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ORIGINAL RESEARCH

Altered Brain Functional Connectivity and Neurometabolite Correlations in Orofacial Chronic Pain with Central Sensitization

Makoto Terumitsu [ID][1,2], Yuhei Takado[3], Ken-Ichi Fukuda[2], Eisuke Kato[2], Sei Tanaka[4], Khin Khin Tha [ID][5]

[1]Division of Dental Anesthesiology, School of Dentistry, Department of Human Biology and Pathophysiology, Health Sciences University of Hokkaido, Hokkaido, Japan; [2]Division of Special Needs Dentistry and Orofacial Pain, Department of Oral Health and Clinical Science, Tokyo Dental College, Tokyo, Japan; [3]Quantum Life Spin Group, Institute for Quantum Life Science, National Institutes for Quantum Science and Technology, Chiba, Japan; [4]Department of Oral and Maxillofacial Surgery, Tokyo Dental College, Tokyo, Japan; [5]Global Centre for Biomedical Science and Engineering, Faculty of Medicine, Hokkaido University, Hokkaido, Japan

Correspondence: Makoto Terumitsu, Division of Dental Anesthesiology, School of Dentistry, Department of Human Biology and Pathophysiology, Health Sciences University of Hokkaido, 1757 Kanazawa, Tobetsu-cho, Ishikari-gun, Hokkaido, 061-0293, Japan, Tel/Fax +81 133 23 1445, Email terumitsu@hoku-iryo-u.ac.jp

Purpose: Central sensitization (CS) is one of the causes of refractory chronic orofacial pain (COFP). Chronic pain reportedly alters resting-state functional connectivity (FC) in the brain. The salience network (SN) overlaps with brain regions involved in chronic pain. Moreover, excitatory and inhibitory neural activities can alter FC. This study investigated the correlations between FC of the SN and other neural circuits, neurometabolites in anterior cingulate cortex (ACC), and CS Inventory (CSI) scores.

Patients and Methods: Participants were 21 COFP patients and 21 healthy sex- and age-matched volunteers. We conducted a combined study of CSI scores, resting-state functional magnetic resonance imaging, and proton magnetic resonance spectroscopy of ACC.

Results: FC between the nucleus accumbens within the mesolimbic system and SN regions was significantly stronger in patients compared with that in controls. In patients, the results revealed positive correlations between CSI scores and FC in four pairs of regions: ACC-right putamen and ACC-right globus pallidus, left insula-right putamen, and right anterior supramarginal gyrus-right lateral prefrontal cortex. Regarding correlations between neurometabolites in ACC and FC between ACC and other cortical regions, the results revealed that bilateral ACC-anterior insula FC was negatively correlated with glutamate, glutamine, and glutathione. Additionally, aspartate, an N-methyl-D-aspartic acid receptor agonist, was negatively correlated with FC of ACC-right parietal cortex in the default mode network. Regarding FC of bilateral ACC-posterior parietal cortex in the frontoparietal network, FC on the right side was positively correlated with glutathione associated with excitatory neuronal activity, whereas that on the left side was negatively correlated with gamma-aminobutyric acid / total creatine associated with inhibitory neural activity.

Conclusion: Connectivity within the SN, and between the SN and the mesolimbic system and other networks, may be involved in CS in COFP. Neurometabolites in the ACC may modulate FC linked to the ACC.

Keywords: chronic pain, orofacial region, central sensitization inventory, resting-state fMRI, MR spectroscopy

Introduction

The incidence of orofacial pain is estimated to range from 16.1%[1] to 33.2%,[2] and the condition becomes chronic and refractory in many cases. A previous study reported that chronic orofacial pain (COFP) was present in 11.2% of older patients who visited dental hospitals.[3] COFP can impair eating and sleep, induce anxiety, and reduce quality of life.[4] Central sensitization (CS) in the nervous system is a possible cause of the transition from acute to chronic pain. CS is a neurophysiological mechanism defined as the amplification of neural signaling within the central nervous system that elicits pain hypersensitivity.[5] This condition is clinically characterized by disproportionate pain, in which the severity of

pain and functional disability are disproportionate to detectable tissue damage and pathophysiology, or which occurs regardless of peripheral lesions.[6] Assessment of CS is critical for the diagnosis and treatment of COFP.

Functional connectivity (FC) in the central nervous system is used as an index for identifying CS and COFP, and can be measured using resting-state functional magnetic resonance imaging (rsfMRI). Regarding macro-scale brain networks,[7] previous studies have reported variable findings regarding which networks are associated with COFP, and a triple network model involving the default mode network (DMN), salience network (SN), and central executive network has been proposed to underlie chronic pain.[8] Among these networks, the SN is considered to modulate pain processing by facilitating the detection and response to nociceptive stimuli and interoception.[9] The brain regions that comprise the SN overlap with the medial pathway that processes the emotional aspects of pain.[10] The anterior cingulate cortex (ACC) and insular cortex (Ins) are included in the SN and act as key nodes, with many previous studies reporting that these two regions play important roles in the sensory processing and chronicity of pain.[11] In one study, enhanced ACC-right Ins FC and ACC-secondary somatosensory cortex FC were reported to be correlated with pain intensity, depression, and anxiety ratings in patients with classic trigeminal neuralgia as a type of COFP.[12] In another study, Ins-thalamus FC was increased in patients with medically refractory classic or idiopathic trigeminal neuralgia.[13] Among these patients, non-responders to surgical treatment exhibited decreased ACC-amygdala and ACC-hippocampus FC. FC within the cortical regions of the SN and the rest of the brain may be deeply involved in the central nervous system mechanisms of COFP.

The SN also includes subcortical regions,[14] which influence chronic pain. In a previous study, the ventrolateral periaqueductal gray and locus coeruleus were found to exhibit increased FC strength with higher brain regions such as the nucleus accumbens (Acb) and ACC in patients with chronic orofacial neuropathic pain following nerve injury.[15] Specifically, the Acb is reported to be involved in the brain reward center as part of the mesolimbic network of the dopaminergic transmission system, which modulates the sensory aspects of nociception, the effects of analgesics, and the emotional symptoms of chronic pain.[16] In addition, the Acb is the ventral extension of the striatum, the main input nucleus of the basal ganglia.[17] Previous studies reported that stronger caudate nucleus-ACC FC was exhibited in chronic migraine patients,[18] increased monthly migraine attack frequency was associated with increased caudate-Ins FC, and longer disease duration was correlated with increased Acb-ACC FC.[19] These findings suggest that COFP and CS may involve changes in FC between the SN and the mesolimbic system.

