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

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Chapter 4

Is the brain of complex regional pain syndrome patients truly different?

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ABSTRACT

Background

In recent years, changes in brain structure and function have been studied extensively in patients with Complex Regional Pain Syndrome (CRPS) following clinical observations of altered central processing of sensory stimuli and motor control. However, concerning MRI data, the evidence is complex to interpret due to heterogeneity in statistical methods and results.

Method

The aim of this study is to determine if CRPS patients exhibit specific, clinically relevant changes in brain structure and function in rest. We do this by presenting MRI data on brain structure and function in 19 chronic, female CRPS patients and age and sex matched healthy controls (HCs). In addition, we analyse and report the data in multiple ways to make comparison with previous studies possible and to demonstrate the effect of different statistical methods, in particular concerning the correction for multiple testing.

Results

Using family-wise error (FWE) correction for multiple testing, in our group of CRPS patients, we find no specific difference in brain structure or function in rest in comparison to healthy controls. In addition, we argue that previous found MRI results in literature are inconsistent in terms of localisation, quantity and directionality of the reported changes in brain structure and function.

Conclusion

Previously published MRI-based evidence for altered brain structure and function in rest in CRPS patients is not consistent and our data suggests that no such phenomenon exists.

INTRODUCTION

Complex regional pain syndrome (CRPS) is a severely disabling pain syndrome characterized by autonomic, sensory, trophic and motor disturbances of the affected limb. Current evidence suggests a multifactorial aetiology that includes aberrant inflammation, vasomotor dysfunction, and neuroplasticity in spinal cord and brain[1]. Aberrant neuroplasticity in the brain has been the focus of many studies in the last decade, following clinical observations of altered central processing of sensory stimuli[48–51] and abnormalities in motor control[52–54]. Specifically, studies reported cortical sensorimotor reorganization of the affected limb[38–40], the unaffected limb[122], changes in local grey matter volume throughout the brain[41–44], and altered activity patterns in rest[45,123]. However, 2 recent systematic reviews concluded that evidence of aberrant neuroplasticity of the somatosensory and primary motor cortex in CRPS is limited and at high risk of bias[124,125]. In addition: the reported MRI results are inconsistent, correlations with clinical measures are lacking or inconclusive and the methods - especially concerning the correction for multiple testing - differ between the studies.

Considering these concerns, the aim of this study is twofold: First, to reproduce previous MRI findings in literature with current advocated statistical methods in a sample of 19 chronic female CRPS patients. Second, to assess the evidence for specific, clinically relevant changes in brain structure and function in CRPS by discussing our results and previous published MRI data in literature. In our patient sample, we first perform an analysis of local Grey Matter Volume (GMV) by Voxel Based Morphometry (VBM) and white matter connectivity by Diffusion Tensor Imaging (DTI) on all voxels of the brain. In a secondary VBM analysis, we focus specifically on the sensorimotor cortex in an attempt to replicate recent findings in this brain area that is thought to be of high importance in CRPS aetiology[43]. Second, we study resting state functional Magnetic Resonance Imaging measurements (rsfMRI) in the sensorimotor, parietal, right and left executive attentional, salient and default mode network based on their role in sensory, pain and motor processing and on previous reports of alterations in CRPS patients[45].

Ultimately, transparently presented, statistical sound results of neuroplasticity in CRPS patients are imperative, since novel therapeutic strategies have been based on these results (e.g.[126–130])and might well be in the future.

METHOD

Participants

In order to make the patient sample as homogeneous as possible, only female CRPS patients followed up at the neurology outpatient clinic of the Leiden University Medical Center (LUMC) in Leiden, the Netherlands, were asked to participate in this study between May 2011 and March 2013. We chose female CRPS patients because the incidence of CRPS among females is 3-4 times higher than among men [4]. All had to fulfil the Budapest clinical criteria for CRPS in an upper limb. If a patient was interested, a patient information sheet was sent to her home 2 weeks before the potential entry in the study. Before entering the study, a neurological examination was performed by the principal investigator (GAJV) and Budapest Criteria[2] were checked for inclusion to the 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 or functional impairment of an upper extremity. A group of healthy pain free controls, age and sex matched at group level to the CRPS patients were additionally investigated. All participants were screened for MRI contraindications before MRI acquisition. The study protocol was approved by the Medical Ethics Committee of the LUMC, and written informed consent was obtained from all patients and control subjects.

