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FUNCTIONAL NEURORADIOLOGY

Role of altered cerebello-thalamo-cortical network in the neurobiology of essential tremor

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Abstract
Introduction Essential tremor (ET) is the most common movement disorder among adults. Although ET has been recognized as a mono-symptomatic benign illness, reports of non-motor symptoms and non-tremor motor symptoms have increased its clinical heterogeneity. The neural correlates of ET are not clearly understood. The aim of this study was to understand the neurobiology of ET using resting state fMRI.
Methods Resting state functional MR images of 30 patients with ET and 30 age- and gender-matched healthy controls were obtained. The functional connectivity of the two groups was compared using whole-brain seed-to-voxel-based analysis.
Results The ET group had decreased connectivity of several cortical regions especially of the primary motor cortex and the primary somatosensory cortex with several right cerebellar lobules compared to the controls. The thalamus on both hemispheres had increased connectivity with multiple posterior cerebellar lobules and vermis. Connectivity of several right cerebellar seeds with the cortical and thalamic seeds had significant correlation with an overall score of Fahn-Tolosa-Marin tremor rating scale (FTM-TRS) as well as the subscores for head tremor and limb tremor.
Conclusion Seed-to-voxel resting state connectivity analysis revealed significant alterations in the cerebello-thalamo-cortical network in patients with ET. These alterations correlated with the overall FTM scores as well as the subscores for limb tremor and head tremor in patients with ET. These results further support the previous evidence of cerebellar pathology in ET.

Keywords Essential tremor · fMRI · Resting state · Functional connectivity

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Introduction

Essential tremor (ET) is a chronic neurological disorder and is one of the common causes of tremor in adults. Kinetic tremor of the upper limbs is the core motor symptom of ET [1]. In addition to tremor of the limbs, patients with ET may develop tremor of the head, voice, tongue, palate, and trunk [1, 2]. However, the motor symptom in ET is not limited to tremor as other motor features such as cerebellar signs, dystonia and disturbances of gait, balance, and speech have been described in ET [3]. Although several non-motor symptoms such as depression, anxiety, apathy, cognitive impairment, and hearing abnormalities have also been reported in ET, majority of the neuroimaging studies have focused on the core symptom of ET, i.e., tremor. While these studies have reported abnormalities in the cerebellar networks and pathological changes in the cerebellum in patients with ET, the exact neural correlates of ET still remain elusive [4, 5].

Several studies based on advanced structural and functional neuroimaging modalities have been conducted in ET [6, 7]. However, resting state functional magnetic resonance imaging

(rs-fMRI), which is an excellent tool for analyzing the neural networks, is relatively less studied in patients with ET. Unlike the task-based fMRI analyses, which provide insight into the neural activities during a task, rs-fMRI analyses provide information independent of the task. As abnormal movements could alter the performance of the motor tasks during image acquisition in task-based fMRI studies, rs-fMRI is particularly useful in patients with disorders of motor function such as ET, Parkinson's disease, and writer's cramp. At the time we started this study, there were only two published articles on application of fMRI in understanding the neural correlates of ET. To the best of our knowledge, to date, there are only five rs-fMRI-based studies in ET. Three of those studies have used independent component analysis (ICA) [8–10] and the other two have used regional homogeneity (ReHo) [11] and low-frequency oscillation-based analysis [12]. The three studies based on ICA have reported decreased functional connectivity of the cerebellar network, whereas the studies based on ReHo and low-frequency oscillations have reported decreased functional connectivity in the cerebellum, thalamus, and motor cortices. However, long-range functional connectivity studies at the whole-brain level (e.g., seed-to-voxel-based analysis) in ET are not yet available. We therefore undertook this study to compare the functional brain connectivity of ET patients at the whole-brain level with that of healthy controls in order to understand the neural correlates of ET.

Methodology

Study population and approval

This prospective case-control study was conducted in a tertiary care neurological institute. Based on the consensus statement of the Movement Disorder Society on tremor, 32 consecutive patients with definite ET were recruited for this study [13]. A single movement disorder specialist (author-PKP) had evaluated all the patients prior to the recruitment. Demographic parameters such as age at presentation, age at onset of tremor, duration of illness, gender, and family history of tremor were documented. Patients under treatment for tremor were instructed to temporarily discontinue the medications. As it takes three to five plasma half-lives to eliminate a drug from the body and half-life of the tremor medications range from 5 to 50 h [14], medications were stopped 2 weeks prior to the respective appointments for clinical evaluation and neuroimaging. Tremor was quantified using the Fahn-Tolosa-Marin (FTM-TRS) tremor rating scale (component A and B) [15]. Component A of FTM measures the severity of rest, postural, and intentional tremor of several parts of the body whereas the component B represents clinical assessment of tremor severity during performance of tremor-inducing tasks such as writing, drawing, and pouring. Mini mental status examination (MMSE) score was used to screen the participants for cognitive impairment. Thirty-two healthy volunteers who did not have family history of any neuropsychiatric illness were recruited from the hospital staffs and spouses or non-family friends of the patients. Based on the Edinburgh handedness inventory, all subjects were documented to be right handed [16].

Both patients and healthy volunteers gave written informed consent for participation in the study. The Institute Ethics Committee had approved the study.

Functional magnetic resonance imaging

Image acquisition

All participants underwent rs-fMRI and structural MR imaging on a 3T-MR scanner (Philips) with a 20-channel head coil. The acquisition parameters for EPI using blood oxygen level dependent (BOLD) contrast were as follows: volumes 192; TR 2000 ms; TE 30 ms; sections 34; slice thickness 4 mm; FOV 192 × 192 mm; resolution 64 × 64; refocusing pulse 90°; and voxel size 3 × 3 × 4 mm. Anatomic images were acquired by using 3D T1-weighted MPRAGE sequences in 192 sections with a TR of 1900 ms, TE of 2.43 ms, TI of 900 ms, FOV of 256 × 256, resolution of 256 × 256, and a slice thickness of 1 mm. Axial FLAIR, T2, and gradient sequences were used to rule out structural abnormalities. Sedation or general anesthesia was not required during the image acquisition in any of the participants. The maximum translational and rotational head movement allowed was 1.5 mm, thus four subjects (two with ET and two healthy controls) were excluded because of higher head movement. Hence, data of 30 patients and 30 healthy controls were analyzed. At the time of MR image acquisition, we minimized the head motion with proper foam padding around the head. The average head motion was not significantly different between the patients and the controls (Table 1), though there was a trend among the controls to have greater head movements than the patients. Although the exact reason is not clear, it is perhaps due to the fact that patients were more compliant to the instructions given prior to MRI compared to the controls.

