

## ORIGINAL ARTICLE

# Altered fMRI resting-state connectivity in individuals with fibromyalgia on acute pain stimulation

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## Conflicts of interest

None declared.

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## Abstract

**Background:** Fibromyalgia is a chronic widespread pain condition, with patients commonly reporting other symptoms such as sleep difficulties, memory complaints and fatigue. The use of magnetic resonance imaging (MRI) in fibromyalgia has allowed for the detection of neural abnormalities, with alterations in brain activation elicited by experimental pain and alterations in resting state connectivity related to clinical pain.

**Methods:** In this study, we sought to monitor state changes in resting brain connectivity following experimental pressure pain in fibromyalgia patients and healthy controls. Twelve fibromyalgia patients and 15 healthy controls were studied by applying discrete pressure stimuli to the thumbnail bed during MRI. Resting-state functional MRI scanning was performed before and immediately following experimental pressure pain. We investigated changes in functional connectivity to the thalamus and the insular cortex.

**Results:** Acute pressure pain increased insula connectivity to the anterior cingulate and the hippocampus. Additionally, we observed increased thalamic connectivity to the precuneus/posterior cingulate cortex, a known part of the default mode network, in patients but not in controls. This connectivity was correlated with changes in clinical pain.

**Conclusions:** These data reporting changes in resting-state brain activity following a noxious stimulus suggest that the acute painful stimuli may contribute to the alteration of the neural signature of chronic pain.

**What does this study/add?:** In this study acute pain application shows an echo in functional connectivity and clinical pain changes in chronic pain.

## 1. Introduction

Fibromyalgia (FM) is a chronic widespread pain condition that severely affects physical functioning (Liedberg et al., 2006; Clauw, 2014), and is associated with sleep problems, memory complaints (Kim et al., 2012) and fatigue (Wolfe, 1990). The underlying

pathophysiology of FM is not completely understood, however, there is accumulating evidence that centralization of pain (Hawkins, 2013) causing abnormal pain processing (Petersel et al., 2011) plays a critical role. FM patients are found to have altered central processing of nociceptive input, believed to

contribute to the presence of chronic pain (Saab, 2013; Smallwood et al., 2013).

The use of magnetic resonance imaging (MRI) in FM has allowed for detection of disease-specific brain abnormalities such as structural (Schmidt-Wilcke et al., 2007) and functional (Cifre et al., 2012; Jensen et al., 2012) differences between FM patients and healthy controls (HCs). For example, increased brain activation within the pain system in response to noxious stimuli is well documented (Gracey et al., 2002; Cook et al., 2004). However the investigation of chronic pain and neural correlates thereof is more challenging than the exploration of experimental pain since chronic pain is difficult to modulate within the scanner environment.

Functional connectivity MRI (fcMRI) is a non-invasive technique applied in a wakeful state either at rest (i.e. resting state) or during a task. Amid resting state analyses, low-frequency (<0.1 Hz) temporal correlations in the MRI signal are assessed across various brain regions. These low-frequency fluctuations are thought to be functionally relevant indices of connectivity between brain regions subserving similar or related functions (Birn, 2007). fcMRI has been used to investigate various chronic pain populations, including chronic low back pain (Loggia et al., 2013), migraine (Schwedt et al., 2013) and temporomandibular disorder (Ichesco et al., 2012). We previously reported fcMRI differences in FM patients (relative to HCs) (Napadow et al., 2010), both within the pain network (Ichesco et al., 2014) and between the default mode network (DMN), a brain network thought to be active during a state of introspection (Buckner et al., 2008) and the pain network. In these studies, FM patients displayed increased fcMRI between the insular cortex and DMN.

While experimental and clinical pain are thought to be related (Giesecke et al., 2005), the correlation between clinical pain intensity and experimental pain thresholds is generally weak, albeit stronger with randomized stimulus presentation (Harris et al., 2006). In this study, we were primarily interested in assessing the state changes of fcMRI before and after the application of experimental pressure pain in FM patients as compared to HCs.

We aimed to perturb the central pain system with individually calibrated pressure pain stimuli and monitor changes in fcMRI induced by this acute pain intervention. We hypothesized that FM patients, relative to controls, would display increased fcMRI in regions involved in pain processing following experimental pressure pain.

## 2. Methods

### 2.1 Subjects

Twelve female fibromyalgia (FM) subjects (mean age  $\pm$  SD;  $38.5 \pm 12.1$  years) and 15 age-matched healthy women (HC) (mean age  $\pm$  SD;  $39.9 \pm 13.0$  years) were recruited for the study.

Criteria for FM inclusion into the study at the time of participation were: having met the 1990 American College of Rheumatology criteria for FM (Wolfe et al., 1990); having disease duration  $\geq 1$  year, and reporting continued pain presence for more than 50% of each day; limiting new medications or treatment introductions for FM symptom control throughout the study; between 18 and 75 years old; right-handed; capable of giving informed written consent. Exclusion criteria for FM patients were: either current or history of opioid or narcotic analgesics use; history of substance abuse; concurrent autoimmune or inflammatory disease that may contribute to pain (e.g. rheumatoid arthritis, systemic lupus erythematosus or inflammatory bowel disease); concurrent participation in other therapeutic trials; being pregnant or currently a nursing mother; having a psychiatric illness (e.g. schizophrenia, major depression with suicidal ideation or substance abuse within the past 2 years).

