Diagnosis and imaging of essential and other tremors

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CHAPTER 4

BILATERAL CEREBELLAR ACTIVATION IN UNILATERALLY CHALLENGED ESSENTIAL TREMOR: AN EMG-FMRI STUDY

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ABSTRACT

OBJECTIVE: Essential tremor (ET) is the most common hyperkinetic movement disorder. Previous research into the pathophysiology of ET suggested underlying cerebellar abnormalities. In this study, we added electromyography as an index of tremor severity to functional Magnetic Resonance Imaging (EMG-fMRI) to study a homogeneous group of ET patients.

METHODS: We included 21 propranolol sensitive patients with a definite diagnosis of ET defined by the Tremor Investigation Group. Simultaneous EMG-fMRI recordings were performed while patients were off tremor medication. Patients performed unilateral right hand and arm extension, inducing tremor, alternated with relaxation (rest). 21 healthy, age- and gender-matched participants mimicked tremor during right arm extension. EMG power variability at the individual tremor frequency was used as a regressor mathematically independent of the motor task in the general linear model used for fMRI analysis, to find specific tremor-related activations.

RESULTS: Task-related activations were found in the classical upper-limb motor network, both for ET patients and healthy participants in motor, premotor and supplementary motor areas. In ET patients, we found tremor-related activations bilaterally in the cerebellum: in left lobules VI and V, and in right lobules V, VI, VIIIa and b, and in the brainstem. In healthy controls we found simulated tremor-related activations in the right cerebellum lobule V.

CONCLUSIONS: Our results expand on previous findings of bilateral cerebellar involvement in et: we have identified specific areas in the bilateral somatomotor regions of the cerebellum. We hypothesize that the cerebellar cortex is disorganized in et, consequently leading to aberrant cerebellar activity.

INTRODUCTION

Although essential tremor (ET) is the most common hyperkinetic movement disorder (1), the underlying disease mechanism is poorly understood. ET has long been considered a benign disorder, but recently opinions about the disabling nature of ET changed (2).

Previous work investigating the pathophysiology of ET can be divided into pathology and neuroimaging studies (3-5). Post-mortem studies have provided interesting but conflicting results, with cerebellar degeneration reported in some (6, 7) but not all studies (8). Neuroimaging results in ET are also incongruent, but do provide support for cerebellar involvement. In structural imaging the most frequent result is cerebellar abnormality in ET although not consistently reported (9). PET experiments and an fMRI study examining motor tasks showed abnormalities in the (bilateral) cerebellum and in some cases in the red nucleus, thalamus and inferior olive (10-13).

Although many of the results point towards the cerebellum, overall studies are inconclusive. One cause contributing to this diversity in findings may be that 'ET' used to be the label for 'tremor not otherwise specified', resulting in a heterogeneous group with high variability in clinical presentation, response to therapeutic intervention and on etiologic level. In this study, we have attempted to define a homogeneous group of ET patients with a clear diagnosis and a positive response to propranolol (14). Moreover, we wish to improve functional imaging in ET by combining EMG and fMRI. This novel approach allows recording tremor simultaneously with brain activity. As cerebellar involvement is a common finding in previous studies, we particularly expect to find cerebellar abnormalities.

METHODS

SUBJECTS

This study was conducted in two academic hospitals in the Netherlands: the University Medical Center Groningen (UMCG) and the Academic Medical Center in Amsterdam (AMC). Patients who had a definite diagnosis of ET according to criteria defined by the Tremor Investigation Group were included (15). All patients had bilateral upper limb tremor, an age at onset <65 years, and a disease duration >5 years. A positive family history was present in most patients (see Table 4.1) but not required for inclusion. Patients had to report a positive subjective response to propranolol. Patients and healthy controls (age- and gender matched) were all right-handed as assessed by the Annett Handedness scale (16). Another inclusion criterion was a score >25 on the Mini Mental State Examination to ensure proper understanding of the task. Exclusion criteria were neurological comorbidity (for patients: other than ET), age < 18 and the use of medication (other than for ET) affecting the central nervous system. The study was approved by the medical ethical committees of the University Medical Center Groningen and the Academic Medical Center Amsterdam. This study was conducted according to the declaration of Helsinki (Seoul, 2008) and all participants gave written informed consent.

