterior IC | Anterior cingulate cortex | VAS | 32 | 29† | –0.952 | 2 | 42 | 6 |
| | Anterior cingulate cortex | MPQ total | 32 | 28† | –0.830 | –4 | 48 | 10 |

Table 2c describes resting-state connectivity results correlated with behavioral data within TMD subjects. An uncorrected voxel level threshold value of $P = .001$ was used.

†Note that this cluster did not survive the a priori determined cluster extent of 82 contiguous voxels.

IC = insular cortex; MNI = Montreal Neurological Imaging; MPQ total = McGill Pain Questionnaire total score; TMD = temporomandibular disorder; VAS = visual analog scale.

$y = 32, z = 12$; Z value = 3.51). Functional connectivity for the pain run correlated with previously determined pressures used to elicit high pain ratings; that is, the more pressure required to elicit high pain (13.5 on the GBS), the more functional connectivity TMD patients showed between the aforementioned structures (left anterior IC and rACC/the medial frontal gyrus [peak voxel: $x = 0, y = 48, z = -6; \rho = 0.838, P = .009$]).

## DISCUSSION

The current study sought to investigate functional connectivity of the IC in TMD patients and HCs. In a first step, we were able to demonstrate a segregated resting-state functional connectivity between subregions of the IC and the medial frontal wall. Within the medial frontal wall, the clusters showing connections with the anterior IC projected anterior to the clusters connected to the posterior IC. More specifically, we found that the anterior IC was functionally connected to the MCC (extending into the posterior ACC), whereas the posterior IC was functionally connected mainly to the SMA, extending into the MCC. A similar segregation has been described by Taylor et al.[11]

When comparing TMD patients and HCs, the left anterior IC was hyper-connected to the rostral

**Table 2d.—Insular Connectivity in TMD Patients and HCs During Elicited Pain (High Pain vs Off)**

| | | | | | Coordinates (MNI) | | |
|---|---|---|---|---|---|---|---|
| Seed Region | Connectivity Region | Brodmann Area | Cluster Size (# of Voxels) | Z Score (Peak Value) | $x$ | $y$ | $z$ |
| Main effect | | | | | | | |
| Left anterior IC | Left SII cortex | 6 | 211 | 4.46 | –56 | –4 | 34 |
| | Left cerebellum | | 82 | 4.10 | –48 | –70 | –24 |
| Left posterior IC | Right DLPFC | 9 | 36 | 3.66 | 54 | 12 | 30 |
| Interaction (pressure × group) | | | | | | | |
| Left anterior IC | Anterior cingulate cortex | 32 | 590 | 3.92 | 4 | 42 | 16 |
| | Right superior frontal gyrus | 10/9 | 427 | 4.85 | 24 | 52 | 28 |
| | Left medial frontal gyrus | 9/10 | 176 | 4.70 | –6 | 56 | 38 |
| Right anterior IC | Right anterior cingulate cortex | 32 | 24 | 3.51 | 18 | 38 | 12 |

Table 2d describes functional connectivity results within an evoked high pain vs off (no pain) block design. Shown are the main effect and the interaction (TMD patients > HCs), at an uncorrected threshold of $P < .001$.

IC = insular cortex; HCs = healthy controls; MNI = Montreal Neurological Imaging; SII cortex = secondary somatosensory cortex; DLPFC = dorsal lateral prefrontal cortex; TMD = temporomandibular disorder.

