Sex, quality of life and brain function in complex regional pain syndrome

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Chapter 5

Noxious heat elicits opposite responses in brain regions that mediate salience and affection in complex regional pain syndrome

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ABSTRACT

Background: The nature of altered processing of sensory stimuli and motor control in complex regional pain syndrome (CRPS) patients is poorly understood. In an earlier study in patients with CRPS we found no convincing alterations of brain structure or function in rest. In this study we therefore investigated how central brain networks for somatosensory, motor and behavioural phenomena in CRPS respond to external stimuli and applied a painful heat stimulus, which elicits hyperalgesia, a key characteristic of CRPS.

Method: During functional MRI scanning, we administered the heat stimulus to the affected hand of CRPS patients and the right hand of healthy controls. Brain activations were compared between the groups. Activation patterns that significantly differed between the groups were further analysed by measuring their functional connectivity with other brain areas using psychophysiological interaction analyses.

Results: Fifteen female CRPS patients and 16 female healthy controls were included in the final analysis. Patients rated the evoked pain significantly higher than healthy controls. In both groups, a significant bilateral activation of the insula, thalamus, anterior cingulate cortex and the secondary somatosensory cortex was seen. Additionally, in comparison to healthy controls, we found an activation of the left temporal parietal junction (TPJ) in CRPS patients, a brain area involved in salience detection. Furthermore, brain activations in the left TPJ were negatively correlated with activity in prefrontal cortices in CRPS patients, not in healthy controls.

Conclusion: While experiencing a painful heat stimulus, CRPS patients display increased salience detection in combination with opposite activation of brain regions involved in reducing the affective burden of pain.

INTRODUCTION

Complex regional pain syndrome (CRPS) is a neuropathic pain syndrome characterized by autonomic, sensory, trophic and motor disturbances of the affected limb. The pathophysiology is thought to encompass a pathologic host response to tissue injury, involving both the immune and nervous system, that in time leads to aberrant neuroplasticity of the spinal cord and brain[1,157].

In CRPS, findings on processing of sensory stimuli[49,158–160] and motor control[52–54] in the brain while patients are at rest, are inconsistent. Interestingly, several studies in CRPS reported widespread cerebral activation in somatosensory, attentional- and motor brain areas during mechanically induced allodynia[161,162] and altered emotional processing in response to electrically induced pain[158,163]. However, 2 of these studies[161,162] were uncontrolled and all studies presented results insufficiently corrected for multiple comparison. Therefore, we investigated brain responses to a moderately painful heat stimulus in CRPS patients and healthy controls.

The heath stimulus is designed to elicit slow temporal summation of C-fibre-evoked responses of dorsal horn neurons which induce hyperalgesia. This process, termed windup[164,165], is mediated by an upregulation of the N-methyl-D-aspartate (NMDA) receptor[166] which is thought to play a key role in the chronification of pain and a target for pain relief in CRPS[167]. Using this stimulus, we expect to be able to study networks involved in somatosensory, motor and behavioural processing adequately.

METHODS

Part of the method section has been published before[160]. In short:

Participants

Between May 2011 and March 2013, female CRPS patients followed up at the neurology outpatient clinic of the Leiden University Medical Center (LUMC) in Leiden, the Netherlands, who met at least the Budapest clinical criteria for CRPS type 1[168] in an upper limb were asked to participate in this study. Participants were excluded if they suffered from (serious) neurological illness, were younger than 18 years, male, had known psychiatric disorders or suffered from any condition other than CRPS that is associated with pain of functional impairment of an upper extremity.

A group of healthy, pain-free controls, age and sex matched to the CRPS patients were additionally included. Many were hospital staff from other departments, or (PhD) students not linked to our research group.

All participants were screened for MRI contraindications before MRI acquisition.

The study protocol was approved by the Medical Ethics Committee of the LUMC (protocol nr NL34614.058.11), and written informed consent was obtained from all patients and control subjects.

Demographic data and pain measurements

During the week prior to the investigation, patients completed questionnaires measuring pain (McGill Pain Questionnaire, MPQ)[77] and dexterity of the affected hand (Radboud skills questionnaire, RSQ)[131]. On the day of examination we collected data on demographic variables, pain severity experienced in the past week (numeric rating scale (NRS) 0-10, with 10 reflecting the worst pain imaginable), CRPS (CRPS severity score)[75] and loss of voluntary motor control such as dystonic postures (Burk-Fahn-Marsden scale)[132], decreased active range of motion, weakness and slowness of movement of the affected hand.