A combination of methods is needed to assess CS. The CS Inventory (CSI) score is commonly used as a self-report questionnaire tool.[20] In addition, several studies have combined rsfMRI with measurements of cortical neurometabolites using MR spectroscopy (MRS) in chronic pain patients, indicating correlations between FC and the levels of neurometabolites.[21–23] Our previous MRS study revealed that levels of aspartate (Asp), an excitatory neuronal metabolite, and glutathione (GSH), which is associated with increased excitatory neuronal activity, are elevated in ACC in COFP patients.[6] Additionally, we found a negative relationship between CSI scores and gamma-aminobutyric acid (GABA) / total creatine (tCr), and a positive relationship for glutamine in the ACC.[6] Therefore, in the current study we hypothesized that neurometabolites associated with excitatory and inhibitory neuronal activity in the ACC, a key node of the SN, would affect FC linked to the ACC. In this study, we combined FC data from rsfMRI, CSI scores, and our previous MRS results in the ACC to examine CS in COFP patients.

Materials and Methods
Participants
We recruited 21 patients (19 women and two men) with COFP from the pain clinic of Tokyo Dental College at Suidobashi Hospital, Japan. The patients had spontaneous and persistent pain with widespread dysesthesia and/or static allodynia. Criteria for CS included disproportionate pain regardless of peripheral lesions[24] as determined by imaging examinations such as computed tomography, conventional MRI, MR neurography (three-dimensional volume rendering MR neurography: 3DVR-MRN),[25] and 3D anisotropy contrast on the basis of diffusion-weighted imaging.[26] In addition, pain control was difficult, and patients were unresponsive or only temporarily responsive to conventional pain therapies such as various medications, nerve block anesthesia, and local anesthetic injection into myofascial trigger points, strongly

suggesting CS. The patient group consisted of 20 patients from our previous MRS study[6] plus one additional 68-year-old female patient. However, because of poor MRS data, only rsfMRI data were added to the additional patient data. Patients' mean age was 53.1 ± 12.9 years (mean ± standard deviation [SD]). Pain duration ranged from 0.5 to 25 years, with a mean duration of 7.6 ± 8.1 years (mean ± SD).

The patients were classified into three groups according to the International Classification of Orofacial Pain categories:[27] 1) 4.1.2.3 post-traumatic trigeminal neuropathic pain (n = 13), 2) 2.1.2 chronic primary myofascial orofacial pain (n = 4), and 3) coexistence of 4.1.2.3 and 2.1.2 (n = 4).

A total of 21 sex- and age-matched healthy volunteers (19 women and two men) without pain, medication for central nervous system disorders, or cerebral nervous disease participated in the study as a control group. The mean age of the control group was 53.2 ± 12.9 years (mean ± SD), and there was no significant difference in age between the control and patient groups (p = 0.61, Student's *t*-test).

Written informed consent was obtained from all participants. The cross-sectional study protocol was approved by the Ethics Committee of Tokyo Dental College (#923). The study complied with the Declaration of Helsinki.

Questionnaire Evaluation

The CSI was used as a self-report questionnaire tool.[20] Patients completed the Japanese version of the CSI[28] prior to MRI data acquisition. Scores were calculated using a five-point rating for 25 items in Part A of the CSI, and total scores ranged from 0 to 100. We were unable to obtain CSI scores for two patients.

Resting State Functional Magnetic Resonance Imaging

Image Acquisition

All experiments were performed using a 3.0 T Ingenia Philips MRI scanner (Philips Healthcare, Best, the Netherlands) with a 32-channel receive head coil and a multi-transmit body coil. Images for rsfMRI were obtained using a single-shot T2*-weighted echo-planar imaging sequence (repetition time: 1000 ms; echo time: 30 ms; flip angle: 70°; matrix: 64 × 64; field of view: 197×197 mm; 13 slices; slice thickness: 5.0 mm; slice gap: 2.5 mm; voxel size: 3.1 mm × 3.1 mm × 5.0 mm; 300 volumes; scan time: 5 min). The alignment for the fMRI images was at the anterior to posterior commissure line. During the scan, subjects were instructed to keep their eyes open, relax, and look at the ceiling of the MRI bore without moving or falling asleep. A high-resolution three-dimensional anatomical image was acquired using a T1-weighted gradient-echo pulse sequence (repetition time: 6.3 ms; echo time: 3.5 ms; flip angle: 8°; inversion time: 950 ms; matrix: 512 × 512; field of view: 256 mm × 256 mm; voxel size: 0.5 mm × 0.5 mm × 1.5 mm).

Functional Connectivity Analysis

The rsfMRI data were analyzed using SPM12 (Welcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/), CONN version 20.b (Functional Connectivity Toolbox; http://www.nitrc.org/projects/conn), and Matlab version 9.7 (R2019b; MathWorks, Natick, MA, USA). The parameters for analysis were the default values shown below. After discarding the first three images to eliminate any signal decay, preprocessing was performed, including realignment, slice-timing correction, outlier detection, co-registration to the anatomical image, segmentation of the anatomical image (gray matter [GM], white matter [WM], and cerebrospinal fluid [CSF]), normalization with the Montreal Neurological Institute standard brain, and smoothing with an 8-mm Gaussian kernel. After preprocessing, signal and motion artifacts (global signal z-value threshold ≥ 5; composite motion threshold ≥ 0.9 mm) were removed from the data using the CompCor strategy,[29] and the data were band-pass filtered (0.008–0.09 hz) to reduce the influence of noise.

Subsequent region of interest (ROI)-to-ROI connectivity analyses were performed using ROIs that were part of CONN's default networks and atlas ROIs. The ROIs were set in regions that constitute the SN in relation to chronic pain, including the ACC, Ins, rostral prefrontal cortex (RPFC), orbitofrontal cortex (FOrb), supramarginal gyrus (SMG), Acb, putamen (Put) and globus pallidus (GP), and that consist of frontoparietal network which overlaps with the SN, including the lateral prefrontal cortex (LPFC). A standard second-level general linear model analysis of FC MRI was used to compare the patient and control groups using parametric statistics on the basis of random field theory.[30] Fisher-

transformed correlation coefficients were calculated between each of the ROIs. The threshold for statistical significance was determined at p < 0.05 for the cluster-level false discovery rate (FDR).