Image analysis

Preprocessing

The functional and structural MR imaging preprocessing was performed using the statistical parametric mapping software SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) [17]. To maintain the magnetization equilibrium, the first five images were discarded. The data were realigned for motion correction by registration to the mean image. The functional images of all the subjects were transformed to the

Table 1 Demographic and clinical characteristics of the subjects

Parameters	HC (n = 30)	ET (n = 30)	Significance (p)
Women/men	10:20	11:19	0.78
Age (mean ± SD years)	43.4 ± 9.2	45.4 ± 13.7	0.51
MMSE	30.0 ± 0.0	29.9 ± 0.6	0.20
Handedness (R/L)	30:0	30:0	–
Age at onset of tremor (mean ± SD, years)		38.2 ± 16.1	–
Mean FTM score			
Total	–	36.0 ± 15.8	–
FTM (A + B)	–	26.7 ± 6.7	–
FTM (C)	–	9.3 ± 5.4	–
Family history of tremor (%)	0	56.7	–
Response to alcohol	–	6/9	–
Body parts with tremor			
Upper limb (%)	–	30/30 (100)	
Lower limb (%)	–	11/30 (36.6)	
Head (%)	–	13/30 (43.3)	
Voice (%)	–	12/30 (40.0)	
Magnitude of head motion during MRI			
Transverse axis (mm)			
Right	0.50 ± 0.32	0.55 ± 0.52	0.67
Forward	0.83 ± 0.61	1.07 ± 0.38	0.08
Up	0.68 ± 0.39	0.9 ± 0.61	0.11
Rotational axis (°)			
Pitch	0.012 ± 0.013	0.02 ± 0.021	0.14
Roll	0.006 ± 0.005	0.008 ± 0.006	0.24
Yaw	0.008 ± 0.009	0.009 ± 0.01	0.65

The magnitude of head motion in different directions is provided in mean ± SD in millimeters

ET essential tremor, *HC* healthy controls, *NS* not significant, *S* standard deviation, *R* right, *L* left, *MMSE* mini mental status examination, *FTM* Fahn-Tolosa-Marin score

Montreal Neurological Institute standard space by using the deformation field derived in the new segmentation procedure (voxel size 3 × 3 × 3 mm). In addition, the structural data were segmented for gray matter, white matter, and CSF for nuisance regression of the signals related to white matter and CSF. In the functional connectivity toolbox (CONN, http://www.nitrc.org/projects/conn), rigid-body transformation for head motion correction was performed using three translational and three rotational parameters as covariates. The BOLD time series for each subject was extracted and band-pass filtered (0.009–0.09 Hz). The CompCor algorithm was used on the segmented white matter and CSF to correct for physiologic noise [18].

Anatomical parcellation

The fMRI data were segmented into 132 cortical, subcortical, and cerebellar anatomic regions of interest (ROI/seeds). The cortical (n = 91) and subcortical (n = 15) ROIs were obtained from FSL Harvard–Oxford cortical and subcortical atlas (HarvardOxford-cort-maxprob-thr25–1 mm.nii, HarvardOxford-sub-maxprob-thr25–1 mm.nii), whereas the 26 cerebellar ROIs were obtained from AAL atlas [19].

Functional connectivity analysis

Connectivity maps were generated by extracting BOLD time series from the 132 seeds/ROIs, followed by calculation of the correlation coefficients between the seed time course and the time courses of all other voxels in the brain. Bivariate correlations were calculated as reflections of connections. The correlation coefficients were converted to z-scores using Fisher's r-to-z transformation. The general linear model was designed to determine significant BOLD signal correlation between the mean time series from each seed ROI and that of every other brain voxel, at the individual subjects' level (first-level analysis). Second-level random-effects analysis was used to create within-group statistical parameter maps for each network, to investigate connectivity within each group, and to identify regions with differential connectivity between the two groups of participants.

Statistical analysis

Descriptive statistics for clinical and demographic variables was performed using SPSS (Version 21). Student's *t* test was used for comparing the continuous variables and chi-square test was used for the categorical variables. The statistical level of significance was set at $p < 0.05$.

Voxel wise two sample *t* test analyses were performed to detect regions with significant differences in connectivity between the two groups. Statistical parameter maps between the groups were in threshold at whole-brain cluster-level-corrected α value of 0.05 for voxel wise *p* value of <0.05 with false-discovery rate (FDR) correction (with a minimum cluster extent of 10 contiguous voxels). The corrections for multiple comparisons were carried out using FDR-corrected *p* value of 0.05. Linear Pearson correlation analysis was carried out between the FTM scores and the connectivity strengths in the regions that were significantly different between the ET and control groups. The regions showing significant brain connectivity difference were represented in multi-slice brain images using *xjView* (http://www.alivelearn.net/xjview8/).

Results

Demographic and clinical evaluation

There were no significant differences in the mean age, gender distribution, and MMSE scores between the patients and the controls. The mean age at onset of tremor was 38.2 ± 16.1 years and the mean total FTM score was 36.0 ± 15.8. A positive family history of tremor was documented in 56.7% of the patients with ET. Nine of the 30 patients used to take alcohol occasionally and six of them had reported improvement of tremor with alcohol. All the patients had tremor of both the upper limbs. Tremor of the head was observed in 43.3% of the patients whereas 40.0% had voice tremor. Tremor of the lower limbs was observed in 36.6% of the patients. The details of demography and clinical characteristics of the subjects are provided in Table 1.

Functional connectivity

The seed-to-voxel-based analysis of the resting state images of patients with ET and controls revealed significantly reduced functional connectivity in multiple seeds in patients with ET compared to the controls. With a FDR-corrected statistical threshold of $p < 0.05$, we observed significantly decreased cerebello-cortical and increased cerebello-thalamic connectivity in the patient group. The results of the seed-to-voxel-based connectivity showing significant changes between the ET group and the controls (Table 2) are detailed below. We also analyzed the correlation between the strength of connectivity and FTM scores and observed significant correlations with the connectivity across several seeds.

Functional connectivity of the cerebellum

We observed significant alterations in the connectivity of the cerebellar seeds with several regions of the brain. There was reduction in the cross cerebello-cortical functional connectivity of seeds from the right cerebellar hemisphere (crus I and II) with (i) the left medial prefrontal cortex and (ii) the left supplementary motor cortex. Similarly, the seeds in lobules 8 and 10 on the right side also revealed reduced connectivity with the left precentral gyrus (primary motor cortex). However, there was increased connectivity of the crus I and II (right side) with both thalami and the vermis (Fig. 1).

Functional connectivity of the thalamus

We observed significant increase in the functional connectivity of the bilateral thalami with multiple seeds in both anterior (lobules 4 and 5) and posterior (lobules 8, 6, and 9; crus II and I in the right side) lobes of the cerebellum as well as the vermis (Fig. 2).

Functional connectivity of the left precentral gyrus

Compared to healthy controls, the patients with ET had significantly reduced connectivity of the left precentral gyrus (primary motor area) with two seeds in the posterior lobe of the right cerebellar hemisphere (lobules 8 and 10) and with the supramarginal gyrus of the dominant hemisphere (Fig. 3).