Pain administration

During fMRI scanning, repetitive heat pulses were applied to the affected hand in CRPS patients and the right hand of healthy controls. If CRPS signs were present in both hands, then the most affected hand was used. The heat pulses were applied by CHEPS (Contact heat evoked Potential stimulator, Medoc Advanced Medical Systems, Ramat Yishai, Israel). This device is capable of delivering extremely fast heating and cooling stimulation rates of the skin, 70 C°/sec and 40 C°/sec, respectively, due to a Heat foil Peltier thermode (HP). The HP thermode can stimulate a circular skin area of 27-mm diameter (5,73 cm2) and is composed of 2 layers: (1) an external layer that is composed of a very thin, fast heating foil with 2 electronic thermal sensors that can measure skin and thermode temperature and (2) a second layer consisting of a Peltier element. The rapid heating is induced in the first layer, the cooling in the second.

To elicit maximal windup, 8 trains of 9 repetitive heat pulses of 47 C° from a baseline of 30 C° were applied on glabrous skin on the dorsal side of the affected limb[164,165,169]. Before every run, 40 seconds of baseline activity was measured followed by 3 seconds of repetitive heat pulses (3Hz) and 47 seconds of rest. Thus, in total 90 seconds per run and 12 minutes of fMRI acquisition. We used the same temperature settings for all participants because we were interested in possible differences in responsiveness of brain activity to the same sensory stimulus. The maximum temperature of 47 C° was based on a previous study in fibromyalgia

patients[165] and was validated in a small trial (not published) in 6 healthy controls to validate pain scores and fMRI activity in response to the stimulus.

Pain ratings

Before and outside the MRI scanner, participants were asked to rate the pain induced by the thermode with 10 seconds apart using the NRS. The average of 3 measurements was recorded as pain score elicited by the thermode before the scanning procedure.

At the end of fMRI acquisition, patients were asked to report the mean pain score of the last heat pulse train using the NRS scale.

MRI acquisition

All scanning sessions were in the beginning of the evening. To prevent hearing loss due to loud scanner noise, participants received earplugs and wore headphones. Before commencing the experiment, first T_1-weighted, DTI and resting state fMRI scans were made of which the results were published previously[160].

Imaging data was acquired on a Philips 3.0 T Achieva MRI scanner using a 32-channel SENSE head coil (Philips Medical Systems, Best, The Netherlands). Structural T_1-weighted gradient-echo imaging (for registration purposes) was acquired with the following parameters: slices: 140, voxel size: 1.17 x 1.17 x 1.2mm, repetition time (TR) 9.8ms, echo time (TE) 4.6ms, flip angle 8, in-plane matrix resolution 256 x 256 slices, field of view 224. fMRI imaging was done with 38 slices, voxel size 2.75 x 2.75 x 2.75mm without a gap, TR 2400ms, TE 30ms, flip angle 80°, field of view 220.

MRI analysis

For the fMRI statistical analysis we used FSL v5.0, Oxford, UK[133–135] with FMRIB Expert Analysis Tool (FEAT). For the primary analysis, pre-processing of fMRI images incorporated mirroring ("flipping") the hemispheres of patients affected in (and stimulated on) the left arm to stack all "affected" hemispheres onto the same, left hemisphere (toolbox: FSLswapdim). A secondary analysis was done using the "non-flipped data" to rule out a "flipping bias" since the flipping of hemispheres is performed in 7 patients, and not in healthy controls. Motion correction was done using FLIRT[147], removing of physiological or scanner-related artefacts using MELODIC and Fsl_Regfilt [149,170], brain extraction with BET[140], spatial smoothing with a Gaussian kernel of 6-mm full width at half maximum and a high-pass temporal filtering of 0.01Hz. Images were registered to the high resolution T1-weighted images (12 df) and subsequently to standard space MNI-152[148]. Due to limited range of view, the cerebellum was not completely scanned in all participants, yielding incomplete data. Therefore, these data were excluded from further analysis.

We used FMRIB's Improved Linear Model (FILM)[171] for first level (individual) analysis of the pain stimulus with cluster z-statistic threshold 2.3, p<.05. For group level analysis we used FMRIB's Local Analysis of Mixed Effects (FLAME) stage 1[172] with covariate age. Single group averages (one sample T-test) and unpaired 2-group differences (two sample unpaired T-tests) were calculated. Correction for multiple testing was done at the cluster level using Family-Wise Error (FWE) with pre-threshold masking of the two group activation averages and z threshold >2.3 and $p < 0.05$.

