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Title: Inflammation is associated with pro-nociceptive brain connections in rheumatoid arthritis patients with concomitant fibromyalgia

Running Header: Neurobiological interactions between inflammation and fibromyalgia in RA

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ABSTRACT:

Objective:

Rheumatoid arthritis (RA) patients with comorbid fibromyalgia (FM) manifest alterations in brain connectivity synonymous with central sensitization. Here we consider how peripheral inflammation, the principal nociceptive stimulus in RA, interacts with brain connectivity in RA patients with comorbid FM.

Methods:

RA patients with (FM+, n=27) and without (FM-, n=27) comorbid FM completed functional connectivity magnetic resonance imaging. Seed to whole-brain functional connectivity analyses were conducted using left mid/posterior insula and left inferior parietal lobule (IPL) seeds, regions previously linked to FM symptoms and inflammation respectively. The association between functional connectivity and erythrocyte sedimentation rate (ESR) was assessed in each FM group separately, followed by post-hoc analyses to test for interaction effects. Significance was set at a cluster-level family-wise error (FWE) rate of $p < 0.05$.

Results:

RA patients with and without FM did not differ by age, gender or ESR ($p > 0.2$). In FM+ RA patients, increased insula - left IPL, left IPL - dorsal anterior cingulate and left IPL - medial prefrontal cortex functional connectivity correlated with higher levels of ESR (all $p < 0.05$ FWE). Post-hoc interaction analyses largely confirmed that the relationship between ESR and connectivity changes as FM scores increase.

Conclusion:

Here we provide the first neurobiological evidence that comorbid FM in RA may be linked to peripheral inflammation through pro-nociceptive patterns of brain connectivity. In patients with such 'bottom-up' pain centralization, comorbid symptoms may partially respond to anti-inflammatory treatments.

INTRODUCTION:

Rheumatoid Arthritis (RA) is known for its heterogeneous clinical presentation, with some patients reporting pain localized to affected joints and others reporting widespread hyperalgesia, fatigue, and cognitive difficulties. This latter phenotype bears remarkable similarities to fibromyalgia (FM) and indeed the prevalence of comorbid FM ranges between 12-48% in RA compared to 2-8% in the general population [1]. Moreover, we recently showed that one of the chief neurobiological signatures of FM, increased default mode network (DMN) to insula functional connectivity, also occurs in RA patients in proportion to their comorbid FM symptoms [2].

In the context of RA, it has long been hypothesized that peripheral inflammatory nociceptive processes sensitize the central nervous system (CNS) via pro-nociceptive CNS pathways and drive the appearance of comorbid FM [3]. This suggests that individual differences in how the brain responds to inflammation may be critical to understanding concomitant FM in RA.

We recently provided some of the first evidence that functional brain connections are substantially altered in RA patients with high levels of peripheral inflammation [4]. Here we expanded that effort by exploring the interaction between FM symptoms and inflammation on functional brain connectivity in the same cohort of RA patients. These analyses were designed to identify patterns of brain connectivity in RA that promote core symptoms of FM in response to inflammation. We hypothesized that higher inflammation would be associated with distinct changes in connectivity from the insula (a pro-nociceptive brain region) and left inferior parietal lobule (IPL; a region associated with the neural response to peripheral inflammation in RA patients) to other brain regions in patients with high levels of FM symptoms.

METHODS:

Participants:

RA patients were approached through a UK regional rheumatology service. Participants were considered eligible if they met the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria [5]. This study was designed to examine the neural correlates of fatigue in RA, therefore patients had to have a clinically significant level of fatigue for at least 3

months (defined as a score > 3 on the Chalder fatigue binary scale). The analyses presented here address alternate hypotheses regarding inflammation and centralized pain in RA. Exclusion criteria were contra-indications to MRI, left-handedness, or an alternative medical explanation for fatigue. A total of 73 patients fulfilled these criteria, and 54 RA patients (41 female) completed the entire study. The North of Scotland Research Ethics Committee gave ethical approval for the study. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Clinical evaluation:

All consenting participants underwent a clinical evaluation and a functional MRI (fMRI) brain scan. FM diagnosis was assessed using the ACR FM survey criteria which combines a measure of widespread pain (number of painful sites [0-19]) with a symptom severity scale (e.g., fatigue, subjective cognitive problems, headache, poor mood; scores range from 0-12) [6]. A combined score of 13 or greater was used as the diagnostic cutoff for FM [6]. 27 RA patients met criteria for FM (FM+) and 27 RA patients did not (FM-). Inflammation was measured using the erythrocyte sedimentation rate (ESR), a measure of systemic inflammation, which is commonly used to diagnosis and monitor chronic inflammatory diseases such as RA. A trained phlebotomist performed a venous blood draw during normal clinic hours (9 a.m. – 5 p.m.) and the blood was immediately processed and analyzed for the calculation of ESR (Westergren method).

fMRI Data Acquisition and Preprocessing:

fMRI data were collected using a 3 Tesla scanner (Achieva X-series, Philips Medical, Best, The Netherlands) and 8 channel phased array head coil. Each participant completed an 11-minute functional scan while performing the Paced Auditory Serial Attention Test (PASAT), a validated measure of cognitive function. The PASAT was given in a block design with 3x3 minute 'on' periods, interspersed with 4x30 second rest or 'off' periods. During the 'on' periods, a series of numbers were audibly presented. Participants were asked to sum consecutive numbers and to press a button every time the numbers summed to 10. Concurrently, patients were shown a distracting visual stimulus of random, rapidly changing numbers meant to increase task difficulty. The functional images were acquired by a using a three-dimensional (3D) T2*-weighted gradient-echo single-shot echo-planar imaging pulse sequence with the following parameters: TR = 3000 ms, TE = 30 ms, flip angle (FA) = 90°, in-plane SENSE acceleration 2, field of view (FOV) = 240 mm, matrix size 128×128 with 30 slices, 1.88×1.88×5 mm^3 voxels and 226 volumes. A high-resolution structural T1-weighted fast-field echo 3D structural scan was collected for normalization (TR = 8.2 ms, TE = 3.8 ms, TI = 1018 ms, FA = 8°, FOV = 240 mm, matrix size 240×240 with 160 slices, and 0.94×0.94×1 mm^3 voxels).

fMRI data were preprocessed using SPM8 (Wellcome Department of Cognitive Neurology, London, United Kingdom) running on MATLAB R2014a (Mathworks, Sherborn, MA, USA), as previously described [2, 4]. Briefly, the first four volumes were discarded to avoid equilibration effects, and the remaining 222 functional images were realigned to the first. The structural image was co-registered to a mean functional image and then segmented. The structural and functional scans were normalized to the standard SPM Montreal Neurological Institute template grey prior probability map via the individuals segmented grey matter image. Functional scans were then smoothed with an 8-mm full-width half-maximum Gaussian kernel.

Seed to whole brain connectivity analysis:

In these same RA patients, we have shown that DMN - left mid/posterior insula connectivity is associated with the FM survey criteria score [2], and that the left IPL is more connected to multiple brain networks in patients with high levels of inflammation [4]. To determine how FM and inflammation interact in the brain, we used these same left insular and IPL clusters as seed regions (Fig 1A and Fig 2A). The preprocessed functional data were entered into the Functional Connectivity Toolbox (CONN; Cognitive and affective neuroscience laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA; www.nitrc.org/projects/conn) v15. As previously described [2, 4], a nuisance regression model using the CompCor method was performed with six subject-specific motion parameters, the signal from white matter and CSF, and their first order derivatives included as confounds. A band pass filter (0.01-0.1 Hz) was applied to remove linear drifts and high frequency noise in the data. Task and rest onset periods were modelled and concatenated in first-level analyses to generate beta maps representing connectivity between the insula and IPL and the rest of the brain during task and rest conditions. The resulting beta maps were passed onto second-level group analyses in SPM8. Rest periods were not analysed at the group level because the summed resting condition (2 minutes) is too short for reliable connectivity estimates [7].