Demographic data and pain measurements

At home on the day before research-day, patients completed questionnaires evaluating pain (McGill Pain Questionnaire, MPQ)[77] and manual activity of the affected hand (Radboud skills questionnaire, RSQ)[131]. On the day of examination we collected data on demographic variables, pain severity (numeric rating scale, NRS), CRPS (CRPS severity score)[75] and loss of voluntary motor control due to dystonic postures (Burke-Fahn-Marsden scale)[132]. Decreased active range of motion, weakness and slowness of movement of the affected hand were assessed during neurological examination.

MRI acquisition

For standardization purposes, all scanning sessions were carried out in the early evening. To prevent hearing loss due to loud scanner noise, participants used earplugs and headphones. During the rsfMRI protocol, which always followed the T_1-weighted and DTI imaging scanning protocols, participants were requested - and checked afterwards - to close their eyes, but not to fall asleep.

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 VBM analysis) were 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. DTI images were acquired using echo planar imaging in 60 slices with voxel size: 2 x 2 x 2mm; TR: 6580ms; TE: 71ms; flip angle: 90°; in-plane matrix resolution: 112 x 110; field of view: 224 x 224; b_o:800s/mm^2; in 32 diffuse directions and one non-diffusion weighted slice for head registration and head motion correction. RsfMRI imaging was done in 38 slices, voxel size: 2,75 x 2,75 x 2,75mm without a gap; TR: 2200ms; TE: 30ms; flip angle 80°; field of view: 220; acquisition matrix: 80x79, acquisition time: 7minutes and 30 second.

MRI analysis

We analysed all MRI data twice using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) v5.0 (Oxford, UK) [133–135]: one analysis with mirroring of the hemispheres of patients affected in the left arm to stack all "affected" hemispheres onto the same, left hemisphere (toolbox: FSLswapdim). This data will be referred to as "flipped" data. Additionally, the analysis was performed with the original, non-flipped images to investigate possible asymmetrical differences, in particular in the right parietal lobe, since this region has been hypothesised to play a role in CRPS symptom aetiology[136].

Brain volume

Total grey matter volume was calculated using the FSL-tool: "Structural Image Evaluation using Normalisation of Atrophy". The results were later used for correlation analysis with age, disease duration and fractional anisotropy (DTI analysis) and included in fMRI analysis as a nuisance variable.

Local GMV analysis was performed using the optimized VBM protocol in FSL-VBM[137–139]. First, structural images were brain extracted using Brain Extraction Tool (BET)[140] and grey matter segmented before being affine registered to the Montreal Neurological Institute (MNI) 152 standard space using non-linear registration with FMRIB's nonlinear Image Registration Tool[141]. Next, the resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Subsequently all native grey matter images were non-linearly registered to this study-specific template and modulated to correct for local expansion due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 4mm. We used permutation based threshold-free cluster-enhancement (TFCE) for thresholding of significant clusters. This method has the advantage that it preserves the sensitivity of cluster-based-thresholding (in contrast to voxel-based thresholding which is more conservative), while there is no need for a predetermined cluster-forming threshold which is always arbitrary[142]. A voxel-wise general linear model (GLM) was applied with

permutation-based non-parametric testing[143]. Results were Family Wise Error controlled (FWE) for multiple testing across space: $p < .05$. Age was included as nuisance variable. Group differences were tested against 5000 random permutations. In a secondary analysis we focussed specifically on the primary sensorimotor cortex by using a mask of the primary sensorimotor cortex derived from the MNI152 standard space. Subsequent statistical analyses were performed only in this area thereby increasing the power of detecting group-differences.

Lastly, in the CRPS group, we studied the effect of pain intensity (NRS) and disease duration (in months) onGMV.

White matter structural connectivity (DTI)

Voxel-wise statistical analyses of fractional anisotropy (FA) was carried out using tract-based spatial statistics (TBSS)[144]. FA is a measure of mean diffusivity along white matter tracts which represents the structural connectivity of those tracts[145].

DTI images were first converted to "Neuroimaging Informatics Technology Initiative" files using a ExploreDTI v4.8.3 toolbox[146]. Next, subject movements and eddy current induced distortions were corrected with FSL-eddy. Subsequently we extracted the brain[140] and fitted a diffusion tensor at each voxel of the images. The resulting FA data were aligned into a common space[141]. The individual FA data were concatenated into a mean FA skeleton representing the centre of all common white matter tracts per group (CRPS and HC). Lastly, we used permutation-based non-parametric testing with TFCE (FWE $p < .05$).

In a separate analysis we correlated the individual whole brain FA data to whole brain GMV to replicate previous results of a disrupted relationship between white matter connectivity and GMV in chronic CRPS patients[41].

Resting state functional magnetic resonance imaging (rsfMRI)

For rsfMRI analysis we used the graphical user interface of FSL- Multivariate Exploratory Linear Optimized Decomposition into Independent Components 3.12. Pre-processing of rs-fMRI images incorporated motion correction[147], brain extraction[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 T_1-weighted images (12 degrees of freedom) and subsequently to standard space MNI-152[147,148].

