The Somatosensory Link in Fibromyalgia

Functional Connectivity of the Primary Somatosensory Cortex Is Altered by Sustained Pain and Is Associated With Clinical/Autonomic Dysfunction

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Objective. **Fibromyalgia (FM) is a chronic functional pain syndrome characterized by widespread pain, significant pain catastrophizing, sympathovagal dysfunction, and amplified temporal summation for evoked pain. While several studies have demonstrated altered resting brain connectivity in FM, studies have not specifically probed the somatosensory system and its role in both somatic and nonsomatic FM symptoms. Our objective was to evaluate resting primary somatosensory cortex (S1) connectivity and to explore how sustained, evoked deep tissue pain modulates this connectivity.**

Methods. **We acquired functional magnetic resonance imaging and electrocardiography data on FM patients and healthy controls during rest (the rest phase) and during sustained mechanical pressure–induced pain over the lower leg (the pain phase). Functional connectivity associated with different S1 subregions was calculated, while $S1_{leg}$ connectivity (representation of the leg in the primary somatosensory cortex) was contrasted between the rest phase and the pain phase and was correlated with clinically relevant measures in FM.**

Results. **During the rest phase, FM patients showed decreased connectivity between multiple ipsilateral and cross-hemispheric S1 subregions, which was correlated with clinical pain severity. Compared to the rest phase, the pain phase produced increased $S1_{leg}$ connectivity to the bilateral anterior insula in FM patients, but not in healthy controls. Moreover, in FM patients, sustained pain–altered $S1_{leg}$ connectivity to the anterior insula was correlated with clinical/behavioral pain measures and autonomic responses.**

Conclusion. **Our study demonstrates that both somatic and nonsomatic dysfunction in FM, including clinical pain, pain catastrophizing, autonomic dysfunction, and amplified temporal summation, are closely linked with the degree to which evoked deep tissue pain alters S1 connectivity to salience/affective pain-processing regions. Additionally, diminished connectivity between S1 subregions during the rest phase in FM may result from ongoing widespread clinical pain.**

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Patients with chronic pain feel pain as a primarily somatosensory sensation, although it is well understood that clinical pain is much more than somatic and encompasses multiple affective and cognitive domains. Fibromyalgia (FM) is a prototypical functional pain syndrome characterized by multidimensional symptomatology. Symptoms include widespread pain, mood disturbance with significant pain catastrophizing, cognitive and physical fatigue, dysfunction of autonomic activity, and amplified sensitivity and temporal summation to experimental pain stimuli (1). Multiple neuroimaging studies have supported the theory that FM is primarily a multisystem disorder of central nervous system (e.g., brain) processing. However, the precise linkage between the circuitries processing somatic sensation and those underlying broader affective and cognitive domains remains unknown.

Functional connectivity magnetic resonance imaging (MRI) is an adaptation of functional MRI (fMRI) that may help assess brain circuitry supporting spontaneous clinical pain. While spontaneous clinical pain (2) and negative affect (3) components of FM have been linked to altered resting (or intrinsic) functional brain connectivity, previous studies have not systematically probed the primary somatosensory cortex (S1)—a potentially integral brain area for somatic symptomatology such as pain. In FM, decreased secondary somatosensory cortex (S2) connectivity to the primary motor cortex (3) and reduced connectivity between S2 and S1 (4) were also recently reported. Interestingly, S1 connectivity is also sensitive to sustained experimental pain stimulation in healthy adults (5), suggesting malleable state–like properties for S1 connectivity networks. This view is consistent with a previous report that functional brain connectivity can reflect both state and trait processes (6). Such state processes may even underlie the hyperalgesia, allodynia, and temporal summation commonly noted in patients with chronic pain, as region-specific changes in S1 connectivity may support maladaptive changes in central processing of somatosensory afference.

Our current study was undertaken to investigate evoked pain state–induced alterations in S1 connectivity in chronic pain patients with FM. We also explored how altered S1 connectivity is associated with clinically relevant variables such as pain intensity and pain-related catastrophizing, key determinants of FM morbidity. Furthermore, we linked evoked deep tissue pain–modulated S1 connectivity with temporal summation of pain and core nonsomatic aspects of FM pathophysiology, including altered autonomic modulation. Investigation of the nonsomatic aspects of FM follows past studies that have demonstrated autonomic dysfunction in FM patients (7), linking such dysfunction with clinically relevant parameters (7,8). We hypothesized that multisystem pathology, common to FM, is supported by altered functional S1 connectivity at rest and/or in response to evoked nociceptive stimuli, e.g., deep tissue pain, that are highly relevant in FM.

PATIENTS AND METHODS

Participants. All participants in the study provided written informed consent in accordance with the requirements of the Human Research Committee of the Massachusetts General Hospital. To be included in the study, patients had to be ages 18–70 years, had to be diagnosed as having FM as confirmed by physician and medical records, and had to meet the American College of Rheumatology preliminary diagnostic criteria for FM (9). Exclusion criteria for the FM group were a history of significant neurologic disorders, a history of anxiety disorders or significant anxiety symptoms interfering with MRI procedures, a history of significant cardiac events, a history of significant head injury, current treatment with opioids, self-reported current use of recreational drugs, and typical contraindications for MRI. Healthy controls in the same age range were also studied; exclusion criteria for the healthy control group were the same as those for the FM group, in addition to chronic or acute pain. Data on 35 FM patients (32 women and 3 men, mean ± SD age 44.94 ± 12.02 years) and 14 healthy controls (10 women and 4 men, mean ± SD age 44.21 ± 14.26 years) were included for statistical analyses. The distributions of sex ($P = 0.091$ by Fisher's exact test) and age ($P = 0.86$ by 2-sample *t*-test) did not differ between the groups. Special statistical considerations were used when fMRI analyses included groups of different sample sizes (see below).