Functional connectivity of the left postcentral gyrus

Compared to controls, the left postcentral gyrus (primary somatosensory area) in patients with ET had significantly decreased functional connectivity with several regions of the brain other than the cerebellar crus I and II (right) that has been described earlier. These regions include (i) the bilateral superior and middle frontal gyrus, and (ii) the bilateral posterior division of superior temporal gyrus. In addition, we also observed a significantly higher connectivity of the left postcentral gyrus with the right supplementary motor cortex (Fig. 4).

Correlation of functional connectivity with the severity of tremor

Correlation analyses were carried out between the strengths of connectivity of the aforementioned seeds with the following: (i) FTM (A + B); (ii) subscores of the head, voice, and upper limb and lower limb tremor; and (iii) duration of tremor.

Table 2 Results of seed-to-voxel-based connectivity showing significant changes between the ET group and the controls

ET > controls (seed region)	Connectivity region	*X*	*Y*	*Z*	Connectivity	p_1 value	β	*T*	Cluster size	*r*	p_2 value
Cerebellum (R) (crus I and II)	Left thalamus	−6	−34	10	Increase	0.02	0.30	5.5	92	0.46	0.01*
	Right thalamus	6	−39	6	Increase	0.02	0.30	5.5	81	0.36	0.04
	Medial prefrontal cortex (L)	−4	68	−12	Decrease	0.001	−0.35	−5.05	285	−0.25	0.17
	Supplementary motor cortex 14	−4	−16	61	Decrease	<0.001	−0.31	−5.14	187	−0.03	0.87
	Vermis	18	−52	−44	Increase	0.04	0.33	5.69	32	0.12	0.50
Bilateral thalami	Cerebellar lobules 4 and 5 (R)	30	−46	−42	Increase	<0.001	0.35	6.68	386	0.58	<0.01*
	Cerebellar lobule 6 (R)	30	−46	−42	Increase	<0.001	0.35	6.68	197	0.33	0.07
	Cerebellar lobule 8 (R)	18	−36	−48	Increase	<0.001	0.35	6.68	637	0.45	0.01*
	Cerebellar lobule 8 (L)	−12	−33	−53	Increase	<0.001	0.35	6.68	426	0.13	0.47
	Cerebellar lobule 9 (R)	48	−61	−26	Increase	<0.001	0.35	6.68	179	0.75	<0.01*
	Cerebellar lobule 9 (L)	−26	−31	−49	Increase	<0.001	0.35	6.68	293	0.13	0.48
	Vermis	−30	−46	−42	Increase	0.007	0.39	5.17	102	0.13	0.47
Precentral gyrus (L)	Cerebellar lobule 8 (R)	18	−36	−48	Decrease	<0.001	−0.21	−6.11	269	−0.41	0.02
	Cerebellar lobule 10 (R)	30	−38	−44	Decrease	<0.001	−0.21	−5.27	55	−0.51	<0.01*
	Supramarginal gyrus (L)	−60	−40	−46	Decrease	0.003	−0.20	−5.73	281	−0.35	0.05
Postcentral gyrus (L)	Cerebellar crus I (R)	50	−68	−34	Decrease	<0.001	−0.23	−5.97	422	−0.68	<0.01*
	Cerebellar crus II (R)	50	−68	−34	Decrease	<0.001	−0.22	−5.49	264	−0.57	<0.01*
	Supplementary motor cortex 14	–	2	52	Increase	0.02	0.18	4.51	73	0.45	0.01*
	Superior temporal gyrus (R)	68	−24	−4	Decrease	0.002	−0.02	−5.3	220	−0.08	0.65
	Superior temporal gyrus (L)	−66	−26	−2	Decrease	0.004	−0.18	−5.14	107	0.01	0.92
	Superior frontal gyrus (R)	42	24	50	Decrease	<0.001	−0.24	−6.02	363	−0.06	0.73
	Superior frontal gyrus (L)	6	56	40	Decrease	0.01	−0.19	−5.19	473	−0.09	0.63
	Middle frontal gyrus (R)	42	24	50	Decrease	<0.001	−0.23	−5.49	503	−0.08	0.65
	Middle frontal gyrus (R)	42	24	50	Decrease	<0.001	−0.23	−5.49	503	−0.08	0.65
	Middle frontal gyrus (L)	−38	22	52	Decrease	0.01	−0.19	−5.19	473	−0.01	0.92

β value represents the Fisher-transformed correlation coefficient values. *T* values represent the strength of connectivity between the source seed region and connectivity regions (correlated-voxels regions). Cluster size represents the number of voxels correlated in the brain region along with the seed region. p_1 value represents the significance level (FDR corrected) of seed region with connectivity to the brain region, *r* represents the Pearson correlation coefficient, and p_2 value is the significance level of the Pearson correlation which was computed with FTM (A + B) score and connectivity strength of individual seed-to-voxel ROI connections. The *asterisk* in p_2 value represents the significance between the FTM (A + B) score and the connectivity strength of individual seed-to-voxel ROI connections after multiple comparisons using FDR

FTM (A + B) scores correlated positively with the connectivity of (i) the left thalamus with the right cerebellar crus I and II (Fig. 5a) and (ii) the bilateral thalami with right cerebellar lobules 4, 5, 8, and 9 (Fig. 5b–d). FTM (A + B) had a negative correlation with the connectivity of (i) the left precentral gyrus with right cerebellar lobule 10 (Fig. 5e), (ii) the left postcentral gyrus with the supplementary motor cortex (Fig. 5f), and (iii) the left postcentral gyrus with the right cerebellar crus I and II (Fig. 5g). Connectivity of the right cerebellar crus I and II with the right thalamus (Fig. 5h) and right cerebellar lobule 8 with the bilateral thalami (Fig. 5i) had positive correlations with head tremor. Upper limb tremor had positive correlation with the connectivity of both the thalami with right cerebellar lobule 9 (Fig. 5j) whereas it had negative correlation with the connectivity of the left postcentral gyrus with the right cerebellar crus II (Fig. 5k). Lower limb tremor had positive correlation with the connectivity of the bilateral thalami with right cerebellar lobule 9 (Fig. 5l).

