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RESEARCH ARTICLE

Using Structural Equation Modeling to Investigate the Neural Basis of Altered Pain Processing in Fibromyalgia with Functional Magnetic Resonance Imaging

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ABSTRACT
Participants with fibromyalgia (FM) and healthy controls (HC) experienced an identical 'threat/safety' experimental pain paradigm while undergoing functional magnetic resonance imaging (fMRI) to investigate the differences in pain processing between the two groups. In the 'threat' (Pain) imaging runs, participants were told that they would receive noxious heat stimuli to their right hands, calibrated to elicit subjectively moderate levels of pain. In the 'safety' (No-Pain) imaging runs, no stimulus was given. This design enabled the study of both continuous and reactive components of pain processing, as well as brain activity associated with anticipation and reward. The fMRI data were analyzed with a data-driven structural equation modeling approach, and significant group-level connectivity differences were identified in both study conditions, in both time periods of interest (Expectation, Stimulation). Group-level connectivity differences in the No-Pain condition occurred mainly during the expectation of pain, and involved regions associated with emotion and reward, suggesting FM may involve altered affective/reward processing. Group-level connectivity differences in the Pain condition occurred mainly during stimulation, with the FM group having decreased connectivity between the anterior cingulate cortex (ACC) and the amygdala, and increased connectivity between the posterior cingulate cortex (PCC) and the thalamus. The decreased ACC→Amygdala connectivity supports previous findings, suggesting FM likely involves altered responses in motivational-affective aspects of pain processing. The increased PCC→Thalamus connectivity may suggest the FM group experienced heightened saliency toward the noxious stimuli, which may contribute toward the mechanism which causes hyperalgesia in FM.

Keywords: human, pain, fibromyalgia, neuroimaging, MRI, functional MRI

Introduction

Fibromyalgia (FM) is common chronic wide-spread pain disorder that affects 2 to 4% of the population, most often people aged 30 to 50 years old, but its etiology is still not known.[1,2] Pain responses in FM patients have been studied previously with functional magnetic resonance imaging (fMRI).[3–17] However, as we recently demonstrated, the different findings in these studies may depend on the fMRI analysis methods used.[18] Specifically, studies using model-driven analyses of pain responses have found little to no significant differences in brain activity between healthy control (HC) and FM patients when sensitivity-adjusted levels of pain stimuli were given.[5–9,12,15,17] This method involves predicting the expected blood oxygenation-level dependent (BOLD) time-course response based on the stimulation paradigm, and identifying the voxels where this expected response occurs. In contrast, while utilizing similar experimental paradigms, studies using data-driven analyses, which do not rely on predicting the expected BOLD response pattern in time, have identified significant differences between the two study groups.[4,10,11,13] These results indicate that BOLD responses may not be as predicted based on the timing of a stimulation paradigm, and may vary across anatomical regions. The results also suggest that FM and HC groups activate pain-related brain regions differently. Importantly, in all of these studies, regardless of the type of noxious stimulus used, the FM patients required lower stimulus intensities than the HC groups to report equivalent levels of pain,[3–17] emphasizing the phenomenon of hyperalgesia, a hallmark of FM.[19]

Interestingly, most group differences identified by previous studies using data-driven analyses involved brain regions that are more likely to be associated with motivational-affective aspects of pain processing.[4,10,11,13] In 2009, Pujol et al. found that FM and HC participants had similar brain connectivity in regions involved with sensory-discriminative aspects of pain processing, while the FM group had significantly higher connectivity in regions associated with the motivational-affective aspects of pain, such as in the insular cortex (IC) and anterior cingulate cortex (ACC).[11] In two separate studies, Jensen et al. (2012, 2014) reported decreased functional connectivity between the rostral ACC and other brain regions, such as the hippocampus (Hipp) and amygdala (Amg), in FM participants compared to controls.[4,10] Interestingly, in 2015, Kim et al. identified increased connectivity between the primary somatosensory cortex and IC (i.e., regions thought to be more involved in the sensory-discriminative aspects of pain processing) of the FM group compared to controls.[13] Beyond these findings, FM has also been consistently reported to be highly comorbid with anxiety and depression, as well as autonomic dysfunction.[20–26] The pattern of these findings suggests the underlying mechanism of FM likely involves dysfunction in emotional and autonomic pathways, which largely contribute to the motivational-affective aspects of pain processing.

To further investigate this underlying mechanism in the present study, HC and FM participants underwent an experimental 'threat/safety' pain paradigm while undergoing fMRI. Introducing instances of perceived threat (imaging runs with painful stimulation) and perceived safety (imaging runs without painful stimulation) in a randomized order enabled us to investigate how the continuous aspects of pain processing differed between the two study groups during the anticipation and the experience of pain. In contrast to most previous studies that used static pain stimuli, we used dynamic heat stimuli which predominantly activate C-fibers and thus are more relevant for clinical pain.[27,28] The fMRI data were analyzed using a data-driven structural equation modeling (SEM) approach (i.e., there were no assumptions made regarding the BOLD responses associated with the anticipation and experience of pain). Using a partial dataset from this current study and full datasets from previous fMRI pain studies conducted in our lab, we have previously shown that this analytical approach is more sensitive than the standard, model-driven approach for identifying differences in pain processing between HC and FM study groups in the brain, brainstem and spinal cord.[18,29,30] We hypothesized there would be significant differences in brain connectivity between the HC and FM groups, and these differences would involve regions associated with the motivational-affective components of pain processing.

Methods

The study was reviewed and approved by our institutional research ethics board, and all participants provided written, informed consent prior to the onset of their first study session. The data that support the findings of this study are available from the corresponding author upon reasonable request.

PARTICIPANT RECRUITMENT

The participants included in this study were part of a larger study to investigate pain processing in the brain, brainstem, and cervical spinal cord of individuals with FM. The brain and brainstem/spinal cord imaging experiments were separated into two different sessions, and the sessions were typically

separated by one week to avoid sensitization to the noxious stimuli. Twenty-two women with previous diagnoses of FM and 18 healthy women were recruited from the local community using various online and physical advertisements. Participants were not asked to withhold from taking their medications, but were only recruited for the study after their medication dosages had been stabilized for 3 months (Table 1). Of the total number of participants recruited, complete brain fMRI datasets included 20 FM (age range = 24-64, M_{age} = 48.8 ± 12.7; mean ± std) and 17 HC (age range = 21-59, M_{age} = 37.9 ± 10.4; mean ± std) participants.

Table 1. List of medications being taken by study participants in each group.

	Healthy Controls n = 17	**Fibromyalgia** n = 20
Types of Medications	Group Totals	Group Totals
Anticonvulsants	0	5
Antidepressants		
NDRI	1	2
SARI	0	2
SNRI	1	10
SSRI	0	2
TCA	0	1
Antihistamines	2	5
Antipsychotics	0	1
Anxiolytics	0	2
Body Regulators		
Blood Pressure Lowering	0	4
Glucose Regulating	0	2
Hormonal		
Birth Control	1	2
Estrogen Replacement Therapy	0	1
Thyroid Replacement Therapy	2	4
Lipid Lowering	0	1
Proton Pump Inhibitors	0	4
Pain Relief		
Analgesic/Antipyretic	0	8
NSAID	1	4
Opioidergic		
Agonist	0	1
Antagonist	0	2
Other		
Antimalarial	0	1
Cannabis/CBD oil	1	5
Muscle Relaxants	0	1
Steroids	1	2
Stimulants	0	1
Sleep Aids	0	2
Supplements		
Dietary	1	7
Herbal	0	2
Vitamin	1	7

Note: NDRI = norepinephrine-dopamine reuptake inhibitor. **SARI** = serotonin antagonist and reuptake inhibitor. **SNRI** = serotonin-norepinephrine reuptake inhibitor. **SSRI** = selective serotonin reuptake inhibitor. **TCA** = tricyclic antidepressant. **NSAID** = nonsteroidal anti-inflammatory drug. **CBD** = cannabidiol.

QUESTIONNAIRES

To better understand the relationship between autonomic and motivational-affective function and pain processing, participants were asked to complete a series of questionnaires which were subsequently related to the group-level fMRI results. To quantify psychological health, participants completed the State-Trait Anxiety Inventory (STAI) and the Beck Depression Inventory-II (BDI-II). The STAI measures an individual's current (state) and general (trait) anxiety,[31] and the BDI-II measures an individual's current level of depression.[32] To quantify autonomic symptoms, participants completed the Composite Autonomic Symptom Score 31 (COMPASS-31). The COMPASS-31 measures autonomic symptom severity across six

domains (orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, pupillomotor), and higher scores indicate higher symptom severity.[33] Evidence both for and against the relationship between autonomic dysfunction and FM has been reported in the literature.[23,24,34–37] The COMPASS-31 questionnaire was included to investigate this possible relationship, and to determine if it could contribute to explaining abnormal pain processing in FM. Participants also completed the Social Desirability Scale (SDS) and the Pain Catastrophizing Scale (PCS). The SDS measures an individual's need for social approval,[38] and the PCS quantifies how an individual copes with pain.[39] The SDS was included to determine if group-level pain ratings may have been influenced by differences in social desirability. The PCS was included to determine whether or not group-level differences in catastrophizing exist, and if these differences could potentially explain the hyperalgesia associated with FM.

