Imaging the effects of anti-tumour necrosis factor treatment on pain processing in rheumatoid arthritis

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1. **Introduction**

Rheumatoid arthritis (RA) is a chronic inflammatory disease with a relatively well-understood pathology. Pain is the most disabling symptom of RA, and for the majority of patients it is the main symptom they would like to reduce [1].

Proinflammatory cytokines, especially tumor necrosis factor (TNF), play a crucial role in the pathogenesis of RA [2] and are also important in the generation of pain [3]. TNF inhibition blocks the pro-inflammatory cytokine cascade and results in a significant improvement in general wellbeing, pain, joint tenderness and joint swelling within days from the initiation of treatment [2].

Functional magnetic resonance imaging (FMRI) is a non-invasive technique, which is used to study the mechanisms involved in perception and modulation of pain [4]. It has been suggested that these mechanisms are altered in chronic pain conditions [5, 6]. There have been few neuroimaging studies on pain processing in arthritis. Hess and colleagues reported increased pressure-evoked brain activation in five RA patients [7]. Jones and Derbyshire [6] described reduced heat-evoked brain activation in RA patients but other researchers did not observe any differences in response to heat between arthritis patients and healthy controls [8, 9].

TNF inhibition is very effective clinically but its central neurobiological effects are not well understood. The aim of this study was to investigate the modulatory effect of anti-TNF treatment on pain processing in RA and to examine whether neuroimaging can be used as a marker of treatment effect and a predictor of response to treatment. We expected that anti-TNF treatment would result in a reduction in brain activation in response to mechanical and thermal stimuli related to a decrease of inflammation and sensitisation.

2. Methods

2.2 Patients

Patients with active RA being considered for initiation of anti-TNF were consecutively recruited from the Nuffield Orthopaedic Centre, Oxford, UK. All patients had seropositive, erosive arthritis and fulfilled the American College of Rheumatology criteria for RA [10].

The study was approved by the Oxfordshire Research Ethics Committee and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all the participants at the beginning of the first visit.

Patients were excluded for the following reasons: 1) any neurological, psychiatric or medical condition that could affect the results of the study other than depression, which is a common co-morbidity in chronic pain [11]; 2) any medication acting on the CNS other than low dose antidepressants, such as amitriptyline < 25mg per day; and 3) contraindications for MRI.

At the time of the study, there were three TNF inhibitors approved for clinical use in the UK: infliximab (Remicade®), etanercept (Enbrel®), and adalimumab (Humira®). All three have comparable efficacy, despite differences in their structure and in their action at the molecular level [12]. Patients in this study received anti-TNF drugs either alone or in combination with non-biological disease modifying anti-rheumatic drugs. All patients were previously naïve to anti-TNF medication. The patients were on stable doses of the non-biological disease modifying anti-rheumatic drugs during the time of the study with exception of two patients: in one the dose of leflunomide was reduced, and in one leflunomide was stopped. One patient was diagnosed with depression and was treated with amitriptyline at stable doses. Patients were asked not to take their non-steroidal anti-inflammatory drugs for 24 hours before each scan.

2.3 Study design

This was an observational study of patients with active rheumatoid arthritis treated with anti-TNF drugs. The eligibility for the treatment and subsequent response to therapy was assessed by rheumatologists from the Nuffield Orthopaedic Centre. At three months, the rheumatologists classified the patients as responders or non-responders depending on reduction of DAS28-ESR and low current disease activity [13]. The researchers responsible for the imaging part of this study were not involved in patients' care and the treating physicians where blinded to the imaging results.

Patients were scheduled for scanning on three occasions: at the baseline (BL) visit before the anti-TNF treatment, at the short-term follow-up visit (ST) scheduled between two and four weeks after starting treatment, and at the long-term follow-up visit (LT) between six and ten months after the start of the treatment. The time windows for the follow-up visits were chosen based on the results of clinical studies that have demonstrated a significant reduction in inflammation after two weeks [14] and the maximum effect of treatment usually by six months [15].