Discussion

To the best of our knowledge, this is the first seed-to-voxel-based functional connectivity study in patients with ET. The principal result of our study is the alteration in the connectivity of the components of the cerebello-thalamo-cortical network in patients with ET. We observed (i) a significantly decreased right cerebellar functional connectivity with the left primary motor cortex, supplementary motor cortex, and primary somatosensory cortex, correlating negatively with the FTM scores; (ii) increased functional connectivity of the thalamus with the cerebellum and of the supplementary motor cortex with the left postcentral gyrus correlating positively with the FTM score and subscores for head and limb tremor; (iii) decreased connectivity of the left precentral gyrus with cerebellar lobules 8 and 10 (right side) and left supramarginal gyrus; and (iv) reduced connectivity of the left postcentral gyrus with

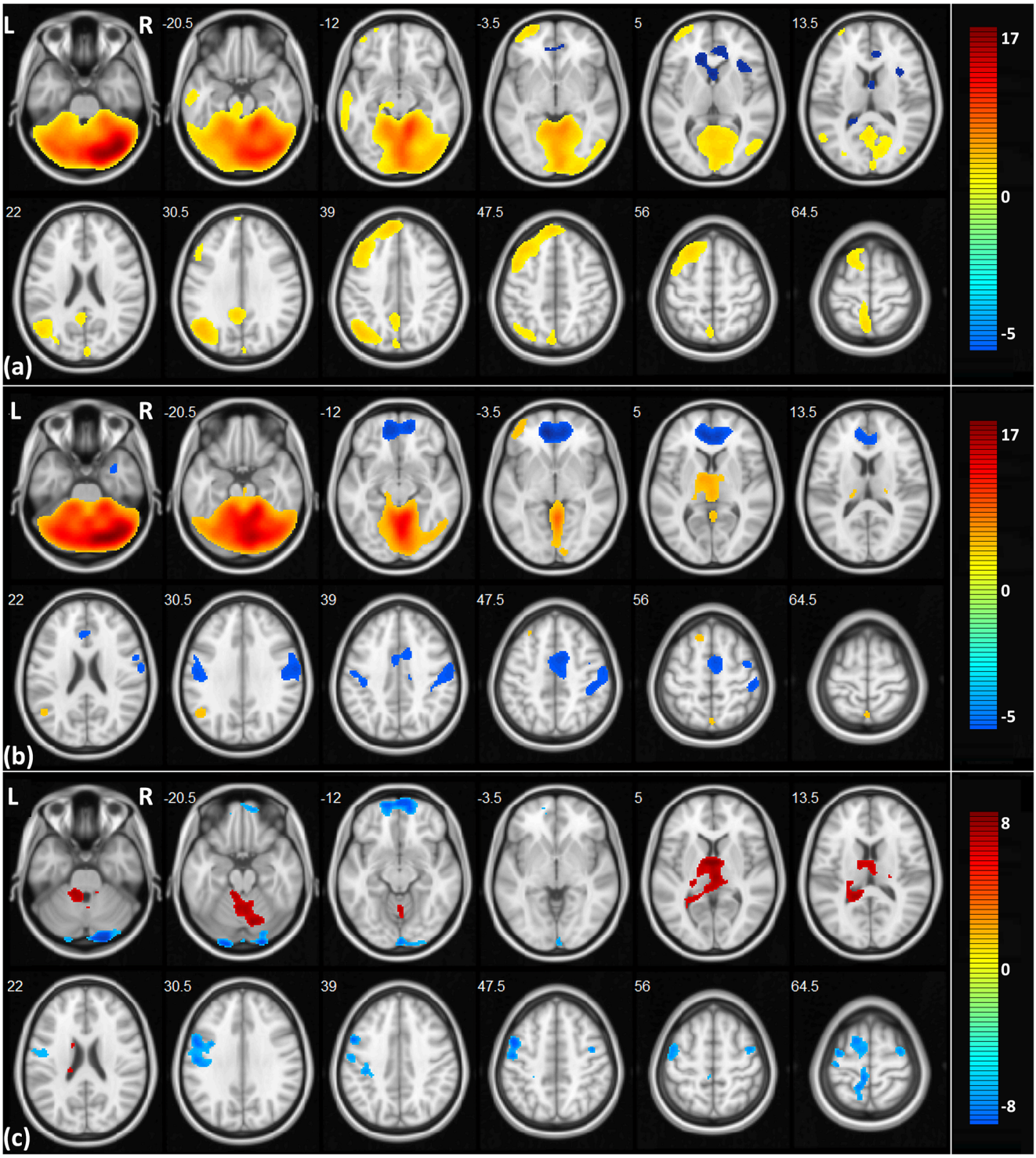

Fig. 1 Seed-to-voxel-based connectivity of the right cerebellum (crus I and II) in **a** healthy controls, **b** patients with ET, and **c** patients with ET > healthy controls rendered on MNI 152 T1 structural template. The *color bars* indicate the changes in connectivity strength

the bilateral superior frontal gyrus, middle frontal gyrus, and posterior division of superior temporal gyrus and increased connectivity with the right supplementary motor cortex.

Previous studies based on histopathology, neuroimaging, and electrophysiology have emphasized the role of the cerebellum in ET [4, 5]. In ET, in addition to the reduction of Purkinje cells in the cerebellum, there is abnormal morphology of these cells (axonal swellings referred to as torpedoes) [20]. In addition, abnormalities of the GABA receptors of the dentate nucleus have been described in ET [21]. Because of