We also included the Revised Fibromyalgia Impact Questionnaire (FIQR), and the Short-Form McGill Pain Questionnaire-2 (SF-MPQ-2) to measure the severity and quality of pain in our participant. The FIQR is a psychometric test which assesses the function, overall impact, and symptom severity of FM.[40] Participants in the HC group were given the same questionnaire, except the word 'fibromyalgia' was altered to the word 'symptom'. For this questionnaire, FM participants reported how FM has affected their activities of daily living, as well as the intensity of their FM symptoms, over the past seven days. In contrast, HC participants reported how much difficulty they had with activities of daily living, as well as the intensity of common medical symptoms they may have had, over the past seven days. The SF-MPQ-2 assesses the quality of pain experienced by an individual based on a total score and four interpretable subscales (continuous pain, intermittent pain, predominantly neuropathic pain, and affective descriptors) over the past week.[41] Finally, because FM inclusion was based on a prior diagnosis by a physician, we included the 2016 Fibromyalgia Survey Questionnaire (FSQ) to determine which participants (FM or HC) currently met the most recent classification criteria for FM.[42]

PARTICIPANT TRAINING

Prior to their first fMRI session, each participant received one hour of training to become accustomed to the experimental protocol and the fMRI environment. The training sessions began with an algometry test (i.e., tender-point examination). The tender-point examination was a critical part of the 1990 American College of Rheumatology (ACR) criteria for the classification of FM, but it is difficult to perform in a consistent manner, and the practice was not standardized (i.e., physicians did not utilize the same measurement tools or techniques).[42–44] It was included in this study to determine if it could provide another means of differentiating between the HC and FM groups. The original tender-point diagram included nine pairs of pressure-points across the body.[43,44] For simplicity, we only evaluated the 12 points above the waist (bilateral occiput, bilateral lateral epicondyle, bilateral low cervical, bilateral supraspinatus, bilateral trapezius, and bilateral second rib). We also evaluated a control point in the middle of the forehead. The participants were instructed to inform the researcher when the sensation produced by the algometer changed from pressure to pain. For each pressure-point, the researcher applied the algometer (FPK 10 pain test algometer, Wagner Instruments, Greenwich, Connecticut) with an increasing pressure of 1 kg/s until the participant reported pain, or until the maximum pressure of 4 kg was reached. The pressure at which pain was reported by the participant was recorded.

Participants were then introduced to the standardized numerical pain intensity scale (NPS), which they subsequently used to describe their pain. The scale ranged from 0 to 100 in increments of 5, with verbal descriptors at increments of 10. Because the noxious stimulation was administered in the form of thermal heat, the verbal descriptors were described in terms of heat: 0 = no sensation, 10 = warm, 20 = a barely painful sensation, 30 = very weak pain, 40 = weak pain, 50 = moderate pain, 60 = slightly strong pain, 70 = strong pain, 80 = very strong pain, 90 = nearly intolerable pain, 100 = intolerable pain. While this scale is not typically used for clinical assessments, it is widely used for pain research because it provides the ability to assess non-painful sensations as well as pain.[45,46] To reduce anxiety related to the noxious heat stimuli, participants were ensured that they would not be experiencing temperatures that could cause bodily harm, and that the objective of the study was not to induce pain ratings above 70 on the NPS. Participants were also repeatedly informed that they were always in control of the study, and if they ever felt that the noxious stimulation was too intense, they could simply remove their hand from it.

After participants understood how to use the NPS, they were introduced to the MRI-compatible Robotic Contact-Heat Thermal Heat Stimulator (RTS-2) which was used to administer noxious heat stimuli in this study. The RTS-2 pneumatically raises and lowers a heated aluminum thermode to make contact with participants' skin, and the timing and temperature of the heat contacts were precisely

controlled using software written in MATLAB (MathWorks Inc., Natick, MA). For this study, the RTS-2 was set up to make contact with the thenar eminence of the participants' right hands both during the training session and the fMRI experiments. Several tests were performed to familiarize participants with the stimulation device and NPS. The temperature was kept constant during each test, and each test used a different temperature. First, participants were given three initialization tests which consisted of 3 consecutive brief heat contacts each. The temperatures for these sets were 45 °C, 47 °C, and 46 °C, respectively. After these initial tests, the participants were given four calibration tests which consisted of 10 consecutive brief heat contacts over 30 seconds (0.33Hz) to mimic what they would experience during the fMRI experiments. The temperatures for these four tests were 46 °C, 50 °C, 44 °C, and 48 °C, respectively. In some instances, the temperatures for these four tests had to be lowered for the FM participants because of excessive pain. For both the initialization and calibration tests, participants were instructed to report their NPS ratings out loud as the heat contacts occurred. The calibration tests were also used to determine the temperature to be used during the fMRI experiments to elicit subjectively moderate levels of pain (50 ± 10 NPS units) in each participant. Therefore, if after the four calibration tests an optimal temperature was not identified, extra tests were performed to identify the optimal temperature. Participants were blinded to this objective, and to the temperatures that were used, but stimulus temperatures never exceeded 52 °C to prevent tissue damage. The training sessions were completed with at least one practice run of the experimental fMRI protocol using our sham MRI scanner.

FMRI PARADIGM

To better understand the anticipatory aspects of pain processing, a 'threat/safety' stimulation paradigm was employed (Figure 1). In this paradigm, fMRI runs were separated into two study conditions: 'Pain' and 'No-Pain', which were delivered in a randomized order. In the Pain condition, participants were informed at the 1-minute mark of the run that they would be experiencing the thermal heat stimulus during that run. At the 2-minute mark of the run, the stimulation began. Similar to the training session, the stimulation consisted of 10 brief heat contacts over a span of 30 seconds (0.33 Hz) at the calibrated temperature which elicited a moderate level of pain in the participant. After the 30-second stimulation period was over, participants were instructed to remember their first and last pain ratings for the remaining 2 minutes. At the end of the Pain runs, participants informed the researchers of their pain ratings for the first and last heat contacts over the MRI intercom. In the No-Pain condition, participants were informed at the 1-minute mark that they would not be experiencing the thermal heat stimulus during that run. Instead, they were instructed to lie still for the remaining 3.5 minutes. In both study conditions, aside from when the participants were being provided with information, the rear-projection screen continuously displayed the NPS from the 1-minute mark to the end of the imaging run.

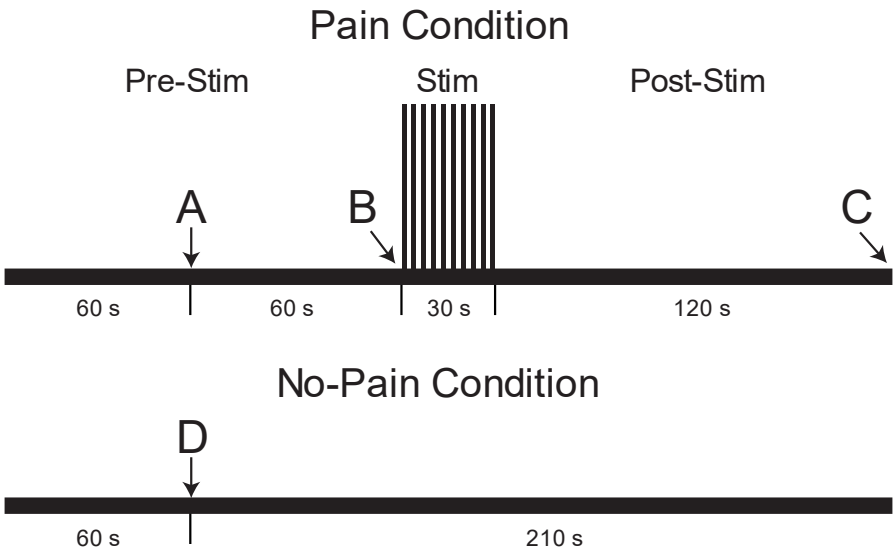

Figure 1. Experimental 'threat/safety' fMRI paradigm. Both the Pain and No-Pain imaging runs were 270 seconds long. In both runs, participants were informed 60 seconds into the run what to expect. A = "You will feel the heat stimulus during this run." B = "The stimulus will start in 3…2…1…" C = "Please give us your first and last heat contact pain ratings for this run." D = "You will not feel the heat stimulus during this run."

