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Resting state functional MRI reveals abnormal network connectivity in orthostatic tremor

Julián Benito-León, MD, PhD[a,b,c,*], Elan D. Louis, MD, MSc[d,e,f], Eva Manzanedo, PhD[g], Juan Antonio Hernández-Tamames, PhD[g], Juan Álvarez-Linera, MD, PhD[h], José Antonio Molina-Arjona, MD, PhD[a], Michele Matarazzo, MD[a], Juan Pablo Romero, MD, PhD[a,i], Cristina Domínguez-González, MD[a], Ángela Domingo-Santos, MD[a], Álvaro Sánchez-Ferro, MD[a,j,k]

Abstract

Very little is known about the pathogenesis of orthostatic tremor (OT). We have observed that OT patients might have deficits in specific aspects of neuropsychological function, particularly those thought to rely on the integrity of the prefrontal cortex, which suggests a possible involvement of frontocerebellar circuits. We examined whether resting-state functional magnetic resonance imaging (fMRI) might provide further insights into the pathogenesis on OT. Resting-state fMRI data in 13 OT patients (11 women and 2 men) and 13 matched healthy controls were analyzed using independent component analysis, in combination with a "dual-regression" technique, to identify group differences in several resting-state networks (RSNs). All participants also underwent neuropsychological testing during the same session. Relative to healthy controls, OT patients showed increased connectivity in RSNs involved in cognitive processes (default mode network [DMN] and frontoparietal networks), and decreased connectivity in the cerebellum and sensorimotor networks. Changes in network integrity were associated not only with duration (DMN and medial visual network), but also with cognitive function. Moreover, in at least 2 networks (DMN and medial visual network), increased connectivity was associated with worse performance on different cognitive domains (attention, executive function, visuospatial ability, visual memory, and language). In this exploratory study, we observed selective impairments of RSNs in OT patients. This and other future resting-state fMRI studies might provide a novel method to understand the pathophysiological mechanisms of motor and nonmotor features of OT.

Abbreviations: DMN = default mode network, fMRI = functional magnetic resonance imaging, FSL = FMRIB Software Library, HC = healthy control, MNI = Montreal Neurological Institute, MRI = magnetic resonance imaging, OT = orthostatic tremor, RSN = resting-state network, SD = standard deviation, TFCE = threshold-free cluster enhancement, WAIS-III = Wechsler Adult Intelligence Scale-Third Edition. WMS-III = Wechsler Memory Scale-Third Edition.

Keywords: case–control study, functional connectivity, magnetic resonance imaging, orthostatic tremor

1. Introduction

The term "orthostatic tremor" (OT), also known as "shaky legs syndrome,"[1] was first coined in 1984 by Heilman,[2] although there may have been earlier descriptions of this entity.[3] This is an intriguing and rare condition, characterized by tremor and unsteadiness when standing that is relieved when sitting or walking. OT can be idiopathic or secondary.[4–6] Gerschlager et al[4] suggested the subdivision of OT into 2 broad groups – those with "primary OT" with or without postural arm tremor, and those with "OT plus," in whom there are additional associated movement disorders, mainly Parkinsonism.

The pathogenesis of OT is poorly understood. Clinical and neuroimaging data suggest that it could arise from a central

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[a] Department of Neurology, University Hospital "12 de Octubre", Madrid, [b] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), [c] Department of Medicine, Complutense University, Madrid, Spain, [d] Department of Neurology, Yale School of Medicine, [e] Department of Chronic Disease Epidemiology, Yale School of Public Health, [f] Center for Neuroepidemiology and Clinical Neurological Research, Yale School of Medicine and Yale School of Public Health, New Haven, CT, USA, [g] Neuroimaging Laboratory, Center for Biomedical Technology, Rey Juan Carlos University, Móstoles, [h] Department of Radiology, Hospital Ruber International, [i] Faculty of Biosanitary Sciences, Francisco de Vitoria University, Pozuelo de Alarcón, Madrid, Spain, [j] Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, MA, USA, [k] Movement Disorders Laboratory, HM CINAC, HM Hospitales, Móstoles (Madrid), Spain.

[*] Correspondence: Julián Benito-León, Avda. de la Constitución 73, portal 3, 7º izquierda, E-28821 Coslada, Madrid, Spain (e-mail: jbenitol67@gmail.com).

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generator in the cerebellum or brainstem.[4,6,7] Furthermore, we have recently observed that OT patients have deficits in specific aspects of neuropsychological function, particularly those thought to rely on the integrity of the prefrontal cortex, which suggests a possible involvement of frontocerebellar circuits.[8] Notwithstanding, very little is known about the underlying causes and brain networks involved in OT, and further study is needed.