For the additional analysis of task-specific functional connectivity between different brain areas, we imputed the significant clusters found in the primary analysis as seeds in the psychophysiological interaction (PPI) analysis[173174]. PPI is a statistical MRI analysis that measures task-specific correlations of brain activity -positive or negative- between different brain areas and is therefore a measure of 'functional connectivity'.

In order to extract the BOLD fMRI signal time-course of the seed per participant, we first non-linearly transformed the region of interest (ROI) from standard space to native space. We then ran a new first level FEAT analysis with three regressors: the block design as the psychological regressor, the time course of the ROI as physiological regressor and lastly the product of the first two regressors ("interaction"). Using the acquired results, we performed a group level analysis as described above, cluster corrected for multiple correction using FWE at $p < 0.05$.

RESULTS

Participants

Data are presented as means ± standard deviation when not stated differently.

Nineteen female CRPS patients (Table 1) and 19 age-matched healthy female controls were included in the study. One patient (nr 19) could not complete the full scanning protocol due to nausea in the MRI scanner; one patient (nr 10) and one healthy control had to be excluded from the analysis due to significant motion artefacts; one patient (nr 11) refused to participate during the MRI procedure due to fear of significant increase of pain; in one patient (nr 13) and two healthy controls the fMRI protocol could not be completed due to technical errors. Therefore 15 CRPS patients (age 47.9 ± 10.9 years) and 16 healthy controls (age 49.0 ± 15.4 years: $t(30)=0.35$, $p=.80$) remained for the fMRI analysis.

All patients that completed the fMRI protocol had chronic CRPS, with a median [and inter quartile range] disease duration of 6.6 [IQR 2-12,5] years, and were affected in at least one

Table 1 Patient characteristics

CRPS patients	Age	hd	Affected side	Disease duration (months)	CRPS severity score (0-17)	Budapest criteria	BFM	Loss VMC	NRS (0-10)	MPQ (0-63)	RSQ (0-5)	Pain- and centrally acting medication
1	54	R	R	120	12	Research	16	yes	5	26	3.68	Paracetamol, tramadol, etoricoxib, pregabalin
2	67	L	R	63	14	Research	16	yes	8	15	3.80	Oxycontin, gabapentin, fexofenadine, betahistine
3	48	L	R	141	16	Research	29	yes	7	42	2.82	Temazepam, clonazepam
4	27	R	L	25	10	Clinical	0	no	7	24	2.82	Tramadol
5	34	R	R	69	6	Research	16	yes	6	18	3.26	Diclofenac
6	35	L	L	80	10	Clinical	32	yes	9	39	4.67	Oxycontin, baclofen, temazepam, oxycodon
7	56	L	R	35	12	Clinical	17	yes	6	29	3.36	Pregabalin, zolpidem
8	50	R	R	150	11	Clinical	18	yes	10	51	4.56	Morphine, diazepam, clonazepam
9	51	R	R	82	10	Clinical	12	yes	8	25	3.91	-
10★	64	L	L	121	11	Clinical	11	yes	8	18	3.20	Amitriptyline, paracetamol, diazepam
11★	37	R	L	84	14	Research	28	yes	8	28	3.80	Pregabalin, baclofen, paracetamol
12	50	L	L	324	12	Research	17	yes	8	26	2.93	Gabapentin, baclofen, zolpidem
13★	33	R	R	204	11	Research	33	yes	5	38	4.19	Pregabalin, paracetamol, diazepam
14	51	R	L	10	9	Clinical	0	no	8	29	2.59	Pregabalin, paracetamol, tramadol temazepam
15	55	R	L	360	13	Research	14	yes	5	27	2.76	Pregabalin
16	60	R	L	13	14	Research	12	Yes	7	25	3.77	Tramadol, gabapentin
17	37	R	R	24	8	Clinical	0	no	6	30	2.00	Pregabalin, amitriptyline
18	42	R	L	18	11	Research	0	no	9	-	2.63	Amitriptyline
19★	61	R	L	9	12	Research	4	yes	7	-	-	Paracetamol
Mean ± SD	48.1 ± 11.6			100.8 ± 100.5	11.5 ± 2.0				7.1 ± 1.5	28.8 ± 9.2	3.6 ± 1.1	

CRPS=complex regional pain syndrome; HD=hand dominance; BFM=Burk-Fahn-Marsden scale; Loss VMC = loss of voluntary motor control; NRS=numeric rating scale; MPQ=McGill pain questionnaire; RSQ=Radboud skills questionnaire; L=left, R=right

hand. The mean (and SD) pain intensity of the examined hand in rest of the patients was 7.1 ± 1.4 (NRS).