At their BL visit, all participants underwent a comprehensive medical assessment including co-morbidities and medication. At the BL and the follow-up visits, the patients were asked to rate their average daily pain intensity (DPI) on an 11-point verbal Numerical Rating Scale (NRS) where 0 represented "no pain" and 10 "the worst pain imaginable" [16]. Disease activity was assessed using the Disease Activity Score in 28 joints (DAS28-ESR) [17], which includes tender joint count (TJC), swollen joint count (SJC), the erythrocyte sedimentation rate (ESR), and the patient's subjective rating of their general health (GH). The blood samples for ESR were taken at the end of each visit to avoid the effect of a venepuncture pain on the scanning session. The duration of early morning stiffness of the joints (EMS) was also recorded [18]. All participants were asked to complete the Beck Depression Inventory (BDI) [19] and the Pain Catastrophising Scale (PCS) [20] questionnaires as both depression and catastrophising may influence the pain experience [16].

2.4 Stimulation

Mechanical pressure of the most tender joint of the patient's right hand was used to evoke clinically-relevant pressure pain. This joint was compressed with a purpose-built, MRI-compatible device, which consisted of a 1 cm^2 rubber probe attached to a spring and a piston. The intensity of the stimulus was identified by using the method of limits [21] so that it reliably evoked moderate pain (PRp) of 5–6 on the 11-point NRS. This stimulus intensity was the same for all the visits.

Heat stimulation was used as a control condition to examine changes in pain processing that were independent of the peripheral disease process. Heat stimulation was delivered using an in-house built, MRI-compatible thermal resistor with a contact area 1.5 x 2 cm and a fast ramp time (from 30° to 60° Celsius in 0.8 s). The thermode was attached to the volar surface of a patient's right forearm. The pressure and temperature required to evoke moderate pain 5-6 on the NRS was established outside the scanner at the baseline visit using the method of limits and was stimulus-locked for all the visits[21].

During the scanning session the pressure and heat stimuli were repeated 10 times (duration 2 s, inter-stimulus interval jittered between 50 and 70 s to avoid the effect of anticipation and habituation). After each scan, subjects were asked to rate the average intensity of pain evoked by the noxious stimulation on the 11-point NRS. After the functional scans with noxious stimulation, the structural scan was acquired for anatomical reference. The imaging session ended with a visual stimulation used as a simple sensory paradigm to assess non-specific effects of medication on brain activation. It consisted of viewing of 10 blocks of black and white checkerboards flickering for 15s at a frequency of 8 Hz alternated with blocks of rest for 15s. The stimulus was generated using Presentation software v.11.0.

2.5 MRI data acquisition

All imaging was performed at the Oxford Centre for Clinical Magnetic Resonance Imaging Research (OCMR) at the John Radcliffe Hospital, Oxford, UK. Data were acquired using a 3T Tim Trio Siemens MR scanner with a single channel head coil. A structural scan was acquired using an MP-RAGE sequence with the following parameters: TR = 2,040 ms, TE = 5.56 ms, TI = 1,100 ms, flip angle 8°, field of view 192x160mm, voxel size 1x1x1 mm.

Functional data were acquired using a standard whole-brain gradient echo EPI sequence with the following parameters: TR = 3 s, TE = 30 ms, flip angle 90°, 36 axial slices covering the whole brain, field of view 192x256 mm, matrix size 64x64, voxel size 3x3mm in plane and 3.3 mm slice thickness.

2.6 Analysis of clinical, psychological, and psychophysical data

Treatment-related changes between the BL, ST and LT visits were analysed using the Wilcoxon Signed Ranks test (2-tailed). For comparisons between the patient groups, the Mann-Whitney U test (2-tailed) was used. Non-parametric tests were chosen because pain ratings were ordinal and many of the other measures were non-normally distributed.

2.7 Analysis of imaging data

The FMRI data were analysed using FEAT v.5.98, a software tool for model-based FMRI data analysis. FEAT is part of the image analysis package FSL v.4.1.7 (www.fmrib.ox.ac.uk/fsl)[22].

Single subject analysis

The non-brain structures were removed from the images using BET [23]. Then, the data were spatially smoothed with a Gaussian kernel with FWHM of 5 mm and normalised to the same mean intensity by a single scaling factor. A high-pass filter cut-off of 100 s was used to remove low frequency artefacts. Motion correction was performed with MCFLIRT [24], which aligns all data applying the rigid body transformation with 6 degrees of freedom (DOF) [24]. Furthermore, motion artefacts were identified and removed with MELODIC [25]. For each stimulus type, an expected response model was created by convolving the stimulus function with a standard Hemodynamic Response Function and then entered into the General Linear Model (GLM) and fitted to the data. This yielded a set of parameters quantifying the response to each of the stimuli. Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of $P=0.05$ [26].