The FM+ and FM- groups were assessed independently using multiple regression models in SPM8. In each group we assessed the relationship between seed-to-whole brain connectivity and ESR with age and sex included as covariates of no interest. The resulting maps were thresholded at an uncorrected voxelwise level of $p < 0.001$, and significance was set at $p < 0.05$ family wise error (FWE) cluster corrected for multiple comparisons. The average fisher transformed r values of significant clusters were extracted from the first level beta maps for each subject for secondary analyses to test for interaction effects.

Post-hoc Interaction Analyses:

To formally test the possibility of interaction effects, we examined whether the relationship between extracted connectivity values from significant clusters (as described above) and ESR changes as a function of FM total scores. Total scores, rather than FM clinical cutoffs were used as we have previously shown that this construct can be used continuously[2]. ESR and FM survey scores were standardized and centered. These analyses were conducted with general linear models in R version 3.4.4.

Finally, we performed post-hoc Spearman rank-order correlations between functional connectivity and CRP to confirm the relationships seen with ESR.

RESULTS:

Clinical characteristics:

The clinical characteristics of this sample has been described previously [2, 4] and are shown in Supplementary Table 1 for FM+ and FM- groups separately. 27 RA patients met criteria for FM. The mean FM score in the FM+ RA patients was 17.89 ± 4.77, and 8.52 ± 3.19 in the FM- patients. There was no significant difference between FM+ and FM- RA patients in age, gender, or ESR.

Seed to whole-brain connectivity analyses:

In FM+ RA patients, higher levels of peripheral inflammation were associated with increased functional connectivity between the insula and the left mid frontal gyrus (MFG) and the left IPL (Fig 1B-D, Table 1). Further, in FM+ RA patients, higher peripheral inflammation was associated with increased functional connectivity between the left IPL and multiple cortical regions, including the dorsal anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), right MFG, right superior frontal gyrus/supplementary motor area and left IPL (adjacent to the seed IPL region) (Fig 2B-D, Table 1). Of the seven connections associated with ESR in FM+ patients, four were significant or marginally significantly associated with CRP (Supplementary Table 2).

In FM- RA patients, higher levels of peripheral inflammation were correlated with lower functional connectivity between the insula and right IPL, and higher functional connectivity between the IPL and left superior temporal gyrus (Table 1).

It is possible that other aspects of disease activity, such as the swollen and tender joint counts (SJC, TJC) that are incorporated into the DAS28, could be affecting the results. We therefore conducted partial correlation analyses between the extracted

connectivity values and ESR while controlling for SJC and TJC, separately in the FM + and FM – groups. There were no appreciable changes in the strength of the association between ESR and connectivity in these analyses (data not shown).

Additionally, adjusting for BMI did not alter the associations between ESR and connectivity in either group (data not shown).

Post-hoc Interaction Analyses:

There was a significant interaction effect between FM scores and ESR for each extracted connectivity value (all $p < .05$; Table 1) except for the IPL to R MFG ($p = .317$), suggesting that the interaction between FM symptoms and inflammation is important whether or not a patient meets epidemiological criteria for FM.

DISCUSSION

In RA, peripheral inflammation appears to interact with FM status on measures of brain functional connectivity. In our previous study [2] we demonstrated that a neurobiological signature of pain centralization, enhanced insula to DMN connectivity, is also present in RA. Importantly, across the overall sample, this connectivity was not significantly associated with peripheral inflammation. Here we show for the first time that inflammation is neurobiologically associated with other more established patterns of pro-nociceptive connectivity in RA, but selectively in those patients with more FM symptoms. These effects were apparent when FM was measured as a continuous construct, suggesting that FM symptoms may have important neurobiological correlates whether or not a patient meets clinical criteria for FM as we have previously reported [2]. This suggests that there is an interaction between peripherally enhanced sensitization and centrally-maintained sensitization that contributes to FM symptoms in RA. Put simply, *how* the brain responds to peripheral inflammation may be critical for understanding why some RA patients have high levels of comorbid FM symptoms while others do not.