Among various advanced magnetic resonance imaging (MRI) techniques, functional MRI (fMRI) allows one to explore the dynamics of cortical functional reorganization, mainly using activation paradigms evoked by simple motor tasks or cognitive tasks. In task-related fMRI studies, however, there is some difficulty interpreting results due to large intersubject variability in task performance.[9] This limitation of task-related fMRI studies is not a feature of a more recent approach – the acquisition of fMRI data during resting state conditions (i.e., with participants awake, but relaxed and not involved in any task).[9] In this setting, spatially distributed networks of interest can be detected that can characterize resting-state networks (RSNs).[9] These RSNs have demonstrated high reproducibility across participants, time, and research sites, and could serve as surrogate biomarkers for several neurological diseases, including paroxysmal kinesigenic dyskinesia, focal hand dystonia, essential tremor, Alzheimer disease, and Parkinson disease, among others.[10–14]

With respect to the motor features of OT, both the cerebellum and sensorimotor networks could be involved. Aside from their possible involvement in these motor symptoms, RSNs alterations might be involved in the pathogenesis of nonmotor manifestations associated with OT. These latter broader networks include the default mode network (DMN) and executive, frontoparietal, auditory/language, and visual networks. However, overall, RSNs integrity in OT patients has not previously been reported.

The present study, using fMRI, compares resting-state functional connectivity in OT patients and healthy controls (HCs) and specifically assessed the following RSNs: DMN, executive network, 2 frontoparietal networks (left- and right-lateralized), as well as sensorimotor, cerebellar, auditory/language, and visual networks. Our a priori study hypotheses were as follows: OT patients will show changes relative to HC in the cerebellar and sensorimotor networks; and several additional RSNs will be impaired in OT patients relative to HC, including the DMN, executive, and the frontoparietal networks (i.e., RSNs that are involved in cognitive processes).[15,16]

2. Methods

2.1. Participants

Patients with OT were consecutively recruited from December 2011 to May 2013 from the outpatient neurology clinics of the University Hospital "12 de Octubre" in Madrid (Spain). Four neurologists, with expertise in movement disorders (JB-L, JPR, MM, and ÁS-F), examined these patients, who were referred to the outpatient neurology clinics with a subjective feeling of unsteadiness when standing, which was absent while walking, seated, or supine. Diagnoses of OT were assigned by the 4 neurologists using the Consensus Statement on Tremor by the Movement Disorder Society.[17]

Of 21 eligible OT patients, 7 were excluded from the final cohort because they did not complete the neuropsychological testing or the MRI procedures. Finally, a strict criterion for head movement assessment was adopted (maximal absolute head movement less than 1.0 mm and 1.0° in the x, y, and z directions). One OT woman who failed to meet this criterion was excluded for this reason. No HC was excluded due to incomplete neuropsychological evaluation or refusal to perform MRI.

OT cases were 1:1 frequency-matched with HC. Frequency-matching was based on age, sex, and years of education.

HC were recruited from either relatives or friends of the health professionals working at the University Hospital "12 de Octubre" of Madrid (Spain) or among the relatives of patients who came to the neurological clinics for reasons other than OT (e.g., headache, dizziness). None reported having a 1st- or 2nd-degree relative with OT or essential tremor. Each control was examined by 2 neurologists (JPR and ÁS-F), to further rule out any neurological conditions, and by a neuropsychologist, as noted above.

According to a recently published comorbidity score developed in ambulatory care settings,[18] a comorbidity index was calculated. The presence of several conditions (atrial fibrillation, nonmetastatic cancer, metastatic cancer, chronic obstructive pulmonary disease, depression, dementia, diabetes, epilepsy [treated], heart failure, myocardial infarction, psychiatric disorders, renal disease, and stroke) resulted in the assignment of more points than others, and the score ranged from 0 to 28 (i.e., all conditions present).[18]

2.2. Neuropsychological testing

All participants underwent a detailed neuropsychological assessment covering the domains of attention, executive function, verbal memory, visual memory, visuospatial ability, and language. These tests have previously been described.[8] No patients were being treated with medication for OT (i.e., clonazepam, dopaminergic agonists, or barbiturates) at the time of the neuropsychological testing because all patients were newly diagnosed at inclusion in the cohort. Neuropsychological tests were conducted in a single session by an experienced clinical neuropsychologist (VP, see acknowledgments) who was blinded to the clinical status during an interview in the week in which the participants completed the below MRI examination.

Raw scores of neuropsychological tests were transformed into z scores based on the mean and standard deviation values from HC. Higher z scores indicated better performance. The severity of depressive symptoms were measured by the original 17-item version of the Hamilton Depression Rating Scale.[19]

The tasks from the neuropsychological assessment were z-standardized, averaged, and compiled to create 6 composite scores (attention, executive function, visuospatial ability, verbal memory, visual memory, and language) for each participant. Each composite score was then employed as a continuous variable in subsequent regression analyses.

2.3. MRI procedure

Patients and controls were positioned in the scanner and were told to relax with their eyes closed. They were immobilized with a custom-fit blue bag vacuum mold (Medical Intelligence, Schwabmünchen, Germany) to prevent motion artifacts. Earplugs and noise-reduction headphones were used to attenuate scanner noise. The functional run required 6 minutes to complete.