During a behavioral training session (on a different date from the fMRI procedure), subjects were familiarized with pressure-induced pain and rating procedures and requested to provide information about pain catastrophizing, depression, and chronic pain using the Pain Catastrophizing Scale (PCS) (10), the Beck Depression Inventory (BDI) (11), and the Brief Pain Inventory (BPI) (12), respectively. Functional MRI sessions included a 6-minute resting-state run (the rest phase), 5-minute block design pain stimuli runs (used as a functional localizer), and a 6-minute continuous pain–state run (the pain phase), in that order. The rest phase always preceded the pain phase in order to negate any potential carryover effects of sustained pain provocation.

Pressure–pain stimuli. Painful pressure stimuli using cuff pain algometry were applied on the left lower leg (over the gastrocnemius muscle belly) with a velcro-adjusted pressure cuff connected to a rapid cuff inflator (Hokanson). Such cuff pressure stimuli have been shown to preferentially target deep tissue nociceptors and can be applied for extended periods of time without damaging tissue (13). Our group

has successfully used cuff pressure algometry with neuroimaging both in healthy adults and in patients with chronic pain (5,14).

MRI session. For the runs during the rest, pain, and block design phases, fMRI data were acquired using a 3.0T TIM Trio MRI System (Siemens) equipped for echo-planar imaging with a 32-channel head coil. A whole-brain T2*-weighted gradient-echo blood oxygen level–dependent echo-planar imaging pulse sequence was used (repetition time [TR]/echo time [TE] 2 seconds/30 msec, flip angle 90°, 37 anterior commissure–posterior commissure–aligned axial slices, voxel size 3.1 × 3.1 × 3.6 mm). In addition to fMRI data, we collected anatomic data using a T1-weighted multi-echo magnetization-prepared rapid gradient-echo pulse sequence (TR 2,530 msec/TE1 1.64 msec/TE2 3.5 msec/TE3 5.36 msec/TE4 7.22 msec, flip angle 7°, voxel size 1 mm isotropic).

For runs in both the rest phase and the pain phase, subjects were instructed to relax and lie still with their eyes open, which has been shown to improve resting connectivity estimation (15). Subjects were asked to verbally rate their clinical pain intensity after the rest phase. A 0–100 numerical rating scale (NRS) was used, where 0 was labeled "no pain" and 100 was labeled "the most intense pain tolerable."

Block design fMRI cuff pain runs were used to localize the contralateral S1 subregion associated with the cortical representation of the left lower leg for seed correlation analysis of pain phase data (i.e., functional localizer). Subjects received 2 cuff pain stimuli per run, which elicited a pain intensity rating of ~40/100. While robust S1 activation was noted (further information is available at https://www.dropbox.com/s/w0vztgmyccuj9a0/SFigure1_Groupmap_v1.0.tif?dl=0), relatively long (duration of 75–105 seconds, interstimulus interval of 52–72 seconds) pressure–pain blocks were used for a separate study hypothesis. Thus, within-subject generalized linear model (GLM) analysis was performed with a regressor of interest modeling pressure–pain onset. Regressors of no interest modeled the variance explained by pressure–pain offset and entire-duration cuff pressure block. Following the scan, subjects rated how well they were able to keep their attention focused on such lengthy pain stimuli on a scale of 0–100, where 0 was "not at all" and 100 was "extremely well." This value served as an interindividual measure of attentiveness to sustained cuff pain.

For the pain phase run, the cuff pressure level was set to target ~40/100 pain intensity. Following the pain phase run, subjects were asked to rate cuff pain intensity using a 0–100 NRS. Subjects rated overall pain intensity for the entire 6-minute pain phase run, as well as separate pain intensity for each of the 2-minute periods at the beginning, middle, and end of this 6-minute run. A variety of methodologies have shown that individuals are generally proficient at remembering pain intensity levels over spans of time ranging from minutes (16) to days (17,18), although the latter may be more controversial. Moreover, previous cuff algometry studies using continuous ratings have shown relative stability of sensation over a 2-minute period (19).

Physiologic data were collected simultaneously with all fMRI runs. Electrocardiography (EKG) data were collected with an MRI-compatible patient monitoring system (Invivo Research). Respiration data were collected using a custom-built pneumatic MRI-compatible belt placed around the subject's ribcage.

Temporal summation. Using the ratings of the 2-minute periods from the pain phase run described above, we also evaluated temporal summation (potential sensitization or habituation) to the sustained cuff pain by calculating a temporal summation index (see equation below). This index was defined as the "end" period pain intensity on an NRS divided by the "beginning" period pain intensity on an NRS. In order to control for individual differences in subjects' sensitivity to cuff pain, the result was further divided by the pressure level (in mm Hg) used to elicit target pain, i.e., [(pain intensity$_{end}$/pain intensity$_{beginning}$)/pressure level in mm Hg] × 100.

Physiologic data analyses. The EKG beat annotation and respiration data time series were used for cardiorespiratory artifact correction using RETROICOR (20), while nuisance regressors were formed by convolving these time series with cardiac and respiratory response functions (21). Additionally, autonomic response to cuff pain in FM patients and healthy controls was estimated using heart rate variability (HRV) analyses. HRV estimation was performed using previously validated Kubios HRV software (22). Normalized high-frequency (0.15–0.40 Hz) spectral power was computed to estimate cardiovagal modulation (23). Spectral power was calculated for the entire 6-minute rest phase and pain phase runs, as well as for the 2-minute periods at the beginning, middle, and end of these runs.

Functional connectivity analyses. Functional MRI data were preprocessed using FSL (FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl/), AFNI (Analysis of Functional NeuroImages; http://afni.nimh.nih.gov/afni), and FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) software packages. Data were corrected for physiologic artifacts, slice timing, and affine head motion, and brain extraction was performed. As recent studies suggest that head motion can significantly influence functional connectivity estimation (24,25), root mean square relative motion estimates were calculated. We found no significant differences in mean relative motion between the rest and pain phases or between FM patients and healthy controls for either phase. In addition, motion during the rest and pain phases was further reduced by independent component analysis, which filtered out components whose time series demonstrated significant motion-relevant spikes (comparing to estimated motion parameters) and spatial distribution consistent with motion artifacts.