Registration

All images were registered to a standard structural space (MNI152). The registration of the functional data to a structural image was carried out by linear registration using FLIRT [24] with 6 DOF. The registration to the standard image was performed using FLIRT with 12 DOF and a non-linear registration method, FNIRT [27]. In order to improve the registration between the sessions, the functional data were co-registered between the visits and registered non-linearly to the standard space using one common transform. This resulted in a better realignment of data as the fit was similar for all visits.

Group analysis

The higher-level analysis was performed using FLAME2 with automatic outlier detection [28, 29]. To assess effects of treatment on brain activation between the baseline and the follow-up visits we performed a two-sample paired t-test in all 15 responders. Furthermore, we investigated the correlations between changes in brain activation and changes in clinical and psychological variables using a three-level analysis; for each patient a difference between the baseline and the follow-up visit were analysed using a paired, fixed-effects model, and then the results were entered into a higher-level model together with changes in the variable of interest. To control for possible non-specific drug effects on brain activation, a response to the visual task was compared using a paired t-test between the baseline and follow-up visits.

3. Results

3.1 Patients' characteristics

Twenty-three RA patients (18 female; median age 63 years, interquartile range - IQR 22; median disease duration 17 years, IQR 38.5) completed the

BL and the ST visit (Table 1). Three patients withdrew from the study for non-clinical reasons after the ST follow-up visit and were lost to follow-up. Five patients did not respond to the prescribed anti-TNF therapy and had their medication changed by their treating physicians after three months of treatment. The remaining 15 patients (11 female; median age 64 years, IQR 18; median disease duration 17 years, IQR 16) continued the treatment with the original anti-TNF drug and attended the LT follow-up visit. The median time between the BL and the ST visit was 18 days (IQR 7), and between the BL and the LT visit was 6 months (IQR 4). All patients scored over 26 on the Mini Mental State Examination [30] and did not show any signs of cognitive impairment.

3.2 Treatment-related improvement in clinical and psychological measures

In the 15 patients who responded to the treatment and completed the study, all outcome measures decreased significantly with anti-TNF treatment (Table 2). There was an early reduction in all the measures, except for the catastrophising. A further marked decrease at the LT visit was present for all the measures except for PRp, PRt, SJC, and EMS.

Decrease in pain ratings for pressure and thermal stimulation between the BL and the ST visit did not correlate with changes in any of the clinical or psychological measures. Measures of improvement, such as the Patients' Global Impression of Change score and a change in general health, correlated positively with a reduction in DAS28-ESR (Spearman's rho=0.69 p=0.004 and rho=0.74 p=0.002, respectively). There was also a strong positive correlation between a reduction in ESR and a reduction in BDI (rho=0.72 p=0.002) and a trend for a positive correlation between a decrease in DAS28-ESR and in BDI (rho=0.44 p=0.1).

Between the BL and LT visit, there was a correlation between reduction in DAS28-ESR and decrease in PRp (rho=0.58 p=0.02) as well as a trend for correlation with a reduction in GH (rho=0.48 p=0.07) and in ESR (rho=0.46 p=0.08). A decrease in ESR also correlated with a reduction in general health (rho=0.66 p=0.007).

The pressure stimulus was no longer painful (PRp $\leq$1) for three patients at the ST follow-up visit and for further four patients at the LT visit. There were no differences between the patients with and without pain at the LT visit, except a significantly lower PRp at the LT visit in the group without pain (N=7) (the Mann-Whitney test Z=-3.3 p<0.0005) and a larger decrease in DAS28-ESR and in PRp between the BL and the LT visit (the Mann-Whitney test Z=-2.5 p=0.009 and Z=-3.2 p=0.001, respectively) as well as a significantly lower PRp at the LT visit (the Mann-Whitney test Z=-3.3 p<0.0005).

Five patients who did not respond to the treatment did not differ from the 15 responders in the baseline values of any of the clinical and psychological measures except for a significantly higher TJC and EMS in non-responders (the Mann–Whitney test Z=-2.4, p=0.015 and Z=-2.1 p=0.04). Reduction in DAS28-ESR between the BL and the ST visit was significant in responders and non-responders (the Wilcoxon signed rank test Z=-3.4 p=0.001 and

Z=-2.0 p=0.04, respectively). As the non-responders had their anti-TNF medication stopped or altered they were excluded from the LT follow-up analysis.