Pain scores CHEPS

Before the scanning procedure, CRPS patients rated the evoked pain significantly higher than healthy controls (NRS; CRPS: 5.3 ± 2.1, HC's 3.5 ± 1.5; t(30)=-2.78, p=.009).

Patients, and particularly healthy controls, rated the last stimulus train slightly higher, which resulted in a non-significant group difference in pain ratings (CRPS 5.8 ± 2.5, HC's 4.4 ± 1.6; t(30)=-1.78, p=.084). These pain scores in patients did not correlate with the MPQ or the NRS pain severity in the last week.

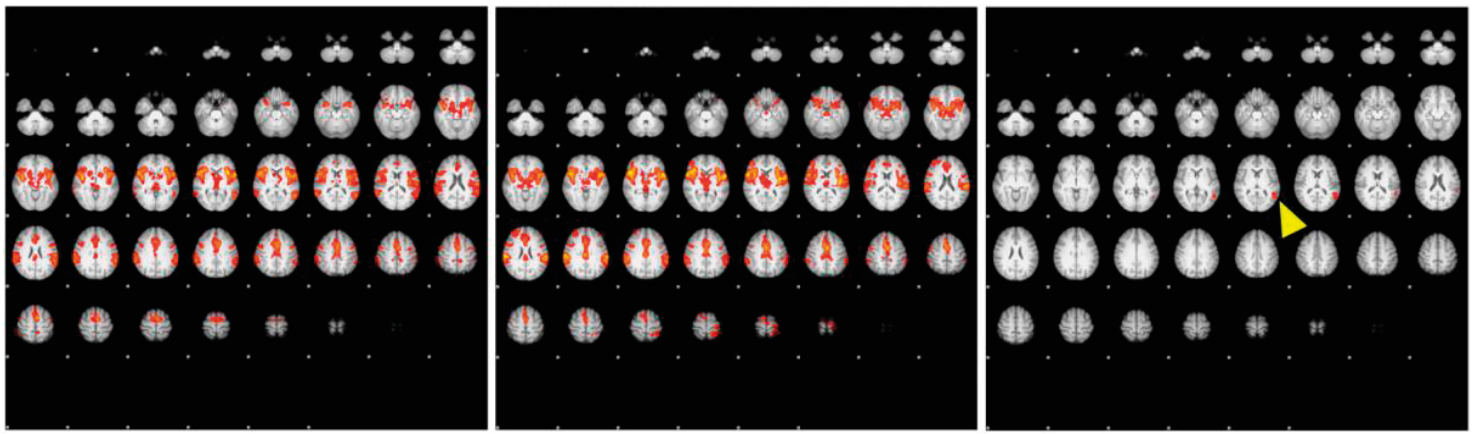
fMRI results

During stimulation of the affected or right hand, CRPS patients and healthy controls had significant and robust activation of bilateral insula, thalamus, anterior cingulate cortex, and bilateral activation of the secondary somatosensory cortex (figure 1a, table 2). In CRPS patients an additional bilateral activation of the temporoparietal junction (TPJ) was seen, of which the cluster on the left side was significantly more activated in comparison to healthy controls (figure 1b). The mean activation in this cluster correlated with disease duration (Pearson's r = .55, p=.03) in CRPS patients. No significant correlation was found with any of the pain scores.

In both groups, using the cluster of the left TPJ as a seed, positive functional connectivity was found with bilateral secondary somatosensory cortex, anterior cingulate cortex (ACC) and insula. Negative functional connectivity was found bilaterally in the posterior cingulate cortex, precuneus, inferior parietal lobule and occipital cortex. In contrast to healthy controls, CRPS patients showed an additional negative functional connectivity with bilateral medial and lateral frontal cortices next to more extensive activation of bilateral operculum (figure 2, table 3).