Cortical surface reconstruction was completed to improve structural-functional coregistration using FreeSurfer's bbregister tool (26). Functional data were then registered to standard Montreal Neurological Institute (MNI) space using FMRIB's nonlinear coregistration tool. Data were then resampled to 2-mm isotropic voxels and spatially smoothed (6-mm full width at half maximum), followed by high-pass temporal filtering (f = 0.006 Hz). We chose to retain fMRI signal at high frequency (i.e., no low-pass filtering), as our recent study highlighted the importance of fMRI signal at higher frequencies (24), while other groups have reported altered cortical dynamics at higher frequencies in patients with chronic pain (27,28).

Functional connectivity was computed using seed-based correlation analysis (29). For rest phase data, seed locations within S1 were defined based on the block design pain fMRI results (representation of the leg in the primary somatosensory cortex [S1$_{leg}$]; see below) and based on other evoked-stimulation fMRI studies that showed S1 activation. These latter studies included somatosensory stimuli applied to the back

Table 1. Clinical and behavioral data on the study subjects*

	Healthy controls (n = 14)	FM patients (n = 35)	P†
Age, years	44.2 ± 14.3	44.9 ± 12.0	NS
No. of women	10	32	NS
Symptom duration, years‡	–	9.76 ± 8.56	NA
PCS score, 0–52	5.4 ± 5.8	22.2 ± 12.9	<0.01
BDI score, 0–63	2.8 ± 3.8	13.5 ± 8.2	<0.05
BPI scores, 0–10			
Pain severity	0.3 ± 0.6	5.1 ± 2.0	<0.01
Pain interference	0.0 ± 0.0	5.2 ± 2.1	<0.01
Clinical pain at time of MRI scan, 0–100	0.0 ± 0.0	29.9 ± 22.6	<0.01
Cuff pressure at which target pain level was perceived during pain phase run, mm Hg	180.4 ± 91.4	105.4 ± 64.4	<0.01
Score for attention to cuff pain, 0–100	84.7 ± 14.1	77.9 ± 17.0	NS
Intensity of cuff pain, 0–100			
Overall (6 minutes)	45.2 ± 17.6	55.7 ± 17.8	NS
Beginning 2-minute period	34.4 ± 15.0	46.7 ± 13.8	<0.01
Middle 2-minute period	43.1 ± 14.5	50.0 ± 16.1	NS
End 2-minute period	42.9 ± 22.3	57.1 ± 19.4§	<0.05
Temporal summation index	0.9 ± 0.6	1.5 ± 0.8	<0.05
Change in nHF_{HRV}, mean ± SEM¶			
Overall (6 minutes)	−6.5 ± 3.8	−7.8 ± 2.5#	NS
Beginning 2-minute period	−7.5 ± 4.7	−1.9 ± 3.5	NS
Middle 2-minute period	−8.4 ± 5.7	−6.5 ± 3.4	NS
End 2-minute period	0.5 ± 4.6	−9.7 ± 3.4#	NS

* Except where indicated otherwise, values are the mean ± SD. See Patients and Methods for description of rest and pain phases. FM = fibromyalgia; NS = not significant; NA = not applicable; PCS = Pain Catastrophizing Scale; BDI = Beck Depression Inventory; BPI = Brief Pain Inventory; MRI = magnetic resonance imaging; nHF_{HRV} = normalized high-frequency component of heart rate variability.
† By 2-group *t*-test.
‡ Based on date of diagnosis.
§ $P < 0.05$ versus beginning 2-minute period, by Dunnett's test.
¶ From the pain phase through the rest phase.
# $P < 0.01$ versus rest phase, by paired *t*-test.

(±18, −44, 64 mm in MNI coordinates [30]), chest (±18, −36, 64 [31]), hand (±28, −30, 50 [32]), finger (±50, −16, 50 [33]), and face (±60, −14, 40 [34]). Seeds were mirrored across the midsagittal plane for analysis. For rest phase data analyses, we averaged fMRI signal from a 4-mm radius sphere centered on each coordinate above. These time series were used to calculate a correlation matrix covering S1 subregions across both brain hemispheres. Correlation matrices were transformed by a Fisher's r-to-z transformation to impose a normal distribution, followed by an omnibus *t*-test contrasting FM patient and healthy control matrices.

Whole-brain voxelwise correlation analyses were focused on the $S1_{leg}$ seed contralateral to the leg experiencing cuff pain. In order to use an unbiased seed location, the $S1_{leg}$ seed was defined by a 4-mm radius sphere centered on the peak activation voxel (8, −38, 68 mm in MNI coordinates) from the group map of the block design pain runs, combined over both FM patients and healthy controls. The average fMRI time series from this seed was used as a GLM regressor for both rest and pain phase data. Nuisance regressors included fMRI signals from deep cerebral white matter, fMRI signals from cerebral ventricles using previously validated masks (15), and cardiorespiratory artifacts defined by convolving the heart rate and respiratory signal with appropriate transfer functions (21). Notably, we did not include the global fMRI signal in this GLM. Resultant connectivity maps, and their variance, from each individual were passed up to group-level analyses to explore differences between the rest and pain phases, for both FM patients and healthy controls, using FMRIB's Local Analysis of Mixed Effects (FLAME1+2) using Metropolis-Hastings Markov Chain Monte Carlo sampling for improved mixed-effects variance estimation, which is recommended in group comparisons that involve unequal sample sizes.

We also performed whole-brain voxelwise linear regression analysis to investigate the link between pain-altered $S1_{leg}$ connectivity and clinical and behavioral/autonomic measures. PCS scores were controlled for depression (BDI), similar to procedures used in previous studies (for example, see ref. 35), to estimate the specific influence of catastrophizing above and beyond generalized depression. All brain maps were thresholded using cluster correction for multiple comparisons (Z score >2.3 and a cluster-size threshold of $P < 0.05$).

All clinical and behavioral data were compared between groups using 2-tailed *t*-tests for independent samples in SPSS version 22. Analysis of variance models were computed for functional connectivity values taken from significant clusters' peak voxels, in order to test for interactions between group factors (FM patients and healthy controls) and scan factors (rest phase and pain phase) that were significant at $P < 0.05$.