3.3 Decrease in pressure-evoked brain activation after treatment

Between the BL and the ST visit, 20 patients (15 responders and 5 non-responders) showed a reduction in pressure-pain evoked brain activation bilaterally in the primary somatosensory and primary motor cortex, the right secondary somatosensory cortex, and the dorsal part of the anterior cingulate cortex (Figure 1A). Between the BL and the LT visit, responders (N=15) demonstrated a marked decrease in brain activation bilaterally in the primary somatosensory and primary motor cortex, the secondary somatosensory cortex, the premotor cortex, the insular cortex, the dorsal part of the anterior cingulate cortex, the thalamus, the brainstem as well as the hippocampus and the amygdala (Figure 1B). There was no increase of pressure-evoked brain activation after treatment. There were no significant correlations between changes in the pressure-evoked activation and changes in DAS28-ESR between the baseline and the follow-up visits.

We also used the presence of pain in response to pressure stimulation at the LT visit as a covariate of interest in an analysis in the responders group (N=15). Between BL and the LT visit, patients without pain (N=7) had more extensive reduction of activation bilaterally in the amygdala, the hippocampus, the brainstem, the secondary somatosensory cortices, the dorsal part of the anterior cingulate cortex, the thalamus, the precuneus, the posterior cingulate cortex as well as the primary somatosensory and primary motor cortices (Figure 1C). In patients who reported pain (N=8) a decrease in activation was less extensive and changes were present in the right amygdala, the right insular cortex, the left secondary somatosensory cortex, and bilaterally in the primary somatosensory and primary motor cortex (Figure 1D). The statistical analysis was not sensitive enough to detect differences in treatment-related activation changes between patients with and without pain. At the LT visit, patients with pain had a stronger activation in the right posterior insular cortex, the right hippocampus, and the precuneus.

We also analysed changes in pressure-pain evoked activation at the baseline using the response as a covariate of interest. For standard mixed-effects analysis there were no significant differences between the responders (N=15) and non-responders (N=5) in the activation at the baseline. However, for fixed-effects analysis, there was a stronger activation in the responders group in the right amygdala and the right anterior insular cortex as well as bilaterally in thalamus, the primary and secondary somatosensory cortices (Figure 1E). Therefore, despite lower TJC and no significant differences in other clinical measures, responders demonstrated more extensive pressure-evoked brain activation at baseline. It seems likely that the mechanisms that are targeted by the anti-TNF are enhanced in the patients who respond to the treatment. Although fixed-effects results cannot be generalised to the whole patient population, brain activation at baseline may potentially be used to identify patients who will respond to the treatment. This effect seems to be specific to clinically relevant pressure-induced pain as it was not present for the heat-evoked pain.

3.4 Increase of heat-evoked brain activation after treatment

There were no changes in heat-evoked brain activation between the BL and the ST visit (N=20). In fifteen responders the activation was significantly stronger at the LT visit than at the BL visit in the precuneus, the posterior cingulate cortex, as well as left prefrontal and temporal cortices. These changes in brain activation did not correlate with changes in DAS28-ESR.

3.5 No treatment effect on visual task

There were no differences in the activation in response to the visual task after the treatment, which suggests that anti-TNF did not have a global effect on brain activation.

4. Discussion

We used brain imaging to study the effects of anti-TNF treatment on processing of evoked pain in RA patients and to investigate whether neuroimaging could potentially be used for identifying patients who will respond to treatment.

As expected, within the first month of treatment, there was a marked reduction in ESR, DAS28-ESR and DPI, which is consistent with the results of earlier clinical studies [14]. The subjective measures of improvement, Patients' Global Impression of Change and change in general health, correlated positively with a reduction in DAS28-ESR. There was also a relationship between a decrease in ESR and BDI. What is remarkable, at the ST visit, changes in pain ratings did not correlate with decrease in any of the assessed clinical and psychological variables. A reduction in pain ratings for pressure as well as for heat at the ST visit may be partly related to reporting bias as patients expecting an improvement give lower pain ratings to match their expectations [31]. This was an observational study; therefore, there was no placebo arm and the results may be confounded by patients' expectations. Between the BL and the LT visit, a decrease in PRp correlated with a reduction in DAS28-ESR. However, there was no direct relationship with a change in ESR, which may be explained by the complex effects of anti-TNF on pain and inflammation. A decrease in ESR correlated with a reduction in PRt and general health, suggesting that the reported pain and general health depend on disease activity and inflammation.