Correlation analysis was performed between each CSI score as a covariate and FC (ROI-to-ROI analysis) for the patients, using CONN at the significance level (cluster-wise FDR corrected p < 0.05). Each significant FC value and correlation coefficient were extracted. The normality test for the samples was evaluated with the Shapiro–Wilk test using SPSS (IBM SPSS Statistics version 29, IBM Corp, Armonk, NY, USA). The results revealed that all samples were normally distributed. Pearson's correlation coefficients (r) were then calculated. Correlation coefficients were interpreted as follows: > 0.75, good to excellent relationship; 0.50 to 0.75, moderate to good relationship; 0.25 to 0.50, fair relationship; and 0.00 to 0.25, little or no relationship.[31]

Magnetic Resonance Spectroscopy

Single Voxel Spectroscopy

The MRS protocol included a single-voxel point-resolved spectroscopy (PRESS) sequence with chemical-shift selective water suppression. The MRS acquisition parameters were as follows: repetition time / echo time = 2000 / 35 ms, complex points for the spectral data = 1024, and total number of acquisitions = 128. The linewidth of the water spectrum in magnitude mode became less than 18 hz after shimming. The current study used the MRS data we obtained in our previous research.[6]

Voxel Location

Hydrogen 1 magnetic resonance spectroscopy voxels of interest (VOIs) were set in the ACC (20 mm × 30 mm × 20 mm).[6] The VOIs in the ACC were positioned parallel to and above the corpus callosum, starting from the genu of the corpus callosum and extending 3 cm posteriorly, and covered left and right ACC[32] in patients and controls.

MRS Data Processing and Measurement

MRS data were processed using a linear combination model (LCModel version 6.3, Stephen Provencher, Inc., Oakville, ON, Canada).[33] Tissue composition within the VOI was calculated on the basis of the segmentation of 3D T1-weighted images using the Gannet3.0 open-source tool (https://www.gabamrs.com/gannet).[34] Water concentrations used in the LCModel analysis were calculated on the basis of the volume fractions of WM, GM, and CSF. Metabolite concentrations were then divided by the WM and GM fractions to correct for CSF within the VOI, because metabolites are primarily present in the WM and GM.[35] Water scaling and eddy current correction were performed, and metabolites were fitted in the chemical shift range (0.2–4.2 ppm).

We measured glutamate + glutamine (Glx), aspartate (Asp) / tCr, GABA / tCr, and glutathione (GSH). The tCr (Cr + PCr) was used for normalization because tCr is widely used as an internal reference in human studies.[36]

Metabolites processed by LCModel with Cramer-Rao lower bounds < 25% for GSH and 15% for the others were analyzed. Mean signal-to-noise ratio (SNR) ± SD values for patients and controls were 27.35 ± 6.67 and 31.29 ± 2.97 in the ACC, respectively. Mean full-width at half maximum ± SD values for the patients and controls were 0.033 ± 0.010 ppm and 0.037 ± 0.006 ppm in the ACC, respectively. No significant differences in full-width at half maximum in the region were observed between patients and controls (F[1, 78] = 2.99, p = 0.088, two-way analysis of variance [ANOVA]). Patients' data showed lower SNR compared with controls' data in the ACC (F[1, 78] = 3.99, p = 0.049, Tukey's honestly significant difference test for post hoc test: p = 0.014 [ACC]). Statistical analyses were performed using Statistica version 13 (TIBCO Software Inc. CA, USA).

Correlation Analysis Between Neurometabolites and FC

Correlation analysis was performed between each neurometabolite level as a covariate and FC setting a seed in ACC for the patients, using CONN at the significance level (cluster-wise FDR corrected p < 0.05). Each significant FC value and correlation coefficient was extracted for the patients, and the FC value and correlation coefficient in each FC that was significant in patients were also extracted for controls. Spearman correlation analysis was used to determine statistical significance in non-normally distributed data (ie, Asp / tCr and ACC-right lateral parietal FC in the DMN in patients, Glx and ACC-left posterior SMG FC and ACC-left anterior Ins (aIns) FC in both groups, and GABA / tCr and ACC-left
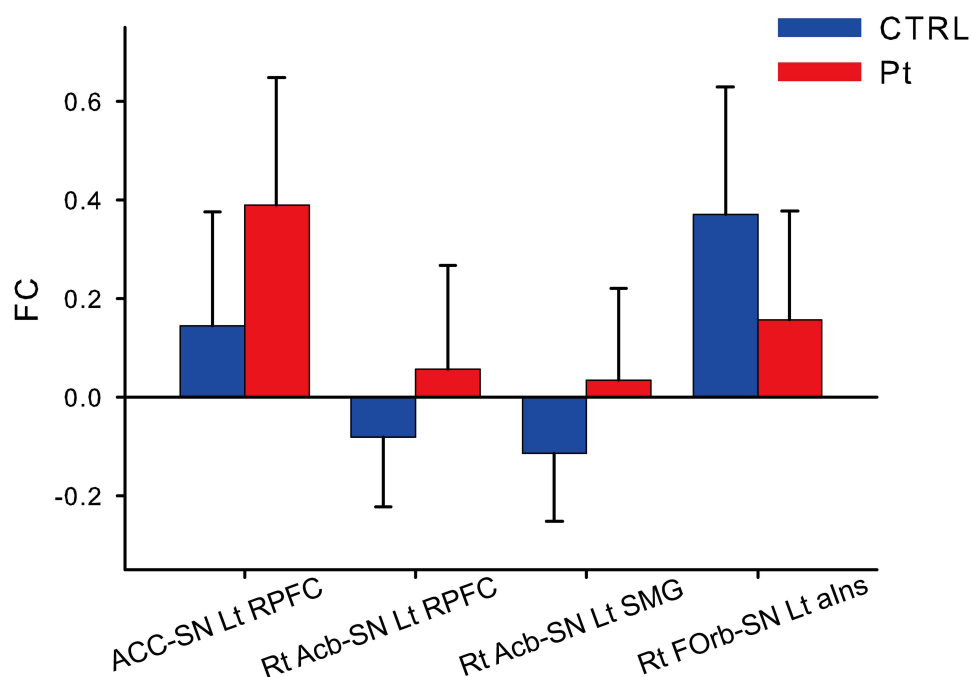
Figure 1 Significant functional connectivity between patients and controls.
Notes: A functional connectivity (FC) analysis was performed between the patients and controls, using ROI-ROI analysis. Axis of ordinate (FC value) and axis of abscissa (FC region). Blue bar (control group) and red bar (patient group). P < 0.05 (corrected by false discovery rate). Error bar (standard deviation).
Abbreviations: FC, functional connectivity; ACC, anterior cingulate cortex; SN, salience network; Lt, left; Rt, right; RPFC, rostral prefrontal cortex; Acb, nucleus accumbens; SMG, supramarginal gyrus; FOrb, orbitofrontal cortex; aIns, anterior insula.

posterior parietal cortex (PPC) FC in controls). The rest of the data, which exhibited a normal distribution, were evaluated using Pearson's correlation analyses.

Because of the exploratory nature of this study, correction for multiple comparisons was not performed.