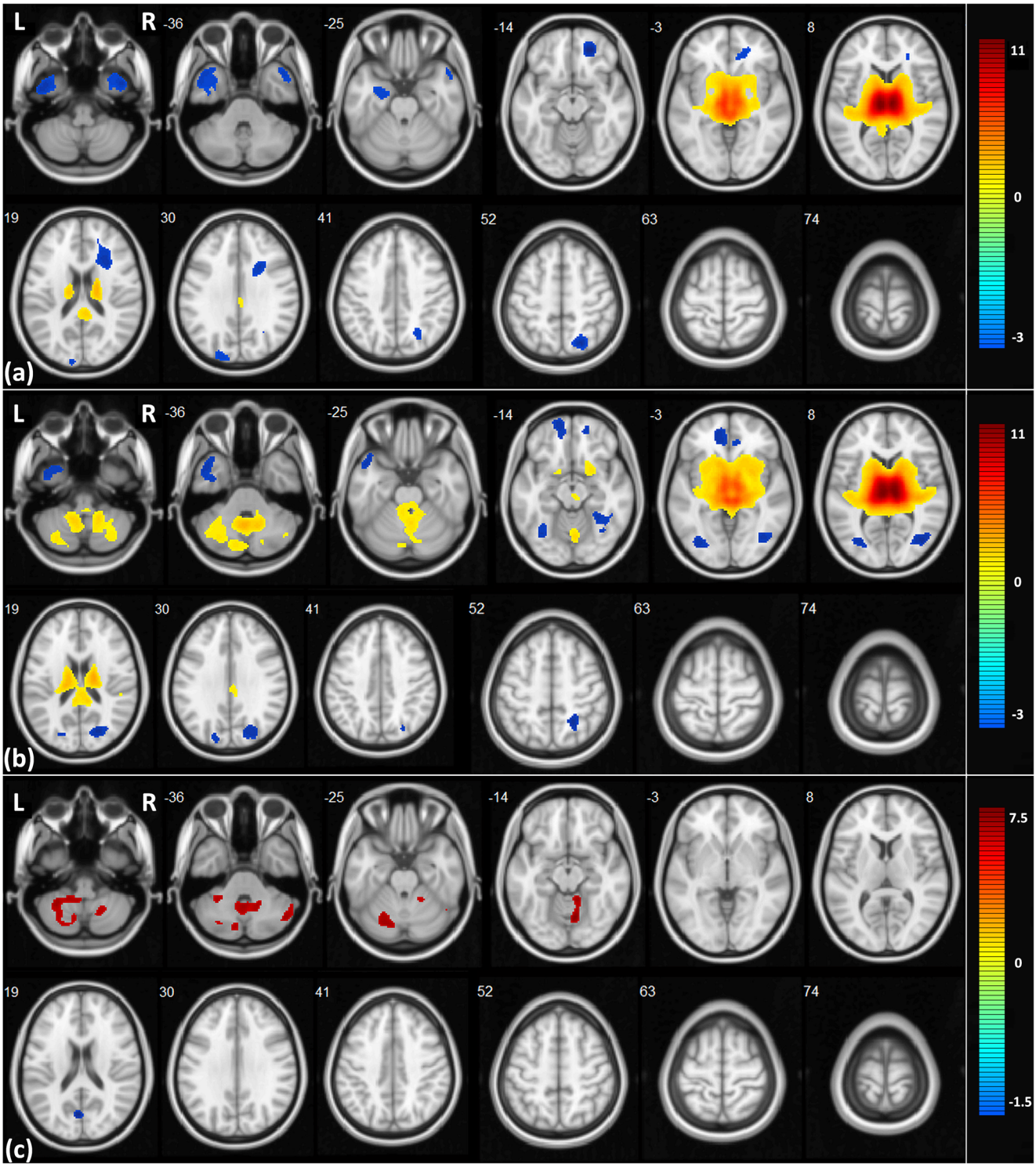

Fig. 2 Seed-to-voxel-based connectivity of the bilateral thalami in **a** healthy controls, **b** patients with ET, and **c** patients with ET > control rendered on MNI 152 T1 structural template. The *color bars* indicate the changes in connectivity strength

the pacemaker-like activity, the dentate nucleus has the ability to produce an inhibitory post-synaptic potential, which is dependent on the GABAergic Purkinje cell inputs. Hence, it has been postulated that the neurodegeneration and abnormal neurotransmission in the cerebellum might alter the GABAergic output resulting in the disinhibition of the dentate nucleus [21].

We observed reduced cross cerebellar functional connectivity of seeds in the posterior lobe (lobules 8 and 10, crus I and II) of the right cerebellum with the primary motor cortex,

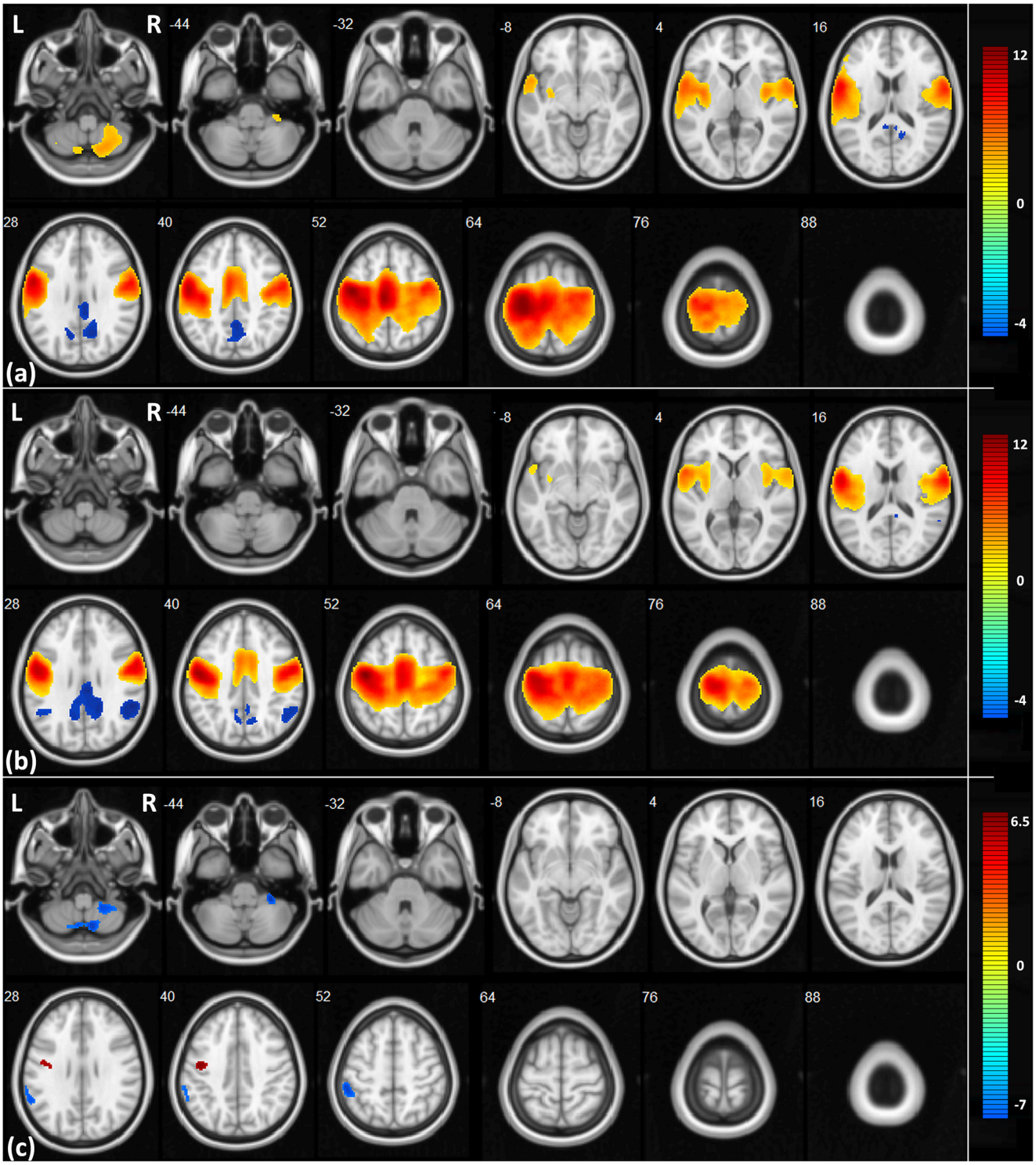

Fig. 3 Seed-to-voxel-based connectivity of the left precentral gyrus in **a** healthy controls, **b** patients with ET, and **c** patients with ET > control rendered on MNI 152 T1 structural template. The *color bars* indicate the changes in connectivity strength

supplementary motor cortex, and postcentral gyrus of the dominant hemisphere. Connectivity of these seeds had negative correlations with the FTM scores. Although the exact association cannot be established, it may be speculated that the altered cerebello-cortical connectivity is partially indicative of the disease process. In a recent task-based fMRI study in patients with ET, Buijink et al. (2015) have also reported significantly reduced functional coupling between the cerebellum and the cortical motor areas [22]. This shows that the cerebello-cortical network is altered in both resting