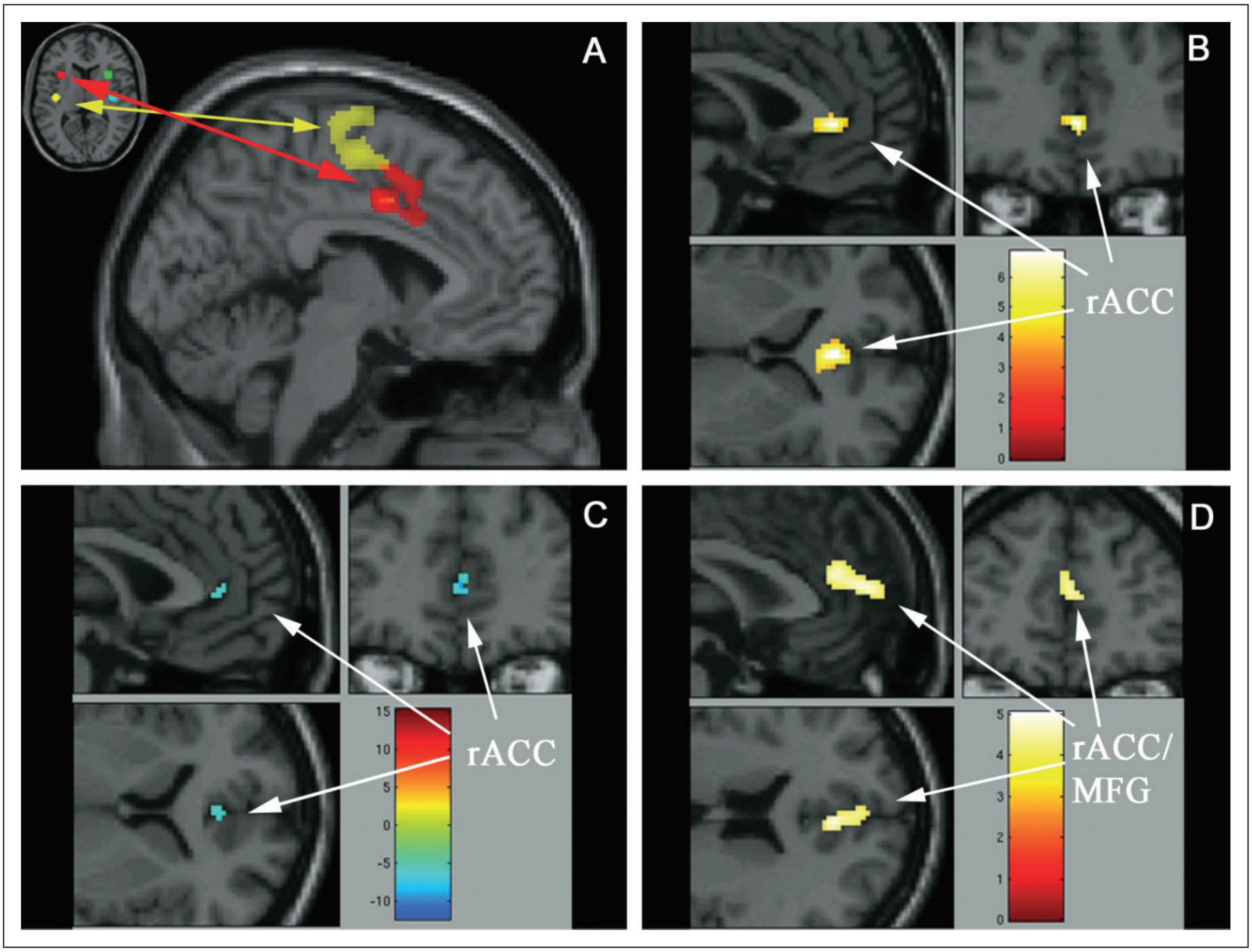

**Fig 3.—Insular cortex connectivity maps during resting state and pain runs. (A) Functional connectivity between the anterior and posterior insular cortex and the cingulate cortex (Analysis 2a). (B) The resting-state hyper-connectivity in temporomandibular disorder (TMD) patients between the left anterior insular cortex and the rostral anterior cingulate cortex (rACC; Analysis 2b). (C) Negative correlation between visual analog scale scores (clinical pain) and the resting-state functional connectivity among TMD patients (Analysis 2c); color bar: red represents positive values (positive correlation) and blue represents negative values (negative correlation). (D) Hyper-connection in TMD patients compared to healthy controls between left anterior insular cortex and anterior cingulate cortex (ACC)/medial frontal gyrus (MFG) in evoked pain (high pain vs off condition, Analysis 2e). Clusters are displayed at a *P* value <.001, uncorrected.**

(pregenual) ACC in the patients. At the same time there was a negative correlation between left IC–ACC connectivity and pain intensity within the TMD group; that is, those patients with decreased connectivity had relatively higher pain scores. Finally, we showed that TMD patients, compared to HCs, had an increased functional connectivity between the anterior IC and ACC when painful pressure stimuli were applied to the facial region.