Inclusion criteria for HCs at the time of study participation were: between 18 and 75 years old; right-handed; capable of giving written informed consent; willing to complete all study procedures. Criteria for excluding healthy control subjects were: having any chronic medical illness, including a psychiatric disorder; diagnosed with a chronic pain disorder; or current pregnancy or nursing. In addition, current, habitual, or previous use within the last 12 months of artificial nails or nail enhancements covering the thumbnail was an exclusion criterion for both groups to control for tactile quantitative sensory testing on the thumbnail. All subjects were also asked to refrain from taking over-the-counter analgesics at least 8 h prior to the study visit.

The study protocol and consent documents were approved by the medical institutional review board of the University of Michigan. All subjects read and gave informed consent prior to study participation.

### 2.2 Behavioural Data

Clinical symptom data and pain ratings were collected and subsequently utilized for correlation

analyses with connectivity measures obtained from FM individuals. These included (1) a pain Visual Analogue Scale (VAS), collected outside of the scanner, measuring clinical pain on a 0–100-mm scale (this scale served as an assessment of clinical pain immediately before and after scanning) with 0 anchored by the words ‘no pain’ and 100 anchored by the words ‘worst possible pain’, (2) The Positive and Negative Affect Scale (PANAS) (Watson et al., 1988), measuring mood-related affective measures and (3) the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983), recording levels of anxiety and depression, which were all collected within 6 h before a subject’s MRI scan (See Table 1 for values; Fig. 1 for timeline of collection for behavioural measures).

Experimental pressure pain testing was also conducted within 6 h prior to the MRI session using the Multi-modal Automated Sensory Testing (MAST) system (Harte et al., 2013). This device delivered a series of computer-controlled pressure stimuli to the right thumbnail. Experimental pain evoked by thumbnail pressure has been shown to be associated with overall body tenderness (Petzke et al., 2001), clinical pain (Harris et al., 2006; Geisser et al., 2007) and functional neuroimaging (Gracely et al., 2002; Hampson et al., 2013; Harris et al., 2013). Furthermore, the thumb is ideal for quantitative sensory testing because it represents a ‘neutral site’ that is not associated with any chronic pain condition (e.g.

it is not a FM tender point), and also implicitly implies that tenderness observed in FM is neither due to muscle sensitivity nor confined to muscles but, rather, is a property of deep tissue, with the tenderness of FM being generally expressed over the entire body. Each pressure application was 5 s in duration and delivered in 20-s intervals, beginning with 0.50 kg/cm<sup>2</sup> and increasing in ascending order in 0.5 kg/cm<sup>2</sup> increments thereafter to either a maximum of 10 kg/cm<sup>2</sup> or to tolerance. Subjects rated each pressure on a 0–100 Numerical Rating Scale (NRS) displayed on a touch-screen interface. Zero was defined as ‘no pain’ and 100 was defined as ‘worst pain imaginable’. These pain ratings were used to interpolate each individual’s Pain40, defined as the amount of pressure that evokes 40% of the individual’s maximal tolerated pain response recorded from the aforementioned ascending series. The Pain40 pressure level was utilized in the scanner to produce a moderate level of pain that could be tolerated for the duration of testing.

### 2.3 fMRI data acquisition

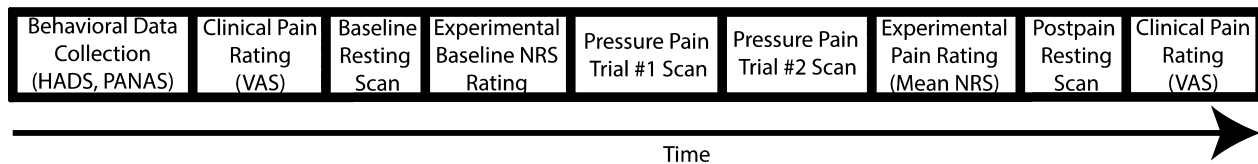
Resting state fMRI data were acquired using two different scanners: a General Electric (GE) Discovery 3.0 T scanner (10 FM, 11 HC) and a GE Signa 3.0 T scanner (2 FM, 4 HC) 9.0, VH3 with quadrature birdcage transmit-receive radio frequency coil. Both scanners used a custom T2\*-weighted spiral-in sequence (TR = 2.0s, TE = 30 ms, FA = 90°, matrix size 64 × 64 with 43 slices, FOV = 20 cm and 3.12 × 3.12 × 3 mm voxels). Importantly each subject underwent the baseline and post-pain scan on the same scanner; fMRI scanning occurred during the same day as clinical assessment.