Images were acquired on a General Electric Signa 3T MR Scanner (General Electric Healthcare, Fairfield, CT) using a whole-body radiofrequency coil for signal excitation and quadrature 8-channel coil for reception. Resting-state fMRI data consisted of 120 volumes of a repeated gradient-echo echo planar imaging T2*-weighted sequence whose parameters were

repetition time = 3 seconds, echo time = 28 milliseconds, voxel dimensions = 2.7 × 2.7 × 2.8 mm, 39 oblique ACPC-oriented slices, flip angle = 90°, and 6 dummy scans.

For the structural image, a high-resolution, 3-dimensional T1-weighted gradient Echo-SPGR was acquired with the following parameters: repetition time = 9.2 milliseconds, echo time = 4.128 milliseconds, inversion time = 500 milliseconds, field of view = 240 mm, acquisition matrix = 240 × 240, slice thickness = 1 mm, full brain coverage, resolution = 1 × 1 × 1 mm, flip angle = 120°, and 166 sagittal slices.

2.4. Image preprocessing

Resting-state fMRI images were analyzed using FMRIB Software Library (FSL; available at: www.fmrib.ox.ac.uk/fsl) and Analysis of Functional NeuroImages (available at: http://afni.nimh.nih.gov/afni/).[20,21] The preprocessing included the following steps: despiking, slice-timing correction, motion correction, field map correction, spatial smoothing (full-width half maximum = 6 mm), temporal high pass filtering (cut-off of 100 seconds), functional to anatomical image registration, and normalization to the atlas space of the Montreal Neurological Institute (MNI) 152 T1 2 mm template. Despiking was performed using Analysis of Functional NeuroImages, and the remainder of the steps of the preprocessing pipeline were performed with FSL.

2.5. Image analysis

Resting-state fMRI data were analyzed using independent component analysis, in combination with a "dual-regression" technique.[22] This method automatically determines the most consistent RSNs, based on an assessment of the similarity of predefined templates.[22]

In order to obtain the group independent spatial maps identifying RSNs across all participants, we used the multivariate exploratory linear optimized decomposition into independent components toolbox in FSL. A Temporal Concatenation Group Independent Component Analysis restricting the number of components to 25 was performed to study large-scale spatial networks.[23] Data from all subjects, patients and controls, were concatenated for this analysis.

The 25 independent components were sorted into 2 broad classes: biologically plausible/functionally relevant components or RSNs, and scanner/physiological artifactual components (cerebrospinal fluid, white matter, head motion, and large vessels artifacts). The inspection was made visually based on each component's spatial profile and time course following criteria purposed by Kelly et al.[24] Eight RSNs previously related to functionally relevant brain functions[25] were identified: DMN, executive network, 2 frontoparietal networks (left- and right-lateralized), and sensorimotor, cerebellar, auditory/language, and visual networks.

These 8 independent components spatial maps were used as the RSN spatial map templates in the 1st step of the subsequent dual regression analysis.

The image analysis was performed in 2 steps with FSL-dual regression:[22] each RSN spatial map template was used as a mask in a spatial regression against each individual fMRI dataset in order to obtain a subject specific time course associated to that RSN; and the obtained individual time courses related to each RSN spatial map template in the 1st regression were used in a temporal regression to estimate a subject-specific spatial correlation map per RSN. After this dual regression, spatial maps of all subjects were collected for each original RSN.

Permutation statistics were computed with FSL-randomize to evaluate functional connectivity differences between the 2 groups in each RSN using the previously obtained subject specific spatial maps (number of permutations = 1000). We statistically accounted for effects of age and sex by including these variables as covariates in the statistical model. The dual regression considered the whole brain, not only the areas where each RSN was strongly manifested.[22] Results were considered significant for $P < 0.005$ uncorrected using a threshold-free cluster enhancement.[26] The following information was provided for the clusters whose size was greater than or equal to 10 voxels (80 mm^3): maximum uncorrected threshold-free cluster enhancement P value of the cluster (permutation statistics); cluster size; MNI coordinates of the maximum of the cluster; Talairach atlas label of this region; and the corresponding Brodmann area or the most probable lobule reported in the cerebellar atlas in MNI152 space, after normalization with FMRIB Linear Image Registration Tool.

All procedures were approved by the ethical standards committees on human experimentation at the University Hospital 12 de Octubre (Madrid). Written (signed) informed consent was obtained from all enrollees.

2.6. Sample size and statistical analyses of clinical and neuropsychological data

In several recent resting-state fMRI studies of other rare movement disorders, a sample size of 13 to 15 in each group has been sufficient.[10,11]

Statistical analyses for the clinical and neuropsychological measures were conducted using SPSS 21 (Statistical Package for the Social Sciences). Mean scores (age and neuropsychological variables) were compared using 2 independent sample t tests for continuous and normally distributed data, and Mann–-Whitney U test for nonnormally distributed data, where appropriate. The χ^2 test was used to analyze group differences in sex and smoking status.