Probabilistic independent component analysis[149] was performed using multi-session temporal concatenation with 25 components pre-set, and independent component map threshold of 0.5 (probability that a voxel belongs to a resting state network and not to background noise).

From these 25 networks we selected the sensorimotor, parietal, right and left executive attentional, salient and default mode network for further statistical analysis based on their role in sensory, pain and motor processing and on previous reports of alterations in CRPS patients[45,123] We then made a mask of these networks (14,5% of total brain volume) and computed functional connectivity of each voxel within the mask with each of the 6 networks. Next, we performed dual regression[150]. Lastly, we used statistical between-group analysis with permutation-based non-parametric testing with TFCE (FWE $p < .05$) to find significant group differences. A GLM was used with mean GMV and age as nuisance variables.

RESULTS

Participants

Data are presented as means ± standard deviation when not stated differently. Nineteen female CRPS patients (48.1 ± 11.6 years) and 19 female healthy controls of similar age (49.4 ± 14.3 years; age: $t(37)=0.31$, $p=.76$) were included in the study. One patient (nr 19) could not complete the full scanning protocol due to nausea in the MRI scanner; therefore only structural images are available for this patient. RsfMRI and DTI data of one HC and rsfMRI data of patient 10 had to be excluded from the analysis due to significant motion artefacts. Therefore 19 patients and 19 HC's were included in the VBM analysis, 18 patients and 18 HC's in the DTI analysis, and 17 patients and 18 controls in the rsfMRI analysis.

Characteristics of the CRPS group can be found in Table 1. All patients had chronic CRPS, median 6.7 [2-11,75] years and were affected in at least one hand. The pain intensity in the examined hand was 7.1 ± 1.5 on a scale of 0 to10.

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	Zaldiar, arcoxia, lyrica,
2	67	L	R	63	14	Research	16	yes	8	15	3.80	Oxycontin, gabapentin, fexofenadin, betahistin
3	48	L	R	141	16	Research	29	yes	7	42	2.82	Temazepam, Rivotril
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, temazapam, oxynorm
7	56	L	R	35	12	Clinical	17	yes	6	29	3.36	Lyrica, zolpidem
8	50	R	R	150	11	Clinical	18	yes	10	51	4.56	Morfine, diazepam, rivotril
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, Panadol, diazepam
11	37	R	L	84	14	Research	28	yes	8	28	3.80	Lyrica, 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	Lyrica, paracetamol, diazepam
14	51	R	L	10	9	Clinical	0	no	8	29	2.59	Lyrica, paracetamol, tramadol, temazepam
15	55	R	L	360	13	Research	14	yes	5	27	2.76	Lyrica
16	60	R	L	13	14	Research	12	Yes	7	25	3.77	Tramadol, gabapentine
17	37	R	R	24	8	Clinical	0	no	6	30	2.00	Lyrica, 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

MRI data

Total and local grey matter volume

Total brain grey matter did not differ between the two groups (CRPS: 752.01 ± 46.6 cm3 vs HC: 754.77 ± 43.1 cm3; t(38)=0.14, p=.89).

Local GMV analysis between CRPS patients and HC's using the flipped and non-flipped data did not result in significant FWE corrected differences between the two groups. Table 2 reports clusters with a minimum cluster volume of 45 mm3[42] with uncorrected p-values (p<.001) and the corresponding FWE corrected p-values (p<.05). Subsequent analysis of only the sensorimotor cortex did not result in any significant cluster difference. Including total grey-matter in the analysis as an additional nuisance variable did not changed the results.

In the patient group, local GMV did not correlate significantly with pain or disease duration in the flipped and non-flipped data set. Total brain grey matter correlated negatively with age (CRPS: r=-.556, p=.013; HC: r=-.684, p=.001), but the strength of the correlation did not differ significantly between the groups (z(38)=0.59, p=.55).

White matter connectivity

No regional difference in FA was found between CRPS patients and HCs (table 3).

Mean whole brain FA of white matter correlated significantly with total GMV in both groups equally (CRPS: r=.722, p=.001 vs HC: r=.612, p=.007; z(36)=.55, p=.58)) (Figure 1a&b).

Resting state networks

Table 4 reports in total 10 clusters with one-tailed FWE corrected (per rs-network) differences between CRPS patients and HCs. Table 5 reports cluster differences with a minimum cluster size of 150mm^2, uncorrected for multiple testing (p<.001)[151].