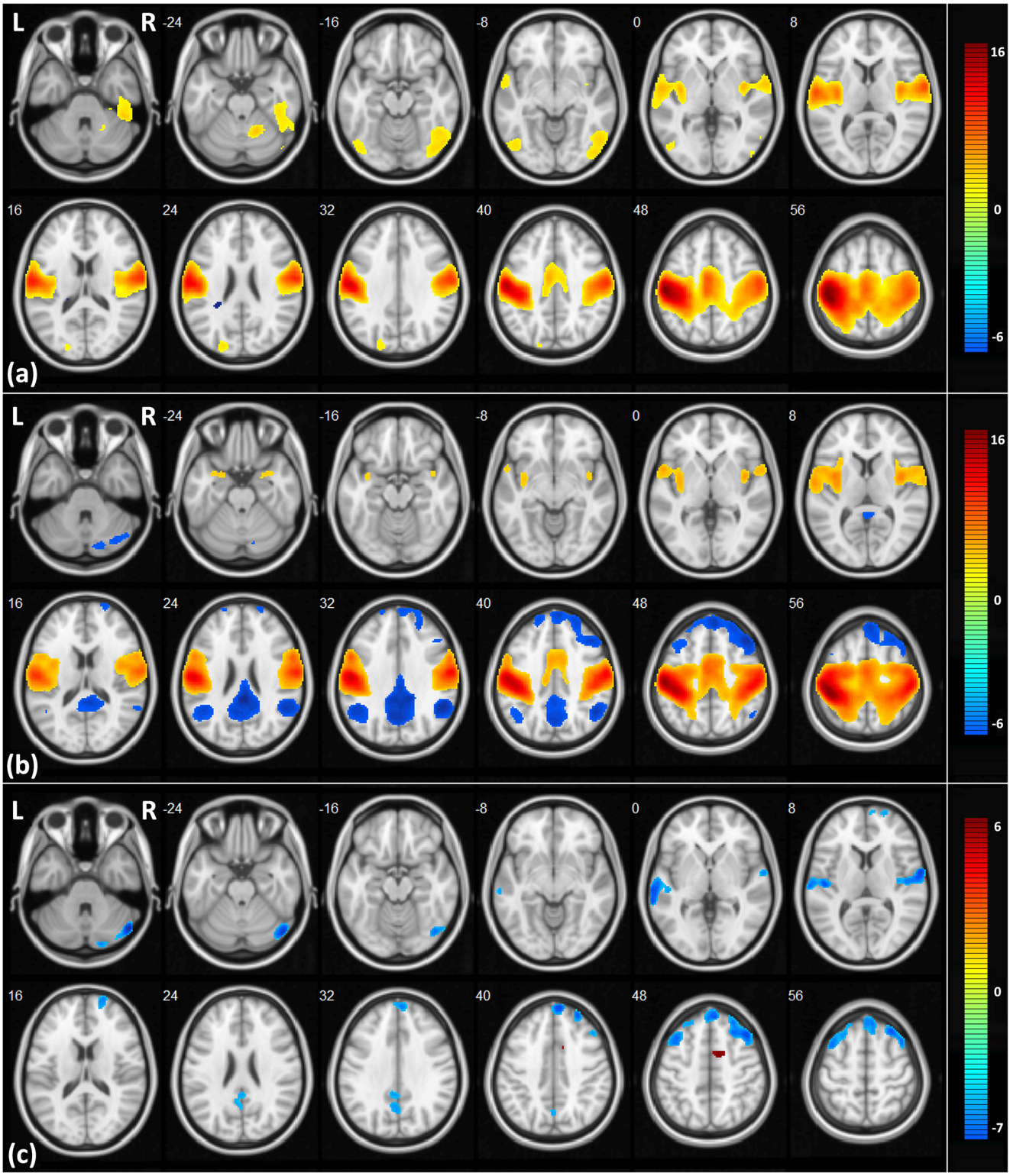

Fig. 4 Seed-to-voxel-based connectivity of the left postcentral gyrus in **a** healthy controls, **b** patients with ET, and **c** patients with ET > control rendered on MNI 152 T1 structural template. The *color bars* indicate the changes in connectivity strength

state and while performing a task. However, studies designed for both rs-fMRI and task-based fMRI in the same group of patients may provide more insights into the alterations in the cerebello-cortical connectivity.

Contrary to the pattern of connectivity of the cerebellar seeds with the cortical seeds, we observed a significantly higher functional connectivity with both thalami. The FTM (A + B) score and subscore for head tremor had positive