For the RSNs that were significant after group comparison, the mean z scores of the clusters whose size was greater than or equal to 50 voxels were regressed against disease duration, and each 1 of the 5 different cognitive composite measures, and the 17-item HAMD score. A value of $P < 0.05$ was considered statistically significant.

Failure of any of the test was defined as a z score $\leq$1.5 standard deviation compared to HC. Cognitive impairment was defined as failure on at least 3 tests.

3. Results

3.1. Clinical and neuropsychological testing results

All 13 OT patients were right-handed (mean age 65.5, range 37–81). There was a female preponderance (N = 11, 84.6%) with a mean age of onset at 55.9 (range 17–74) years. On diagnosis, 8 (61.5%) of patients presented with primary OT and 5 (38.5%) had additional neurological features (mild parkinsonian signs). Ten (76.9%) patients reported a progressive course. Structural brain MRI was unremarkable in all patients; none had cerebellar atrophy. Routine blood and chemistry tests including thyroid function tests, serum protein electrophoresis, and vitamin B12 levels were also in the normal range in all patients. No patients were being treated with medication for OT (i.e., clonazepam, dopaminergic agonists, gabapentin, or barbiturates) at the time of the neuropsychological testing.

Table 1

Comparison of demographic, clinical and neuropsychiatric domains of orthostatic tremor patients versus healthy controls.

	Orthostatic tremor patients (N = 13)	Healthy controls (N = 13)	*P*
Age, years	65.5 (68.4) ± 14.3	63.8 (64.0) ± 14.3	0.762*
Sex (female)	11 (84.6%)	11 (84.6%)	1.0†
Education, years	7.5 (8.0) ± 4.7	9.3 (9.0) ± 3.8	0.286*
Comorbidity index‡	1.0 (0.0) ± 1.1	0.4 (1.0) ± 0.9	0.123§
Current smoker	1 (7.7%)	0 (0.0%)	0.308†
17-Item Hamilton Depression Rating Scale total score	7.5 (6.0) ± 6.8	6.3 (6.5) ± 4.8	0.616*
Age at onset, years	55.9 (60.0) ± 15.3	–	
Tremor duration, years	9.6 (7.4) ± 7.4	–	
Cognitive domains			
Attention			
Direct Digit Span subtest from the WAIS-III	5.1 (5.0) ± 1.4	6.2 (6.5) ± 1.4	0.068*
Coding-Digit Symbol subtest from the WAIS-III	34.5 (24.0) ± 28.7	52.8 (48.5) ± 19.5	0.086*
Executive function			
Stroop Color–Word Trial	**23.4 (20.0) ± 13.9**	**34.2 (34.5) ± 10.1**	**0.042***
Similarities subtest from the WAIS-III	**10.7 (9.0) ± 4.4**	**17.9 (18.5) ± 4.9**	**0.001***
Indirect Digit Span subtest from the WAIS-III	**3.0 (3.0) ± 1.3**	**4.2 (4.0) ± 0.7**	**0.008***
Controlled Oral Word Association Test	**22.4 (23.0) ± 17.4**	**37.5 (39.0) ± 14.7**	**0.028***
Tower of London (time of execution in seconds)	575.5 (556.0) ± 306.0	362.1 (298.0) ± 210.2	0.056*
Frontal Battery Assessment	**14.1 (14.0) ± 3.3**	**17.0 (17.0) ± 0.6**	**0.014§**
Visuospatial ability			
Benton Judgment of Line Orientation Test	8.0 (8.0) ± 3.1	9.8 (10.0) ± 2.7	0.150*
Hooper Visual Organization Test	**27.9 (29.0) ± 14.7**	**39.2 (37.0) ± 10.5**	**0.043***
Verbal memory			
WMS-III Word List			
Learning trials total	26.0 (23.0) ± 7.4	29.1 (29.0) ± 7.7	0.318*
Immediate recall	**4.8 (4.0) ± 2.4**	**7.1 (6.5) ± 2.9**	**0.047***
Delayed recall	**4.4 (4.0) ± 2.8**	**6.8 (7.0) ± 2.6**	**0.035***
Recognition	**19.8 (21.0) ± 4.1**	**22.5 (22.5) ± 1.2**	**0.039***
Visual memory			
Brief Visuospatial Memory Test-Revised			
Learning trials	**14.0 (10.0) ± 12.1**	**29.3 (32.0) ± 7.1**	**0.003***
Delayed recall trial	**5.1 (4.0) ± 4.8**	**10.1 (11.0) ± 2.4**	**0.004***
Recognition trial	11.7 (12.0) ± 0.5	11.8 (12.0) ± 0.4	0.574§
Language			
Boston Naming Test	**40.2 (36.0) ± 11.3**	**52.7 (54.0) ± 6.2**	**0.004***
Total number of animals as possible in 1 minute	**13.9 (13.0) ± 6.7**	**22.1 (20.5) ± 7.9**	**0.010***

Mean (median) ± SD and frequency (%) are reported. SD = standard deviation, WAIS-III = Wechsler Adult Intelligence Scale-Third Edition. WMS-III = Wechsler Memory Scale-Third Edition, Significant values are in bold font.