The fMRI study design consisted of one baseline resting state scan, two evoked pressure pain scans, and one post evoked-pain resting-state scan (see Fig. 1). Each of the two resting scans were 8 min in duration, where a fixation cross was presented on the screen. Subjects were instructed to remain awake with their eyes open while fixating on the cross, and to ‘clear their minds of any particular thoughts’. Minimal cognitive tasks such as staring at a cross typically do not disrupt resting state networks (Greicius et al., 2003). Immediately following baseline resting-state scanning, subjects were asked to rate the degree of pain using a 0–100 NRS in order to obtain a baseline measure of pain intensity. Subjects were then asked to rate the degree of experimental pain and unpleasantness after each pressure pain administration, using the NRS scale, to ensure

**Table 1** Demographics, mood and pain report.

	FM (Mean ± SD)	HC (Mean ± SD)	<i>p</i> -value
Age	38.5 ± 12.1	39.9 ± 13.0	0.772
Mood			
HADS anxiety	10.0 ± 4.1	2.4 ± 4.4	<b>&lt;0.001</b>
HADS depression	6.9 ± 3.9	2.0 ± 5.4	<b>0.014</b>
PANAS positive affect	28.6 ± 8.9	34.5 ± 10.3	0.130
PANAS negative affect	23.2 ± 7.9	14.3 ± 8.3	<b>0.009</b>
Clinical pain			
VAS pre scan	52.6 ± 20.5	1.1 ± 2.9	<b>&lt;0.001</b>
VAS post scan	71.8 ± 23.1	2.6 ± 4.1	<b>&lt;0.001</b>
Experimental pain			
Rest 1 NRS (pre-experimental pain)	9.3 ± 13.0	17.3 ± 23.7	0.306
Pressure pain mean NRS (post-experimental pain)	52.7 ± 18.3	34.0 ± 19.8	<b>0.019</b>

Demographics table with subject-specific age, mood and experimental/clinical pain ratings. Values in bold type indicate significant differences between groups. FM = fibromyalgia; HADS = Hospital Anxiety and Depression Scale; HC = healthy control; NRS = Numerical Rating Scale; PANAS = Positive and Negative Affect Scale; VAS = Visual Analogue Scale.



**Figure 1** Scanning protocol. Diagram representing scanning paradigm; resting scans were performed before and after the two pressure pain scans; Mood variables (HADS, PANAS) were collected within 6 h of a subject's first MRI scan; Clinical pain ratings were collected immediately prior to and after scanning session; a mean NRS rating of experimental pain was calculated following both pressure pain scans.

that subjects received adequate pain (See Fig. 1 for timeline; Table 1 for values).

Evoked-pain stimulation was delivered in the form of pressure pain. The two evoked-pain scans were 10 min in length each and administered pressure to the left thumbnail. Three pressure conditions were presented during the evoked-pain scans: (1) a 'rest' condition consisting of 30 non-painful faint touch pressures ( $0.1 \text{ kg/cm}^2$ ), (2) an 'equal pressure' condition consisting of 15 stimuli maintained at  $1.5 \text{ kg/cm}^2$  for all subjects and (3) an 'equal pain' condition consisting of 15 stimuli at the Pain40 pressure intensity calculated for each individual during behavioural testing. Both the equal pressure ( $1.5 \text{ kg/cm}^2$ ) and equal pain (Pain40) conditions were 5 s in duration. These pain conditions were pseudo-randomized throughout the scan and interleaved with varying durations (7.5, 10 or 12.5 s) of the non-painful rest condition (see supporting information Fig. S1 for detailed pain pressure paradigm). After each pain scan, participants were asked to rate the overall pain intensity (NRS 0–100) evoked by the pressure stimuli to the thumbnail. The mean NRS value for the two pressure pain scans was compared to the pre-experimental pain baseline NRS (acquired after first resting state scan) to determine the magnitude of acute pain evoked by the experimental pain paradigm. Functional blood-oxygen level-dependent (BOLD) data from the pressure pain scans will be interpreted in a subsequent analysis.

During all functional scans, physiological data were collected simultaneously as cardiac and respiratory fluctuations are known to influence fMRI intrinsic connectivity (Birn, 2012). Respiratory volume data were collected by securing a GE magnetic resonance-compatible chest plethysmograph around each subject's abdomen. Cardiac data were collected using an infrared pulse oximeter (GE) attached to the subject's right middle finger. Participants motion was minimized using foam pads placed around the head along with a forehead strap.

In addition, high-resolution structural images were acquired (TR = 10.5 ms, TE = 3.4 ms, TI = 200 ms,

FA =  $25^\circ$ , 24 cm FOV,  $256 \times 256$  matrix,  $0.94 \times 0.94 \times 1.5 \text{ mm}$  voxels, yielding 106 slices) using a spoiled gradient echo (SPGR) inversion recovery sequence. Inspection of individual T1 MR-images revealed no gross morphological abnormalities for any participant.

#### 2.4 fMRI data pre-processing and statistical analysis

Raw data acquired from fMRI were corrected for physiological motion effects using RETROICOR method (Glover et al., 2000). Resulting data were pre-processed using the standard protocol in the SPM8 software (Functional Imaging Laboratories, London, UK) through Matlab 7.5b (Mathworks, Sherborn, MA, USA), including motion correction via realignment to the first volume in the series, normalization to the Montreal Neurological Institute (MNI) template and smoothing with a 8-mm FWHM Gaussian Kernel. Motion during the scan was assessed using three translations and rotations for each scan. Thresholds for translation were set to  $\pm 2 \text{ mm}$ , and rotation at  $\pm 1^\circ$ . Subjects exceeding these thresholds were removed from further analyses.