FMRI DATA ACQUISITION

Image data were acquired using a 3 tesla whole-body MRI system (Siemens Magnetom Trio and Siemens Prisma; Siemens, Erlangen, Germany). Participants were positioned supine and were supported by foam padding as needed to ensure comfort and minimize bulk body movement. Structural images were acquired at the beginning of the scanning session using a sagittal, T1-weighted MPRAGE sequence (TR = 1760 ms, TE = 2.2 ms, Inversion Time = 900 ms, Flip Angle = 9°, Resolution = 1 x 1 x 1 mm^3). To relate brain and brainstem/spinal cord fMRI data, the imaging window spanned from the top of the C1 vertebra to the top of the cortex (although the degree of brain inclusion was dependent on each participants' head size). Functional images were acquired in 66 contiguous axial slices using a GE-EPI sequence (TR = 2 s, TE = 30 ms, Flip Angle = 84°, simultaneous multi-slice (i.e., multiband) factor of 3, 7/8 partial Fourier sampling of k-space, FOV = 180 mm x 180 mm, matrix = 90 x 90, resolution = 2 x 2 x 2 mm^3). A 32-channel head coil was used for detection of the MR signal, with a body coil for transmission of RF pulses. A total of 135 volumes were acquired for each imaging run of 4.5 minutes, and each participant experienced 4 to 5 Pain and 4 to 5 No-Pain runs.

FMRI DATA PREPROCESSING AND ANALYSIS

Data were preprocessed using SPM-12 software (Wellcome Institute of Cognitive Neurology, London, UK) to remove physiological noise and correct for motion artefacts. Data were converted to NIfTI format, were motion and slice-timing corrected, co-registered to their anatomical images, and normalized to the MNI template (Montreal Neurological Institute, Montreal, Quebec). Approximately midway through data collection, the Queen's University MRI facility was upgraded from a Siemens Trio to a Siemens Prisma. To ensure the data pre- and post-upgrade were comparable, test scans involving our experimental paradigm were performed on two participants before and after the upgrade, and the data quality were compared. No significant differences in BOLD activity between the datasets were found, therefore all fMRI data were subsequently analyzed together. Of the 20 FM participant datasets included in this study, 15 datasets were collected before the MRI upgrade, and 5 were collected post-upgrade. Of the 17 HC participant datasets included in this study, 12 datasets were collected pre-upgrade, and 5 were collected post-upgrade.

The preprocessed data were analyzed using a structural equation modeling (SEM) method that has been optimized by our lab, with custom software written in MATLAB, and more recently implemented in python (https://www.queensu.ca/academia/stromanlab/dr-patrick-stroman/fmri-analysis-software).[18,29,47,48] This analysis method requires a predefined anatomical model. Because of our interest in pain processing, our predefined anatomical model consisted of regions known to be associated with pain, motivational-affective, and autonomic processing, including: anterior cingulate cortex (ACC), amygdala (Amg), Heschl's gyrus (HG), hippocampus (Hipp), hypothalamus (Hyp), insular cortex (IC), locus coeruleus (LC), nucleus accumbens (NAc), nucleus gigantocellularis (NGc), nucleus raphe magnus (NRM), nucleus tractus solitarius (NTS), parabrachial nucleus (PBN), periaqueductal gray (PAG), prefrontal cortex (PFC), posterior cingulate cortex (PCC), thalamus (Thal), and ventral tegmental area (VTA) (Fig. 2).[49] The anatomical regions of interest were identified based on spatially-normalized anatomical atlases matching the MNI template, as provided in the CONN15e software package.[50,51]

Structural equation modeling (SEM) analyses were conducted on data from each participant (FM and HC) in each study condition (Pain and No-Pain). Time-series data were extracted from voxels within the 17 anatomical regions of interest. Using data combined across the FM group, each region was divided into 7 subregions based on voxel time-series properties, by means of k-means clustering except for the VTA, which was only divided into 4 subregions due to its smaller volume. The choice of 7 subregions was based on prior studies which demonstrated that this provided a balance between flexibility in separating subregions with different signal properties, and limiting the number of subregions to be tested. The 17 regions included in the predefined model are associated with multiple functions, thus, it is unlikely that all BOLD signalling from these regions was involved with the anticipation and processing of pain. By dividing the regions into subregions based on BOLD time-course properties in the FM group, we were able to separate the regions based on function.[29,48] For consistency, the same subregion definitions were used for both study groups and both study conditions during the analyses. The result of this process provided time-series responses for each subregion for each of the repeated runs in each participant, which were then used for subsequent analyses.

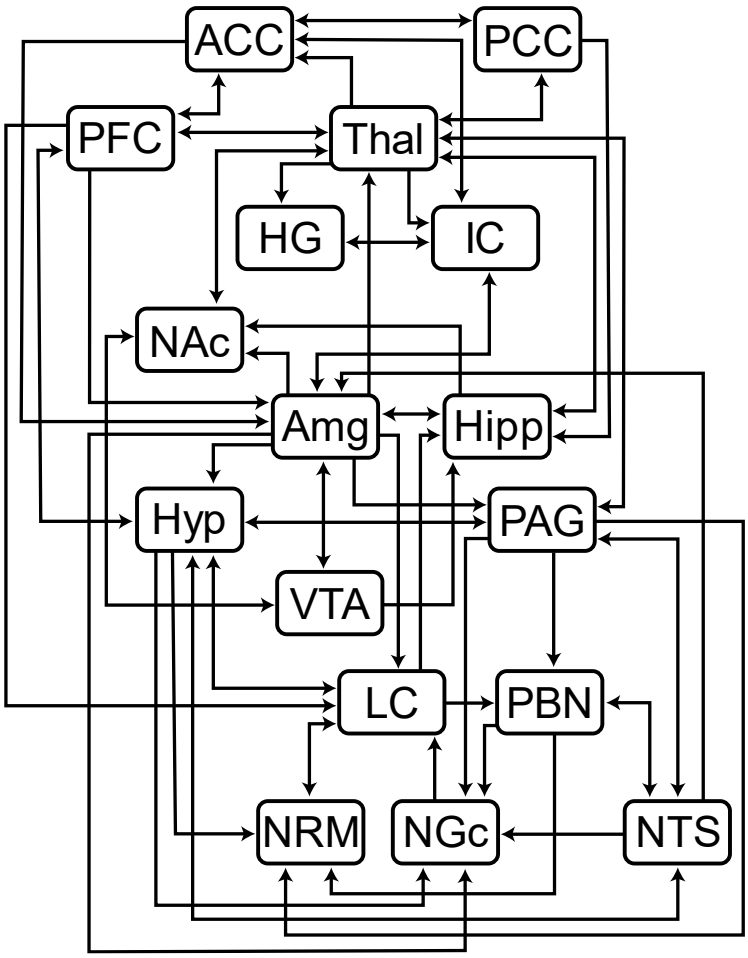

Figure 2. Pre-defined anatomical pain model for structural equation modeling (SEM) analysis. Lines indicate regions known to be functionally connected. Lines depict source regions; arrows depict target regions. Lines with arrows on each end indicate reciprocal connections. ACC = anterior cingulate cortex. Amg = amygdala. HG = Heschl's gyrus. Hipp = hippocampus. Hyp = hypothalamus. IC = insular cortex. LC = locus coeruleus. NAc = nucleus accumbens. NGc = nucleus gigantocellularis. NRM = nucleus raphe magnus. NTS = nucleus tractus solitarius. PBN = parabrachial nucleus. PAG = periaqueductal gray. PFC = prefrontal cortex. PCC = posterior cingulate cortex. Thal = thalamus. VTA = ventral tegmental area.

The SEM analysis consisted of fitting BOLD time-course responses in each target region to the BOLD responses from the other modeled regions (sources) by means of a general linear model (GLM). In this instance, the linear weighting factors, referred to as β_{SEM}-values, represent the connectivity strengths from each source region to the target region. For example, if region A is known to receive input signalling from regions B and C, and the BOLD time-course responses in these regions are S_A, S_B, and S_C, then $S_A = \beta_{AB}S_B + \beta_{AC}S_C + e_A$, where e_A is the residual signal variation that cannot be explained by the SEM fit. Connectivity values were determined for one target region at a time. However, every combination of subregions within the predefined anatomical model were investigated, and the subregions with connections that provided the best fits to the target were identified.