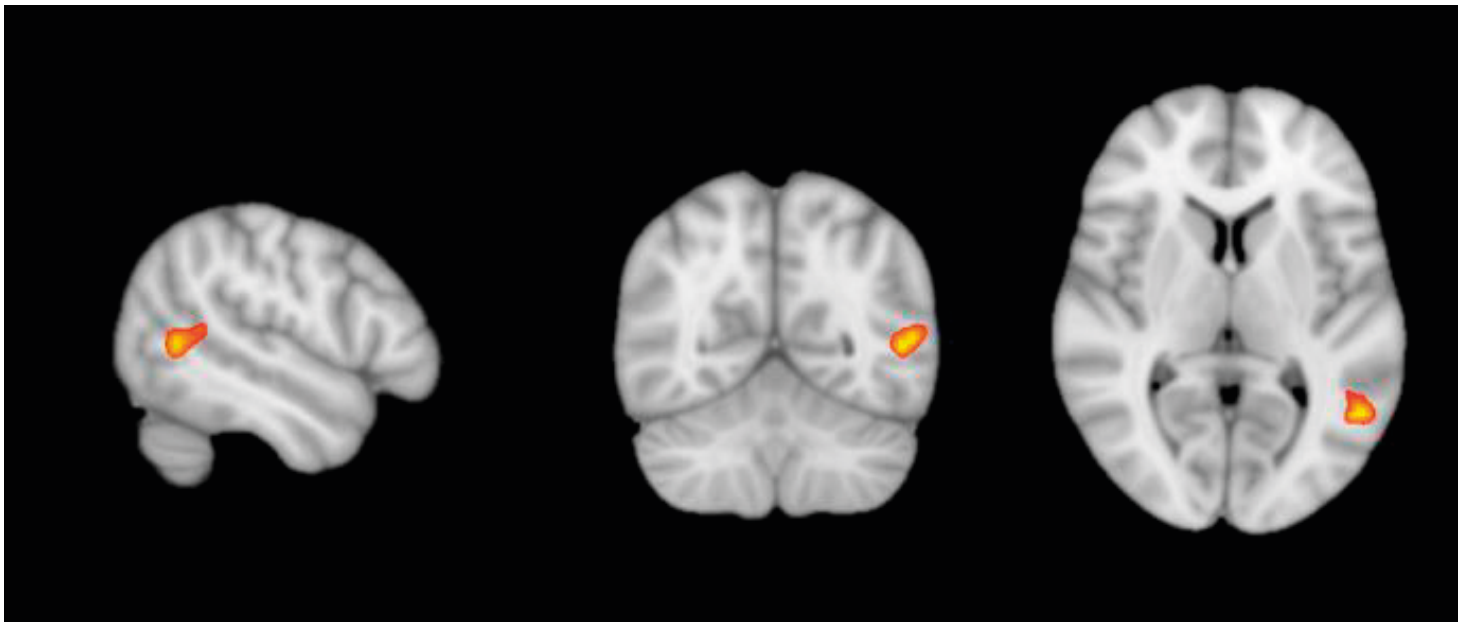
The secondary analysis of the "non-flipped" data showed a similar activation bilaterally in the TPJ, although no significant group difference was found. The correlation with disease duration was significant for the left TPJ (Pearson's r = .55, p=.034), but not for the right TPJ (Pearson's r = .48, p=.068). PPI analysis with the left TPJ as seed showed only in CRPS patients a negative connectivity with bilateral medial frontal cortex and left lateral frontal cortex, although this difference between the groups was non-significant. In this analysis, the difference in negative connectivity with the bilateral operculum decreased and was non-significant.

Figure 1a



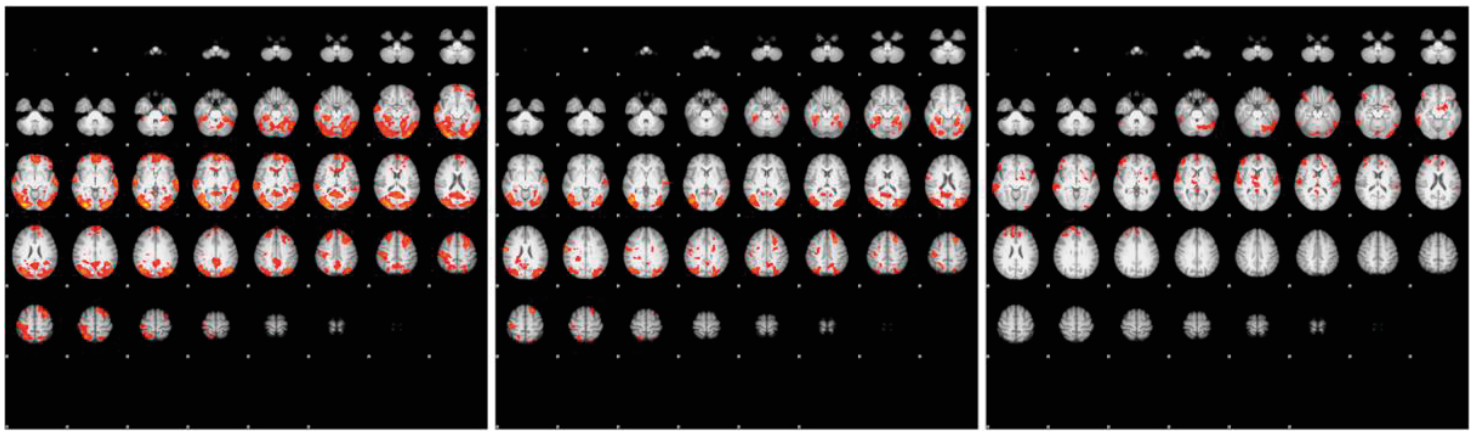
Brain activation during heat stimulus. Cluster threshold $z>2.3$, $p < .05$. In both groups robust activation of the bilateral insula, thalamus, anterior cingulate cortex, and secondary somatosenory cortices. Significant group difference was found in the left temporal parietal junction (yellow errow), see also figure 1b.