STUDY SET-UP

Patients quit their medication for a minimum of three days before participating in the study. Tremor was assessed off medication using the Fahn-Tolosa-Marin Tremor Rating Scale (TRS)(17) and a visual analog scale (VAS). In all patients, propranolol was washed in again at the end of the study, according to a personalized schedule.

TABLE 4.1. PATIENTS AND HEALTHY PARTICIPANTS CHARACTERISTS

Patients	Age	Gender	Mean Tremor frequency (Hz)	Age at onset (years)	Duration (years)	Family history	Propranolol use (mg)	VAS-score off medication
1	21	Male	10	10	11	+	40	5.4
2	22	Male	7	12	10	-	20	5.2
3	27	Male	7.5	0	27	-	160	8.7
4	30	Female	8	15	15	+	20	2.9
5	32	Female	7	3	29	+	40	6
6	35	Male	8	7	28	+	80	7.8
7	46	Male	7.5	5	41	+	80	4.4
8	47	Male	7	15	32	+	40	6
9	48	Female	7	10	38	+	120	5.4
10	53	Female	7.5	28	25	+	30	7.8
11	53	Male	8	16	37	+	50	8.6
12	57	Female	7	22	40	+	10	4
13	62	Female	8.5	5	57	+	100	8.5
14	63	Male	7	43	20	+	40	3.4
15	63	Female	7.5	39	24	+	80	7.4
16	64	Male	6.5	12	52	+	20	4
17	65	Female	7.5	60	5	+	80	2.7
18	69	Male	7.5	40	29	+	40	9.2
19	72	Male	6	10	62	+	320	9.2
20	74	Male	9	50	24	-	80	6.6
21	80	Female	6	60	20	+	80	6.9
Mean (SD)	**51.6 (17.8)**	**M: 12 F: 9**	**7.5 (0.9)**	**22 (18.9)**	**29.8 (15)**		**72.9 (67.8)**	**6.2 (2.1)**

VAS: Visual Analogue Scale, range 0-10. SD: Standard deviation. HP: healthy participants.

MOTOR TASKS DURING EMG-FMRI

An fMRI scan was performed, while EMG was recorded simultaneously, off-medication. During scanning patients executed a motor task in which they were instructed to alternate 21 periods of 30 seconds rest with 20 periods of 30 seconds right hand and arm extension without supporting the hand and arm. An additional silent reading task was presented during half of all action blocks. Only the action blocks without the silent reading tasks were analysed in this study, i.e. 10 blocks of 30 seconds. Healthy controls only participated in this part of the study and mimicked a tremor during right arm extension by self-paced wrist flexion extension. Before scanning, participants were instructed and practiced the task outside the scanner. All subjects received visual task instruction using slides.

TABLE 4.1. (CONTINUED)

HP	Age	Gender	Mean Tremor frequency (Hz)
1	20	Male	5
2	22	Male	3.5
3	27	Male	5
4	30	Female	5
5	33	Female	3.5
6	36	Male	7.5
7	47	Male	6
8	49	Male	4
9	52	Male	6.5
10	52	Male	4
11	56	Male	3.5
12	57	Female	6
13	59	Female	5
14	59	Female	4
15	60	Male	5.5
16	60	Female	4
17	62	Male	4.5
18	68	Male	5.5
19	68	Male	5.5
20	72	Female	7
21	74	Male	6
Mean (SD)	**50.6 (16.4)**	**M: 14 F: 7**	**5.1 (1.2)**

EMG-FMRI ACQUISITION

Images were acquired on a Philips 3-T MR scanner (UMCG: Intera, AMC: Intera and Achieva, Philips, Best, The Netherlands) with SENSE-32 channel (UMCG) and SENSE-16 channel (AMC) head coils. In both centres, T2*-weighted, 3D functional images were obtained using multislice echo planar imaging (EPI) with an echo time (TE) of 30 ms and a repetition time (TR) of 2000 ms. Per TR, 39 axial slices, with a field of view (FOV) of 224 mm, flip angle of 5˚ with a 64 X 64 matrix and isotropic voxel size of 3.5 x 3.5 x 3.5 mm were acquired. To provide anatomical information, additional T1-weighted 3D anatomical scans with an axial orientation and a matrix size of 256 x 256 mm were obtained (isotropic voxel size 1 X 1 X 1 mm). EMG was recorded simultaneously (BrainProducts GmbH, Munich, Germany (UMCG) and MicroMed, Italy (AMC)) from five right arm muscles. To verify absence of left arm movement during the tasks, EMG was recorded from three left arm muscles as well (see Supplementary Materials for more details).