In order to understand how connectivity changes between pain anticipation and pain perception, dynamic variations in β_{SEM}-values were also identified by analyzing data from 45-second windows (23 volumes from each fMRI run). The two epochs of interest were 'Expectation', a 45-second window between the 1-minute and 2-minute mark after participants were told what to expect; and 'Stimulation', a 45-second window containing the entire stimulation period during Pain runs. Although no stimulation was administered in the No-Pain runs, the same two epochs were analyzed in both the Pain and No-Pain conditions so that appropriate brain connectivity comparisons between the two study conditions could be made (i.e., so we could determine how connectivity differs in each study group, based on presence or absence of external stimulation). The goodness-of-fit was determined by computing the amount of variance in each target

region that was explained by the fit, expressed as the R^2-value. The significance of the fit to each target region was estimated by converting the R-value to a Z-score by means of the Fisher Z-transform. Network components were inferred to be significant at a family-wise error rate (FWER) corrected $p_{FWER} < 0.05$, accounting for the total number of network combinations that were tested across the combinations of anatomical subdivisions. The significance threshold was confirmed with repeated simulations of the analysis procedure on null data consisting of normally distributed random values. Additionally, to determine the importance of each source region in the fit, one source region at a time was omitted from the network and the fitting process was repeated, and F-values were computed.

To identify differences in connectivity between the HC and FM groups, the SEM results were analyzed using Student's *t*-tests. Between-group analyses were performed on the SEM results from both the Pain and No-Pain conditions, at both time periods. Similar to the SEM analyses, the *t*-tests were inferred to be significant at $p_{FWER} < 0.05$ which was Bonferroni corrected to account for multiple comparisons. The results from these analyses were further analyzed by correlating β_{SEM}-values from each significantly different connection in each study group during the Pain imaging runs with questionnaire scores and normalized pain scores. Participants' normalized pain scores were calculated by dividing each participant's average final pain ratings by the average stimulus temperature they received during the Pain imaging runs. The group-level correlations were inferred to be significant at $p_{FWER} < 0.05$. Group-level correlation coefficients were also compared using the Fisher Z-transform, and these correlations were inferred to be significantly different at $p < 0.05$.

Participant characteristics such as demographics, questionnaire scores, algometry results, and normalized pain scores were also compared using Student's *t*-tests to determine if there were significant differences between the study groups that could potentially explain the fMRI results. *T*-tests were inferred to be significant at $p < 0.05$. Finally, group-level correlations between questionnaire scores and normalized pain scores were calculated, and the group-level correlation values were compared using Fisher Z-transform. The group-level correlations were inferred to be significant at $p_{FWER} < 0.05$, and the correlation comparisons between groups were also inferred to be significant at $p < 0.05$.

Results

PARTICIPANT CHARACTERISTICS

Student's *t*-tests revealed several significant differences between the FM and HC participant groups, in terms of the participant characteristics (Table 2). A significant difference in age was identified ($p = 7.1 \times 10^{-3}$), with the FM group being approximately 11 years older on average than the HC group. There was no significant difference in body mass index (BMI; $p = 0.64$). The only questionnaire scores that did not significantly differ between the two study groups were the STAI ($p_{STAI\text{-}Y1} = 0.13$, $p_{STAI\text{-}Y2} = 0.13$) and the SDS scores ($p = 0.82$). The remaining questionnaires revealed significantly greater scores in the FM group (Table 2). Although the scores were significantly different, there was a high degree of variability in questionnaire scores within each study group, most notably for the FIQR/SIQR and SF-MPQ-2 questionnaires (Table 2). Furthermore, due to participant error and the fact that some of the questionnaires were added after the beginning of the study, not every participant completed all questionnaires. Based on the 2016 criteria for FM,[42] only 13 of the 18 FM participants and zero of the 14 HC participants who completed the FSQ met the criteria for FM. Nonetheless, all the participants included in the FM group had self-reported as being previously diagnosed with FM by their physician. Our subsequent analyses were designed to accommodate individual differences in responses, in the event that some participants had weaker effects related to FM.

Table 2. Group-level comparisons of participant characteristics.

	Controls n = 17		**Fibromyalgia** n = 20		**P-value**
Demographics					
Age	37.94 ± 10.35		48.80 ± 12.73		**7.10×10^{-3}**
BMI	27.68 ± 4.16		28.56 ± 6.85		0.64
Questionnaire Scores		#		#	
STAI					
State (20-80)	31.29 ± 12.31	17	37.35 ± 11.50	20	0.13
Trait (20-80)	35.94 ± 13.14	16	42.10 ± 9.67	20	0.13
SDS (0-33)	19.65 ± 5.28	17	20.05 ± 5.67	20	0.82
BDI-II (0-63)	6.53 ± 9.08	17	16.90 ± 11.74	20	**4.60×10^{-3}**

	Controls n = 17		Fibromyalgia n = 20		P-value
PCS					
Total (0-52)	7.27 ± 6.19	15	21.32 ± 12.97	19	**2.91×10^{-4}**
Rumination (0-16)	3.60 ± 3.29	15	8.00 ± 4.80	19	**3.40×10^{-3}**
Magnification (0-12)	1.07 ± 1.16	15	3.89 ± 2.64	19	**2.95×10^{-4}**
Helplessness (0-24)	2.60 ± 2.53	15	9.42 ± 6.59	19	**3.59×10^{-4}**
FIQR/SIQR					
Total (0-100)	10.45 ± 11.88	17	54.58 ± 16.85	20	**7.31×10^{-11}**
Function (0-30)	0.98 ± 2.47	17	14.28 ± 7.61	20	**1.46×10^{-7}**
Impact (0-20)	1.71 ± 3.57	17	10.75 ± 4.34	20	**4.35×10^{-8}**
Symptom (0-50)	7.76 ± 7.06	17	29.55 ± 7.64	20	**1.32×10^{-10}**
COMPASS-31					
Total (0-100)	11.86 ± 9.23	17	40.19 ± 17.67	20	**7.68×10^{-7}**
Orthostatic (0-40)	4.94 ± 6.71	17	15.21 ± 10.43	20	**1.00×10^{-3}**
Vasomotor (0-5)	0.00 ± 0.00	17	1.91 ± 1.42	20	**9.16×10^{-6}**
Secretomotor (0-15)	1.14 ± 2.02	17	7.53 ± 3.97	20	**6.88×10^{-7}**
Gastrointestinal (0-25)	4.51 ± 3.20	17	10.48 ± 4.43	20	**3.64×10^{-5}**
Bladder (0-10)	0.39 ± 0.86	17	2.49 ± 2.77	20	**3.80×10^{-3}**
Pupillomotor (0-5)	0.89 ± 0.91	17	2.59 ± 0.99	20	**4.53×10^{-6}**
SF-MPQ-2					
Total (0-220)	12.72 ± 10.94	11	92.25 ± 49.06	20	**5.38×10^{-7}**
Continuous (0-60)	5.55 ± 4.78	11	31.05 ± 14.58	20	**1.58×10^{-7}**
Intermittent (0-60)	1.82 ± 3.60	11	24.40 ± 15.87	20	**3.75×10^{-6}**
Neuropathic (0-60)	3.27 ± 5.72	11	22.50 ± 13.93	20	**9.84×10^{-6}**
Affective (0-40)	2.09 ± 3.59	11	14.30 ± 9.70	20	**2.90×10^{-5}**
FSQ					
FS Total (0-31)	4.14 ± 3.42	14	19.56 ± 6.66	18	**4.97×10^{-9}**
Widespread Pain Index (0-19)	1.29 ± 1.07	14	10.56 ± 5.59	18	**1.64×10^{-6}**
Symptom Severity Scale (0-12)	2.86 ± 2.82	14	9.00 ± 1.94	18	**5.46×10^{-7}**
Quantitative Sensory Results					
First Pain Ratings	26.04 ± 13.35		33.81 ± 14.74		1.02×10^{-1}
Last Pain Ratings	38.93 ± 12.07		44.22 ± 12.04		1.93×10^{-1}
Temperature	50.89 ± 1.04		47.51 ± 2.72		**2.55×10^{-5}**
Normalized Pain Scores	0.77 ± 0.24		0.94 ± 0.28		5.37×10^{-2}

Note: All values are indicated as mean ± standard deviation. **BMI** = Body Mass Index. **STAI** = State-Trait Anxiety Inventory. **SDS** = Social Desirability Scale. **BDI-II** = Beck Depression Inventory-II. **PCS** = Pain Catastrophizing Scale. **FIQR** = Fibromyalgia Impact Questionnaire – Revised. **SIQR** = Symptom Impact Questionnaire – Revised (the SIQR is the equivalent to the FIQR for healthy controls). **COMPASS-31** = Composite Autonomic Symptom Score. **SF-MPQ-2** = Short-Form McGill Pain Questionnaire-2. **FSQ** = Fibromyalgia Survey Questionnaire. **#** = the number of participants who filled out each questionnaire. Independent *t*-tests were conducted to determine if any significant group-level differences between the healthy control and fibromyalgia groups existed. Bold p-values indicate significant differences between study groups at $p < 0.05$.