Figure 1b



Brain activation during heat stimulus, CRPS > HC. Cluster threshold z>2.3, p < .05. Coordinates: MNI (mm) X=-54,Y=-58, Z=8

Figure 2



Negative functional connectivity with left temporal parietal junction (see figure 1a &b; CRPS > HC). Cluster threshold z>2.3, p < .05. In contrast to healthy controls, the left temporal parietal junction in CRPS patients shows a negative functional connectivity with many frontal brain areas during the application of the heat stimulus, most noticeably with medial and lateral frontal cortices and bilateral operculum.

Table 2 Clusters of brain activation during heat stimulus, z-threshold > 4

CRPS

Cluster nr	Cluster size in voxels	Peak cortical region	Brodmann area	Maximum Z score	MNI (mm) max			FWE Corrected p-value
					X	Y	Z	
1	1720	l. insula	13	6.19	-34	14	4	<.000
2	1436	r. COP	13	5.78	44	18	4	<.000
3	1058	l. SMC/ ACC	6	5.43	-6	2	54	<.000
4	345	l. SMG	40	5.20	-60	-24	20	<.000
	321	r.SMG	40	4.89	68	-42	30	<.000
5	128	l. TPJ	39	5.05	-54	-58	8	<.000
6	82	r. TPJ	39	4.98	64	-52	14	<.000
7	38	l. FT	38	4.43	-32	8	-22	.004
8	20	r. SMC/ ACC	6	4.27	56	2	50	.015
9	14	r. amygdala	53	4.49	28	6	-22	.025
10	13	r. insula	13	4.34	40	-14	16	.028
11	13	l. SMC/ ACC	6	4.43	-48	0	38	.028
12	11	l. insula	ND	4.34	-36	-14	-10	.03
13	8	l. putamen	ND	4.28	-14	8	-6	.04

Clusters of brain activation during painful stimulus. For mean activation scores per group a z-threshold >4 was used to illustrate the plurality of regions in the brain that are active during the heat stimulus. Group *differences* are depicted with z-threshold of >2.3. CRPS = complex regional pain syndrome; MNI = Montreal neurological institute (brain model derived from mean 152 healthy persons); SMC=supplementary motor cortex; ACC=anterior cingulate cortex; SMG = supramarginal gyrus; TPJ = temporal parietal junction, FT = frontal temporal; COP = central opercular cortex; IFG=inferior frontal gyrus; M = mesencephalon; ND = not defined; FWE = family wise error

Healthy controls

Cluster nr	Cluster size in voxels	Peak cortical region	Brodmann area	Maximum Z score	MNI (mm) max			FWE Corrected p-value
					X	Y	Z	
1	2653	l. COP	6	6.35	-54	0	4	<.000
2	2192	r. COP	44	6.15	58	8	6	<.000
3	1137	r. ACC	24	5.87	0	-2	42	<.000
4	554	r.SMG	40	5.83	64	-24	24	<.000
6	273	l. amygdala	53	5.14	-20	0	-16	<.000
7	138	r. amygdala	53	4.95	18	0	-10	<.000
9	27	r. M.	ND	5.09	12	-26	-12	.009
10	23	r. IFG	46	4.36	44	42	8	.012
11	9	r IFG	44	4.12	56	12	22	.04
12	8	pons	ND	4.3	0	-20	-22	.04

CRPS > Healthy controls (z-threshold >2.3)

Cluster nr	Cluster size in voxels	Peak cortical region	Brodmann area	Maximum Z score	MNI (mm) max			FWE Corrected p-value
					X	Y	Z	
1	371	l.TPJ	39	3.92	-54	-58	8	.04