EMG-FMRI ANALYSIS

EMG data were corrected for scanning artefacts using the MR correction algorithms embedded in Brain Vision Analyser (Imaging Artefact Reduction method (18); UMCG data) and FARM (fMRI artefact reduction for motion (19); AMC data). After correction, data was further analyzed in Matlab (Matlab R2007a, Mathworks, Natrick, USA) using custom-made scripts. We calculated the frequency spectrum and total spectral power in a 5Hz symmetrical band around the individual (mimicked) tremor peak frequency for every two seconds of EMG data and exported these values as a vector for each right arm muscle. The vectors of the three muscles with the highest total power were averaged. This procedure resulted in an EMG power vector with one entry for every 2 second scan. This vector was orthogonalised, element-wise multiplied with the task vector, convolved with the canonical haemodynamic response function (HRF) and scaled by their respective SDs (20). This vector is referred to as residual EMG or r-EMG vector and was used as a regressor in the fMRI design matrix in addition to the task regressor. It represents tremor variation

over time during the motor task, independently of this motor task. See Supplementary Materials for more details about the analysis.

fMRI data was analysed using SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Preprocessing consisted of standard realignment and coregistration steps. A group-specific anatomic template was created using DARTEL (diffeomorphic anatomical registration through exponentiated lie algebra) for a more precise inter-subject alignment to take age-related changes in anatomy into account (21). Individual functional data was normalized and smoothed using the DARTEL template and an 8-mm full-width half maximum (FWHM) Gaussian kernel. To reduce movement artefacts, the six movement parameters derived from realignment corrections were entered as covariates in each individual analysis. Inspection of the EMG was used to correct the task regressor for actual on- and offsets of the motor task. Each single-subject first-level model thus consisted of a regressor for the motor task (task-related activations), a residual-EMG regressor (r-EMG, tremor related activations) and the six movement regressors. By constructing the design matrix in this manner, variation in activation due to 'pure' task execution will be mostly explained by the task regressor, whereas variation in activation due to tremor will be mostly explained by the r-EMG regressor, thereby overcoming the problem in traditional designs, where task- and tremor-related activations are mixed.
Second level within-group comparisons for the task and r-EMG contrasts, and between-group comparisons for each individual contrast were made on whole brain level. Activations were considered significant at a threshold of $p<0.05$ (FWE corrected) and an extent threshold (k) of 20 voxels.

As we hypothesized cerebellar involvement in ET, we additionally performed an analysis focused on the cerebellum using the Spatially Unbiased Infratentorial Template (SUIT) toolbox (22) (see Supplementary materials). Contrasts were thresholded at voxel level $p<0.001$, uncorrected, applying a cluster size of 20 voxels.

RESULTS

SUBJECT CHARACTERISTICS

A total of 40 ET patients were initially included in this study. Data of twenty-one ET patients and twenty-one age- and gender-matched healthy controls were analysed. Reasons for exclusion of patients from further analysis were either too much head-movement during scanning (one patient), insufficient tremor during fMRI data collection (16 patients), failure of equipment during scanning (one patient) or incorrect normalisation to the DARTEL template (one patient). Analyzed ET patients (12 male) had a mean age of 51.6 (SD 17.8) years and mean disease duration of 29.8 (SD 15) years. See Table 4.1 for characteristics of patients and healthy controls. Healthy controls (14 male) had a mean age of 50.6 (SD 16.4) years. Age and gender did indeed not differ between the analysed groups ($p=0.86$ and $p=0.35$, respectively). Patients had a mean TRS score of 25.7 (SD 10.8) and a mean VAS score of 6.2 (SD 2.1) off medication. No left arm movement was seen in the EMG signal. Head movement during scanning did not differ between ET patients and healthy controls (see Supplementary Material).