Results

Compared with the control group, patients with COFP exhibited significantly stronger FC of ACC-left RPFC in the SN, left RPFC-right Acb, and right Acb-left SMG in the SN. In contrast, the patient group exhibited significantly weaker FC of left aIns in the SN-right FOrb (Figure 1, Figure 2A, and Table 1).

In patients, positive correlations were found between CSI scores and FC in four pairs of regions (Figures 2B and (3A–D, and Table 2): ACC-right Put (Figure 3A), ACC-right GP (Figure 3B), left Ins-right Put (Figure 3C), and right anterior SMG-lateral prefrontal cortex (LPFC) in the frontoparietal network (Figure 3D).

Table 1 ROI-ROIs in Patients Vs Normal Controls

ROI	MNI			ROI	MNI			FC		T	unc p	p*
	X	Y	Z		X	Y	Z	CTRL	Pt			
ACC	1	18	24	SN Lt RPFC	−32	45	27	0.15 ±.23	0.38 ±.26	3.18	0.0028	0.014
Rt Acb	9	12	−7	SN Lt RPFC	−32	45	27	−0.08 ±.14	0.06 ±.21	2.44	0.019	0.048
				SN Lt SMG	−60	−39	31	−0.12 ±.14	0.03 ±.19	2.95	0.0053	0.027
Rt FOrb	29	23	−16	SN Lt aIns	−44	13	1	0.37 ±.26	0.15 ±.22	−2.83	0.0073	0.036

Notes: Significant functional connectivity between the patients and controls, using ROI-to-ROI analysis. P* < 0.05 (corrected by false discovery rate).
Abbreviations: MNI, Montreal Neurological Institute coordinates; FC, functional connectivity values (mean ± SD); T, T score; unc p, uncorrected p-value; ACC, anterior cingulate cortex; SN, salience network; Lt, left; Rt, right; RPFC, rostral prefrontal cortex; Acb, nucleus accumbens; SMG, supramarginal gyrus; FOrb, orbitofrontal cortex; aIns, anterior insula.
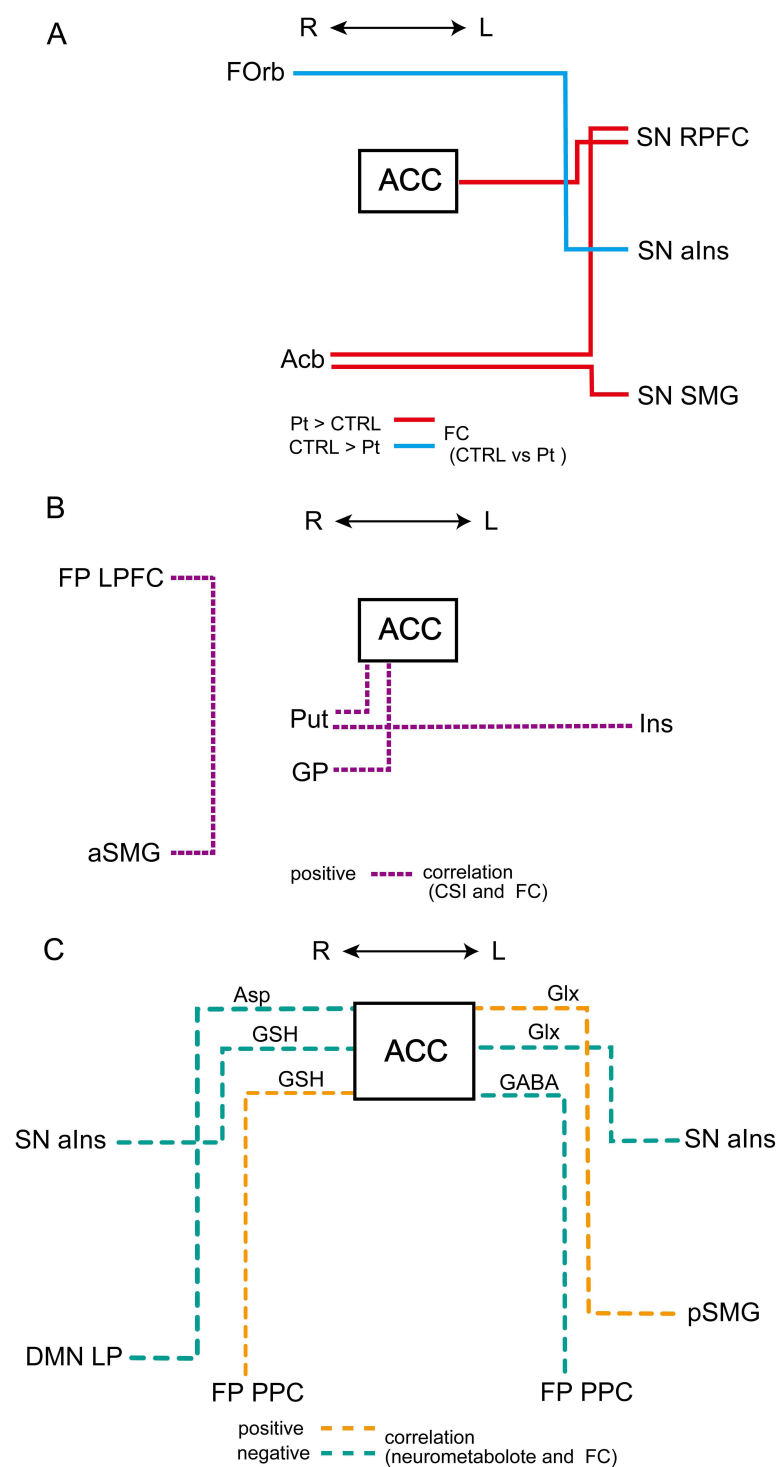
Figure 2 (**A**–**C**) Schematic diagram of functional connectivity.
Notes: (**A**) Functional connectivity (FC) comparisons between patients and controls. Red and blue lines represent significantly stronger and weaker FC, respectively, in patients compared with that in controls. (**B**) Correlations with Central Sensitization Inventory (CSI) score and FC. Purple dotted line represents significantly positive correlation in patients. (**C**) Correlations with Neurometabolites and FC. Orange and blue green dashed lines represent positive and negative correlations, respectively, between each neurometabolite and FC with ACC as a seed.
Abbreviations: ACC, anterior cingulate cortex; SN, salience network; Lt, left; Rt, right; RPFC, rostral prefrontal cortex; Acb, nucleus accumbens; SMG, supramarginal gyrus; FOrb, orbitofrontal cortex; aIns, anterior insula; Put, putamen; GP, globus pallidus; Ins, insula; aSMG, anterior supramarginal gyrus; FP, frontoparietal network; LPFC, lateral prefrontal cortex.
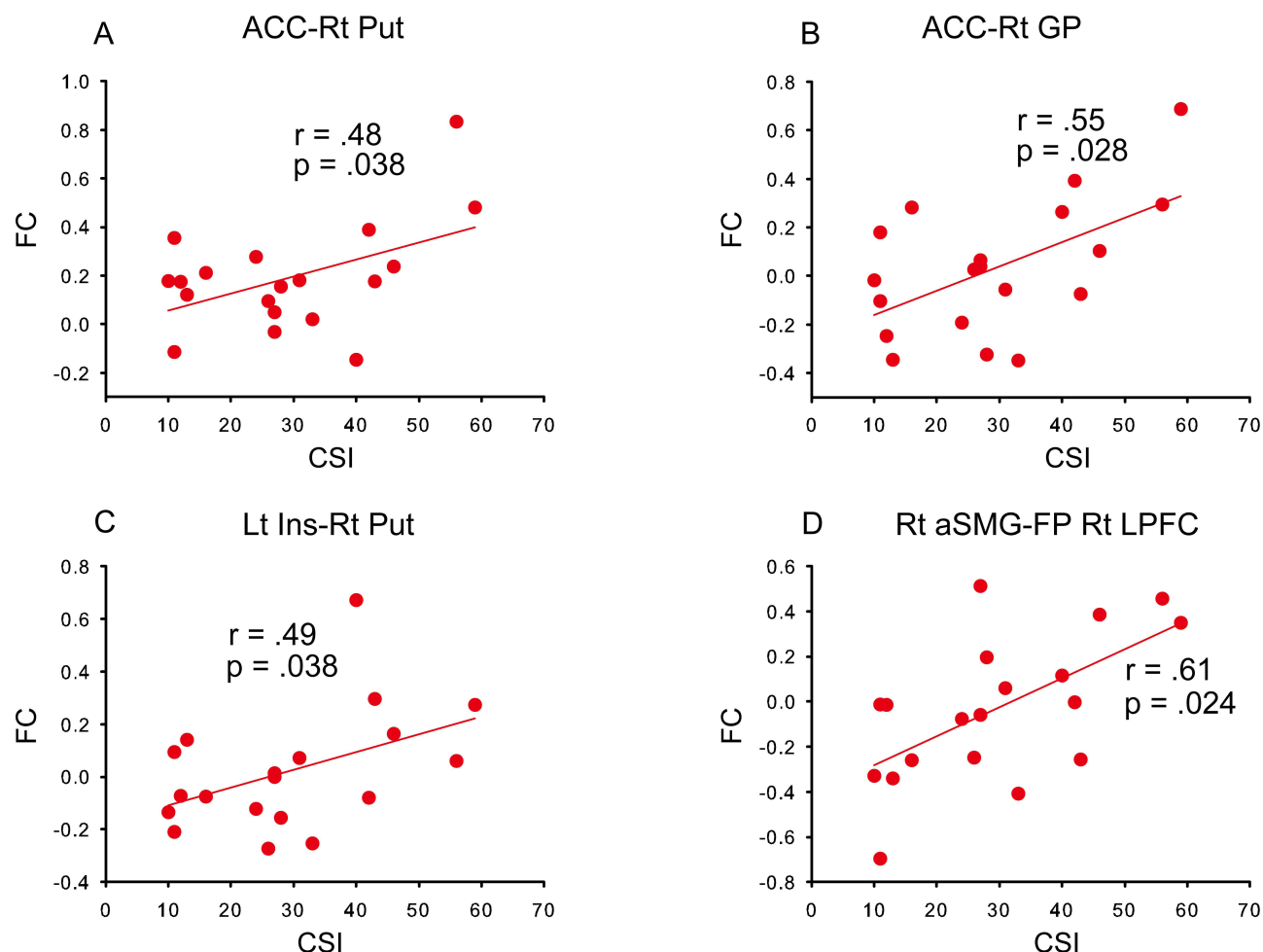
Figure 3 Correlations between Central Sensitization Inventory scores and functional connectivity.