The largest and most significant cluster was found in the non-flipped data in the left posterior cingulate cortex (1232 mm^3, p=.006) (Figure 2). In CRPS patients, this cluster showed positive connectivity with the left executive attentional network (mean z-score: 1.48± 1.05), while in HCs this connectivity was negative (z=-4.19±1.12). No correlation between connectivity scores and pain scores, disease duration or CRPS severity was found.

Table 2 Local grey matter volume differences (VBM)

Data and contrast	Cortical region	Side	N voxels ($2mm^3$) in cluster	Volume cluster mm^3	MNI (mm) max Z	X	Y	Local max t-value	Uncorrected p-value	FWE Corrected p-value
Flipped data										
HC > CRPS	Temporal lobe	R	194	1552	28	-26	-32	2.280	<.001	.300
	Cerebellum	R	55	440	18	-32	-48	2.124	”	.378
	Temporal lobe	L	39	312	-30	-22	-22	2.021	”	.451
	Cerebellum	L	30	240	-16	-80	-46	3.084	”	.127
	Occipital fusiform gyrus	R	13	104	36	-64	-8	2.773	”	.160
Non-flipped data										
CRPS > HC	Frontal lobe	R	184	1472	46	48	-6	3.234	<.001	.417
HC > CRPS	Temporal lobe	L	102	816	-30	-24	-24	2.537	”	.347

VBM = voxel based morphometry; HC = healthy control; CRPS = complex regional pain syndrome; MNI = Montreal neurological institute (brain model derived from mean 152 healthy persons); FWE = family wise error

Table 3 White matter connectivity (FA)

Data and contrast	Cortical region	Side	N voxels in cluster	Volume cluster mm^3	MNI (mm) max Z	X	Y	Local max t-value	Uncorrected p-value	FWE Corrected p-value
Flipped data										
CRPS > HC	Frontal	R	4	4	22	47	26	3.540	<.01	1
HC > CRPS	Inferior frontal gyrus	L	24	24	-42	14	22	1.163	”	.778
	Secondary somatosensory cortex	L	6	6	-41	-22	22	2.768	”	1
Non-flipped data										
HC > CRPS	Temporal lobe	R	15	15	25	0	-30	2.028	<.01	1

FA = fractional anisotropy; HC = healthy control; CRPS = complex regional pain syndrome; MNI = Montreal neurological institute (brain model derived from mean 152 healthy persons); FWE = family wise error

Table 4 Differences in resting state network activation, FWE corrected

Data and contrasts	Cortical region	Connectivity with resting state network	Side	N voxels in cluster	Volume cluster mm^3	MNI (mm) max			Local max t-value	FWE Corrected p-value = one tailed
						Z	X	Y		
Flipped data										
CRPS > HC	Precentral gyrus	r-EAN	R	12	96	12	-16	48	5.06	.032
Non-flipped data										
CRPS > HC	PCC	l-EAN	L	154	1232	-4	-26	44	5.66	.006
	Postcentral gyrus	l-EAN	R	25	200	4	-38	72	4.17	.038
		l-EAN	L	14	112	-8	-34	72	4.41	.039
		l-EAN	L	11	88	-14	-42	70	4.04	.044
	Precentral gyrus	l-EAN	L	3	24	-16	-26	74	4.46	.044
	Superior frontal gyrus	l-EAN	R	15	120	12	36	50	4.48	.039
	Frontal pole	l-EAN	R	13	104	16	38	42	5.25	.027
	Superior frontal gyrus	l-EAN	R	12	96	4	26	48	4.47	.043
	Lateral occpital lobe	l-EAN	R	5	40	22	-60	38	5.83	.038

HC = healthy control; CRPS = complex regional pain syndrome; MNI = Montreal neurological institute (brain model derived from mean 152 healthy persons); FWE = family wise error; PCC = posterior cingulate cortex; r-EAN = right executive attentional network; l-EAN = left executive attentional network