* Student *t*-test.

† χ^2 test for sex, and current smoker.

‡ Comorbidity included 13 conditions: atrial fibrillation, nonmetastatic cancer, metastatic cancer, chronic obstructive pulmonary disease, depression, dementia, diabetes, epilepsy (treated), heart failure, myocardial infarction, psychiatric disorders, renal disease, and stroke.

§ Mann–Whitney *U* test.

The 13 right-handed OT patients (11 women and 2 men) were compared with 13 right-handed HC (11 women and 2 men). The 13 OT patients did not differ to a significant degree from the 13 HC in terms of age, sex, years of education, comorbidity index, current smoking, and depressive symptoms (Table 1). The results of neuropsychological testing are shown in Table 1. In most domains, OT patients' cognitive performance was significantly worse than that of the HC. These differences involved selected tests of executive function, visuospatial ability, verbal memory, visual memory, and language (Table 1).

3.2. Resting-state fMRI results

All results for the RSNs, which showed between-group functional connectivity differences, including MNI coordinates and *P*-values for peak voxels of all statistically significant clusters, are summarized in Table 2 and visualized in Fig. 1. Overall, OT patients showed changes relative to HC in the cerebellar and sensorimotor networks and in those major RSNs that might be involved in nonmotor symptoms, mainly cognition, including the DMN, executive, and the frontoparietal networks.

In additional analyses, we excluded OT plus cases (N = 5) (i.e., those associated with mild parkinsonian signs on examination), and the results were similar (data not shown). We also excluded 4 OT cases with cognitive impairment (defined as failure on at least 3 tests) (Table 3). In these analyses (Table 3), major RSNs that might be involved in both motor and nonmotor symptoms (i.e., cognition) were altered. However, fewer brain areas were involved in comparison with the analyses that included all OT cases (Table 3).

3.3. Relationships between functional connectivity, duration of disease, and cognition

These correlations were calculated in OT patients only, and only for the voxels that showed differences between patients versus HC (see Table 1). There was an association between disease

Table 2

Regions that showed statistically significant differences in functional connectivity between all OT patients versus healthy controls.

Resting state networks	Brodmann area/lobule	*P*	Number of voxels	Montreal Neurological Institute coordinates		
				x	y	z
Default mode network						
Patients > controls						
Left anterior lobe of cerebellum (culmen)	V	0.001	2846	−6	−64	−2
Left lingual gyrus	18	0.001		−10	−74	−2
Right anterior lobe of cerebellum (culmen)	I–IV	0.002		4	−50	−2
Left middle temporal gyrus	21	0.001	395	−64	−28	−16
Right fusiform gyrus	37	0.001	371	54	−64	−18
Left inferior temporal gyrus	37	0.002	354	−46	−70	2
Left superior frontal gyrus	11	0.003	298	−24	44	−24
Right middle temporal gyrus	39	0.003	225	50	−70	14
Right middle occipital gyrus	19	0.001	193	46	−74	8
Left superior frontal gyrus	6	0.002	104	−12	36	54
Right superior frontal gyrus	6	0.003	79	6	34	54
Left middle frontal gyrus	11	0.004	56	−44	40	−22
Right inferior frontal gyrus	47	0.003	33	48	26	−20
Left precuneus	19	0.003	26	−34	−74	48
Right superior parietal lobule	7	0.002	18	30	−74	52
Left superior frontal gyrus	8	0.001	16	−32	26	50
Left middle occipital gyrus	18	0.001	11	−10	−96	20
Right superior frontal gyrus	8	0.003	10	12	44	46
Executive network						
Patients > controls						
Left middle temporal gyrus	21	0.002	52	−62	0	−22
Left posterior lobe of cerebellum (pyramis)	VIII	0.001	48	−30	−88	−32
Left posterior lobe of cerebellum (tonsil)	IX	0.002	20	−46	−62	−34
Left middle temporal gyrus	21	0.003	13	−68	−16	−8
Right frontoparietal network						
Patients > controls						
Right medial frontal gyrus	11	0.001	1135	8	62	−24
Right middle frontal gyrus	10	0.003	166	48	44	8
Right superior frontal gyrus	9	0.002	127	18	60	32
Left anterior cingulated	32	0.004	66	−2	22	−16
Left superior frontal gyrus	10	0.004	35	−28	62	−4
Right inferior occipital gyrus	18	0.001	28	42	−92	−12
Right superior parietal lobule	7	0.005	20	44	−56	54
Right middle occipital gyrus	18	0.002	20	42	−92	8
Right anterior lobe of cerebellum (culmen)	V	0.002	16	26	−52	−20
Left posterior lobe of cerebellum (declive)	VI	0.001	11	−50	−72	−20
Left frontoparietal network						
Patients > controls						
Left middle frontal gyrus	11	0.002	26	−34	40	−24
Cerebellar network						
Controls > patients						
Right subcallosal gyrus	47	0.001	1746	20	18	−18
Left middle frontal gyrus	10	0.002		−4	42	−16
Right inferior frontal gyrus	11	0.002		16	36	−28
Right middle frontal gyrus	10	0.002	530	24	70	16
Right middle temporal gyrus	21	0.001	367	70	−18	−8
Left thalamus	–	0.002	227	−6	−4	6
Right claustrum	–	0.001	145	28	18	−10
Right parahippocampal gyrus	19	0.001	108	36	−46	−4
Left superior frontal gyrus	10	0.003	97	−8	68	10
Right superior temporal gyrus	22	0.001	75	70	−44	8
Right insula	13	0.004	62	46	4	−8
Left lateral globus pallidus	–	0.003	38	−22	−10	−8
Right insula	13	0.002	28	46	−18	8
Right middle frontal gyrus	47	0.001	27	52	38	−12
Right inferior frontal gyrus	13	0.002	19	48	24	2
Left middle temporal gyrus	39	0.003	16	−54	−78	16
Left middle temporal gyrus	21	0.002	11	−66	−52	2