Table 3 PPI, negative functional connectivity with the left TPJ, z-threshold > 2.3

CRPS

Cluster nr	Cluster size in voxels	Peak cortical region	Brodmann area	Maximum Z score	MNI (mm) max			FWE Corrected p-value
					X	Y	Z	
1	33158	r.OC	18	5.27	28	-88	-12	<.000
2	5366	l.FPC	10	4.46	-26	68	4	<.000
3	2080	l.MFG	6	4.28	-28	16	56	<.000
4	1172	l.PC	7	3.7	-2	-38	68	<.000
5	1093	r.STG	41	4.35	60	-8	0	<.000

Healthy Controls

Cluster nr	Cluster size in voxels	Peak cortical region	Brodmann area	Maximum Z score	MNI (mm) max			FWE Corrected p-value
					X	Y	Z	
1	4737	r.OC	19	4.94	40	-72	2	<.000
2	4683	l.OC	19	4.31	-42	-74	8	<.000
3	937	l.SFG	8	4.5	-20	24	54	.001
4	868	r.PC	23	3.38	12	-54	10	.002
5	525	r.TFC	37	3.85	36	-36	-18	.043
6	521	l.STG	ND	4.01	-56	-6	-10	.044

CRPS > Healthy controls

Cluster nr	Cluster size in voxels	Peak cortical region	Brodmann area	Maximum Z score	MNI (mm) max			FWE Corrected p-value
					X	Y	Z	
1	4484	l.ITG	37	3.98	-52	-60	-24	<.000
2	2195	r.MFG	9	3.76	30	38	26	<.000
3	1279	l.COP	6	4.08	-56	0	2	<.000
4	892	r.COP	41	3.76	54	-8	6	.002
5	699	l.PCG	5	3.25	-16	-32	44	.010
6	660	l.PCC	10	3.21	-34	54	10	.013

TPJ = temporal parietal junction; CRPS = complex regional pain syndrome; MNI = Montreal neurological institute (brain model derived from mean 152 healthy persons); PPI = psychophysiological interaction analysis; l. = left; r. = right; COP = central opercular cortex; MFG = middle frontal gyrus; PCC = posterior cingulate cortex; ND = not determined; ITG = inferior temporal gyrus; OC = occipital cortex; PC = precuneous cortex; STG = superior temporal gyrus; SFG = superior frontal gyrus; TFC = temporal fusiform cortex; PCG = precentral gyrus; FWE = family wise error

DISCUSSION

In this study we evaluated central processing of a moderately painful heat stimulus in CRPS patients in comparison to healthy controls using functional MRI of the brain.

As expected, the initial heat stimulus was more painful in CRPS patients than healthy controls, who still rated the stimulus as moderately painful. This finding is in line with the results of other studies, suggesting that CRPS patients have lower pain thresholds and hyperalgesia[175,176], although the results on *heat* hyperalgesia have been less consistent across studies[177]. During the scanning period which followed the administration of multiple stimuli, pain scores in both groups increased slightly, in healthy controls even more than CRPS patients.

During the administration of the heat stimulus both groups showed a robust activation of bilateral insula, thalamus, anterior cingulate cortex (ACC) and the secondary somatosensory cortex. This finding has been reported by others in response to a variety of heat stimuli in healthy controls[178] and subjects with other pain syndromes (meta-analyses[179,180]). This brain activation pattern reflects circuits involved in processing pain perception and attention to a salient external stimulus[181].

We also found a significant bilateral activation of the temporal-parietal junction (TPJ) in CRPS patients. TPJ activation differed statistically from controls for the left side only. TPJ activation correlated positively with disease duration, but not with pain or any of the other clinical variables. This finding was unexpected since the TPJ is not involved in the *pain matrix* which includes the somatosensory cortices, ACC and insula[182]. There are several potential explanations for this finding. First, the TPJ is part of a multi-modal (nociceptive or non-nociceptive) sensory network that is involved in salience detection (right>left TPJ). The TPJ regulates sensory salience with top-down attentional control (left>right TPJ)[183–185] and negative emotions in relation to pain(left TPJ)[186]. (Of note, in the study of Orenius et al.[186] the TPJ was included in a cluster called the "secondary somatosensory cortex"). Further, the left TPJ has been shown to have a negative functional connectivity with brain areas involved in the default mode network (DMN)[184]. This brain network is associated with internally oriented attention when the brain is not engaged in any specific task and therefore considered the counterpart of externally directed cognition[187]. Indeed, next to a robust positive functional connectivity between the left TPJ and brain areas active during pain administration, we found a negative functional connectivity with brain areas associated with the DMN, that is, the bilateral precuneus and inferior parietal cortices. These brain areas are associated with recollecting prior experiences, consciousness and interpretation of sensory information[187,188]. However, only in CRPS patients we found an additional negative connectivity with the prefrontal cortex. The ventral medial component of the prefrontal cortex (VMPC) is part of the