Within the first month of treatment, there was a reduction in pressure-pain evoked activation but not in the heat-pain evoked activation. The effect of anti-TNF on mechanical hyperalgesia may be a result of reduction of inflammation in the periphery, including a decrease in swelling and tenderness of the stimulated joint, or systemic changes, including a reversal of inflammation-related augmentation of pain [14]. Moreover, some of the effects of anti-TNF treatment may be related to its direct action on nerve fibres or CNS, as the effects of the treatment on mechanical hyperalgesia occur faster and are stronger than its effects on inflammation [7, 32]. In this study, treatment resulted in early changes in the pressure-evoked pain, mainly in the regions encoding the sensory dimension of pain [33]. However, there was no reduction of activation in response to heat-evoked pain suggesting that anti-

TNF did not change central pain processing in general. Furthermore, PRp decreased early in the treatment but there was only a small further reduction at the LT visit, despite the fact that the DAS28-ESR and ESR were further reduced. A similar pattern was present for SJC corresponding to the amount of the inflamed joint tissue and effusion [34]. This further supports the role of the peripheral rather than central mechanisms during the first month of treatment.

Between the BL and the LT visit, there was a distinct decrease in pressure-evoked brain activation in all responders. Changes were present in the primary and secondary somatosensory cortices as well as the insular cortex, i.e., regions engaged in the representation of touch and pain [33]. The primary sensory cortex is thought to be involved in processing the discriminatory components of painful stimuli, as well as the perceived stimulus intensity, whereas the secondary somatosensory cortex encodes the noxious nature of the stimulus and has a somatotopy for nociceptive inputs [35]. The secondary somatosensory and insular cortices are also activated during hyperalgesic states [36] including clinical pain [37] and are involved in behavioural response to inflammation in chronic pain [38]. Long-term changes in pressure-evoked brain activation were present not only in the regions encoding the sensory aspect of pain but also in the thalamus, the brainstem, and bilaterally in the hippocampus and the amygdala. These changes could not be explained by a reduction in disease activity score or depression. A reduction of activation in the limbic structures as well as the brainstem suggests that treatment was also associated with changes in central pain modulation.

We also investigated whether the long-term changes in pressure-evoked brain activation were different in patients who did not report pain during pressure stimulation and those for whom the stimulus was still painful. In the group with pain, there was a decrease of activation mainly in the regions encoding the sensory dimension of pain. In the group with no pain, treatment resulted in a reduced activation not only in the somatosensory and the insular cortices, as in the group with pain, but also in the dorsal part of the anterior cingulate cortex, i.e., a region involved in the affective and motivational aspect of pain as well as bilaterally in the amygdala and the hippocampus. For these patients the stimulus was not only qualitatively different, i.e., less painful, but also quantitatively different, i.e., not painful and not unpleasant. At the LT visit, patients without pain showed less activation in the right hippocampus and the right posterior insular cortex. These regions have been previously reported to be involved in encoding the sensory aspect of pain [39]; therefore, it is pertinent that they differentiated patients with and without pain on joint stimulation.

The mixed-effects analysis was not sensitive enough to detect differences in baseline activation between patients who responded to treatment and those who did not. However, the fixed-effects analysis showed a stronger response to pressure in the right amygdala and the right insular cortex in the responders. This indicates that brain activation may be potentially useful to identify patients who respond to treatment.

The treatment-related effect in the amygdala and hippocampus is particularly

interesting as these regions are involved in processing of the affective dimension of pain [40] and their activation is typically reported in studies on anxiety or fear [41]. We could not investigate the effects of anxiety, as it was not specifically assessed in our study. However, we observed that the changes in the limbic regions became evident only later in the treatment and were present for the pressure-evoked but not for the heat-evoked pain. Moreover, patients with no pain at the LT visit showed less activation in the amygdala. Therefore, we suggest that the observed effects are specific for the pressure-evoked pain and are probably related to the stimulus not being painful and therefore, no longer threatening for patients, rather than to reduction of anxiety related to scanning or learning effect at the follow-up visit [42]. In addition to that, the amygdala and the hippocampus mediate the effects of proinflammatory cytokines on mood [43]. Correlations between activation in the amygdala and depression have been described in subjects with fibromyalgia [37] but were not apparent in our study. Finally, the significant reduction of pressure-evoked signal in the amygdala may also be related to improvement in more general sense. The amygdala is involved in processing sensory-discriminative aspects of pain, complex pain behavior, autonomic responses as well as in descending pain modulation, especially during chronic inflammation [44-47]. Activation in this region reflects changes in arthritic pain and pain behaviour after treatment [8, 46].