(*continued*)

Table 2

(continued).

Resting state networks	Brodmann area/lobule	*P*	Number of voxels	Montreal Neurological Institute coordinates		
				x	y	z
Medial visual network						
Patients > controls						
Left supramarginal gyrus	40	0.002	103	−62	−54	30
Right middle temporal gyrus	39	0.003	96	56	−62	26
Left precuneus	19	0.003	90	−44	−74	40
Right precuneus	19	0.002	57	36	−76	46
Left superior temporal gyrus	22	0.003	22	−62	−54	12
Left cuneus	7	0.004	20	−2	−64	36
Sensorimotor network						
Controls > patients						
Left fusiform gyrus	19	0.003	53	−54	−72	−12
Right precentral gyrus	44	0.004	26	54	20	2
Right anterior lobe of cerebellum (culmen)	V	0.001	26	56	−50	−32
Right middle temporal gyrus	21	0.001	14	70	−40	−2
Right posterior lobe of cerebellum (declive)	VI	0.002	12	48	−68	−22
Auditory/language network						
Patients > controls						
Left superior frontal gyrus	8	0.001	797	−20	54	38
Right middle frontal gyrus	9			34	52	34
Left inferior frontal gyrus	47	0.001	141	−48	16	−10
Left limbic lobe (anterior cingulate cortex)	25	0.001	129	−2	32	−4
Right superior temporal gyrus	22	0.001	69	42	10	−18
Right inferior frontal gyrus	47	0.002	52	42	28	−24
Left posterior lobe of cerebellum (tuber)	Crus II	0.001	42	−44	−84	−30
Right precuneus	7	0.001	41	6	−66	50
Left medial frontal gyrus	11	0.003	40	−2	52	−24
Left precuneus	7	0.003	37	−8	−72	58
Left superior temporal gyrus	22	0.003	27	−68	−20	4
Left superior frontal gyrus	9	0.002	10	−4	52	30

Results were considered significant for TFCE $P < 0.005$ uncorrected (cluster size ≥ 10 voxels). For the biggest clusters, up to 3 local maxima are shown. OT = orthostatic tremor, TFCE = threshold-free cluster enhancement.

duration and connectivity in the DMN and the medial visual network (Table 4). In addition, connectivity in 2 RSNs (DMN and medial visual network) was associated with cognitive processes (attention, executive function, visuospatial ability, visual memory, and language) (Table 4).

4. Discussion

We investigated functional connectivity in a sample of OT patients and HC. Overall, relative to HC, OT patients showed increased connectivity in RSNs involved in cognitive processes (DMN, as well as in executive and frontoparietal networks), and decreased connectivity in motor control (cerebellum and sensorimotor networks). Changes in network integrity were associated not only with duration (DMN and medial visual network), but also with cognitive function. Moreover, in at least 2 networks (DMN and medial visual network), increased connectivity was associated with worse performance on different cognitive domains (attention, executive function, visuospatial ability, visual memory, and language).

At 1st glance, the presence of increased connectivity seems counterintuitive; this is also found in early multiple sclerosis, mild cognitive impairment, essential tremor, and diabetes.[27–29] In general, RSNs are functionally connected, and dysfunction in 1 network may lead to dysfunction in the other networks.[30] Reduced functional connectivity is thought to reflect dysfunction of the network, and increased functional connectivity has been interpreted as a compensatory mechanism or reorganization of the network.[30]

Although the sensorimotor and the visual and auditory networks involve cortical regions normally engaged in sensorimotor, visual, and auditory processes, respectively, the DMN and the executive and the frontoparietal networks are the RSNs most relevant for cognition.[15,16] We found increased functional connectivity in the DMN, as well as executive and frontoparietal networks in OT patients. Of additional interest, we found the right insula to be less connected to the cerebellum network in OT patients. Recent neuroimaging data reveal that the insular cortex is involved in essential tremor or in various neuropsychiatric diseases.[31,32] One may speculate that these insular changes in OT might be an early marker of cognitive impairment in OT. However, this possibility requires further study.