Notes: Scatter plot and regression line between Central Sensitization Inventory scores (abscissa) and functional connectivity values (ordinate) in the patients. r as Pearson's correlation coefficient; p < 0.05 (corrected for false discovery rate). (**A**) ACC-Rt Put, (**B**) ACC-Rt GP, (**C**) Lt Ins-Rt Put, (**D**) Rt aSMG-FP Rt LPFC.

Abbreviations: ACC, anterior cingulate cortex; Lt, left; Rt, right; Put, putamen; GP, globus pallidus; Ins, insula; aSMG, anterior supramarginal gyrus; FP, frontoparietal network; LPFC, lateral prefrontal cortex.

Regarding the relationships between neurometabolites and FC (Figure 2C Figure 4A–F, and Table 3), Asp / tCr was negatively correlated with FC of ACC-right lateral parietal in the DMN in patients (Figure 4A). GSH was positively correlated with FC of ACC-right PPC in the FP network (Figure 4B), and negatively correlated with FC of ACC-right aIns in the SN (Figure 4C). Glx was positively correlated with FC of ACC-left posterior SMG (Figure 4D) and negatively correlated with FC of ACC-left aIns in the SN (Figure 4E). GABA / tCr was negatively correlated with FC

Table 2 Functional Connectivity with Significant Correlation with Central Sensitization Inventory Score

ROI	MNI			ROI	MNI			FC	T	unc p	p*
	X	Y	Z		X	Y	Z				
ACC	9	12	−7	Rt Put	25	2	0	0.19 ±.22	2.25	0.038	0.038
				Rt GP	20	−4	−1	0.031 ±.27	2.74	0.014	0.028
Lt Ins	−36	1	0	Rt Put	25	2	0	0.17 ±.26	2.29	0.035	0.038
Rt aSMG	58	−27	38	FP Rt LPFC	41	38	30	−0.034 ±.32	3.17	0.006	0.024

Notes: Coordinates of FC exhibiting significant correlations with Central Sensitization Inventory scores in patients, using ROI-to-ROI analysis. P* < 0.05 (corrected by false discovery rate).