Neither the first pain ratings, nor the last pain ratings, differed significantly between the two study groups ($p_{FirstPain} = 0.10$ and $p_{LastPain} = 0.19$) during the fMRI sessions, indicating successful administration of subjectively equivalent levels of pain in both study groups. Consistent with previous studies, the stimulus temperatures were significantly different between the two groups ($p = 2.55 \times 10^{-5}$), approximately 3.4 °C lower, on average, in the FM group to elicit the same pain (Table 2). Although stimulus temperatures significantly differed across groups, normalized pain scores did not ($p = 5.37 \times 10^{-2}$); however, the difference approached significance. Comparison of algometry results revealed significant group differences at every pressure-point except the forehead control point ($p = 0.25$; Table 3). At every significantly different pressure point, the average pressure required to elicit pain was lower in the FM group than the HC group (Table 3).

Table 3. Group-level comparison of algometry results.

	Healthy Controls N = 17		Fibromyalgia N = 20		
Pressure Points	**Felt Pain (%)**	**Pressure (kg)**	**Felt Pain (%)**	**Pressure (kg)**	**P-value**
Forehead (Control)	47	3.47 (± 0.78)	90	3.17 (± 0.79)	2.50×10^{-1}
Occiput (Left)	65	**3.45 (± 0.63)**	95	**2.40 (± 0.89)**	**1.92×10^{-4}**
Occiput (Right)	65	**3.40 (± 0.73)**	100	**2.23 (± 0.90)**	**1.03×10^{-4}**
Lateral Epicondyle (Left)	29	**3.71 (± 0.61)**	80	**2.70 (± 0.92)**	**5.78×10^{-4}**
Lateral Epicondyle (Right)	35	**3.74 (± 0.61)**	85	**2.79 (± 1.15)**	**4.60×10^{-3}**
Low Cervical (Left)	94	**2.09 (± 0.83)**	100	**1.28 (± 0.56)**	**2.00×10^{-3}**
Low Cervical (Right)	88	**2.12 (± 0.83)**	100	**1.35 (± 0.63)**	**3.40×10^{-3}**
Supraspinatus (Left)	18	**3.93 (± 0.29)**	75	**3.13 (± 1.04)**	**4.10×10^{-3}**
Supraspinatus (Right)	18	**3.90 (± 0.31)**	85	**2.75 (± 1.13)**	**2.59×10^{-4}**
Trapezius (Left)	35	**3.83 (± 0.40)**	80	**2.94 (± 0.92)**	**5.39×10^{-4}**
Trapezius (Right)	29	**3.80 (± 0.40)**	90	**2.82 (± 0.92)**	**1.90×10^{-4}**
Second Rib (Left)	47	**3.40 (± 0.76)**	100	**2.18 (± 0.78)**	**2.82×10^{-5}**
Second Rib (Right)	53	**3.44 (± 0.74)**	100	**2.34 (± 1.05)**	**7.09×10^{-4}**

Note: 'Felt Pain' values indicate the percentage of participants in each group that experienced pain before/during the cut-off pressure value of 4.0 kg. 'Pressure' values are indicated as mean ± standard deviation. Independent t-tests were conducted to determine if any significant differences existed between the two study groups at each pressure point. **Bold values** indicate significant differences between the healthy control and fibromyalgia participant groups at $p < 0.05$.

STRUCTURAL EQUATION MODELING COMPARISONS

Structural equation modeling identified extensive networks in both study groups in all study conditions and time periods, and *t*-tests of these SEM results revealed significant group-level connectivity differences in both study conditions and time periods (Figures 3 and 4). In the No-Pain condition, 11 connections were found to be significantly different between the two study groups in the Expectation time period (Figure 3). The majority of these connections involved the thalamus (five connections), PAG, hypothalamus, or amygdala (four connections each). Three Amg→Thal connections, and one connection each between ACC→Amg, NTS→Hyp, PAG→Hyp, and PAG→NGc were stronger in the FM group than controls (i.e., the β_{SEM}-values were higher in the FM group than the HC group). The other connections, Hipp→Thal, Hyp→NGc, PAG→Hyp, and PAG→Thal, were stronger in the HC group. During the Stimulation-equivalent period in the No-Pain condition, four connections were identified as being significantly different between the two study groups, and all of these involved the thalamus (Figure 3). Only one connection was stronger in the FM group, NAc→Thal. The other three connections (two Thal→ACC and one Thal→Hipp) were stronger in the HC group.

The Pain condition comparison identified six connections in the Expectation time period, with the amygdala being involved in four of these connections (Figure 4). Three of these amygdalar connections were stronger in the HC group (ACC→Amg, Amg→NGc, and Amg→PAG), and the other three connections were stronger in the FM group (Hipp→Amg, PCC→Thal, VTA→Hipp). In the Stimulation time period, 11 significantly different connections were identified (Figure 4). There were five connections that were stronger in the HC group (ACC→Amg, HG→IC, PFC→Thal, Thal→IC, VTA→Amg), and six connections that were stronger in the FM group (Hipp→Thal, NAc→Thal, and four PCC→Thal)).

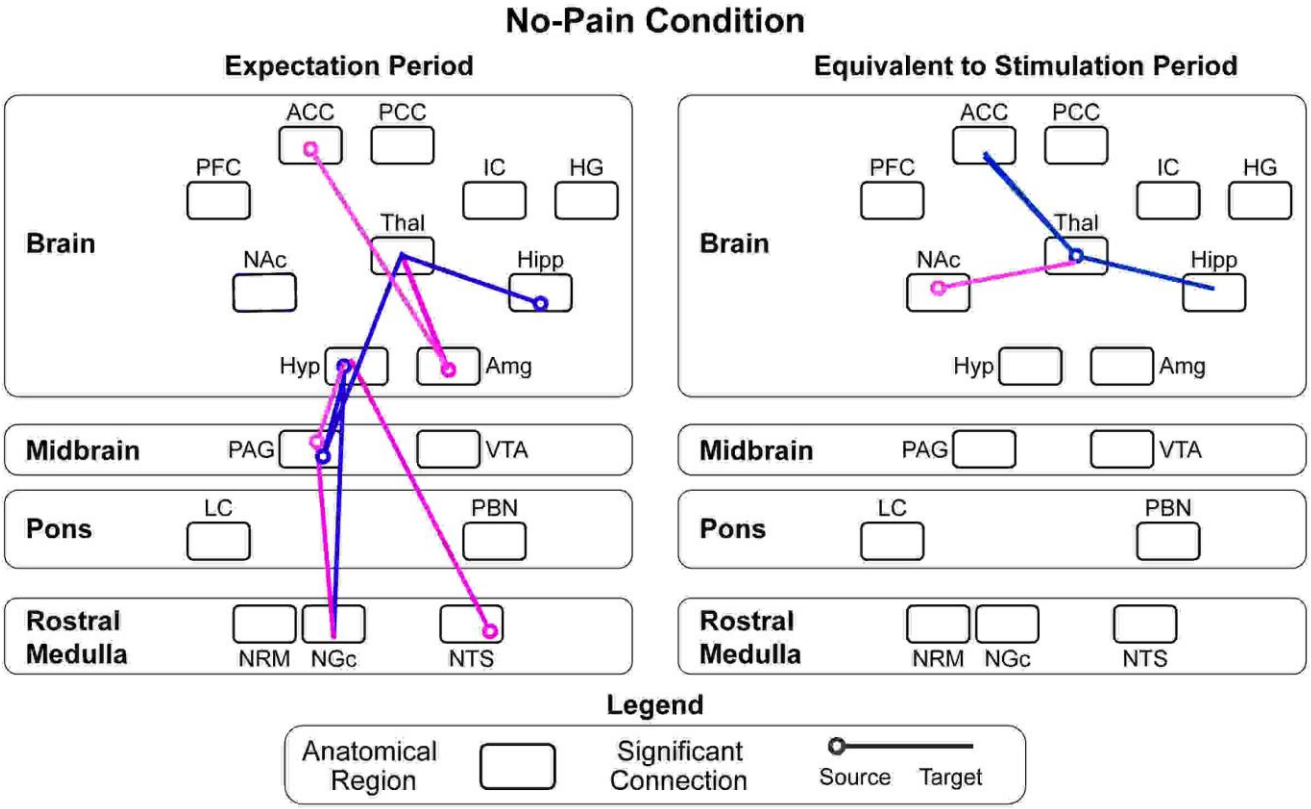

Figure 3. Group comparison of SEM results from the No-Pain condition. Lines indicate which connections from the SEM calculations were significantly different between the HC and FM groups (blue for HC > FM, pink for FM > HC). Only significant connections are shown, at a family-wise error rate (FWER) corrected $p_{FWER} < 0.05$. Expectation = 45 second window between 1-minute mark and 2-minute mark. Stimulation = 45 second window encompassing the stimulation period of Pain runs. ACC = anterior cingulate cortex. Amg = amygdala. HG = Heschl's gyrus. Hipp = hippocampus. Hyp = hypothalamus. IC = insular cortex. LC = locus coeruleus. NAc = nucleus accumbens. NGc = nucleus gigantocellularis. NRM = nucleus raphe magnus. NTS = nucleus tractus solitarius. PBN = parabrachial nucleus. PAG = periaqueductal gray. PFC = prefrontal cortex. PCC = posterior cingulate cortex. Thal = thalamus. VTA = ventral tegmental area.