Interestingly, we did not observe marked decrease in pain ratings or in brain activation in response to thermal stimuli. This suggests that anti-TNF treatment and marked reduction in inflammation do not change processing of heat pain. The results of neuroimaging studies on heat pain in pain conditions are inconsistent. Increased responses to thermal stimuli have been reported in patients with fibromyalgia but not in patients with lower back pain or arthritis [8, 48, 49]. In contrast, Jones and Derbyshire [6] reported less extensive heat-evoked brain activation in RA patients and suggested that pain processing is altered by the ongoing inflammation. In our study, there was an increase in activation after treatment that was present mainly in the regions typically engaged in processing of attention, self-awareness, and integration of sensory information in relevance to self [50]. The posterior cingulate is also involved in pain processing in chronic pain states; however, it encodes the valence of stimuli rather than nociception [51]. The increase in the heat-evoked brain activation may be a result of patients paying more attention to the experimental pain and perceiving it as more self-relevant when the clinical pain decreases after treatment. These changes seem to be specific to the thermal pain as this effect was not present for the pressure or visual stimulation.

In this study, we demonstrated that TNF inhibition has different effects on processing of pressure- and heat-evoked pain stimuli. The effect of treatment on mechanical pain processing was present within the first month of treatment. Moreover, the activation at the baseline could potentially be used to identify responders. The analysis in this study was limited by small number of patients and lack of placebo control. Further studies are needed to understand mechanisms involved in clinical and experimental pain processing in chronic pain patients.

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Figure 1. Brain activation in response to pressure stimuli. Mixed-effects analysis, $Z > 2.3$, $p < 0.05$. *Z*-score bar for the parametric activation maps and axial slice numbers are shown at the bottom of the figure. Images are presented in radiological convention with the right side being on the left. **A.** Reduction in activation between baseline and the short-term follow-up visit in 20 patients (15 responders and 5 non-responders). Significant changes were present bilaterally in the primary motor (M1) and primary somatosensory (S1) cortex, the dorsal part of the anterior cingulate cortex (dACC), and the right secondary (S2) somatosensory cortex. **B.** Reduction in activation between baseline and the long-term follow-up visit in15 patients who responded to treatment. A significant decrease in activation was present bilaterally in the primary (S1) and secondary (S2) somatosensory cortices, the insular cortex (IC), the dorsal part of the anterior cingulate cortex (dACC), the thalamus (THAL), the premotor cortex (PREM), the precuneus (PRECUN), the amygdala (AMYG), the hippocampus (HIPP), and in the brainstem (BST). **C.** Reduction in activation between baseline and the long-term follow-up visit in 7 patients who responded to treatment and for whom the stimulation was not painful at the long-term visit. There was a decrease in brain activation bilaterally in the primary (S1) and secondary (S2) somatosensory cortex, the primary motor cortex (M1), the precuneus (PRECUN), the posterior cingulate cortex (PCC), the dorsal part of the anterior cingulate cortex (dACC), the thalamus (THAL), the amygdala (AMYG), the hippocampus (HIPP), and the brainstem (BST). **D.** Reduction in activation between baseline and the long-term follow-up visit in 8 patients who responded to treatment and for whom the stimulation was painful at the long-term visit. There was a decrease in brain activation in the right insular cortex (IC), and the right secondary somatosensory cortex (S2), the right amygdala (AMYG), and bilaterally in the primary motor (M1) and the primary somatosensory (S1) cortex. **E.** More extensive activation at baseline in responders (N=15) versus non-responders (N=5). Mixed-effects analysis, $Z > 2.3$, $p < 0.05$. Differences were present bilaterally in the primary (S1) and secondary (S2) somatosensory cortices, the primary motor cortex (M1), the thalamus (THAL), the right insular cortex (IC), and the right amygdala (AMYG).