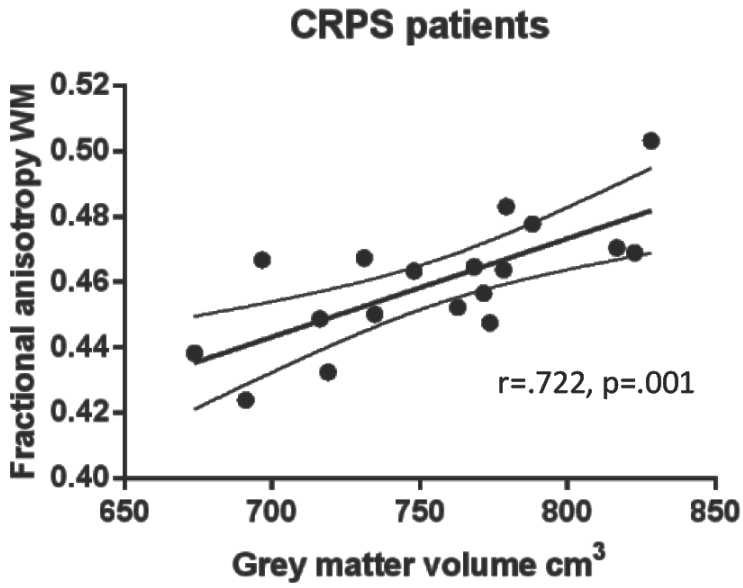

Figure 1a

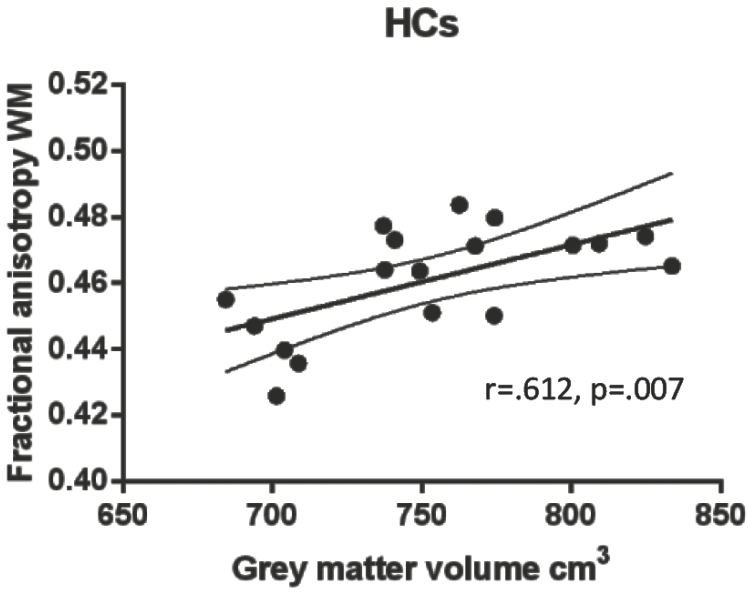

Figure 1b

(a) Correlation between white matter connectivity and grey matter volume. (b) Correlation between white matter connectivity and grey matter volume. Interpolate line with 95% confidence interval; CPRS = complex regional pain syndrome; HCs = healthy controls; WM = white matter

Table 5 Differences in resting state networks activation, FWE uncorrected

Data and contrasts	Connectivity with resting state network	Side	N voxels in cluster	Volume cluster mm^3	MNI (mm) max Z	X	Y	Local max t-value	Uncorrected p-value	FWE Corrected p-value
Flipped data										
CRPS > HC	DMN	R	37	240	30	-40	-12	5.17	<0.001	.477
		L	108	864	-24	18	34	4.77		.116
		R	63	504	34	8	30	4.09		.220
	l-EAN	R	35	280	66	-54	-2	4.27		.547
		L	32	256	-6	-60	60	4.38		.145
		L	30	240	-42	-58	58	3.23		.428
		R	20	160	54	4	28	3.92		.457
	Salience	L	80	640	-40	40	-16	4.28		.374
		L	33	264	-44	-56	-16	4.43		.653
	r-EAN	R	180	1440	24	-66	44	3.72		.152
		L	177	1416	-18	-76	38	3.83		.174
		R	**142**	**1136**	**12**	**-16**	**48**	**5.06**		**.032**
		R	95	760	36	-56	54	4.39		.101
		R	64	512	18	34	42	3.16		.425
		L	47	376	-12	-12	48	3.55		.155
		L	42	336	-4	62	28	3.91		.220
		L	32	256	-18	50	24	3.36		.241
		L	26	208	-26	38	28	3.29		.276
		-	25	200	0	20	52	3.35		.379
	S1M1	L	25	200	-4	-38	28	4.32		.227
		R	20	160	14	-50	18	3.56		.514

Table 5 Differences in resting state networks activation, FWE uncorrected (continued)