RESULTS

Clinical, behavioral, and autonomic response to sustained pain. Compared to healthy controls, patients with FM demonstrated significantly higher scores on

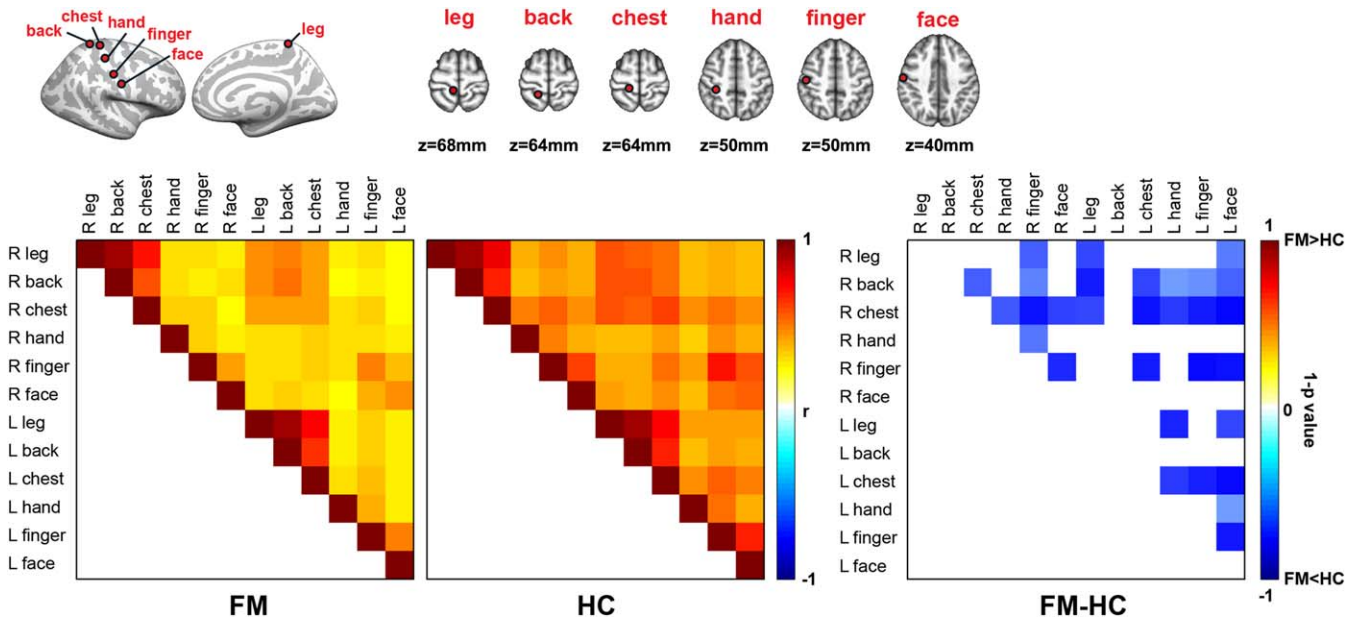

Figure 1. Diminished resting-state primary somatosensory cortex (S1) functional connectivity within S1 regions in patients with fibromyalgia (FM). Correlation analysis using different S1 regions of interest demonstrated disrupted interregional functional correlation (blue squares) at rest in FM patients as compared to healthy controls (HC). FM>HC and FM<HC denote differences in resting-state S1 connectivity within S1 regions from FM patients through healthy controls. Montreal Neurological Institute coordinates are shown. R = right hemisphere; L = left hemisphere.

the PCS ($P < 0.01$), BDI ($P < 0.05$), and BPI ($P < 0.01$) (Table 1). FM patients reported, on average, mild-to-moderate clinical pain (~30/100 on an NRS) at the MRI session (Table 1).

For the pain phase run, cuff pressures were calibrated individually to ~40/100 on an NRS just prior to the run. Pain intensity ratings at this calibration did not differ between FM patients and healthy controls (mean ± SD 43.13 ± 7.97 versus 43.63 ± 8.09, respectively; $P = 0.86$). Cuff pressure over the lower leg during the pain phase run produced, on average, moderate-to-strong pain intensity in both FM patients and healthy controls (see Table 1). The overall pain intensity for 6 minutes of cuff stimulation did not differ significantly between FM patients and healthy controls, although there was a trend toward greater cuff pain in FM patients (55.67 ± 17.83 versus 45.21 ± 17.58; $P = 0.068$) due to temporal summation (see below). All subjects also rated cuff pain intensity for 3 sequential 2-minute periods from this 6-minute pain phase run. Dunnett's test was performed to evaluate sensitization or habituation to the cuff pain, using the beginning 2-minute period as a reference. In FM patients, the end 2-minute period showed significantly greater pain intensity compared to the beginning 2-minute period (57.11 ± 19.42 versus 46.69 ± 13.80; $P < 0.05$), while the middle 2-minute period (50.0 ± 16.1) did not differ from the beginning 2-minute period ($P = 0.61$). In healthy controls, there were no significant differences in the middle or end 2-minute periods (43.1 ± 14.5 and 42.9 ± 22.3, respectively) compared to the beginning 2-minute period (34.4 ± 15.0). Temporal summation was greater in FM patients compared to healthy controls (1.5 ± 0.8 versus 0.9 ± 0.6; $P < 0.05$).

We evaluated cardiovagal activity using HRV analysis and found that, compared to the rest phase, sustained cuff pain reduced the normalized high-frequency component of HRV (nHF_{HRV}; in normalized units) in FM patients (mean ± SEM −7.78 ± 2.48) ($P < 0.01$), while the reduction in healthy controls was not significant (−6.50 ± 3.80 [$P = 0.15$]) (Table 1). In FM patients, the reduction in nHF_{HRV} was also more robust over time (from the pain phase through the rest phase) (beginning 2-minute period −1.85 ± 3.52 [$P = 0.60$], middle 2-minute period −6.47 ± 3.42 [$P = 0.07$], end 2-minute period −9.69 ± 3.39 [$P < 0.01$]). In contrast, in healthy controls, changes in nHF_{HRV} were sporadic over time and were not significant (beginning 2-minute period −7.49 ± 4.72 [$P = 0.31$], middle 2-minute period −8.40 ± 5.65 [$P = 0.11$], end 2-minute period 0.51 ± 4.56 [$P = 0.21$]).