Pre-processed data then underwent connectivity analyses via the Functional Connectivity Toolbox v2.0 (CONN) ([www.nitrc.org/projects/conn](http://www.nitrc.org/projects/conn)) in SPM8. The CONN toolbox utilizes a component-based noise correction method (CompCor), which increases sensitivity and selectivity and also allows for a high degree of interscan reliability (Whitfield-Gabrieli and Nieto-Castanon, 2012). A band pass filter (0.01–0.1 Hz) was used to remove high-frequency noise and linear drift artefacts. Regressors of no interest included motion, white matter and CSF in the first-level analyses, and age and scanner in second-level analyses. Using CONN, we assessed seed-to-voxel whole brain correlations (first-level analysis), including in our analysis-specific regions of interest (ROIs) created as 5-mm spheres. We were specifically interested in two

regions known to play a critical role in both acute pain and chronic pain perception: the thalamus and the insular cortex. Thalamic seeds were placed in the lateral/posterior part of the thalamus, a region that has been identified by pain activation (Andersson et al., 1997; Ab Aziz and Ahmad, 2006), as well as connectivity studies (Beckmann et al., 2005). This region also has been identified in lesion studies to be a central hub in pain transmission and specifically the thalamic seed also comprised the region in the posterior part recently shown to play a critical role in the development of post-stroke pain (of thalamic origin) (Sprenger et al., 2012). Insular seeds were placed in anterior and posterior regions, based on this region's role in pain processing, as well as its demonstrated abnormal connectivity patterns in FM (Napadow et al., 2010, 2012). Seed to voxel connectivity measures (throughout the whole brain) are based on correlation analyses, where Fisher  $z$  transformed  $r$  values represent the degree of connectivity between the ROI and target region.

First-level, subject-specific, connectivity maps for each seed obtained from the CONN toolbox were then used in a second-level analysis where we performed a repeated measures ANOVA (two-factorial design) with the factors being group (FM vs. HC) and time point (pre- vs. post-experimental pressure pain). We then performed three resting-state analyses: *analysis 1*) main effect of group at baseline and post pain, *analysis 2*) main effect of time within groups (FM and HC) and *analysis 3*) a group by time interaction. Age and scanner site were entered as covariates of no interest in the analysis. Results were deemed significant with a family wise error (FWE) cluster-level corrected  $p$  value  $<0.05$  derived from a voxel-wise uncorrected threshold of  $p < 0.001$ . Regions with significant connectivity differences between groups had Fisher transformed  $r$  values extracted from first-level seed-to-whole brain map images for each subject which were correlated with clinical symptoms using SPSS v.21.

### 3. Results

#### 3.1 Behaviour

At baseline, FM subjects reported higher levels of HADS depression ( $p = 0.014$ ) and anxiety ( $p < 0.001$ ) and PANAS negative affect ( $p = 0.009$ ) relative to HCs (Table 1).

For clinical pain (VAS), FM patients reported significantly higher pain ratings post-scanning relative to baseline [mean baseline  $\pm$  SD =  $51.1 \pm 20.8$ ; mean post-pain  $\pm$  SD =  $71.8 \pm 23.1$  ( $p < 0.001$ )]. When performing a group\*time interaction (VAS pre scan vs. VAS post scan) within a multiple regression model in SPSS, FM patients' clinical pain significantly increased compared to HCs (FM mean VAS difference  $\pm$  SD =  $20.7 \pm 15.3$ ; HC mean VAS difference  $\pm$  SD =  $2.9 \pm 4.3$ ;  $p < 0.001$ ).

While the FM group and HC group did not differ significantly with respect to baseline pain reported prior to the experimental pain paradigm (Rest 1 NRS;  $p = 0.306$ , Table 1), the FM group reported significantly greater pressure pain sensitivity after experimental pain administration (FM: Rest 1 NRS  $\pm$  SD =  $9.3 \pm 13.0$ , mean pressure pain NRS  $\pm$  SD =  $52.7 \pm 18.3$ ,  $p < 0.001$ ; HC: Rest 1 NRS  $\pm$  SD =  $17.3 \pm 23.7$ , mean pressure pain NRS  $\pm$  SD =  $34.0 \pm 19.8$ ,  $p = 0.086$ ). In the group\*time interaction analysis FM patients also showed a significantly greater increase in experimental pain as compared to HC (FM mean NRS difference  $\pm$  SD =  $43.3 \pm 23.4$ ; HC mean NRS difference  $\pm$  SD =  $16.6 \pm 34.9$ ;  $p = 0.032$ ).