Our results also indicate that OT patients present a certain increased functional connectivity in medial visual and auditory/language networks. The aberrant functional connectivity of both networks found in our study could be associated with perceptual and language impairments in OT patients. In line with this, OT patients scored worse on the Hooper Visual Organization Test,[33] an instrument that measures visual organizational skills,[33] as well as language tests; however, clinical studies have yet to study or document such changes.

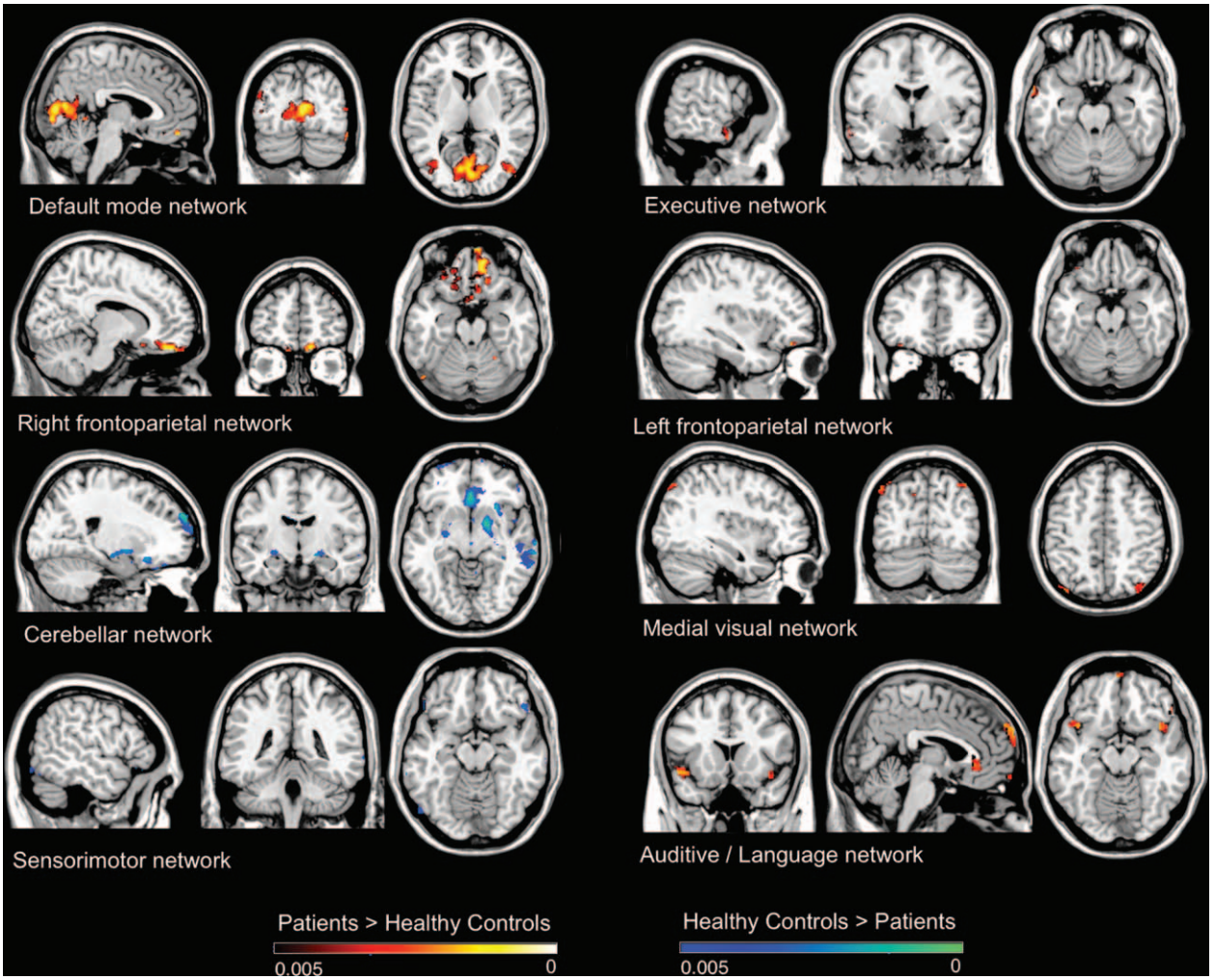

Figure 1. Resting state networks, which showed between-group functional connectivity differences, including Montreal Neurological Institute coordinates and *P*-values for peak voxels of all statistically significant clusters.