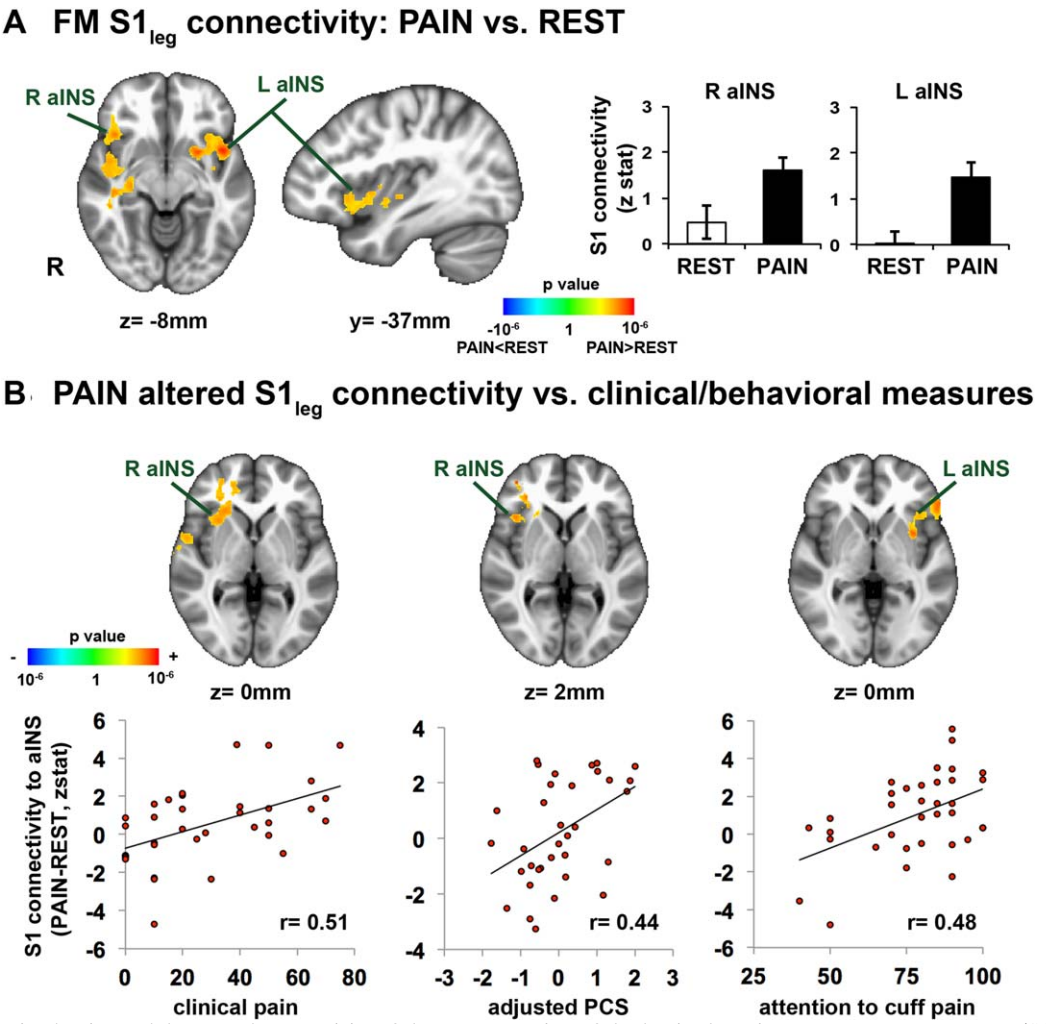

Figure 2. **A,** Sustained pain modulates seed connectivity of the representation of the leg in the primary somatosensory cortex ($S1_{leg}$). In patients with fibromyalgia (FM), the pain phase increased connectivity between $S1_{leg}$ and the bilateral anterior insula (aINS). Values are the mean ± SD. **B,** Shown are associations between clinical/behavioral measures in FM patients and sustained pain–induced changes in $S1_{leg}$ functional connectivity to the anterior insula. Increases in $S1_{leg}$ connectivity (from the pain phase through the rest phase) to the anterior insula were positively correlated with clinical pain intensity at the time of the scan, with Pain Catastrophizing Scale (PCS) scores, and with the score for attention to cuff pain. Montreal Neurological Institute coordinates are shown. Symbols represent individual subjects. See Patients and Methods for description of rest and pain phases. R = right hemisphere; z stat = Z statistic. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.39043/abstract.

We also found a significant association between clinical/behavioral and autonomic measures in FM patients. Temporal summation in FM patients showed a positive correlation with the PCS score (r = 0.53, $P < 0.05$). Thus, FM patients with higher PCS scores were more sensitized to cuff pain over the 6-minute stimulation period. Individually tailored cuff pressure was negatively correlated with the PCS score (r = −0.43, $P < 0.05$). In addition, in FM patients, temporal summation showed a negative correlation with pain-induced decreases in nHF_{HRV} (calculated over the whole 6-minute run) (r = −0.50, $P < 0.01$), suggesting that FM patients with greater temporal summation to sustained deep tissue pain also had greater reductions in cardiovagal modulation.