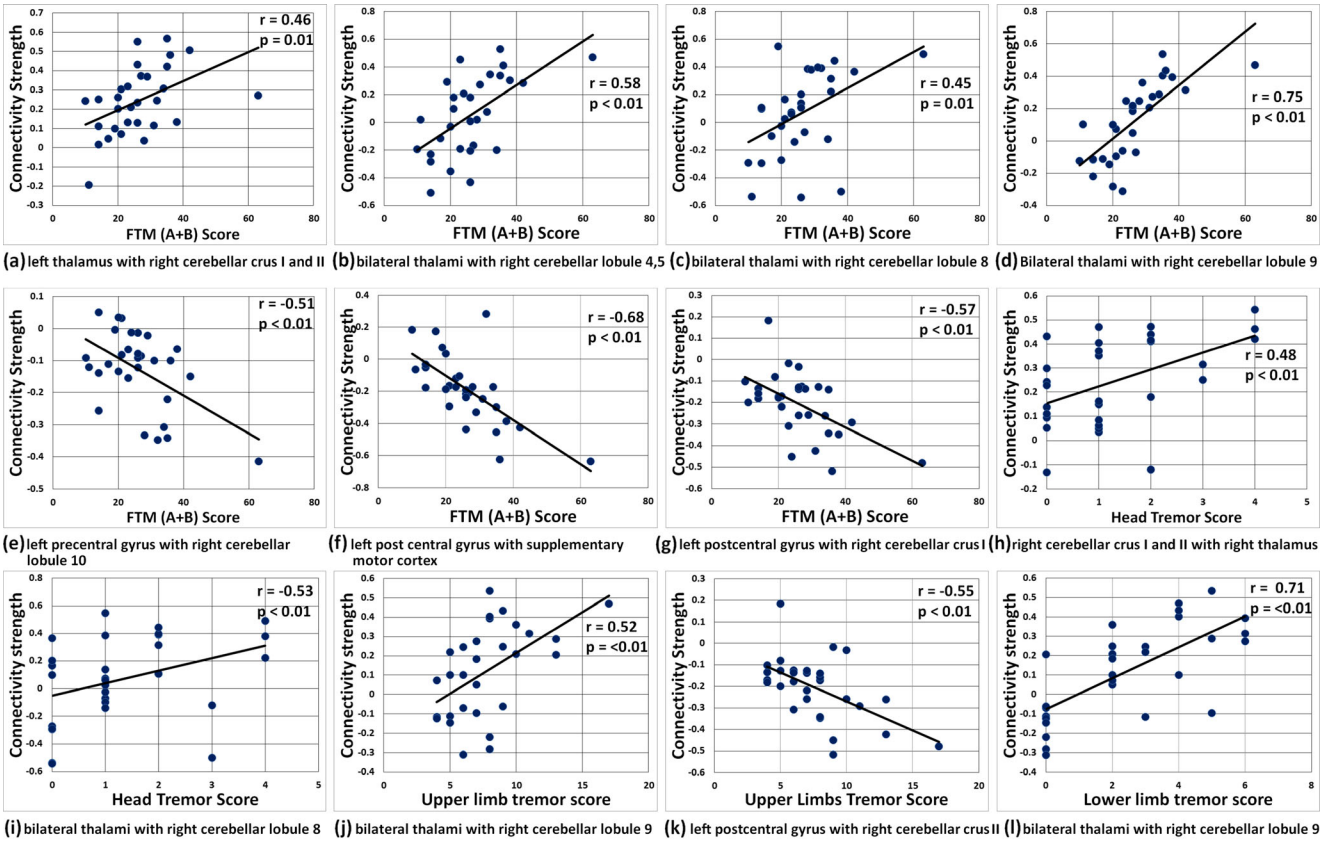

Fig. 5 Correlation of the strength of connectivity across the seed pairs with tremor severity. **a** Positive correlation with FTM (A + B) scores with the connectivity of the left thalamus with right cerebellar crus I and II. **b** Positive correlation with FTM (A + B) scores with the connectivity of the bilateral thalami with right cerebellar lobules 4 and 5. **c** Positive correlation with FTM (A + B) scores with the connectivity of the bilateral thalami with right cerebellar lobule 8. **d** Positive correlation with FTM (A + B) scores with the connectivity of the bilateral thalami with right cerebellar lobule 9. **e** Negative correlation of FTM (A + B) with the connectivity of the left precentral gyrus with right cerebellar lobule 10. **f** Negative correlation of FTM (A + B) with the connectivity of the left postcentral gyrus with the supplementary motor cortex. **g** Negative correlation of FTM (A + B) with the connectivity of the left postcentral gyrus with right cerebellar crus I and II. **h** Positive correlation of FTM subscore for head tremor with the connectivity of right cerebellar crus I and II with the right thalamus. **i** Positive correlation of FTM subscore for head tremor with the connectivity of right cerebellar lobule 8 with the bilateral thalami. **j** Positive correlation of FTM subscore for upper limb tremor with the connectivity of the bilateral thalami with right cerebellar lobule 9. **k** Negative correlation of FTM subscore for upper limb tremor with the connectivity of the left postcentral gyrus with right cerebellar crus II. **l** Positive correlation of FTM subscore for lower limb tremor with the connectivity of the bilateral thalami with right cerebellar lobule 9

correlation with the connectivity of the thalami with ipsilateral cerebellar lobule 8. Similarly, connectivity of the right cerebellar crus I and II with the right thalamus positively correlated with subscore for head tremor and the connectivity of both the thalami with right cerebellar lobule 9 correlated positively with subscores for upper and lower limb tremor. This result supports the possible role of the thalamus in the genesis of tremor in ET as suggested by the previous works [23]. Our result also provides evidence for the possible role of different cerebellar lobules in the genesis of different tremor subtypes. It has been suggested by previous studies that the increased cerebello-thalamic connectivity in ET probably represents the pathological entrainment of the cerebello-thalamic network secondary to the altered cerebello-cortical network [22]. Buijink et al. (2015) also observed increased connectivity of the left thalamus with cerebellar lobules 1 to 4 on the right side which had a positive correlation with the clinical tremor severity [22]. Fang et al. (2013) in a rs-fMRI-based study had reported abnormal regional homogeneity (ReHo) in ventral intermediate and medio-dorsal nucleus of the thalamus in patients with ET [11]. Though the ReHo-based analyses do not provide a clear picture of the long-range connectivity, the study by Fang et al. certainly reinforces the role of the thalamus in the pathogenesis of ET. Increased cerebello-thalamic connectivity has also been reported in other diseases in which tremor is a predominant symptom. Although a pathologically distinct disorder, a recent study reported that patients with tremor dominant Parkinson's disease had increased functional connectivity between the ventral intermediate nucleus of the thalamus and the cerebellum [24]. While several aforementioned studies have implicated the alterations in cerebello-cortical and cerebello-thalamic networks in the neurobiology

of tremor, it is difficult to predict which network gets affected early in the disease process. For this reason, longitudinal studies assessing the functional brain connectivity of ET patients are of paramount importance to get a better insight into the natural course of alterations of the cerebello-thalamo-cortical network.

The cerebello-thalamic connectivity (bilateral thalami to right cerebellar lobule 8, and right thalamus to cerebellar crus I and II) had positive correlations with subscores for head tremor. Interestingly, voice tremor that usually coexists with head tremor in patients with ET did not correlate with connectivity of the thalamic seeds. This perhaps indicates separate neural substrates for tremor of head and voice in ET and warrants studies in future assessing the functional connectivity separately in patients with head and voice tremor.

In addition to the reduced functional connectivity with contralateral cerebellar lobules 8 and 10 that is described earlier, the left precentral gyrus also had reduced connectivity with the ipsilateral supramarginal gyrus. Although the role of the supramarginal gyrus in the genesis of tremor is unclear, it may be speculated to be associated with the non-motor symptoms of ET. A recent rs-fMRI study by Benito-León et al. based on ICA has reported increased connectivity of several components of frontoparietal network (FPN) including the left supramarginal gyrus in ET patients compared to controls [8]. FPN is one of the important resting state networks involved in cognitive processing. Hence, altered connectivity of the supramarginal gyrus, which is a part of FPN, may possibly indicate subclinical impairments in certain cognitive domains. More studies are warranted to understand the connectivity of the precentral gyrus and supramarginal gyrus and their effect of cognitive functions in patients with ET.

The left postcentral gyrus (primary somatosensory cortex) had a significantly reduced connectivity with the right cerebellar crus I and II, bilateral superior temporal gyrus, superior frontal gyrus, and middle frontal gyrus. It also had an increase in connectivity with supplementary motor area. Although the literature on role of the somatosensory cortex in ET is sparse, a study by Restuccia et al. (2003) has reported abnormal gating of somatosensory inputs in ET [25]. As these connections of the primary somatosensory cortex with the cerebellum and supplementary motor area had significant correlations with FTM (A + B) score, further studies on the connectivity of the primary somatosensory cortex in ET are warranted.

Our study has several limitations. We have evaluated the patients only for motor symptoms and could not systematically evaluate the presence and severity of non-motor symptoms. Hence, the altered functional connectivity in ET in our study may not represent the neural correlate of the non-motor symptoms of ET. In the future, neuroimaging studies along with detailed assessment of both motor and non-motor symptoms may provide better insight into the dysfunctions of the neural networks associated with ET. There was heterogeneity in the clinical features as patients had tremor of several parts of the body in addition to the upper limbs. Future studies focusing on comparison of functional connectivity of clinically homogenous cohort of ET patients may provide better insights into the neurobiology of ET. As previous epidemiological and neuroimaging studies have posited ET with and without head tremor to be distinct entities [2, 26], it will be interesting to compare the functional connectivity of these two subtypes of ET. rs-fMRI itself has certain limitations as several physiological factors such as cardiac and respiratory cycles, arterial CO_2 of the subjects may affect the results. A recent study by Driver et al. has revealed that neuronal oscillatory power is strongly linked to arterial CO_2 concentration down to the fine-scale modulations that occur during spontaneous breathing [27].