#### 3.2 Resting state functional connectivity

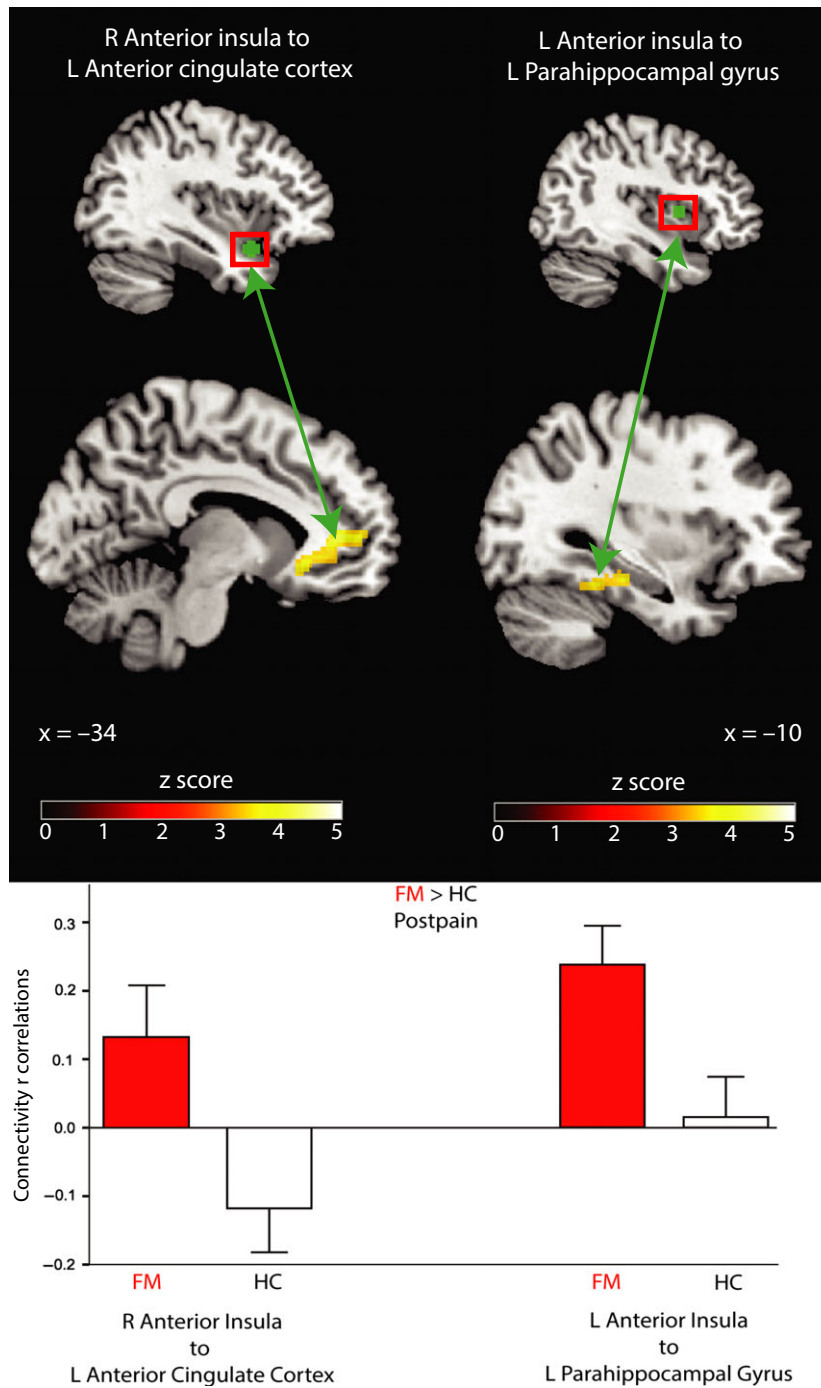
In *analysis 1*, we observed no main effect of group on connectivity at baseline. Following the experimental pain scans, we observed a main effect of group (FM vs. HC) on connectivity between the right anterior insular cortex and the left anterior cingulate cortex (ACC) ( $p_{\text{fwe}} = 0.015$ ) and the left anterior insular cortex and the left parahippocampal gyrus (PHG) ( $p_{\text{fwe}} = 0.036$ ; for more details see Fig. 2, Table 2).

For *analysis 2*, we did not observe any significant main effect of time on fMRI metrics in either group.

In *analysis 3*, we found a significant group\*time interaction (FM vs. HC, post-experimental pain vs. baseline) revealing increased connectivity between the left thalamus and the right superior frontal gyrus ( $p_{\text{fwe}} = 0.019$ ) and the right thalamus and the posterior cingulate/precuneus ( $p_{\text{fwe}} = 0.050$ ; Fig. 3, Table 2). No significant results were found in the same group\*time interaction when HCs were compared to FM patients.

#### 3.3 Correlations

The difference in resting-state connectivity (post-experimental pain minus baseline) between the right



**Figure 2** Increased insular cortex connectivity in FM relative to HC post pain. This figure shows FM patients have increased resting state connectivity following experimental pressure pain stimuli between the right anterior insular cortex and the left anterior cingulate cortex as well as increased connectivity between the left anterior insular cortex and the left parahippocampal gyrus. Red rectangles indicate seed location – seed coloured in green; bar graphs display mean degree of group connectivity with 95% confidence interval error bars. FM = Fibromyalgia; HC = Healthy Controls; L = Left; R = Right.

thalamus and the posterior cingulate/precuneus in FM was positively correlated with the difference in clinical pain (VAS) scores (post-scan minus pre-scan)

( $r = 0.610$ ,  $p = 0.046$ , Fig. 4), such that greater increases in connectivity between the thalamus and the precuneus were associated with greater increases