The insula is a multi-modal sensory processing region and critically involved in pain perception [8]. In FM+ RA patients, increased insula – left IPL connectivity was positively correlated with peripheral inflammation. The IPL is a key node of the DMN and heightened DMN-insula connectivity is an established neurobiological feature of FM [8], and in these same RA patients with high levels of FM symptoms [2]. In FM+ RA patients, we would expect to see increased DMN-insula functional connectivity, but the relationship with peripheral inflammation observed here is novel. Interestingly, this left IPL region overlapped with the IPL cluster we previously identified as being more strongly connected to multiple brain networks in patients with high peripheral inflammation [4].

Using the IPL cluster from our previous study [4] as a seed region, we found that IPL – dorsal ACC and mPFC functional connectivity was positively associated with inflammation only in FM+ RA patients. It has been previously shown that FM patients have higher activity in the dorsal ACC compared to healthy participants during a painful task [9]. A previous study in RA patients found that the dorsal ACC, mPFC and left IPL were activated during a provoked joint pain paradigm, and that the mPFC and left IPL were functionally connected during this painful task [10]. In this context, our results may indicate preliminary evidence of the integration of inflammation-linked brain connectivity and classic pro-nociceptive brain connectivity.

In FM- RA patients, insula – right IPL functional connectivity was negatively correlated with peripheral inflammation, while in FM+ patients there was a positive correlation with the left IPL. There is evidence of hemispheric asymmetry in IPL function [11] and in the susceptibility to neurodegeneration. For instance, the left hemisphere is affected earlier and more severely in Alzheimer's disease and the left (compared to right) IPL shows greater metabolic dysfunction in early dementia [11]. The left IPL may be more susceptible to peripherally enhanced sensitization which is why inflammation-associated connectivity changes in this region are predominately in patients with comorbid FM.

This is the first study to examine patterns of inflammation-associated brain connectivity in the context of concomitant FM in RA. Although the evidence is limited, FM symptoms co-existing with another disorder is thought to constitute two broad processes. First, a 'bottom-up' form of central sensitization driven primarily by ongoing nociceptive input and so somewhat responsive to treatments that target peripheral nociceptive drivers. Second, a 'top-down' central sensitization sub-type which is primarily central in origin and so unaffected by manipulations of peripheral nociception [3]. Consistent with this hypothesized distinction, a previous osteoarthritis study indicated that successful joint replacement surgery resulted in a substantial improvement of responses to experimental pain measures outside the surgical site and patients whose surgery eliminated their pain showed a more normal inhibitory response to evoked pain than they did prior to surgery [12]. In RA, the analogous phenomenon would involve improvement in concomitant FM symptoms following successful inflammatory inhibition. Future studies will focus on patterns of inflammation-associated brain connectivity that predict improvement in concomitant FM after inflammation-dampening therapy.

This study has limitations. First, all of the RA participants in this study had significant levels of chronic fatigue which may not necessarily be representative of all patients and could have biased the sample towards a centralized pain phenotype. Second, we examined functional connectivity during the performance of a cognitive task from two seed regions based on earlier results in the same sample. Future studies should attempt a whole-brain agnostic approach in an independent sample while