Abbreviations: MNI, Montreal Neurological Institute coordinates; FC; functional connectivity values (mean ± SD); T, T score; unc p, uncorrected p-value; ACC, anterior cingulate cortex; Lt, left; Rt, right; Put, putamen; GP, globus pallidus; Ins, insula; aSMG, anterior supramarginal gyrus; FP, frontoparietal network; LPFC, lateral prefrontal cortex.
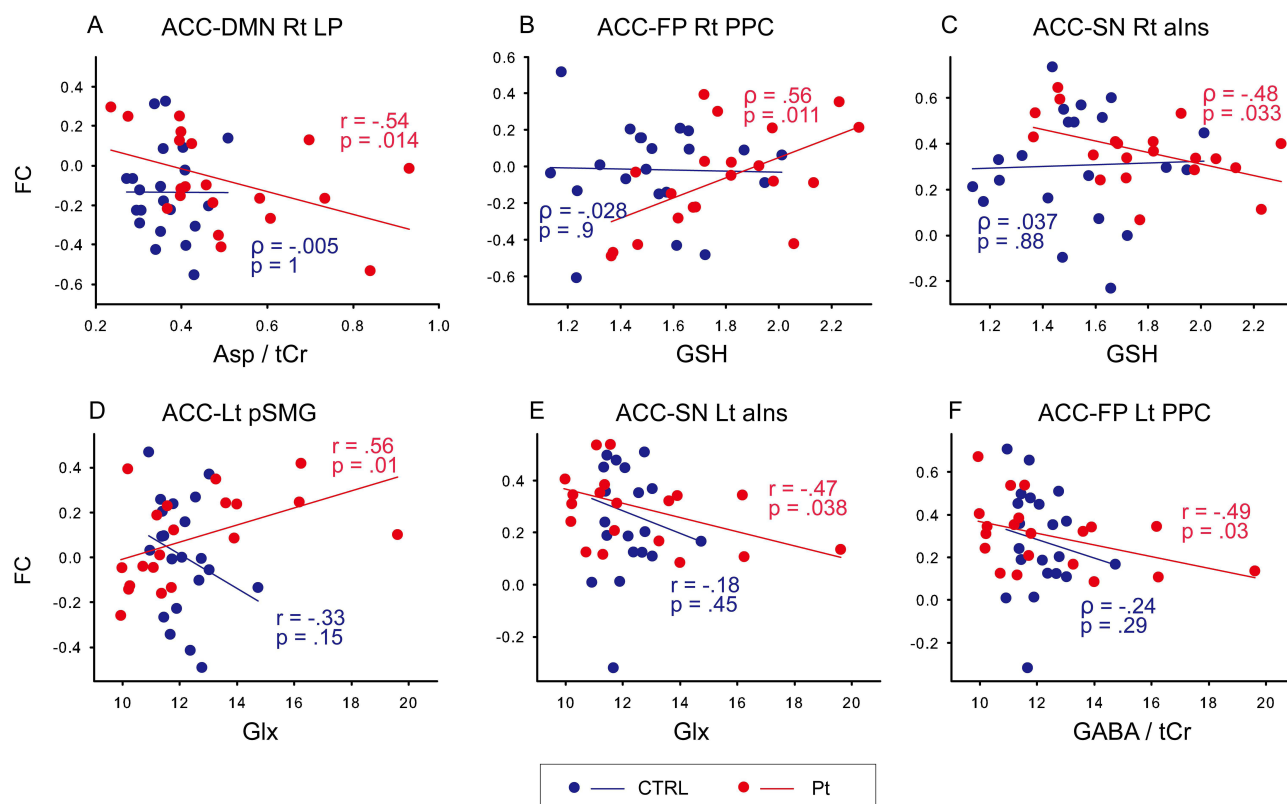
Figure 4 Correlation between neurometabolites and functional connectivity.

Notes: Scatter plot and regression line between neurometabolites (abscissa) and functional connectivity value (ordinate) in controls (blue dot and line) and patients (red dot and line). Pearson's correlation coefficient (r) was calculated when the distribution of the sample was normal, and Spearman correlation coefficient (ρ) was calculated when the distribution of the sample was non-normal. p < 0.05. Multiple comparison test was not performed for the p values of the six combinations of correlation. (**A**) Asp / tCr and ACC-DMN Rt LP, (**B**) GSH and ACC-FP Rt PPC, (**C**) GSH and ACC-SN Rt aIns, (**D**) Glx and ACC-Lt pSMG, (**E**) Glx and ACC-SN Lt aIns, (**F**) GABA / tCr and ACC-FP Lt PPC.

Abbreviations: FC, functional connectivity; Asp, aspartate; GSH, glutathione, Glx, glutamate + glutamine; GABA, gamma-aminobutyric acid; ACC, anterior cingulate cortex; Lt, left; Rt, right; DMN, default mode network; LP, lateral parietal; FP; frontoparietal network; PPC, posterior parietal cortex; SN, salience network; aIns, anterior insula; pSMG, posterior supramarginal gyrus; PPC, posterior parietal cortex.

of ACC-left PPC in the FP network (Figure 4F). In controls, there were no significant correlations in the same six combinations.

Discussion

FC in patients significantly differed from that of controls in SN regions, and between the Acb and SN regions. CSI scores were positively correlated with FC within the SN and between the SN and the mesolimbic system and FP network. Neurometabolites in ACC were correlated with FC between ACC and other regions within the SN and the other DMN and FP networks.

A group comparison of FC between patients and controls showed significant differences in FC between four pairs of regions. First, FC between ACC and left RPFC in SN was stronger in patients. The RPFC corresponds approximately to Brodmann area 10 and is one of the brain regions comprising the SN.[14] Its projections are broad and reciprocal, and the cellular properties of neurons in RPFC are well suited to integrate their inputs.[37] Previous studies have reported that the RPFC and ACC were co-activated or co-deactivated for various pain stimuli. These two regions were co-activated when a painful thermal stimulus was applied to patients with sympathetically mediated chronic pain[38] and to normal volunteers,[39] and during electrically induced tooth pain in normal volunteers.[40] Additionally, a positron emission tomography study reported that the RPFC and ACC were co-deactivated when painful stimuli were applied to patients with chronic central pain after lateral medullary infarction.[41] Co-deactivation was observed following both painful and tactile stimuli.[42] Moreover, several studies indicated that gray matter volume decreased in both RPFC and ACC in

Table 3 Neurometabolite and Functional Connectivity with Significant Correlations

NM	Seed	Region	MNI			Pt	CTRL
			X	**Y**	**Z**	**FC**	**FC**
Asp	ACC	DMN Rt LP	47	−67	29	−0.073 ±.23	−0.13 ±.23
GSH		FP Rt PPC	52	−52	45	−0.070 ±.27	−0.017 ±.26
GSH		SN Rt aIns	47	14	0	0.37 ±.14	0.31 ±.24
Glx		Lt pSMG	−55	−46	33	0.083 ±.20	0.0064 ±.26
Glx		SN Lt aIns	−32	45	27	0.30 ±.16	0.28 ±.24
GABA		FP Lt PPC	−46	−58	49	−0.13 ±.29	−0.16 ±.35