DMN, next to (bi)lateral medial prefrontal cortices (LMPC), and thalamus. The VMPC plays a pivotal role in the processing and relaying of sensory information from the external world to structures such as the hypothalamus, the amygdala and the peri aqua ductal grey of the midbrain[187]. Its activity is influenced by peripheral nerve injury[189], inversely correlated with central hyperalgesia[190], and increased activation reduces nociceptive and affective symptoms of pain and successfully supresses emotional responses to a negative emotional stimulus[191,192]. It therefore plays an important role in pain processing and inhibition[188,189]. Importantly, activity of the LMPC has been found to correlate negatively with hyperalgesia and pain catastrophising[159], and its activity level was shown to normalise after successful pain treatment with cognitive behaviour therapy[193]. In CRPS pain catastrophising (and hyperalgesia) is common[194] and correlates with greater inter-network connectivity between the attention and salience networks[195]. Lastly, other studies have reported decreased thalamic connectivity in chronic pain patients[179] which is assumed to be related to altered thalamocortical connections, causing a disruption of thalamic feedback[193]. In essence, it reflects a shift in chronic pain states from sensory to emotional brain activity[179,196].

We expected a negative influence of pain on motor cortex activity, since patients with CRPS commonly experience a loss of voluntary control of the affected limb[197]. However, we did not find any difference in brain activity of the motor cortices between CRPS and healthy controls during the pain stimulus. In addition, increased saliency did not influence primary motor cortical activity. While this could be due to the absence of a motor task, the fact that eleven of the fifteen patients had abnormal postures due to active muscle spasms rendering this explanation less likely. Alternatively, the lack of altered motor cortex activity may suggest that motor disturbances in CRPS are not directly linked to painful sensory afferent input. Motor disturbances might therefore originate from 'upstream' brain areas such as limbic or frontal cortices, as hypothesized in functional movement disorders[198], or be the result of impaired central processing of proprioceptive Information[55].

Lastly, although the analysis of the cerebellum was not included in this paper, our incomplete data of the cerebellum showed striking differences between the groups in functional connectivity between the left TPJ and cerebellum. Compelling evidence shows an important role for the cerebellum in circuitry involved in motor, emotional and pain processing (reviews[199,200]). Hence, future studies should include the whole cerebellum in field of view.

Collectively, in response to a painful stimulus, CRPS patients activate the TPJ involved in salience detection which, in turn, is negatively correlated with brain areas involved in reducing the affective burden of pain.

Interpreting the results of this study, several points should be considered. First, we flipped the data of CRPS patients affected on the left arm in order to stack all "affected" hemispheres onto the same, left hemisphere. This was necessary in order to interpret the contralateral brain activations in response to the 1x-sided pain stimulus. However, as previously noted, some data suggest that there is a slight difference in function of the right and left TPJ[184]. Because the left TPJ in the CRPS group in the "flipped" data is a compilation of the left and right TPJ, this could mean that the difference between the groups is in fact a difference between the left and right TPJ. However, it is unlikely that this issue has relevant effects on the conclusion of our findings since we did run an analysis of the "non-flipped" data (figure 3, supplementary data), and found similar results of significant bilateral activation of the TPJ in CRPS patients. However, we must emphasize that in that group analysis no significant differences were found. Therefore, preferably, our results should be substantiated using a new, larger cohort of CRPS patients. Second, the PPI results in the "non-flipped" data with the left TPJ as seed resulted in corresponding negative connectivity with prefrontal cortices in CRPS patients, not in healthy controls. In addition, PPI analyses do not allow inferences about the direction of informational flow. Therefore, whether increased activation of the left TPJ resulted in reduced activation of the functional correlated brain areas or vice versa is not known. Finally, as mentioned above, future studies should include the cerebellum in the analyses given its role in motor, emotional and pain processing.

In conclusion, while experiencing a painful stimulus, CRPS patients have increased salience detection in combination with opposite activation of brain regions involved in reducing the affective burden of pain.

Figure 3, supplementary data (unflipped data)