participants are at rest or performing other tasks. We do not know when patients developed FM symptoms relative to the onset of RA; therefore, some patients may be part of a different phenotype wherein 'top-down' central sensitization exists prior to RA diagnosis. We have made the reasonable assumption that our measure of systemic inflammation (ESR) is derived entirely from the well-established peripheral immune dysfunction which characterizes RA. However, considering the evolving evidence supporting a neural inflammatory reflex [13], it is also possible that altered brain connectivity drives peripheral inflammation, perhaps via endocrine pathways. It would be intriguing to evaluate the responsiveness of the identified pro-nociceptive connectivity in the context of a peripherally directed anti-inflammatory therapeutic, as mentioned above. In a small study of TNF inhibition (TNFi) in RA, Rech et al. (2013) demonstrated that pro-nociceptive brain connectivity decreased rapidly (i.e., 3 days) after the commencement of TNFi treatment, and before a positive clinical response was observed [14], providing some support for the concept of bottom-up sensitization. Ultimately, the great clinical value of distinguishing 'bottom-up' mechanisms of central sensitization is the potential to mark out RA patients whose FM symptoms may improve with an enhanced peripheral immunosuppressive approach. In contrast, 'top-down' central sensitization would not be expected to respond to peripherally directed therapies, rather patients may receive benefit from centrally acting interventions. Until more definitive studies are conducted, these remain working hypotheses.

Our finding that peripheral inflammation enhances connectivity in established pro-nociceptive pathways provides preliminary neurobiological evidence for a distinct FM-like sub-type in RA. Identifying the neurobiological patterns associated with different forms of central sensitization of FM in RA may help refine therapeutic targets.

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Figure Legends:

Figure 1. Increased brain connectivity between the left mid/posterior insula (A) and the left IPL (B) was correlated with higher levels of peripheral inflammation in RA patients that met criteria for fibromyalgia (FM+). The inset shows the overlap of the left IPL cluster with previous results [4] showing that numerous brain networks were hyperconnected to the same region of the IPL in RA patients with high inflammation. Scatterplots of the correlations between ESR and insula – IPL connectivity are depicted separately for FM+ and FM- RA patients in (C). There was also a significant positive relationship in FM+ RA patients between insula – left MFG connectivity and peripheral inflammation (D).

IPL, inferior parietal lobule; MFG, middle frontal gyrus; ESR, erythrocyte sedimentation rate; Ins, insula; FC, functional connectivity

Figure 2. In FM+ RA patients, there was a significant relationship between peripheral inflammation and functional connectivity between the left IPL and the dorsal ACC, mPFC, left IPL (adjacent to seed region), right SFG/SMA and right MFG (B). Scatterplots of the correlations between ESR and IPL – mPFC and IPL – dorsal ACC connectivity are depicted in (C) and (D), respectively.

IPL, inferior parietal lobule; dACC, dorsal anterior cingulate cortex; mPFC, medial prefrontal cortex; SFG, superior frontal gyrus; SMA, supplementary motor area;

MFG, middle frontal gyrus; ESR, erythrocyte sedimentation rate; Ins, insula; FC, functional connectivity

Table 1: Associations between seed-to-whole brain functional connectivity and ESR

FM+					
Seed region (direction of association)	MNI coordinates (x, y, z)	Z score	Cluster size (voxels)	p-value, FWE	P value for post-hoc interaction
L Insula					
L mid frontal gyrus (+)	-36, 24, 32	4.38	161	0.007	0.049
L IPL (+)	-50, -50, 40	3.98	132	0.019	0.015
L IPL					
Bilateral Dorsal ACC/mPFC (+)	2 38 36	4.43	239	0.001	0.016
R mid frontal gyrus (+)	30 48 2	4.31	132	0.026	0.317
Bilateral SFG/SMA (+)	8 28 52	4.15	128	0.030	0.040
R mPFC/R Dorsal ACC (+)	12 60 30	3.88	349	0.000	0.002
L IPL (+)	-32 -48 46	3.76	135	0.023	0.016
FM-					
Seed region (direction of association)	MNI coordinates (x, y, z)	Z score	Cluster size (voxels)	p-value, FWE	P value for post-hoc interaction
L Insula					
R IPL/Precuneus/Angular (-)	28, -52, 44	4.51	175	0.002	<.001
L IPL					
L STG (+)	-50 -42 6	4.29	257	0.000	0.024