Data and contrasts	Connectivity with resting state network	Side	N voxels in cluster	Volume cluster mm^3	MNI (mm) max Z	X	Y	Local max t-value	Uncorrected p-value	FWE Corrected p-value
HC > CRPS	DMN	R	62	496	44	26	6	3.79	<0.001	.301
		L	60	480	-48	-70	-10	3.57		.587
		R	36	288	4	-6	-2	4.11		.650
		L	28	224	-40	-82	14	2.84		.644
		R	21	168	40	-4	62	3.23		.623
	S1M1	L	24	192	-38	-60	60	4.16		.511
Non-flipped data										
CRPS > HC	l-EAN	**L**	**461**	**3688**	**-4**	**-26**	**44**	**5.66**	<0.001	**.006**
		L	**427**	**3416**	**-16**	**-26**	**74**	**4.46**		**.044**
		L	135	1080	-44	-24	48	3.88		.099
		L	34	272	-4	-26	64	3.39		.065
	r-EAN	**R**	**247**	**1976**	**16**	**38**	**43**	**5.15**		**.027**
		L	124	992	-28	36	34	3.61		.296
		R	92	736	22	-60	38	5.83		.038
		R	19	152	12	-4	58	3.65		.210
	Salience	R	97	776	20	48	14	5.29		.079
		L	31	248	-14	-88	0	4.95		.999
HC > CRPS	Parietal cortex	R	133	1064	24	4	62	4.12		.188

HC = healthy control; CRPS = complex regional pain syndrome; MNI = Montreal neurological institute (brain model derived from mean 152 healthy persons); FWE = family wise error; S1M1 = primary sensorimotor cortex; r-EAN = right executive attentional network; l-EAN = left executive attentional network

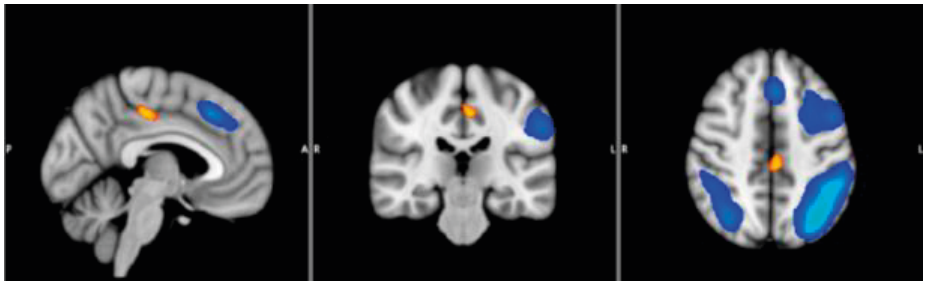

Figure 2

Difference in functional connectivity between CRPS patients and HCs. Non-flipped data. P=posterior; A=anterior; S=superior; I=inferior. Centre of "gravity" for this cluster (mm): x=-4.16, y=-23.3, z=44.3: left hemisphere: posterior cingulate cortex. Activation shown (red-yellow): significant difference in connectivity between CRPS patients (positive) and HCs (negative) with left executive attentional network portrayed in blue.