TASK-RELATED ACTIVATIONS, WHOLE BRAIN

Within-group results

For ET patients, task-related activations (task regressor) were found in the left motor- and premotor cortex, the supplementary motor area (SMA) and the right cerebellum lobules IV and V. Additional activations were

TABLE 4.2. RESULTS FOR TASK-RELATED ACTIVATIONS (TASK REGRESSOR)

CONTRAST	VOXELS (K)	AREA	RIGHT/LEFT	T-VALUE	X[a]	Y[a]	Z[a]
ET patients[1]							
Cerebrum	48	Parietal sup	L	7.54	-26	-44	66
	28	Primary somatosensory cortex	R	7.30	20	-30	60
	3156	Premotor cortex	L	11.21	-30	-14	54
	sc	SMA	M	10.76	-6	-12	54
	sc	Frontal sup	R	10.71	18	0	60
	636	Supramarginal gyrus	R	9.14	56	-36	40
	sc	Supramarginal gyrus	R	9.12	54	-24	32
	sc	Supramarginal gyrus	R	7.33	48	-38	44
	340	Frontal mid	R	9.12	38	26	34
	sc	Frontal mid	R	7.80	32	46	22
	sc	Frontal mid	R	7.40	34	42	30
	493	Frontal inf, oper	R	10.28	56	12	24
	59	Thalamus	R	7.86	20	-14	20
Cerebellum	288	Cerebellum IV, V	R	9.65	20	-46	-22
Healthy participants[1]							
Cerebrum	4290	Premotor cortex	L	15.97	-24	-12	56
	sc	SMA	L	15.16	-4	-10	56
	sc	Medial cingulate gyrus	L	14.20	-4	-4	48
	45	Parietal inf	L	8.44	-52	-22	40
	51	Medial cingulate gyrus	R	10.18	16	-28	38
	1546	Supramarginal gyrus	R	11.15	52	-28	34
	sc	Primary somatosensory cortex	R	11.01	36	-34	50
	sc	Supramarginal gyrus	R	10.53	56	-22	24
	162	Rolandic oper	R	9.04	56	4	18
	sc	Frontal inf, oper	R	8.98	54	10	26
	sc	Premotor cortex	R	8.80	56	4	34
	111	Supramarginal gyrus	L	8.27	-66	-26	18
	sc	Supramarginal gyrus	L	7.98	-52	-26	20
	sc	Supramarginal gyrus	L	7.64	-64	-28	28
Cerebellum	1831	Vermis VII	R	17.33	4	-64	-24
	sc	Cerebellum IV, V	R	15.47	18	-50	-22
	sc	Vermis IV, V	R	14.27	6	-62	-12

TABLE 4.2. (CONTINUED)

CONTRAST	VOXELS (K)	AREA	RIGHT/LEFT	T-VALUE	X[a]	Y[a]	Z[a]
	21	Cerebellum VI	L	7.62	-28	-58	-26
	207	Cerebellum VIII	R	12.53	24	-58	-50
	sc	Cerebellum VIII	R	7.20	14	-70	-50
ET patients> Healthy participants[2]							
	No significiant results						
Healthy participants> ET patients[2]							
Cerebrum	37	Primary somatosensory cortex	L	7.33	-38	-30	56
Cerebellum	133	Cerebellum VI	R	7.33	18	-54	-22

ET: essential tremor; sc: same cluster; R: right; L: left; M: midline; [a]: MNI. [1] Initial voxel-height threshold p<0.05 (FWE corrected, extend threshold k=10 voxels). Coordinates refer to the voxels of maximum activation within clusters of significant activation (p<0.05, FWE whole brain cluster-level corrected). [2] Initial voxel-height threshold p<0.001 (uncorrected, extend threshold k=20 voxels). Coordinates refer to the voxels of maximum activation within clusters of significant activation (p<0.05, FWE whole brain cluster-level corrected).

found in right supramarginal gyrus, frontal areas, primary somatosensory cortex, superior parietal cortex and right thalamus (T>6.49, p<0.05 FWE, k=20, see Table 4.2). In healthy controls, we found task-related activations (task regressor) in the left motor cortex and bilateral premotor cortex, the SMA, and the right cerebellum lobules IV, V, VI and VIII. In addition, activations were observed in the left cerebellum lobule VI, left supramarginal gyrus, the inferior parietal cortex and frontal regions (T>6.95, p<0.05 FWE k=20, see Table 4.2).