Notes: Coordinates and values of FC setting ACC as a seed with significant correlations between neurometabolites in ACC and FC value in patients and controls.
Abbreviations: NM, neurometabolite; MNI, Montreal Neurological Institute coordinates; FC; functional connectivity values (mean ± SD); Pt, patients; CTRL, controls; Asp, aspartate; GSH, glutathione, Glx, glutamate + glutamine; GABA, gamma-aminobutyric acid; ACC, anterior cingulate cortex; Lt, left; Rt, right; DMN, default mode network; LP, lateral parietal; FP; frontoparietal network; PPC, posterior parietal cortex; SN, salience network; aIns, anterior insula; pSMG, posterior supramarginal gyrus.

chronic pain patients.[43–45] Although the role of the RPFC in pain processing is currently unclear, this region is thought to be involved in various forms of pain-related attention, consciousness, modulation, memory, and emotion because of its connections to broad cortical regions and other large-scale brain networks, and its diverse functions.[46]

The RPFC is connected to various subcortical regions, including the striatum.[47] The right Acb, which is part of the ventral striatum, showed increased FC with left RPFC and left SMG in patients in the current study. A previous study reported that Acb-FOrb FC was associated with medication-overuse headaches.[48] Additionally, elevated FC between Acb and the prefrontal cortex (PFC) has been shown to predict persistence and chronicity of pain in patients with chronic low back pain.[49,50] Acb and PFC are part of the mesolimbic system, which is composed of dopaminergic neurons. This circuit plays an important role in the perception and regulation of chronic pain.[51] Acb receives direct afferent input from the contralateral trigeminal nucleus,[52] and is bilaterally connected to SMG and the inferior parietal lobule (IPL; BA 40). Furthermore, a previous study reported that Acb-SMG FC exhibited a negative value in normal volunteers.[53] This finding was consistent with the present results, while the mean value of FC was positive in patients. Although the negative relationship between these regions has not been well elucidated, it appears to occur between functional systems with apparently opposite goals or functions.[54] The IPL has been reported to be generally engaged in selective attention, action observation, and imitating emotions,[55] and the SMG is involved in the ventral attention network.[56] Furthermore, Acb-SMG FC is reported to be decreased in anhedonia with major depression.[57] Changes in FC between these areas suggest that the modulation of attention and negative reward to pain and the control of emotional cognition may be involved in COFP.

Finally, FC of the right FOrb-left aIns in SN was lower in patients compared with that in controls in the current study. The FOrb, together with the Ins and ACC, forms the paralimbic system and is part of the SN.[14] The Ins is highly interconnected with the FOrb (BA12).[58] The FOrb is adjacent to RPFC and also communicates with the somatosensory cortex and striatum, integrating sensation, emotion, learning, and motivation through the reward system.[59] FC of FOrb-Ins was reported to be negatively correlated with pain relief from positive reward in a previous study.[60] Additionally, FC of the FOrb-striatum was found to be reduced in patients with dental phobia.[61] In patients with chronic pain caused by irritable bowel syndrome, thicker gray matter in the FOrb and Ins was found to be correlated with pain inhibition and pain duration.[62] Additionally, FC between these regions has been reported to induce autonomic responses.[63] Therefore, we speculate that the reduced FC observed in this study may affect various aspects of pain-related emotions, cognition, and even behavior and autonomic responses.

The current results revealed that CSI scores were significantly correlated with FC between four pairs of brain regions. CSI scores were positively correlated with FC of ACC-right Put and ACC-right GP, and FC of left Ins-right Put. The Put is part of the ventral striatum and is located adjacent to the Acb in the dorsal striatum. The GP receives input from the

striatum and outputs to other cortical and subcortical regions. Both of these areas are part of the mesolimbic dopaminergic circuitry and are involved in reward-aversion processing[64] and pain processing, such as the sensory-discriminative, emotional/affective, and cognitive dimensions of pain and pain modulation.[65] The Put is consistently activated during acute and chronic pain conditions and is affected by analgesic administration.[65] Previous studies have reported that the GP is activated in response to cold and mechanical allodynia in chronic trigeminal neuralgia pain,[66] and during catastrophizing in fibromyalgia.[67] Decreased FC of ACC-Put and ACC-GP was reported in dental phobia patients.[61] Moreover, pain modulation by neurometabolites or analgesics occurs in the basal ganglia. Pain stimuli elicit significant dopamine release in the Put.[68] The activation of dopamine D_2 neurotransmission was positively associated with individual variation in subjective ratings of sensory and affective qualities of pain.[68] In contrast, decreased dopamine in the Put was reported in patients with burning mouth syndrome, suggesting that reduced dopaminergic inhibition causes chronic pain.[69] Additionally, the basal ganglia contain high levels of endogenous opioids, and high binding of opioid receptors. A previous study reported that oxycodone reduced ACC-bilateral Put FC.[70] Microinjections of morphine into bilateral GP were found to induce analgesia.[71] Inhibiting kappa-opioid receptor in ACC decreased FC of ACC-ventral GP.[72] Because the output of the basal ganglia is inhibitory, we speculate that the CS of COFP involves an inhibitory effect on the connections between the basal ganglia and the limbic system and cortex.

In the current study, FC of right anterior SMG-right LPFC in the SN was positively correlated with CSI scores. The FC of left LPFC-IPL has been reported to be positively correlated with pain intensity in chronic postherpetic neuralgia.[73] FC between SMG and PFC exhibits laterality; compared with the left SMG, the right temporoparietal junction of the SMG (including BA 40) was reported to be more strongly connected with the salience and ventral attention network as well as other regions implicated in salience/pain-related processing.[74] The right-lateralized salience/ventral attention network is considered to encode the prolonged salience of pain.[75] In addition, the mesencephalic-basal ganglia closed loops are reported to contribute to implicitly addressing and modulating selective attention to prioritized stimuli.[76] These findings suggest that FC that is correlated with CSI scores may enhance CS by acting on pain salience/attention networks involving the frontoparietal cortices and the mesolimbic circuit. CS is closely related to category 7 in the International Classification of Orofacial Pain (Psychosocial assessment of patients with orofacial pain), and was evaluated using various questionnaires such as for pain- and function-related constructs, and psychosocial constructs. The FCs correlating to CSI score may indicate brain function associated with this category.

Significant correlations were found between four neurometabolites within ACC and FC in six pairs of regions, setting ACC as a seed. Excitatory neurometabolites include agonists of the N-methyl-D-aspartate (NMDA) receptor, Asp and Glx. GSH, a metabolite of glutamine (Glu) and a major intracellular antioxidant, is approximately twice as abundant in glial cells as in neurons.[77] The fundamental role of GSH is to detoxify reactive oxygen species, which is critical for maintaining the normal function of the human brain.[78] Excessive stimulation of the NMDA receptor and an influx of calcium ions causes the production of excess free radicals and oxidative stress, leading to CS and cellular apoptosis.[79] Thus, GSH may reflect increased excitatory nerve activity.