Altered S1 functional connectivity in FM patients during the rest phase. During the rest phase, correlation matrices for different bilateral somatotopic S1 subregions (leg, back, chest, hand, finger, face) were significantly different between FM patients and healthy controls (omnibus test: t[65] = −17.29, $P < 0.01$), with FM patients showing reduced resting connectivity between multiple different S1 subregions (Figure 1). Moreover, a negative correlation was found between

Table 2. Brain regions showing significant correlations between clinical/behavioral measures and sustained cuff pain–induced $S1_{leg}$ connectivity from the pain phase through the rest phase*

	Side	Size, mm^3	MNI coordinates, mm			Peak Z statistic
			X	Y	Z	
FM patients						
Clinical pain intensity						
Anterior insula	R	8,376	32	18	0	3.46
Dorsal anterior cingulate cortex	–	8,376	0	22	12	4.12
Middle insula	R	688	40	2	−10	2.94
Posterior insula	R	2,576	34	−14	24	3.89
Superior temporal gyrus	R	1,888	54	4	6	4.23
Inferior frontal gyrus	R	1,608	56	16	−14	4.48
Pain catastrophizing scores						
Anterior insula	R	6,552	42	20	2	3.99
Middle frontal gyrus	R	6,552	46	40	−4	3.80
Scores for attention to cuff pain						
Anterior insula	L	9,680	−36	20	0	3.11
Caustrum/middle insula	L	9,680	−34	4	0	3.95
Inferior frontal gyrus	L	9,680	−54	26	0	3.87
Cardiovagal response (nHF_{HRV})						
Anterior/middle insula	R	9,864	34	12	0	−3.19
Middle/posterior insula	R	9,864	42	0	−12	−3.94
Superior temporal gyrus	R	9,864	54	0	−10	−4.70
Inferior parietal lobule	L	67,312	−66	−42	30	−5.02
Cerebellum	L	1,552	−4	−66	−24	−3.81
Temporal summation						
Anterior/middle insula	R	3,472	34	16	−2	2.91
Caudate nucleus	R	3,472	14	4	2	4.39
Putamen	R	3,472	24	10	6	3.36
Premotor	R	4,904	34	0	52	4.16
Middle frontal gyrus	R	3,888	42	40	−14	4.61
Healthy controls						
Temporal summation						
Superior parietal lobule	L	7,496	−36	−76	44	3.71
Superior parietal lobule	R	5,920	38	−72	48	3.78

* See Patients and Methods for description of rest and pain phases. $S1_{leg}$ = representation of the leg in the primary somatosensory cortex; MNI = Montreal Neurological Institute; FM = fibromyalgia; nHF_{HRV} = normalized high-frequency component of heart rate variability.

interregional S1 connectivity and BPI scores (omnibus test following Fisher's r-to-z transformation: t[65] = −12.30, $P < 0.001$). Thus, patients reporting greater clinical pain also showed greater reduction in resting connectivity within S1.

Altered S1 functional connectivity during sustained pain stimuli (the pain phase). In healthy controls, sustained cuff pain over the lower leg produced decreased (compared to the rest phase) $S1_{leg}$ connectivity to S1 subregions outside of the seed's cortical representation, similar to our previous results (5) (further information is available at https://www.dropbox.com/s/79trtzbz0cv66s8/SFigure2_HC_PAIN-REST_uncorr_v1.0.tif?dl=0). In contrast to healthy controls, sustained cuff pain in FM patients elicited increased $S1_{leg}$ connectivity to the bilateral anterior insula (Figure 2A) (further information is available at https://www.dropbox.com/s/aqjnirgznko2dsr/STable1.pdf?dl=0). In fact, we found a significant group (FM patients and healthy controls) by scan (rest phase and pain phase) interaction for $S1_{leg}$ connectivity to the right anterior insula (peak voxel 42, 22, −12 mm in MNI coordinates) (F = 6.98, $P < 0.01$). A whole-brain linear regression analysis in FM patients showed that changes (from the pain phase through the rest phase) in $S1_{leg}$ connectivity to the anterior insula were significantly correlated with clinical pain intensity at the time of MRI scan (r = 0.51), PCS scores (r = 0.44), and attention to cuff pain (r = 0.48) (Figure 2B and Table 2). A whole-brain analysis also showed a positive correlation between changes in $S1_{leg}$ connectivity to the right anterior/middle insula and temporal pain summation (Figure 3A and Table 2). In healthy controls, individual variability in temporal summation was instead positively correlated with changes in $S1_{leg}$ connectivity to the superior parietal lobule (Figure 3B and Table 2).

In FM patients, whole-brain linear regression analysis showed a negative correlation between cuff pain–induced changes in nHF_{HRV} (from the pain phase through the rest phase, the entire 6-minute estimate)