Summary

TNF inhibition has different effects on processing of mechanical and thermal stimuli. Neuroimaging methods have the potential to explain the mechanisms involved in the treatment effects.

Figure

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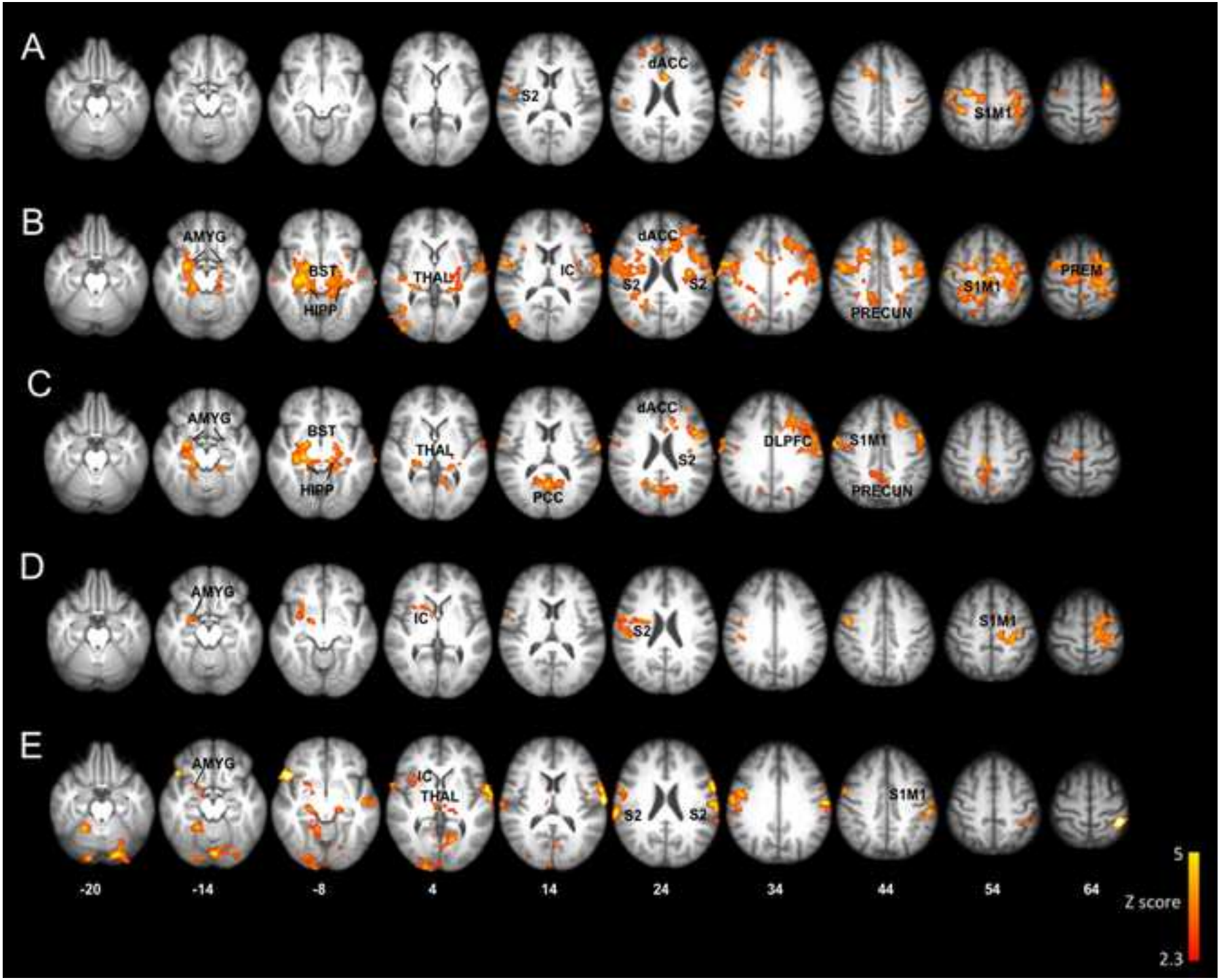