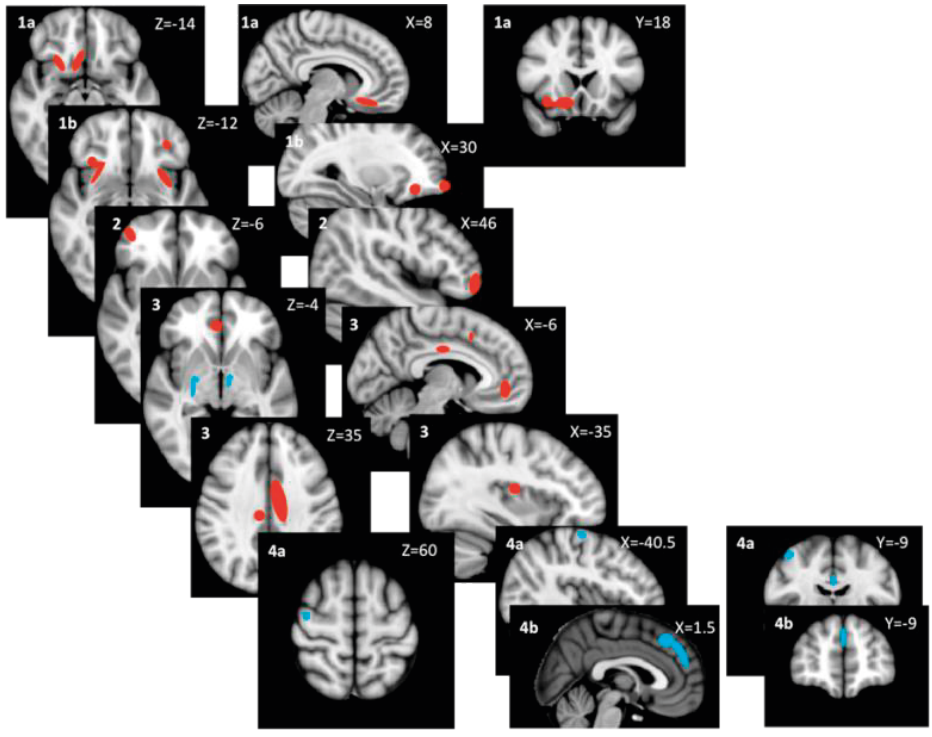

Figure 3

Schematic representation of reported alterations in grey matter volume in CRPS patients and uncorrected data this paper. Red=decreased grey matter volume (GMV), Blue=increased GMV; coordinates (x/y/z) in mm; X=left-to-right axis; Y=posterior-to-anterior; Z=caudal-to-cranial; 1a=Geha et al.[41]; 1b=Baliki et al.[44]; 2=uncorrected data from this paper; 3=Barad et al.[42]; 4a=Pleger et al[43] flipped data; 4b=Pleger et al, non-flipped data

DISCUSSION

Contrary to previous studies, in our sample of chronic CRPS patients GMV and white matter connectivity did not differ with age and gender matched healthy controls. However, using less stringent correction methods in the VBM analysis, structural alterations were seen bilaterally in the cerebellum, temporal lobes, occipital fusiform gyrus and right lateral orbitofrontal cortex. We did find differences in functional connectivity networks, although the statistical and clinical significance of these results need further elaboration.

In the next section we discuss for each analysis the discrepancies between our results and previous published results in literature in adult CRPS patients with special attention to consistencies in terms of localisation, quantity and directionality of the previous reported changes in brain structure and function in rest. In the last section we discus general observations that apply to all MRI analysis used.

Absence of local grey matter volume differences

Our findings showing no FWE corrected differences in local GMV between CRPS patients and HCs contrasts with those of two earlier studies in CRPS showing decreased GMV in the right Anterior Insula (AI)[41] and bilateral AI[44] (see also figure 3). In Geha's paper, decreased GMV in the right AI significantly correlated with pain intensity only in young patients, while in Baliki's paper a reduction of GMV in the primary sensorimotor and insular cortex correlated with pain in patients with disease duration > 5 years. Of note, in our sample, GMV difference in the rAI for both the flipped and non-flipped data was close to zero ($p \approx 1$).

A study of Pleger et al[43] in 20 acute and chronically ill CRPS patients with an affected upper limb, showed increased GMV in the dorsomedial prefrontal cortex (non-flipped data) and in M1 contralateral to the affected hand (flipped data). These changes did not correlate with any clinical feature.

Barad et al[42] studied a group of 15 right-handed CRPS patients with a wide range in disease duration, who all were affected in the right hand. They reported reduced GMV in the dorsal insula, left orbitofrontal cortex and "several aspects of the cingulate cortex" but increased GMV bilaterally in the dorsal putamen and right hypothalamus. Both negative and positive relations between clinical parameters and GMV changes in this study could not be explained by significant differences in GMV between the groups, and therefore authors concluded that the observed abnormalities "are not central to CRPS pathology"[42]. In our patient sample, no correlations were found for pain intensity and disease duration with local GMV.

Collectively, in total 5 studies - including the data presented in this study - on local GMV in CRPS yielded varying or absent GMV changes with no or inconsistent correlations with clinical features. This question the clinical relevance of these findings.

Absence of white matter connectivity changes

We found no difference in white matter connectivity between CRPS patients and HCs.

Only one other study evaluated DTI in CRPS[41] and found a disrupted correlation between total brain GMV and white matter anisotropy. This finding was suggested to indicate diffuse reorganisation of white matter tracts, however, the results did not correlate with clinical parameters. In our data sample, no such dissociation of GMV and white matter anisotropy was found in any of the groups. Collectively, there is so far no compelling evidence for changes in white matter connectivity in CRPS patients.

Alterations in resting state networks

We found 10 "significant" clusters of altered resting state networks, all related to executive attentional networks (Table 4). However, there is a need for caution in interpreting these results. First, next to the absence of a prior hypothesis regarding the direction of activation differences (CRPS>HC or HC>CRPS), all FWE corrected results are 1-tailed tested. Second, the consecutive rs-networks should be considered as multiple tests. Therefore, when full correction for multiple testing is applied, the α would change from $<.05$ to $\leq.0041$ (i.e., .05 / (2 tailed x 6 tests)) (FSL Dual regression user guide. Using this threshold, none of the results were significantly different between the groups. Third, no correlation with clinical parameters was found.

Two papers focussed on resting state networks in adult CRPS patients. First - Bolwerk et al - studied 12 heterogeneous CRPS patients (type 1 and 2) with affected upper and lower limbs[45]. They found significant reductions in default mode network (DMN)[152] activation in CRPS patients and a diffuse increase in connectivity of S1M1 with other brain regions (cingulate cortex, precuneus, thalamus and prefrontal cortex). None of these changes correlated significantly with pain scores. We could not replicate any of these results. In addition, when we used a similar cluster size threshold of $150mm^3$ as Bolwerk et al., numerous clusters in many rs-networks that emerged in FWE uncorrected analysis (uncorrected p-value $<.001$), appeared nonsignificant after correction of multiple comparisons.