**Resting-state Connectivity.—**It has been suggested that the anterior IC–ACC system integrates interoceptive input with its emotional salience, while the posterior IC–MCC system is thought to be more related to environmental monitoring and response selection.[11] On the other hand, with respect to pain perception, there is strong evidence that the anterior IC, as part of the medial pain system, together with the ACC, has a unique role in affective pain processing and learning, while the posterior IC, as part of the lateral pain matrix, together with regions such as the primary and secondary somatosensory cortices, encode pain intensity, laterality, and somatotopy.[23]

This is also supported by a recently published study by Peltz et al investigating IC connectivity during noxious and innocuous thermal stimulation, showing that the anterior IC is more strongly connected to the prefrontal cortex and ACC than is the posterior IC, and that the posterior IC is more strongly connected to the SI and MI cortex.[24] Although in the present study connectivity maps of the anterior and posterior IC were not directly (statistically) compared, we found a strong resting-state connectivity between the posterior IC and the primary somatosensory cortex (SI), supporting the idea of the posterior IC's integration in the lateral pain system.

Differences between groups were found between the left anterior IC–rACC connectivity (TMD patients greater than HCs). Furthermore, anterior IC–rACC connectivity was negatively associated with clinical pain; that is, TMD patients with less connectivity reported higher clinical pain, as assessed by the clinical pain and MPQ total. Just like the IC, the CC is functionally segregated with different parts being involved in different aspects of pain encoding[25] and pain anticipation,[26] but also involved in antinociception[27,28] and habituation.[29] Especially the rACC, as part of the medial prefrontal cortex, has repeatedly been shown to be critically involved in distraction, placebo, and opioid-associated analgesia,[28,30] as well as endogenous hyperalgesia-specific pain modulation.[31] As such, the rACC is strongly connected with the prefrontal cortex and periaqueductal gray, probably serving as a relay between prefrontal and brainstem structures involved in top–down antinociceptive mechanisms. Although there is an increasing body of evidence that suggests that the IC flexibly connects to attentional and emotional brain areas, and that these connections are in fact an important determinant of pain experience,[32] the literature on the capability of the ACC to modulate IC activity in pain conditions, or vice versa, is sparse. Interestingly, in a recently published study by Petrovic et al, the rACC displayed an increased functional connectivity with the orbitofrontal/ventrolateral cortex and anterior IC in the context of placebo analgesia.[33] Given that TMD patients have to deal with an increased nociceptive and/or proprioceptive input to the forebrain (without making any assumptions about the original pain generator), we hypothesize that an increase in anterior IC–rACC connectivity serves antinociception, ie, an adaptive process to down-regulate pain. This would explain the group difference between TMD patients and HCs, with TMD showing an increased functional connectivity. On the other hand, it would explain why those patients with less connectivity showed higher pain scores (clinical pain).

**Pain Run Connectivity.—**We also investigated IC connectivity for the pain runs. Analysis of the main effect showed that the left anterior IC was functionally more connected to the left SII during high pressure pain than during the off condition. This finding is again in line with the study by Peltz et al investigating IC connectivity during noxious and innocuous thermal stimulation, showing that the anterior IC connects more strongly to the SII cortex during pain. The interaction analysis revealed that TMD patients showed a higher connectivity than the HCs between the left anterior IC and the rACC in the high pain condition as compared to the off condition. Within the TMD group, those patients requiring higher pressures to elicit high pain (~13.5 on the GBS—same pain rating across subjects) showed an increased anterior IC–rACC connectivity, when these pressures were applied in the scanner (positive association).