In the current study, ACC-right aIns FC and ACC-left aIns FC were negatively correlated with Glx and GSH, respectively. A previous study reported that Glu in dorsal ACC was associated with connectivity strength in dorsal ACC and aIns in the patients with schizophrenia.[80] In another study, Glu levels in the ACC were reported to be reduced by oxycodone and tapentadol administration, whereas they remained unchanged in the insular cortex.[81] Furthermore, oxycodone has been demonstrated to decrease ACC-aIns FC.[81] Thus, changes in excitatory neural activity of ACC and the decrease in ACC-aIns FC suggest an inhibitory effect on pain.

Generally, activity in the SN is anticorrelated with that in the DMN.[82] In a previous study, the anticorrelation of FC between the two networks was found to be reduced in chronic pain patients compared with that in controls,[83] and was correlated with Glu level in ACC.[84] Additionally, less anticorrelated FC and connectivity between the SN and DMN were reported to be related to pain severity and disease-related symptoms in chronic pain.[83] Asp, an excitatory neurometabolite in ACC, may be involved in cross-network FC.

In the current study, Glx in ACC was positively correlated with FC between ACC and left posterior SMG. Although some previous studies have reported that Glu in ACC is elevated in patients with chronic pain,[84] other studies reported that it is decreased in these patients.[85] In one study measuring Glu in ACC in normal subjects 24 hours after

administration of S-/racemic ketamine, a non-competitive NMDA receptor antagonist, Glu was found to be elevated and FC of ACC-left IPL was decreased.[86] Although differences in the experimental conditions between that study and the present study make direct comparison difficult, the findings suggest that Glx levels in ACC and FC of ACC-left SMG are linked. Furthermore, another study reported that FC between these regions increased with improvement in mindfulness scores when mindfulness-based stress reduction was used to relieve pain and anxiety.[87] Additionally, the correlation between the FC and Glx showed an inverse relationship between the patient and control groups in the current findings, suggesting that this effect is specific to chronic pain.

In the current study, FC of ACC-right PPC in the FP network was positively correlated with GSH, whereas ACC-left PPC in the FP network was negatively correlated with GABA. If GSH reflects increased excitatory neural activity, these findings suggest that FC may be modulated by excitatory and inhibitory nerves, respectively. The right IPL and ACC were previously reported to be associated with anticipation of pain,[88] while patients with both rheumatoid arthritis and fibromyalgia were found to exhibit stronger FC of left IPL-ACC.[89] The present study suggests that differences in neurometabolites within ACC may influence the laterality of ACC-parietal lobes FC.

The current study involved several limitations that should be considered. Chronic pain is more common in women than in men,[90] and most of the subjects in the current study sample were women. In our pain clinic, the vast majority of patients with COFP are women. We asked patients to participate in the study according to our patient selection criteria, which led to this sex difference. Although a random sampling method from a sample population was not performed, this difference may reflect the sex ratio of the COFP patient population. However, the small sample size in the current study makes it difficult to examine sex differences from these results, and this is a topic for future research. GABA / tCr was used in the analysis to quantify GABA because the PRESS method of MRS was used to measure neurometabolites. A Mescher Garwood Point Resolved Spectroscopy (MEGA)-PRESS method should be used to accurately measure direct GABA concentration in future studies. Additionally, we only measured neurometabolites in ACC and calculated the FC of correlated brain regions using ACC as a seed. Neurometabolites in other brain regions that are correlated with ACC should also be measured whenever possible. Correlation coefficients greater than 0.45 and significant FC values were reported in the results, and p-values for correlations were not calculated for multiple comparisons among significant combinations. Therefore, our correlation analyses between neurometabolites and FC values should be considered exploratory. Consequently, further refinement of the data acquisition methods and statistical analysis in future studies may provide additional insight.

Conclusion

A study combining CSI scores, rsfMRI, and MRS was performed in patients with COFP. Significant increases in FC in the patient group were found in ACC-left RPFC in the SN, and Acb in the mesolimbic system-left RPFC and mesolimbic system-left SMG in the SN. In the patient group, CSI scores were positively correlated with FC in four pairs of regions: right LPFC-right anterior SMG, ACC-right GP, and ACC-right Put in the mesolimbic system, and left Ins-right Put.

Regarding the correlations between neurometabolites within ACC and FC of ACC and other cortical regions, bilateral FC of ACC-aIns exhibited negative correlations with Glx and GSH, which are associated with excitatory neuronal activity. Additionally, the results revealed a negative correlation between Asp and FC of ACC-right parietal cortex in the DMN, and in FC of bilateral ACC-PPC in the FP network, in which FC on the right side was positively correlated with GSH, whereas FC on the left side was negatively correlated with GABA / tCr.

Neurocognitive and social cognitive dysfunction and organic brain deficits have been reported to be represented by unique brain network localization.[91] The current results suggested that FC within the SN that exhibited changes in OFP patients occurred in the frontal cortex, ACC, Ins, SMG, and subcortical regions. Additionally, FC between ACC and Ins to the basal ganglia, and FC where the FP network and SN overlap, were identified as regions in which CS, one of the factors of chronic pain, is encoded. Neurometabolites within ACC, particularly those associated with excitatory and inhibitory neural activity, may be involved in altering FC related to COFP.

Patients with COFP are more likely to exhibit complications involving psychosocial disorders such as anxiety, depression, and catastrophizing. COFP can also be classified as a somatic symptom disorder with predominant pain in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).[92] A combination of questionnaires on

psychosocial factors is typically utilized for assessing somatic symptom disorders. A recent study on COFP reported that the CSI used in the current experiment was strongly correlated with many other questionnaires that assess these factors, such as the Hospital Anxiety and Depression Scale (HADS) and the Pain Catastrophizing Scale (PCS).[93] A comprehensive biopsychosocial perspective and approach to the diagnosis and treatment of COFP is essential. Achieving this goal requires an understanding of the alteration of neural networks in relation to psychosocial factors, and the current study provides useful insights into the mechanisms involved. Furthermore, the orofacial region has multiple and important functions, ranging from life-sustaining feeding and swallowing to interpersonal communication. Therefore, multidimensional aspects, complexity and unclear pathology are often present in COFP. This makes diagnosis difficult and can sometimes lead to unnecessary invasive treatment by dentists. If cases are not diagnosed and treated appropriately, 20% of patients with acute orofacial pain may progress to refractory COFP.[92] The accumulation of data using a combination of FC, neurometabolites, and questionnaire methods will provide healthcare providers with greater insight into the central nervous mechanisms of COFP, and constitutes important evidence underpinning the diagnosis, treatment, and prevention of chronicity.

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Disclosure

The authors report no conflicts of interest in this work.

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