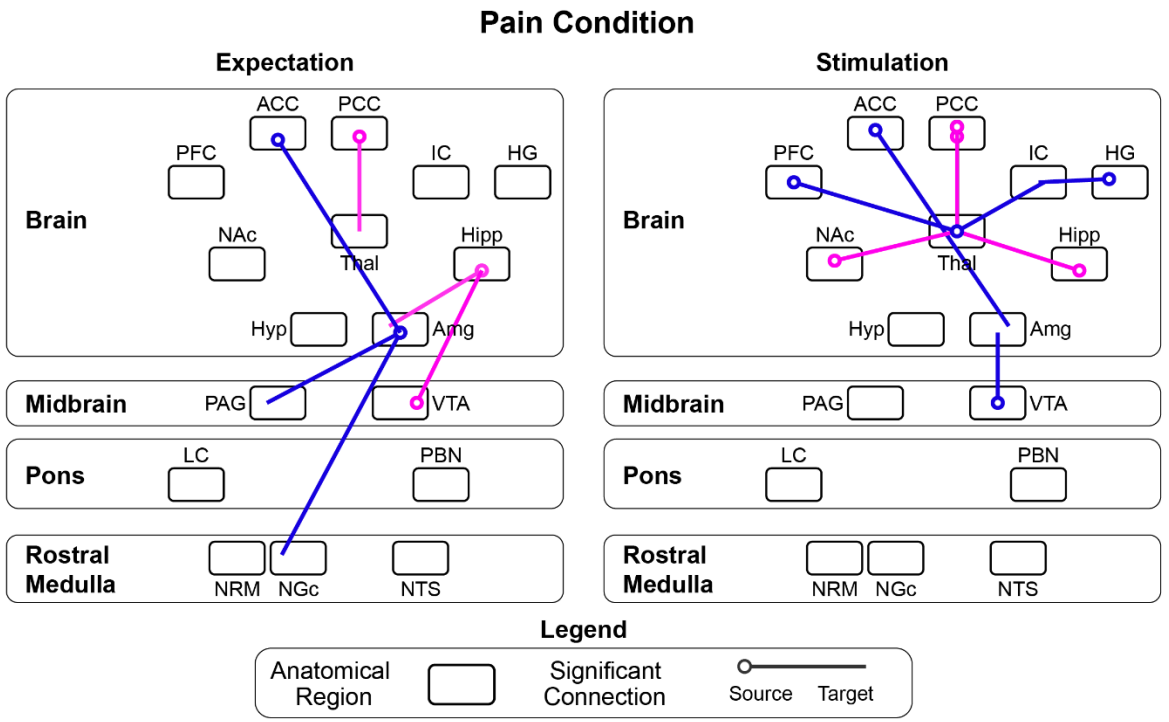

Figure 4. Group comparison of SEM results from the Pain condition. Lines indicate which connections from the SEM calculations were significantly different between the HC and FM groups (blue for HC > FM, pink for FM > HC). Only significant connections are shown, at a family-wise error rate (FWER) corrected $p_{FWER} < 0.05$. Expectation = 45 second window between 1-minute mark and 2-minute mark. Stimulation = 45 second window encompassing the stimulation period of Pain runs. ACC = anterior cingulate cortex. Amg = amygdala. HG = Heschl's gyrus. Hipp = hippocampus. Hyp = hypothalamus. IC = insular cortex. LC = locus coeruleus. NAc = nucleus accumbens. NGc = nucleus gigantocellularis. NRM = nucleus raphe magnus. NTS = nucleus tractus solitarius. PBN = parabrachial nucleus. PAG = periaqueductal gray. PFC = prefrontal cortex. PCC = posterior cingulate cortex. Thal = thalamus. VTA = ventral tegmental area.

CORRELATIONS AMONG PAIN RESPONSES, PARTICIPANT CHARACTERISTICS, AND CONNECTIVITY VALUES

Correlations were computed between normalized pain scores and questionnaire scores related to psychosocial adjustment and autonomic function (STAI, SDS, BDI-II, PCS, COMPASS-31) (Table 4). No significant correlations were found between normalized pain scores and questionnaire scores in either group, after correcting for multiple comparisons. The only correlations that were significantly different between the two study groups were between normalized pain scores and the COMPASS-31$_{Total}$ score (R_{FM} = 0.34, R_{HC} = -0.31, p_{Group} = 3.18 x 10^{-2}), and COMPASS-31$_{Orthostatic}$ sub-score (R_{FM} = 0.32, R_{HC} = -0.30, p_{Group} = 3.76 x 10^{-2}).

Table 4. Group-level correlations between questionnaire and normalized pain scores.

	Healthy Controls		Fibromyalgia		Group Comparison
Questionnaire	**R**	**P-Value**	**R**	**P-Value**	**P-Value**
STAI					
State	0.05	0.84	0.03	0.90	0.47
Trait	-0.01	0.97	0.06	0.80	0.42
SDS	0.03	0.90	-0.05	0.84	0.41
BDI-II	-0.06	0.83	0.44	4.94 x 10^{-2}	6.96 x 10^{-2}
PCS					
Total	0.04	0.88	0.27	0.25	0.26
Rumination	-0.02	0.95	0.22	0.36	0.26
Magnification	0.19	0.46	-0.01	0.98	0.29
Helplessness	0.14	0.59	0.21	0.38	0.43

	Healthy Controls		Fibromyalgia		Group Comparison
Compass-31					
Total	-0.31	0.23	0.34	0.15	**3.18×10^{-2}**
Orthostatic	-0.30	0.24	0.32	0.17	**3.76×10^{-2}**
Vasomotor	NA	NA	0.21	0.36	NA
Secretomotor	-0.01	0.97	0.10	0.68	0.38
Gastrointestinal	-0.09	0.72	0.32	0.17	0.12
Bladder	-0.17	0.52	0.13	0.57	0.20
Pupillomotor	-0.41	0.10	0.12	0.62	6.19×10^{-2}

Note: Bold font indicates significant correlations at a family-wise error rate (FWER) corrected $p_{FWER} < 0.05$, or significant group-level differences in correlation values at $p < 0.05$.

Correlations were also computed between the β_{SEM}-values from the connections that were identified as being significantly different between the two study groups during the Pain condition and participants' normalized pain scores, as well as their STAI, BDI-II and PCS questionnaire scores (Table 5). Many significant differences in correlations between the two groups were identified, but the β_{SEM}-values were not found to be significantly correlated with normalized pain scores in either group (Table 5). In fact, the only significant group-level correlation identified was between the β_{SEM}-values for the ACC→Amg connection in the Stimulation time period and participants' BDI-II questionnaire scores in the FM study group (Table 5). No other correlation in either study group reached significance after correcting for multiple comparisons.

Table 5. Correlations between β_{SEM}-values and questionnaire scores/normalized pain scores for significantly different group connections in the Pain condition.