**Table 2** fcMRI – differences between FM and HC

Seed region	Connectivity region	Brodmann area	Cluster size (# of voxels)	z-score (peak value)	Coordinates (MNI)		
					x	y	z
FM > HC, POST PAIN (ANCOVA with Age and Scanner as covariates of no interest)							
Right Anterior IC	Left rostral ACC	BA 10, 32	343	4.71	-14	44	6
Left Anterior IC	Left parahippocampal gyrus	BA 36, 37	272	4.01	-28	-16	-30
FM > HC, POST PAIN > BASELINE (ANCOVA with Age and Scanner as covariates of no interest) Interaction							
Left Thalamus	Right superior frontal gyrus	BA 10	228	5.00	22	64	6
Right Thalamus	Left Posterior Cingulate/Precuneus	BA 31	187	4.81	-10	-46	44

BA = Brodmann area; fcMRI = functional connectivity magnetic resonance imaging; FM = fibromyalgia; HC = healthy controls; IC = insular cortex; MNI = Montreal Neurological Institute; rACC = rostral anterior cingulate cortex; SFG = superior frontal gyrus.

in clinical pain. Additionally, the change in connectivity (post-experimental pain vs. baseline) between the left thalamus and the superior frontal gyrus in FM was positively correlated with both anxiety and depression (HADS Anxiety,  $r = 0.561$ ,  $p = 0.05$ ; HADS Depression,  $r = 0.618$ ,  $p = 0.032$ ).

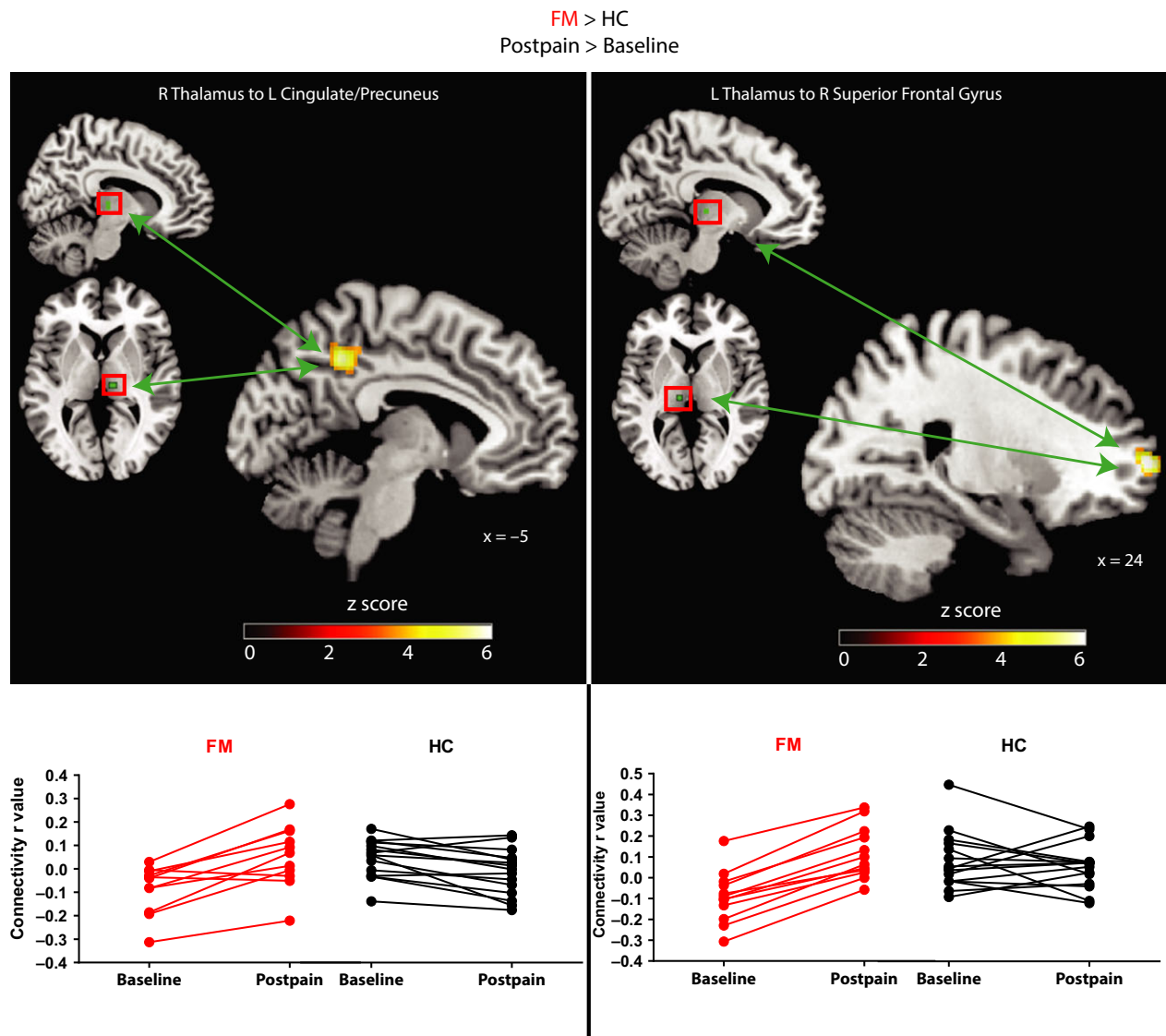
#### 4. Discussion

While many studies have documented both abnormal brain activation in response to experimental pain and abnormal resting-state connectivity related to clinical pain in FM (Napadow et al., 2010, 2012; Jensen et al., 2012), we have expanded upon these approaches by examining resting state networks before and after acute experimental noxious stimulation. Here, we sought to explore state changes in ongoing clinical pain in parallel with changes in functional connectivity strength in brain areas known to be critically involved in evoked and clinical pain processing.