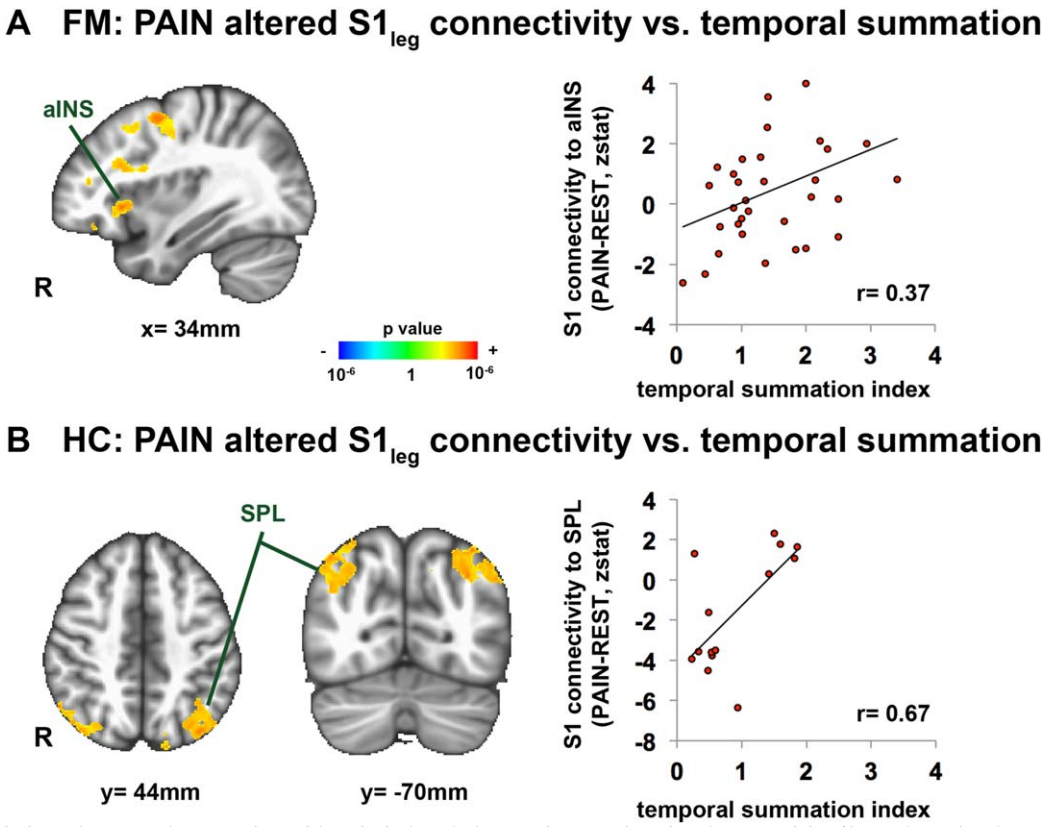

Figure 3. Association of temporal summation with pain-induced changes in $S1_{leg}$ functional connectivity (from the pain phase through the rest phase). **A,** FM patients who were more sensitized to sustained pain showed greater increases in $S1_{leg}$ connectivity to the anterior insula. **B,** Healthy controls (HC) reporting greater temporal summation to sustained pain showed greater pain-induced increases in $S1_{leg}$ connectivity to the superior parietal lobule (SPL). Montreal Neurological Institute coordinates are shown. Symbols represent individual subjects. See Patients and Methods for description of rest and pain phases. See Figure 2 for other definitions. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.39043/abstract.

and changes in $S1_{leg}$ connectivity to the right anterior/middle insula (Figure 4 and Table 2). This suggests that increased $S1_{leg}$ connectivity to the right anterior insula was also associated with more reduced cardiovagal modulation.

DISCUSSION

FM is characterized by multidimensional symptomatology that varies between individuals, while somatic pain remains a consistent core feature of this chronic pain disorder. Our results demonstrated that, compared to healthy controls, FM patients had diminished resting S1 connectivity, both within and between hemispheres. Lower leg cuff pain, compared to the rest phase, produced increased contralateral $S1_{leg}$ connectivity to the bilateral anterior insula in FM. Moreover, in FM, pain-altered $S1_{leg}$ connectivity to the right anterior insula was correlated with clinical pain intensity, pain catastrophizing, temporal summation, and autonomic response to evoked cuff pain, while increased $S1_{leg}$ connectivity to the left anterior insula was correlated with attention to cuff pain. These results highlight the clinically meaningful role of altered S1 physiology, further elucidate the dynamic role of the anterior insula in chronic pain pathophysiology, and suggest that both somatic and nonsomatic aspects of FM pathology are linked by S1 connectivity to non–somatosensory-specific, salience-processing brain regions.

Previous studies have shown altered S1 connectivity in response to noxious afference in healthy adults. Riedl et al found that exposure to repeated noxious stimulation for 10 days produced habituation in terms of pain intensity ratings but increased functional connectivity within the somatosensory motor network (36), suggesting that reduced pain is associated with greater intrinsic sensorimotor network connectivity. The inverse may be true for chronic pain, as we found that greater clinical pain was associated with more reduced resting connectivity within S1. Interestingly, our previous study in healthy adults showed that sustained leg

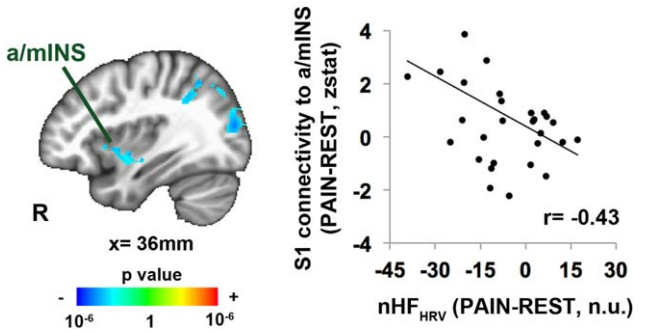

Figure 4. Pain-induced changes in cardiovagal response (normalized high-frequency component of heart rate variability [nHF_{HRV}]) were negatively correlated with changes in $S1_{leg}$ connectivity to the right anterior/middle insula (a/mINS). Thus, a greater decrease in nHF_{HRV} in response to leg cuff pain was associated with greater $S1_{leg}$ connectivity to the right anterior/middle insula. Montreal Neurological Institute coordinates are shown. Symbols represent individual subjects. See Patients and Methods for description of rest and pain phases. n.u. = normalized units (see Figure 2 for other definitions). Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.39043/abstract.

cuff pain decreased $S1_{leg}$ (contralateral to the leg experiencing pain) connectivity to S1 subregions outside the leg representation (5), while in the current study, in FM patients, the pain phase did not reduce $S1_{leg}$ connectivity to these subregions. Thus, we propose that in FM, 1) reduced resting-state connectivity between somatotopically different S1 subregions and 2) lack of cuff pressure–induced reduction of $S1_{leg}$ connectivity to other S1 subregions both resulted from ongoing, widely distributed clinical pain in FM patients that led to a tonic level of elevated somatosensory processing. Regarding the former condition, our hypothesis is supported by the negative correlation found between resting inter-subregion S1 connectivity and clinical pain (BPI) scores, demonstrating that patients reporting greater clinical pain also showed greater reduction in connectivity within S1.