Although experimental pain has been used as a surrogate marker for clinical pain, and frequently a decrease in pain thresholds has been found in chronic pain patients, in- and outside the region of clinical pain,[34-36] the broader concept that experimental pain and chronic pain rely on the same networks has been challenged.[37] To our knowledge, this is the first study to explore functional IC connectivity during resting state and a pain run in a cohort of pain patients and HCs. With respect to IC–CC connectivity, the increased functional connectivity seen during the pain run paralleled the findings during resting state. Again, our data suggest that IC–rACC connectivity subserves an antinociceptive process, especially since those patients with higher connectivity could take more pressure to elicit a certain amount of subjective pain. This in turn would suggest that the anterior IC–rACC system plays a role for both clinical and experimental antinociception. From this perspective, it will be interesting to see whether a decrease in functional connectivity is actually associated with both worsening of clinical pain and a decrease of pain thresholds (increased pain ratings of a given stimulus) in- and outside the region of clinical pain.

**Limitations.—**There are several limitations to our study that need to be addressed. First of all, the study sample with 8 TMD patients and 8 HCs, although thoroughly investigated and carefully matched, is rather small and in these terms, this study needs to be considered a pilot study to be expanded upon.

The patients investigated in the current study are relatively young and only mildly affected. They are thus likely to be at the beginning of the chronification process and/or in a compensated stage. As such, they probably do not represent the clinical picture of "severely disabled" TMD. On the other hand, our results possibly reflect a snapshot of chronic pain in an early or compensated stage. Such study samples might be interesting for future (longitudinal) studies that intend to unravel causes and consequences of chronic pain and to account for symptom heterogeneity among patients. It will be interesting to see whether in some patients the hypothesized antinociceptive mechanism, ie, enhanced anterior IC–rACC connectivity, is "overstressed" with time, and whether this leads to further chronification in terms of more and/or increased clinical pain, as well as decreased reversibility.

A limitation inherent to the cross-sectional design is its inability to resolve conclusively the preexisting vs acquired nature of the observed alterations; that is, it is unclear whether chronic pain leads to the changes described or whether changes in IC connectivity predispose someone to developing TMD pain. Another potential weakness is that we used standardized SR. Subtle (natural) differences in functional anatomy across subjects and differences in brain size (and normalization) might have had an influence on connectivity maps. However, we would assume that variation in functional anatomy is equally distributed between groups and the fact that images had been smoothed prior to analysis helped to correct for such differences. The advantage of this approach lies in the ability to replicate the findings of Taylor et al.[11] Indeed, the fact that the study replicated the findings in previous work[11] provides support for the veracity of our findings, despite the small sample size.

Finally, functional connectivity as assessed by the approach chosen in this study (ie, correlation analyses) allows no assumptions on causality, or on directedness of influence. It is conceivable that functional connectivity between 2 regions is driven by a third region not identified in the analysis. More sophisticated approaches exploring effective connectivity and the relationship between functional and structural connectivity[38] in larger sample sizes will help to overcome such methodological shortcomings in future studies.

## CONCLUSIONS AND OUTLOOK

The identification and investigation of resting-state networks is a promising approach and might in fact turn out to be a stronger tool than approaches using evoked pain paradigms, when it comes to the exploration of internal states, such as clinical pain and mood disturbances that are only insufficiently modeled by external stimuli. Our main goal was to investigate and compare IC connectivity in individuals with TMD and HCs. Our analyses revealed group differences in resting state and an evoked pain run-associated functional connectivity between the IC and the rACC, which we interpret as being indicative of an adaptation of the antinociceptive system early in the chronification process. This might help to further disentangle the neural correlates of chronic pain in TMDs.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Fig S1.**—Gracely Box Scale (GBS).

**Fig S2.**—Correlation between left anterior IC–rACC connectivity and pain intensity (VAS) in TMD patients.

**Fig S3.**—Group difference between left anterior IC–rACC functional connectivity: healthy controls and TMD patients.

**Table S1.**—Seed region central coordinates.

**Table S2.**—Depression/anxiety correlations with clinical pain measures.