Source → Target	**Healthy Controls Correlations (R)**							
Expectation Period	**STAI (State)**	**STAI (Trait)**	**BDI**	**PCS (Total)**	**PCS (Rum.)**	**PCS (Magn.)**	**PCS (Help.)**	**NPS**
ACC 1 → Amg 2	0.16	- 0.05	0.07	0.24	0.26	0.02	0.25	0.27
Amg 1 → NGc 6	0.21	0.11	0.19	- 0.24	- 0.17	- 0.16	- 0.32	- 0.21
Amg 4 → PAG 4	0.26	0.21	0.02	0.10	0.26	- 0.18	- 0.18	- 0.23
Hipp 5 → Amg 6	- 0.16	- 0.10	- 0.19	- 0.02	- 0.05	- 0.04	- 0.18	- 0.01
PCC 2 → Thal 5	- 0.11	0.02	0.36	0.41	0.51	0.20	0.41	- 0.37
VTA 4 → Hipp 5	- 0.19	- 0.23	- 0.39	- 0.28	- 0.26	- 0.01	- 0.11	- 0.09
Stim. Period								
ACC 5 → Amg 5	0.05	0.05	- 0.10	- 0.28	- 0.21	- 0.14	- 0.46	- 0.01
HG 4 → IC 4	0.51	0.43	0.49	0.13	0.20	0.12	0.05	- 0.31
Hipp 6 → Thal 2	0	0	0.16	0.27	0.41	0.40	0.31	0.35
NAc 3 → Thal 5	0.08	0.03	- 0.02	- 0.08	- 0.08	0.04	0.08	0.35
PCC 2 → Thal 5	- 0.37	- 0.40	- 0.37	- 0.45	- 0.49	- 0.44	- 0.27	- 0.14
PCC 6 → Thal 2	0.19	0.24	0.28	- 0.05	- 0.12	- 0.09	- 0.02	- 0.09
PCC 6 → Thal 4	- 0.02	- 0.06	0.08	0.05	0.19	- 0.06	- 0.05	- 0.32
PCC 6 → Thal 5	- 0.12	- 0.25	- 0.18	- 0.05	0.04	- 0.09	0.05	- 0.22
PFC 4 → Thal 1	- 0.17	- 0.02	0	0.30	0.31	0.33	- 0.02	0.09
Thal 2 → IC 6	- 0.13	- 0.08	0.06	- 0.04	- 0.10	- 0.24	0.07	0.33
VTA 1 → Amg 1	0.14	0.07	0.23	0.30	0.24	0.49	0.55	0.43
Source → Target	**Fibromyalgia Correlations (R)**							
Expectation Period	**STAI (State)**	**STAI (Trait)**	**BDI**	**PCS (Total)**	**PCS (Rum.)**	**PCS (Magn.)**	**PCS (Help.)**	**NPS**
ACC 1 → Amg 2	- 0.56	- 0.36	- 0.16	- 0.15	- 0.13	- 0.41	- 0.21	- 0.27
Amg 1 → NGc 6	0.02	0.21	0.48	0.26	0.26	0.11	0.13	0.36
Amg 4 → PAG 4	0.06	0.46	0.25	0.11	0.07	0.05	0.06	0.26
Hipp 5 → Amg 6	- 0.08	0.04	0.39	0.10	0.17	0.12	- 0.01	0.02
PCC 2 → Thal 5	- 0.03	- 0.10	0.11	0.01	0.13	- 0.03	0.05	- 0.06
VTA 4 → Hipp 5	0.02	0.03	0.07	0.18	0.01	- 0.23	0.20	0.09
Stim. Period								

Expectation Period	STAI (State)	STAI (Trait)	BDI	PCS (Total)	PCS (Rum.)	PCS (Magn.)	PCS (Help.)	NPS
ACC 5 → Amg 5	- 0.17	- 0.49	**- 0.62**	- 0.44	- 0.49	- 0.27	- 0.39	- 0.19
HG 4 → IC 4	0.38	0.24	0.20	0.35	0.22	0.24	0.40	0.16
Hipp 6 → Thal 2	0.33	0.42	0.20	0.03	0.10	0.12	- 0.03	0.06
NAc 3 → Thal 5	0.49	0.29	- 0.21	- 0.08	0	0.18	0.13	- 0.36
PCC 2 → Thal 5	- 0.21	0.17	0.24	0.09	- 0.10	- 0.29	0.03	0.11
PCC 6 → Thal 2	- 0.51	- 0.39	0.13	0.19	0.11	0.01	0.12	0.13
PCC 6 → Thal 4	- 0.34	- 0.38	- 0.18	0.07	0.03	- 0.19	- 0.03	- 0.22
PCC 6 → Thal 5	- 0.39	- 0.25	- 0.11	- 0.26	- 0.37	- 0.29	- 0.26	- 0.12
PFC 4 → Thal 1	0.10	- 0.02	0.10	0.10	0.11	0.24	0.12	0.01
Thal 2 → IC 6	0.24	0	- 0.08	0.12	0.15	0.36	0.28	- 0.17
VTA 1 → Amg 1	0.08	0.29	0.27	0	- 0.10	0.03	- 0.03	- 0.05

Note: STAI = State-Trait Anxiety Inventory. **BDI** = Beck Depression Inventory-II. **PCS** = Pain Catastrophizing Scale (**Rum**: Rumination, **Magn**: Magnification, **Help**: Helplessness). **NPS** = Normalized Pain Scores. Bold values indicate significant correlations at a family-wise error rate (FWER) corrected $p_{FWER} < 0.05$. Darkened grey cells indicate significant group-level differences in correlation values at $p < 0.05$.

Due to the significant difference in age between the two study groups, we also computed correlations between age and participants' STAI, BDI-II, and PCS questionnaire scores, participants' normalized pain scores, and β_{SEM}-values from the connections that were identified as being significantly different between the two study groups during the Pain condition. There were no significant correlations between age and the STAI, BDI-II, PCS, or normalized pain scores in either study group after correcting for multiple comparisons, but there was a significant group-level difference in correlation values between age and normalized pain scores ($R_{FM} = 0.45$, $R_{HC} = -0.27$, $p_{Group} = 1.87 \times 10^{-2}$; Table 6a). Finally, age was not significantly correlated with any of the βSEM-values in either group, after correcting for multiple comparisons, but there was a significant group-level difference in correlation values between age and βSEM-values for one of the PCC→Thal connections from the Stimulation time period (RFM = 0.54, RHC = -0.24, pGroup = 9.40 x 10-3; Table 6b).

Table 6a. Correlations between age and questionnaire/normalized pain scores.

	Healthy Controls		Fibromyalgia		Group
	R	**P-value**	**R**	**P-value**	**P-value**
State-Trait Anxiety Inventory					
State	0.05	0.85	-0.24	0.31	0.21
Trait	-0.01	0.97	-0.23	0.33	0.27
Social Desirability Scale	0.25	0.34	-0.06	0.79	0.19
Beck Depression Inventory-II	0.23	0.37	0.43	5.54×10^{-2}	0.26
Pain Catastrophizing Scale					
Total	-0.11	0.68	0.30	0.19	0.12
Rumination	0.08	0.75	0.32	0.17	0.25
Magnification	-0.01	0.96	-0.01	0.96	0.50
Helplessness	-0.02	0.95	0.21	0.37	0.26
Normalized Pain Scores	-0.27	0.30	0.45	4.90×10^{-2}	**1.87×10^{-2}**

Table 6b. Correlations between age and β_{SEM}-values of significantly different connections identified during Pain condition.

Connection	Healthy Controls		Fibromyalgia		Group
Source # → Target #	**R**	**P-Value**	**R**	**P-value**	**P-value**
Expectation Time Period					
ACC 1 → Amg 2	0.29	0.25	0.20	0.40	0.39
Amg 1 → NGc 6	0.05	0.85	0.24	0.30	0.29
Amg 4 → PAG 4	0.21	0.43	-0.13	0.60	0.18
Hipp 5 → Amg 6	-0.13	0.62	0.40	8.42×10^{-2}	6.42×10^{-2}
PCC 2 → Thal 5	0.54	2.39×10^{-2}	0.32	0.17	0.22
VTA 4 → Hipp 5	-0.18	0.50	0.02	0.95	0.30

Stimulation Time Period					
ACC 5 → Amg 5	0.32	0.22	-0.09	0.72	0.13
HG 4 → IC 4	0.41	0.10	-0.14	0.56	5.53×10^{-2}
Hipp 6 → Thal 2	-0.03	0.91	-0.18	0.45	0.34
NAc 3 → Thal 5	-0.12	0.63	-0.50	2.51×10^{-2}	0.12
PCC 2 → Thal 5	-0.17	0.52	0.13	0.57	0.20
PCC 6 → Thal 2	-0.24	0.35	0.54	1.48×10^{-2}	**9.40×10^{-3}**
PCC 6 → Thal 4	0.36	0.15	0.32	0.17	0.45
PCC 6 → Thal 5	0.24	0.36	0.10	0.67	0.35
PFC 4 → Thal 1	0.00	1.00	0.31	0.19	0.19
Thal 2 → IC 6	-0.21	0.42	-0.17	0.47	0.46
VTA 1 → Amg 1	0.18	0.48	-0.10	0.67	0.21

Note: ACC = anterior cingulate cortex. **Amg** = amygdala. **HG** = Heschl's gyrus. **Hipp** = hippocampus. **IC** = insular cortex. **NAc** = nucleus accumbens. **NGc** = nucleus gigantocellularis. **PAG** = periaqueductal gray. **PFC** = prefrontal cortex. **PCC** = posterior cingulate cortex. **Thal** = thalamus. **VTA** = ventral tegmental area. **Group** = group-level difference in correlation values. Bold values indicate significant correlations at a family-wise error rate (FWER) corrected $p_{FWER} < 0.05$, or significant group-level differences in correlation values at $p < 0.05$.