Conclusions

To conclude, our study is the first seed-to-voxel-based rs-fMRI study assessing the long-range connectivity of several structures in ET. We observed significant alterations in the cerebello-thalamo-cortical circuit in patients with ET compared to healthy controls. The major findings were reduction in the cerebello-cortical connectivity and increased cerebello-thalamic connectivity in ET. These results further substantiate the role of an altered cerebello-thalamic-cortical network in the neurobiology of ET. Further studies should evaluate the role of this network in different subtypes of ET and correlation with non-motor symptoms of ET.

Compliance with ethical standards We declare that all human and animal studies have been approved by the ethics committee of the National Institute of Mental Health & Neurosciences (NIMHANS), Bangalore, India, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. We declare that all patients gave informed consent prior to inclusion in this study.

Conflict of interest We declare that we have no conflict of interest.

References

1. Louis ED (2005) Essential tremor. Lancet Neurol 4:100–110. doi:10.1016/S1474-4422(05)00991-9
2. Lenka A, Bhalsing KS, Jhunjhunwala KR, et al. (2015) Are patients with limb and head tremor a clinically distinct subtype of essential tremor? Can J Neurol Sci 1–6. doi: 10.1017/cjn.2015.23
3. Louis ED (2014) "Essential tremor" or "the essential tremors": is this one disease or a family of diseases? Neuroepidemiology 42:81–89. doi:10.1159/000356351
4. Louis ED, Vonsattel JPG, Honig LS et al (2006) Essential tremor associated with pathologic changes in the cerebellum. Arch Neurol 63:1189–1193. doi:10.1001/archneur.63.8.1189
5. Pinto AD, Lang AE, Chen R (2003) The cerebellothalamocortical pathway in essential tremor. Neurology 60:1985–1987. doi:10.1212/01.WNL.0000065890.75790.29

6. Sharifi S, Nederveen AJ, Booij J, Van Rootselaar AF (2014) Neuroimaging essentials in essential tremor: a systematic review. NeuroImage Clin 5:217–231. doi:10.1016/j.nicl.2014.05.003
7. Bhalsing KS, Saini J, Pal PK (2013) Understanding the pathophysiology of essential tremor through advanced neuroimaging: a review. J Neurol Sci 335:9–13. doi:10.1016/j.jns.2013.09.003
8. Benito-León J, Louis ED, Romero JP et al (2015) Altered functional connectivity in essential tremor: a resting-state fMRI study. Medicine (Baltimore) 94:e1936. doi:10.1097/MD.0000000000001936
9. Fang W, Chen H, Wang H et al (2015) Multiple resting-state networks are associated with tremors and cognitive features in essential tremor. Mov Disord 30:1926–1936. doi:10.1002/mds.26375
10. Popa T, Russo M, Vidailhet M et al (2013) Cerebellar rTMS stimulation may induce prolonged clinical benefits in essential tremor, and subjacent changes in functional connectivity: an open label trial. Brain Stimul 6:175–179. doi:10.1016/j.brs.2012.04.009
11. Fang W, Lv F, Luo T et al (2013) Abnormal regional homogeneity in patients with essential tremor revealed by resting-state functional MRI. PLoS One 8:1–11. doi:10.1371/journal.pone.0069199
12. Gallea C, Popa T, Garcia-Lorenzo D et al (2015) Intrinsic signature of essential tremor in the cerebello-frontal network. Brain 138 (Pt10):2920–2933. doi:10.1093/brain/awv171
13. Deuschl G, Bain P, Brin M (1998) Consensus statement of the Movement Disorder Society on tremor. Ad Hoc Scientific Committee. Mov Disord 13(Suppl 3):2–23. doi:10.1002/mds.870131303
14. Shargel L, Wu-Pong S, Yu A (2004) Applied biopharmaceutics and pharmacokinetics, 5th Edition. Ann Intern Med. doi:10.7326/0003–4819–94-6-826_2
15. Stacy MA, Elble RJ, Ondo WG et al (2007) Assessment of interrater and intrarater reliability of the Fahn–Tolosa–Marin Tremor Rating Scale in essential tremor. Mov Disord 22:833–838. doi:10.1002/mds.21412
16. Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113. doi:10.1016/0028-3932(71)90067-4
17. Friston KJ (2006) Statistical parametric mapping: the analysis of functional brain images
18. Behzadi Y, Restom K, Liau J, Liu TT (2007) A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. NeuroImage 37:90–101. doi:10.1016/j.neuroimage.2007.04.042
19. Tzourio-Mazoyer N, Landeau B, Papathanassiou D et al (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15:273–289. doi:10.1006/nimg.2001.0978
20. Louis ED, Yi H, Erickson-Davis C et al (2009) Structural study of Purkinje cell axonal torpedoes in essential tremor. Neurosci Lett 450:287–291. doi:10.1016/j.neulet.2008.11.043
21. Paris-Robidas S, Brochu E, Sintes M et al (2012) Defective dentate nucleus GABA receptors in essential tremor. Brain 135:105–116. doi:10.1093/brain/awr301
22. Buijink AWG, Van Der Stouwe AMM, Broersma M et al (2015) Motor network disruption in essential tremor: a functional and effective connectivity study. Brain 138:2934–2947. doi:10.1093/brain/awv225
23. Hua SE, Lenz FA, Zirh TA et al (1998) Thalamic neuronal activity correlated with essential tremor. J Neurol Neurosurg Psychiatry 64:273–276. doi:10.1136/jnnp.64.2.273
24. Zhang J-R, Feng T, Hou Y-N et al (2016) Functional connectivity of vim nucleus in tremor- and akinetic-/rigid-dominant Parkinson's disease. CNS Neurosci Ther 26:416–423. doi:10.1111/cns.12512
25. Restuccia D, Valeriani M, Barba C et al (2003) Abnormal gating of somatosensory inputs in essential tremor. Clin Neurophysiol 114:120–129. doi:10.1016/S1388-2457(02)00335-8
26. Quattrone A, Cerasa A, Messina D et al (2008) Essential head tremor is associated with cerebellar vermis atrophy: a volumetric and voxel-based morphometry MR imaging study. Am J Neuroradiol 29:1692–1697. doi:10.3174/ajnr.A1190
27. Driver ID, Whittaker JR, Bright MG et al (2016) Arterial CO2 fluctuations modulate neuronal rhythmicity: implications for MEG and fMRI studies of resting-state networks. J Neurosci 36:8541–8550. doi:10.1523/JNEUROSCI.4263-15.2016