Baliki et al[123] compared 5 resting state networks between healthy controls and 3 pain patients groups, including CRPS patients. Only the DMN showed significant differences in connectivity between pain patients and healthy controls, most notably decreased connectivity with

the medial prefrontal cortex and anterior cingulate cortex and increased connectivity with the precuneus. Unfortunately, no distinctive activation pattern for CRPS patients was found.

Collectively, absence of evidence in our sample, absence of distinctive CRPS associated changes in connectivity and absence of significant correlations with clinical features let us conclude that compelling evidence for specific resting state networks changes in CRPS patients is lacking.

General discussion

We did not find compelling evidence for CRPS specific changes in brain structure and function in rest. Most striking was the absence of anticipated changes in somatosensory and limbic areas. Conflicting findings of all studies - including our own uncorrected VBM analysis data - and the absence of consistent clinical correlations questions the clinical relevance of previous MRI findings of reported altered brain structure and function in rest in CRPS patients. However, potential issues explaining the lack of results and discrepancies between the reported data should be considered. First, the lack of results might be the consequence of an underpowered study design, although the sample size did not differ significantly with the other papers. In addition, scanning parameters such as voxel sizes and repetition times differ between the studies and can have influence on the strength of results. Second, sample characteristics (gender, disease duration, CRPS type, applied diagnostic criteria, symptoms, and affected limbs) vary across studies. These differences may contribute to some variability in the magnitude of the results, laterality of findings if different limbs are examined, and spatial representation differences in primary and secondary sensorimotor cortices when affected upper limbs are compared with affected lower limbs. However, when CRPS would encompass uniform changes of the brain, at least the directionality of changes (increase or decrease) would be expected to be consistent between the different data sets as well as clinical parameters (e.g. pain) that correlate to these changes -which is not the case.

Third, changes in brain structure and function at rest may depend on disease stage, i.e. present in acute CRPS (<6months), when symptoms are more pronounced, or change during the course of the disease from nociceptive to emotional circuits[153], decreasing overall group effects. Although our patients were all chronically ill, their disease durations varied which may have influenced the detection of a potential disease duration effect. However, the absence of a correlation between disease duration and MRI data, renders this explanation unlikely.

Fourth, centrally acting drugs could have obscured some changes in brain structure and function, especially in our patient sample with many very long chronically ill patients. However, in all cited papers, patients continued their medication and this therefore is an unlikely explanation for the different findings between studies.

Fifth, diversity in software packages and analysis options in (f)MRI research, in particular the methods used to correct for multiple testing, can have an enormous impact on the results, hampering a reliable comparison of findings between studies[154,155]. We used TFCE to find cluster differences between CRPS patients and healthy controls and based our conclusions on FWE controlled results. One could argue that this is too stringent considering our sample size. However, when we increased the power by limiting the amount of investigated voxels - by focussing solely on the sensorimotor cortex - during the VBM analysis we could still not find significant FWE corrected differences between the groups. For all other analyses, we choose a priori not to focus on one particular brain area since many previous interpretations of event-related fMRI research was based on uncorrected, albeit conservative p-values, making the results liable to type 1 errors[124]. Second; the *un*corrected clusters we found in the VBM whole brain analysis did not correspond with the previous results in literature (figure 3). Collectively, this indicates that data of altered GMV in CRPS patients are inconsistent and questions the evidence for specific and clinically relevant changes in brain structure in CRPS patients.

Lastly, instead of specific changes in brain structure and function associated with CRPS symptoms, some evidence suggests that these symptoms are generated by a mismatch between aberrant afferent signals from the affected limb and the internal state of limb in the brain[55–57]. The resulting aberrant processing likely involves brain circuits containing many parts of the brain and probably changes depending on the afferent feedback -or location in space[156]- of the affected limb. Under such circumstances local grey matter or white matter changes are not to be expected, and common resting state networks would not be involved.

Future MRI studies should focus on this aberrant processing of external stimuli, which may one day result in the elucidation of these *complex* -and fascinating- symptoms.

CONCLUSION

Absence of evidence is not evidence of absence. We therefore cannot prove that changes in brain structure and function in rest are absent in CRPS patients. However, current evidence for altered brain structure and function in rest in CRPS patients is not consistent and in our data in female patients not present. Caution is required when therapeutic strategies are based on these presumed changes of the brain.

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