We also found that evoked pain increased connectivity between the contralateral S1 subregion activated by this stimulus (i.e., $S1_{leg}$) and the anterior insula in FM patients. Notably, while target pain levels were the same between groups, healthy controls experienced far greater cuff pressures to reach these perceptual levels, due to the well-known phenomenon of hyperalgesia in FM. The anterior insula is known as a salience-processing region (37), and it is also a key region for affective and attentional pain processing (38). Thus, our results showing a pain phase–induced increase in S1/insula connectivity in FM suggest a neurobiologic substrate for evoked pain hypersensitivity in this disorder. Specifically, a pain phase–induced increase in S1/insula connectivity may reflect increased salience processing and affective processing attributed to the somatosensory dimension of evoked somatic pain. In fact, we found that changes in $S1_{leg}$ connectivity to the anterior insula during the pain phase were correlated with clinical pain intensity at the time of MRI scan, pain catastrophizing (PCS scores), and reported scores for attention to cuff pressure–induced pain, thus highlighting the clinical relevance of this brain-based response to our experimental pain stimulus. Our previous connectivity studies have demonstrated that resting anterior/middle insula connectivity to default mode network regions is associated with clinical pain intensity (39–41), and the present findings add further evidence that the insula has a dynamic role in both chronic pain perception and hyperalgesic response to experimental mechanical stimuli.

Temporal summation for repeated or long-lasting evoked pain stimuli is also commonly noted in patients with chronic pain, including FM patients (42), and is likely a consequence of central sensitization. While FM patients experienced lower cuff pressures to elicit target (40/100) pain ratings, temporal summation was actually greater than in healthy controls. Previous fMRI neuroimaging studies have implicated several brain regions that support temporal summation, including the posterior (not the anterior) insula and S1, both in healthy adults (43) and in a combined cohort of healthy controls and FM patients (44). Our study used a much longer duration of mechanical pain stimulation and a within-subject–level analysis to show that functional S1 connectivity to the right anterior insula supports greater temporal summation in FM. In healthy controls, temporal summation was instead associated with greater S1 connectivity to the superior parietal lobule, an important somatic attention-processing brain region (45). Hence, our results suggest that in FM patients, enhanced temporal summation (compared to that in healthy controls) may reflect greater linkage between somatosensory and affect/salience-processing brain regions, leading to enhanced emotional attribution to evoked pain stimuli of extended duration. In contrast, temporal summation in healthy controls may reflect enhanced attentional resources attributed to sustained nociceptive afference.

We observed significantly decreased nHF_{HRV} in response to sustained pain stimuli in FM patients. Interestingly, autonomic dysfunction has been demonstrated in FM (46) and is thought to result from patients' chronic pain experience (i.e., reduced cardiovagal activity due to ongoing stress). In our study, reduced cardiovagal modulation was especially pronounced in the final 2-minute period and may have contributed to (or resulted

from) the noted temporal summation, as greater reductions in nHF_{HRV} were correlated with greater temporal summation. We further demonstrated that subjects with greater nHF_{HRV} reductions also showed greater S1 connectivity to the right anterior insula. The anterior insula is known to control autonomic response for both internally driven processes and external sensory stimuli (47,48), and it is a core component of the central autonomic network for both sympathetic and parasympathetic modulation (49). Thus, anterior insula connectivity to S1 appears to play a crucial modulatory role not only in hyperalgesia and temporal summation, but also in autonomic responsiveness to evoked pain, which may reflect elevated levels of clinical pain severity and pain catastrophizing.

Interestingly, while the sustained cuff pressure–induced pain increased $S1_{leg}$ connectivity to the bilateral anterior insula, the association between $S1_{leg}$ connectivity and clinical pain, catastrophizing (affective, emotional dimension), and cardiovagal response was localized to the right anterior insula, and the association between $S1_{leg}$ connectivity and attention to pain (cognitive dimension) was localized to the left anterior insula. Previous studies have suggested that laterality of anterior insula processing may relate to differential autonomic inputs (50), valence of emotional stimuli, and/or the subject's sex (51). Furthermore, the association between S1/insula connectivity and clinical variables such as catastrophizing was not seen during rest, suggesting that a strong affective/somatic input that modulates autonomic outflow is necessary to produce this association between catastrophizing and S1/insula connectivity.

Limitations to our study should also be noted. For instance, while some analyses (e.g., of nHF_{HRV} response to the pain phase) revealed significant effects in FM patients and only trends toward significance in healthy controls, the latter group was composed of fewer subjects. However, we should note that an increasing nHF_{HRV} response to sustained cuff pressure–induced pain over time was seen only in FM patients (and was not a trend in healthy controls) and was correlated with a temporal summation effect specific to the population of patients with chronic pain. Additionally, recent studies have shown altered small-diameter fiber density and hyperexcitable C-fiber nociceptors in FM (52,53). Thus, in addition to the more widely documented central amplification, FM patients may have experienced differential peripheral signaling from cuff stimulation. Future studies are needed to more clearly elucidate the influence of peripheral factors. Nearly half (49%) of the patients were receiving antidepressant therapy, most commonly selective norepinephrine reuptake inhibitors (e.g., duloxetine) or tricyclic antidepressants. A much smaller number were receiving muscle relaxants (16%) or benzodiazepines (9%). Studies with larger patient populations, providing greater statistical power, are needed to explicitly explore the effects of different medications on brain connectivity. Finally, we did not collect clinical pain ratings from FM patients after the pain phase run to understand how the evoked experimental pain interacts with clinical pain. However, the association between pain phase–induced S1/anterior insula connectivity and clinical measures highlights the clinical relevance of the reported brain responses to cuff pain.

In summary, the present results suggest that pain in FM, which is somatic in origin and accompanied by symptomatology covering multiple affective and cognitive domains, may be supported by neural links between somatosensory and affect/cognition-processing brain regions. Our findings highlight the clinically meaningful role of altered S1 physiology in FM, particularly in response to nociceptive afference, and the clear importance of anterior insula connectivity for hyperalgesia, temporal summation, and even autonomic dysfunction in FM.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. J. Kim had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. J. Kim, Loggia, Harris, Wasan, Edwards, Napadow.

Acquisition of data. J. Kim, Loggia, Cahalan, Edwards, Napadow.

Analysis and interpretation of data. J. Kim, Loggia, Beissner, Garcia, H. Kim, Barbieri, Edwards, Napadow.

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