Our findings revealed significantly more resting state brain connectivity in FM patients (compared to HCs), after the administration of painful pressure stimulation, between the right anterior insular cortex and the left ACC, as well as between the left anterior insular cortex and the left parahippocampus. We also found that when investigating differences in connectivity before and after noxious stimulation, FM patients showed more pronounced increases in thalamic connectivity to DMN structures such as the precuneus and posterior cingulate cortex compared to HCs. Furthermore, we find that the degree of thalamic-DMN connectivity in FM was correlated with the change in reported clinical pain (VAS).

Following administration of experimental pressure pain, we observed more insular cortex connectivity to the ACC and parahippocampal gyrus (PHG) in FM

patients as compared to HCs. The insular cortex is known to play a key role in the process of pain perception and emotion regulation (Baliki et al., 2009). The anterior insular cortex in particular is thought to be involved in both self-reflection (Fomberstein et al., 2013) and emotional processing of painful stimuli (Phan et al., 2002). From a systems perspective the anterior insular cortex, together with the ACC, are core structures of the medial pain system which is involved in processing the affective-motivational dimension of pain (Mouraux et al., 2011; Peltz et al., 2011). The insular cortex and ACC display increased activity in response to painful stimuli in FM (Pujol et al., 2009) and in other chronic pain populations (Cook et al., 2004; Giesecke et al., 2004). Structurally, the insular cortex and the cingulate cortex has been studied extensively in primates, which has shown a connection between the anterior insular cortex and the rostral extent of the ACC (Brodmann Area 24) and a dorsal transition area between areas 24 and 6. Furthermore the mid and posterior insular cortex regions have connections with the dorsal cingulate cortex (Brodmann Areas 23 and 24) and the upper banks of the cingulate sulcus and pre-motor cortex (Mesulam and Mufson, 1982). With respect to functional connectivity, Taylor and colleagues found resting-state connectivity topography patterns between the anterior insular cortex and the ACC in healthy control subjects (Taylor et al., 2009), Cifre et al. reported increased resting-state connectivity between the insular cortex and posterior dorsal ACC in FM (Cifre et al., 2012). However, the ACC is functionally heterogeneous, with different regions implicated in pain encoding (Buchel et al., 2002), pain expectation and antinociception (Petrovic et al., 2002; Bingel et al., 2006). In our study, it was the insular cortex – rostral ACC



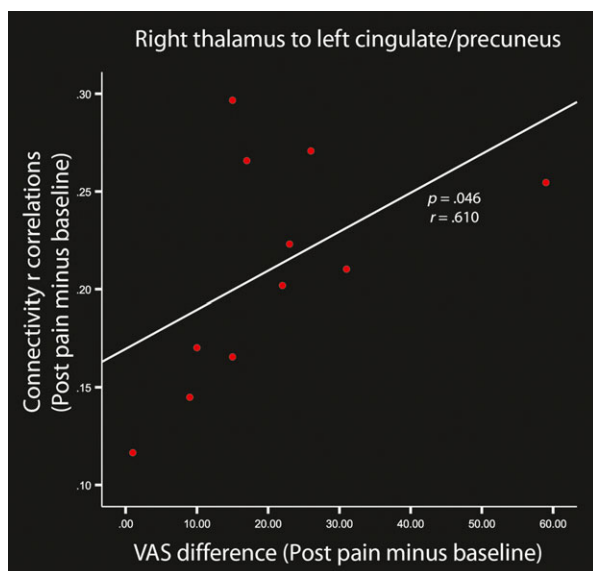
**Figure 3** Increased thalamic connectivity in FM relative to HC post pain versus baseline. This figure displays increased resting-state connectivity when comparing post pain resting-state connectivity to baseline resting-state connectivity in FM patients as compared to HCs between the right thalamus seed (displayed in the sagittal and axial planes) and the left cingulate/precuneus (left display) and the left thalamus seed (displayed in the sagittal and axial planes) and the right superior frontal gyrus (right display). Red rectangles indicate seed location – seed coloured in green; FM = Fibromyalgia; HC = Healthy Controls; L = Left; R = Right.

connectivity that was significantly stronger in FM as compared to HCs after noxious stimulation. Interestingly, the rostral region of ACC is implicated in antinociception and pain distraction (Petrovic et al., 2002; Harte et al., 2011). In a previous study, we also found increased insular cortex – rostral ACC connectivity in temporomandibular disorder (Ichesco et al., 2012). Consistent with the conclusions of that study, we posit that disproportionate insular cortex – rostral ACC connectivity may indicate an increased attempt in patients (more so than in controls) to

engage antinociceptive circuitry – in the present case, following acute noxious stimulation.