Table 1: Patients' characteristics

no	sex	age	dd	treatment	response	depression	DPI	DAS BL	DAS ST	DAS LT
1	F	69	10	Et	++	N	6.5	7	4.4	2.6
2	M	50	10	Ad	++	N	7	6.3	5.9	1.4
3	F	34	12	Et	++	N	3	6.9	4.6	4.1
4	F	46	26	Ad+MTX	++	N	7.5	4.8	2.9	1.8
5	F	53	17	Ad	++	Y	3.5	5.9	3.8	2.8
6	F	61	20	Et	++	N	6.5	5.6	5.7	3.5
7	F	68	18	Et	++	N	8.5	7.3	5.9	3.7
8	F	38	9	Et	+	N	6.5	5.9	5.2	4.2
9	F	66	40	Et	+	Y	6	6.8	4.8	4.1
10	F	65	7	Inf+MTX	+	Y	5	6.7	5.7	5.4
11	M	64	30	Ad	+	N	5	5.1	4	2.6
12	F	70	17	Inf+MTX	+	Y	8.5	5.7	5.6	4.1
13	M	57	35	Et+MTX	+	N	4.5	5.5	3.2	2.3
14	M	67	16	Ad	+	N	4	5.2	5	3.3
15	F	70	20	Et+MTX	+	N	7.5	7	5.7	5.2
16	F	63	9	Ad	-	N	8.5	8.2	6.2	X
17	F	39	1.5	Ad	-	Y	7.5	7.1	6.9	X
18	F	46	5	Et	-	N	5	5.3	4.1	X
19	F	80	18	Ad	-	N	8	6.5	5.9	X
20	F	76	15	Ad	-	N	7.5	6.8	X	X
21	F	38	2.5	Et	ø	N	7	5.7	X	X
22	M	68	25	Ad	ø	N	5	6.2	X	X
23	F	58	20	Et+MTX	ø	N	8	6.4	X	X

Abbreviations: F – female, M – male, dd – disease duration in years; Ad – adalimumab, Et – etanercept, Inf – infliximab, MTX – methotrexate, ++ – responders with no pressure pain at the long-term follow up visit, ++ – responders with pressure pain at the long-term follow up visit, - – non-responder, ø – patients who withdrew, Y – yes, N – no, DPI – daily pain intensity, DAS – disease activity score in 28 joints estimated using ESR; BL – baseline visit; ST – short-term follow-up visit; LT – long-term follow-up visit.

Table 2: Clinical, psychological, and psychophysical measures at each visit

Variable	BL	ST	LT	BL vs. ST	ST vs. LT	BL vs. LT
	Median (IQR)	*Median (IQR)*	*Median (IQR)*	*p value*	*p value*	*p value*
DAS28-ESR	5.9 (1.4)	5.0 (1.7)	3.5 (1.5)	0.001	0.001	0.001
TJC	11.0 (7.0)	10.0 (8.0)	4.0 (8.0)	0.005	0.002	0.001
SJC	11.0 (7.0)	8.0 (5.0)	3.0 (5.0)	0.004	0.07	0.001
ESR, mm/h	34 (29)	23 (23)	13 (11)	0.008	0.002	0.001
DPI	6.5 (3.0)	4.0 (3.0)	2.5 (3.5)	0.004	0.002	0.001
GH	70 (27)	35 (40)	20 (20)	0.001	0.01	0.001
EMS, min	54.7 (45.0)	10.0 (60.0)	0.0 (20.0)	0.005	0.09	0.001
PRp	5.0 (1.1)	3.0 (3.2)	2.0 (3.1)	0.002	0.07	0.001
PRt	6.0 (0.5)	5.5 (2.0)	5.5 (2.0)	0.006	0.4	0.1
BDI	8.0 (6.0)	6.0 (9.0)	4.0 (5.0)	0.02	0.03	0.001
PCS	10.0 (18.0)	11.0 (9.0)	3.0 (5.0)	0.06	0.003	0.005

Absolute values (median and interquartile range) at each visit and significance of changes between visits (p values of the Wilcoxon Signed Ranks test) for clinical, psychophysical and psychological measures in the 15 responders who completed all three visits. Abbreviations: BL – baseline visit; ST – short-term follow-up visit; LT – long-term follow-up visit; DAS28-ESR – disease activity in 28 joints; TJC – tender joint count; SJC – swollen joint count, ESR – erythrocyte sedimentation rate; DPI – daily pain intensity; GH - the global impact of RA on general health; EMS – duration of early morning joint stiffness; PRp – pain intensity of the pressure stimulus; PRt – pain intensity of the thermal stimulus; BDI – Beck Depression Inventory; PCS – Pain Catastrophising Scale.