Discussion

Fibromyalgia and HC study groups underwent identical experimental threat/safety pain paradigms while undergoing fMRI to investigate group-level differences in the continuous and reactive components of pain processing. A number of significant differences were identified between FM and HC participants.

Group-level differences in brain connectivity during the No-Pain condition were mainly identified during the Expectation time period (Figure 3). Informing participants they would not experience the painful stimuli was hypothesized to evoke relief that would potentially be mediated by the brain's reward system,[52] and most of the connections identified as being different between the study groups involved regions associated with reward—such as the ACC, amygdala, hippocampus, and hypothalamus.[53–55] These findings suggest FM may involve altered functioning in reward pathways, however, further investigation into the interplay of pain relief and reward is required, and other explanations for these group level differences do exist. For example, after participants were informed of the run type, the No-Pain condition was similar to a resting-state scan. Differences in resting-state activity between HC and FM groups have been reported, and were thought to be related to the general body pain the FM participants experienced during the time of scanning.[56–59] Because the FM participants also reported being in at least mild pain before scanning, it would be reasonable to conclude that differences in connectivity during the No-Pain imaging runs were caused by general body pain, and not dysfunction in the reward system. In studies of FM that employed resting-state fMRI, most group-level functional connectivity differences were associated with the default mode network (DMN), and between the DMN and insula, which is inconsistent with our current study.[57] We mainly identified connections between the ACC, amygdala, hippocampus, hypothalamus, NGc, NTS, PAG, and thalamus. These regions are implicated more strongly with emotional and autonomic processing than the DMN.[55,60] Furthermore, if the differences were purely related to clinical pain, they would have been equally identified in both time periods studied. Instead, most connections were identified in the Expectation time period (i.e., right after participants were informed there would be no pain in that run), suggesting that connectivity differences were directly related to how the two groups responded to this information, and not due to the general body pain reported by the FM participants before scanning.

To presume these connectivity differences were purely associated with altered reward processing would also be an oversimplification. Two regions known to play significant roles in reward, the NAc and VTA, were included in the predefined anatomical model but were not involved in any of the significantly different connections in the Expectation time period of the No-Pain condition.[61–64] Therefore, more directed investigation will be required to determine whether or not these differences were inherently related to altered function of the reward system in participants with FM.

During the Pain condition, informing participants about the upcoming painful stimuli was hypothesized to evoke an anticipatory/fear response that could be detected by studying brain connectivity during the Expectation time period. Six

group-level connectivity differences were identified during this period, and the amygdala was involved in four of these connections (Figure 4). Interestingly, three of these amygdalar connections (ACC→Amg, Amg→NGc, Amg→PAG) were stronger in the HC group. Signalling from the ACC to the amygdala may be related to aversive behaviour and pain-related emotional processing, and the descending inputs from the amygdala to the NGc and PAG are expected to be associated with mediating the behavioural response to fear.[65] Therefore, if the connectivity in the HC group is considered the normal response to being informed of an upcoming pain, it appears the participants with FM displayed a smaller anticipatory/fear response to this information.

Normal fear responses to upcoming perceived threats can activate descending pain modulatory systems and enable stress-induced analgesia.[66–68] Thus, the smaller anticipatory responses in the FM participants may have resulted in decreased analgesia, which explains why the FM study group required significantly lower temperatures to feel the same level of pain as the controls. However, the Stimulation time period of the Pain condition did not reveal group-level differences in descending brain connectivity (Figure 4), and no significant group-level differences in BOLD signalling in any of the brain regions included in the predefined anatomical model were identified.

This study aimed to elicit moderate levels of pain in both study groups. As participants with FM experience severe pain on a daily basis, the dampened response to being informed of the upcoming pain could be due to group-level differences in how the threat was perceived. Alternatively, there is evidence the autonomic nervous system is persistently hyperactive in people with FM, such that the system does not undergo further augmented responses to stress (i.e., hypo-responsive).[69–71] Further investigation is required to determine if these altered anticipatory responses to upcoming pain reflect decreased descending pain modulatory outputs in participants with FM, and whether or not this mechanism can explain the hyperalgesia associated with FM.

Most of the group-level connectivity differences in the Pain condition were found in the Stimulation time period, when participants were experiencing the noxious heat stimuli. The most striking group-level differences were the ACC→Amg and PCC→Thal connections. The ACC→Amg connection was stronger in the HC group, suggesting FM participants may have experienced a dampened emotional response to the noxious stimulation. Decreased functional connectivity in the rostral ACC during painful stimulation in FM groups have been reported.[4,10] Furthermore, the β_{SEM}-values in this connection were significantly negatively correlated to BDI-II scores in the FM group, and the group-level correlations between β_{SEM}-values and BDI-II scores were significantly different between the two study groups. Although further investigation is required, the current findings, along with the previously reported FM-specific connectivity between the ACC and amygdala suggest that altered communication between the ACC and Amg may significantly contribute to abnormal pain processing in FM.[4,10]

The other prominent group-level differences were the four PCC→Thal connections. All four of these connections involved increased connectivity in the FM group, suggesting increased signalling between the PCC and thalamus during painful stimulation is specific to FM. Although the exact function of the PCC is unknown, it may be the central hub of the DMN and its functions may include directing attention internally/externally, saliency, and memory.[72] There is also evidence for its involvement in pain processing, as it has been associated with pain catastrophizing and pain perception.[73–75] Thus, the PCC may play an important role in saliency detection of external stimuli. If this is the case, the consistently increased connectivity between the PCC and thalamus in the FM group may suggest that FM participants had a heightened awareness to the noxious stimuli, which could explain their observed hyperalgesia. However, none of the β_{SEM}-values in these connections significantly correlated with normalized pain ratings, further highlighting the complexity of altered pain processing in FM.

Although this study has uncovered important differences in pain processing in individuals with FM, there are limitations which need to be addressed. First, the FM group was approximately 11 years older than the control group. A significant difference in group-level correlations between age and normalized pain scores was identified (Table 6a). This age difference may be a confound in this study, however age was not significantly correlated with the fMRI results (Table 6b). More importantly, although the FM participants were previously diagnosed with FM by their physicians, only 13 of the 18 FM participants that completed the FSQ actually met the 2016 criteria for FM. The FM participants that did not meet the criteria typically no longer experienced widespread pain. However, the stimulus temperatures required to elicit moderate pain in these individuals were similar to the other FM participants, suggesting they

resembled the FM participants more than the controls. Because not all FM participants met the 2016 criteria for FM, our findings may only generalize to individuals with chronic musculoskeletal pain. Due to the fact this study was part of a larger study involving the brainstem and spinal cord, there were imaging constraints that prevented us from reliably sampling the somatosensory cortices in each participant. The somatosensory cortex plays a significant role in the sensory-discriminative aspect of pain processing, and must be examined appropriately in future investigations using similar threat/safety paradigms and data-driven analytical techniques. Finally, all participants in this study were allowed to continue using their prescribed medications—as long as their prescriptions were stabilized for at least three months (see Table 1). It is possible these medications impacted the fMRI results; however no clear medication pattern could be discerned by studying the outliers in each significantly different connection in each group.

Conclusions

Significant group-level differences in brain connectivity between HC and FM study groups undergoing an identical threat/safety fMRI paradigm were identified. These differences were found in both the Pain and No-Pain conditions, during both the Expectation and Stimulation (or Stimulation-equivalent) time periods. The connections in the No-Pain condition suggest FM may involve altered emotional responses to pain relief, which may indicate abnormal processing within the endogenous reward system. The connections in the Pain condition suggest FM may be associated with a dampened autonomic/fear response to upcoming noxious stimuli, as well as heightened perception toward the stimuli once it arrives. These results corroborate previous findings, suggesting abnormal pain processing in FM is likely the result of increased saliency toward external noxious stimuli, which is driven by abnormalities within the motivational-affective components of pain processing associated with anticipation, and possibly reward.[4,6,76]

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