Interestingly, major RSNs that might be involved in cognitive function (DMN, executive network, and left frontoparietal network) were altered even in OT patients who were not defined as cognitively impaired. These alterations were, however, subtler than those found when all OT patients were included. We hypothesize that a dysfunction of these RSNs may have a role in the pathogenesis of cognitive dysfunction in OT. Our functional data suggest that there may be an early functional disruption of these RSNs in OT prior to clinical evidence of significant cognitive impairment. This is consistent with evidence in the Alzheimer and Parkinson disease literature, where a functional alteration of the DMN is already present in *APOE*4+ cognitively normal individuals and in cognitively unimpaired Parkinson disease patients, respectively.[34,35] It is also important to note that we defined cognitive impairment conservatively as failure on at least 3 tests rather than failure on 1 or more tests.

The study was not without limitations. First, the sample size was relatively small. The OT literature, however, only includes studies with small sample sizes. One should keep in mind that OT is a very rare disease, and hence it is rather difficult to recruit patients for any case–control study. Although there are no available epidemiological data, in the follow-up evaluation of the Neurological Disorders of Central Spain study,[36] we detected only 1 patient with OT in a cohort of approximately 4000 elderly subjects (data not published). Despite the small sample size, with our sample we could detect a number of differences between the 2 study groups. Second, the recruited sample was quite heterogeneous, including primary and OT plus cases. However, our aim was to examine whether OT patients in general had altered resting state brain networks when compared with matched controls. Furthermore, after exclusion of OT plus cases, the results remained similar. This study also had several strengths. First, this is the first study that has assessed RSN integrity of OT patients. Second, assessments were conducted prospectively in a standardized manner.

In summary, in this exploratory study, we observed selective impairments of RSNs intrinsic functional connectivity in OT patients. This and other future resting-state fMRI studies might provide a novel method to understand the pathophysiological mechanisms of motor and nonmotor features of OT.

Table 3

Regions that showed statistically significant differences in functional connectivity in cognitively unimpaired orthostatic tremor patients (N = 9) versus healthy controls.

				Montreal Neurological Institute coordinates		
Resting state networks	**Brodmann area/lobule**	***P***	**Number of voxels**	**x**	**y**	**z**
Default mode network						
Patients > controls						
Left inferior temporal gyrus	20	0.003	16	−64	−14	−24
Left inferior temporal gyrus	20	0.004	16	−46	−24	−32
Left inferior temporal gyrus	20	0.002	11	−56	−14	−36
Executive network						
Patients > controls						
Right superior temporal gyrus	38	0.002	47	54	14	−16
Right middle temporal gyrus	22	0.002	21	64	−48	4
Left superior temporal gyrus	22	0.004	11	−56	−8	−6
Left inferior temporal gyrus	37	0.001	32	−58	−68	2
Right posterior lobe of cerebellum (pyramis)	–	0.002	16	36	−88	−32
Left frontoparietal network						
Patients > controls						
Left middle frontal gyrus	11	0.003	17	−34	40	−24
Cerebellar network						
Controls > patients						
Right medial globus pallidus	–	0.002	12	18	0	−8
Medial visual network						
Patients > controls						
Right superior temporal gyrus	39	0.002	47	56	−60	20
Left angular gyrus	39	0.002	12	−56	−68	38
Sensorimotor network						
Controls > patients						
Right superior temporal gyrus	42	0.003	13	70	−24	16
Auditory/language network						
Patients > controls						
Right superior frontal gyrus	9	0.003	244	22	60	32
Right orbital gyrus	11	0.001	42	4	46	−28
Left superior temporal gyrus	22	0.003	37	−68	−18	4

Results were considered significant for threshold-free cluster enhancement (TFCE) $P < 0.005$ uncorrected (cluster size ≥ 10 voxels).

Table 4

Associations of disease duration and cognitive variables and the mean z value of the significantly differing voxels of functional connectivity in all orthostatic tremor patients.

	Default mode network (left anterior lobe of cerebellum)	**Default mode network (right middle occipital gyrus)**	**Default mode network (right superior frontal gyrus)**	**Medial visual network (left supramarginal gyrus)**
Disease duration	β = −0.978, t = −4.67, *P* = 0.043	β = −1.176, t = −6.21, *P* = 0.025	β = 0.556, t = 10.88, *P* = 0.008	β = −1.461, t = −6.01, *P* = 0.027
Cognitive domains				
Attention				β = 3.841, t = 5.20, *P* = 0.035
Executive function			β = −1.277, t = −13.94, p = 0.005	
Visuospatial ability			β = 0.664, t = 8.94, *P* = 0.012	
Verbal memory				
Visual memory			β = 1.633, t = 13.28, *P* = 0.006	β = −2.540, t = −4.34, *P* = 0.049
Language		β = −2.173, t = −4.90, *P* = 0.039		β = −2.883, t = −5.06, *P* = 0.037

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