The PHG has been shown to be involved in the processing of pain or pain-related stimuli. Patients with somatoform disorder have been shown to display an increased PHG response to heat stimuli relative to controls, which was attributed to abnormal affective pain processing and perception (Gundel et al., 2008). These results are consistent with the findings of a review that included the PHG as part of a network involved in the processing of psychologi-





**Figure 4** Changes in connectivity for FM patients correlates to changes in clinical pain. This figure shows bivariate non-parametric correlations of resting-state connectivity changes (post-pain minus baseline) to changes in clinical pain before and after experimental pressure pain stimulation. FM patients display increased connectivity between the thalamus and left cingulate/precuneus positively correlates with increases in clinical pain. FM = Fibromyalgia; VAS = Visual Analogue Scale.

cal pain (Meerwijk et al., 2013). Against this background, we speculate that the increased connectivity between the insular cortex and the PHG elicited by pressure pain is in some way related to an abnormal affective-related pain response in FM.

When assessing the effects of experimental pressure pain on resting-state connectivity over time, we find that FM displayed greater connectivity than controls between the left thalamus and the right superior frontal gyrus and also the right thalamus and posterior cingulate/precuneus, target regions that are part of the DMN (Raichle et al., 2001; Fox et al., 2005). The role of the thalamus in pain processing is well documented (Ab Aziz and Ahmad, 2006), and abnormal thalamic involvement in chronic pain, including FM, has frequently been observed (Gracely et al., 2002; Cook et al., 2004; Jensen et al., 2012). Specifically for FM, increased BOLD activity in the thalamus was observed following experimental pain (Burgmer et al., 2009). Here, the change in thalamic connectivity following experimental pain was positively correlated with an increase in clinical pain (VAS), suggesting that thalamic connectivity to the DMN can be modulated by the contribution of experimental pain, which may exert an effect on the way a patient experiences clinical pain.

We were specifically interested in the lateral/posterior aspect of the thalamus, and as this region is involved in the sensory discriminative aspect of pain processing (Andersson et al., 1997; Ab Aziz and Ahmad, 2006), it is plausible that the increased connectivity observed in FM patients following an experimental pain paradigm is caused by increases in nociceptive input. Therefore, we hypothesize that the application of experimental pain to a localized region (i.e. the thumb) is capable of changing (increasing) thalamo-DMN connectivity. We further speculate that this experimental pain stimulus also increases the degree of widespread clinical pain, such that after the experimental pain paradigm clinical pain increased. This thalamo-DMN connectivity may be mediated by the insular cortex. Animal tracer studies have shown structural connections between the thalamus and insular cortex (Craig and Zhang, 2006). Further, it has also been reported that there is strong connectivity between the thalamus and the insular cortex (Wiech et al., 2014). With regard to the DMN, altered connectivity has been commonly noted in FM (Napadow et al., 2010, 2012; Harris et al., 2013). We have previously shown that pregabalin-induced decreases in DMN connectivity to the insula were associated with reductions in clinical pain (Harris et al., 2013). While these studies did not investigate all three pain-related brain regions, it is plausible that a thalamo-insular-DMN connection could be driving our finding. Interestingly, here we reveal the opposite effect, whereby acute noxious stimulation is associated with increased DMN connectivity, in our case to the thalamus, and the degree of this connectivity is associated with increased clinical pain.

#### 4.1 Limitations

Our study has some limitations. Resting on a table for prolonged periods of time during fMRI scanning may cause discomfort in patients and could increase clinical pain and responses to experimental pain. Although each individual subject completed her pre- and post-scans on the same scanner, not all subjects were imaged using the same scanner. Due to equipment upgrades, two different MRI scanners were used during the course of this study. Although the magnetic field strength and the technical acquisition parameters were exactly the same on both scanners, we cannot completely rule out that the scanners might have had an effect on our results. To remedy this, we incorporated the source of the images (scanner) as a nuisance regressor in our second-level analyses. Another limitation is we did not randomize

the order of scans for subjects in this study, so we are unable to look at changes of resting-state connectivity in the absence of a painful stimulus. Finally, connectivity as assessed by the approach chosen in this study (i.e. correlation analyses), allows no assumptions on causality, or on the directness of influence. Against this background it is conceivable that the functional connectivity between two 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 (Damoiseaux and Greicius, 2009) will help to overcome such methodological shortcomings in future studies.

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### Author contributions

All authors of this manuscript have discussed the results and commented on the manuscript.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Figure S1.** Pressure Pain Paradigm Representation during Evoked-Pain fMRI. Diagram representing pressure pain paradigm; light touch stimuli consisted of random-duration (7.5, 10, or 12.5 s) 0.1 kg (light touch) pressure blocks alternating between equal pain and equal pressure stimuli; equal pressure stimuli were of fixed duration (5 s) with constant 1.5 kg of pressure in a pseudo-randomized fashion; Pain-40 pressure consisted of pressure required to elicit a subject-specific 40 pain rating response on NRS scale and duration was set to 5 s.

**Table S1.** Sources and Coordinates for Regions of Interest (ROIs).