Between group comparisons

No significant increased activations were detected in ET patients when compared with healthy controls. Healthy controls had increased activations in the right cerebellum lobule VI and left sensori-motor cortex compared to ET patients (both T>5.34, p<0.05 FWE, k=20, see Table 4.2).

TREMOR-RELATED ACTIVATIONS, WHOLE BRAIN

Within-group results

For ET patients, tremor-related activations (r-EMG regressor) were detected in left cerebellum lobule VI and the left motor-, premotor and somatosensory cortex. Additional activations were found in the bilateral visual cortex, the middle part of the cingulate gyrus and the right motor cortex (T>6.74, p<0.05 FWE, k=20, see Table 4.3). In healthy controls, no significant activations were seen in relation with mimicked tremor (T>7.05, p<0.05 FWE, k=20).

Between-group comparisons

Compared to healthy controls, ET patients showed increased activations in the right motor cortex, middle part of the cingulate gyrus and the left somatosensory cortex (T>5.45, p<0.05 FWE, k=20 see Table 4.3). The reverse contrast (healthy controls > ET patients) was not further investigated because we found no significant mimicked tremor-related activations in healthy controls.

TABLE 4.3. RESULTS FOR TREMOR-RELATED ACTIVATIONS (R-EMG REGRESSOR)

CONTRAST	VOXELS (K)	AREA	R/L	T-VALUE	X[a]	Y[a]	Z[a]
ET patients[1]							
Cerebrum	669	Premotor cortex	L	8.38	-30	-20	68
	sc	Supramarginal gyrus	L	8.28	-52	-22	36
	sc	Premotor cortex	L	8.13	-26	-26	56
	104	Precuneus	R	7.63	6	-40	58
	sc	Primary motor cortex	R	7.34	14	-40	50
	sc	Primary motor cortex	R	7.25	12	-32	54
	119	Medial cingulate gyrus	L	8.71	-12	-34	46
	100	Medial cingulate gyrus	L	7.72	-2	-4	42
	sc	Medial cingulate gyrus	L	7.48	-10	-8	40
	106	Primary somatosensory cortex	L	9.81	-64	-20	16
	sc	Supramarginal gyrus	L	6.85	-52	-24	18
	103	Medial temporal gyrus	L	8.38	-30	-28	12
	794	Primary visual cortex	R	8.80	10	-62	6
	sc	Primary visual cortex	R	8.58	10	-78	6
	sc	Primary visual cortex	L	7.99	-6	-70	6
	74	Associative visual cortex	L	9.16	-40	-86	0
	23	Associative visual cortex	R	7.77	30	-84	-16
Cerebellum	318	Cerebellum VI	L	9.47	-32	-54	-20
	sc	Fusiform gyrus	L	8.93	-32	-60	-14
	sc	Cerebellum VI	L	7.94	-18	-74	-14
Healthy participants[1]		No significant results					
ET patients> Healthy participants[1]							
Cerebrum	92	Primary motor cortex	R	6.47	10	-32	52
	123	Medial cingulate gyrus	L	6.35	-10	-40	52
	24	Primary somatosensory cortex	L	6.00	-48	-24	52
Healthy controls> ET patients[1]		No significant results					

ET: essential tremor; sc: same cluster; R: right; L: left; M: midline; [a]: MNI . [1] Initial voxel-height threshold p<0.001 (uncorrected, extend threshold k=20 voxels). Coordinates refer to the voxels of maximum activation within clusters of significant activation (p<0.05, FWE whole brain cluster-level corrected).

TASK-RELATED ACTIVATIONS, CEREBELLUM (SUIT ANALYSIS)

Task-related activations in the cerebellum of ET patients were found in the right lobule V, VI and VIIIa (T>3.58, p<0.001 uncorrected, see Table 4.4 and Figure 4.1A). Healthy controls showed a large cluster of task-related activations in the right lobules V, VI, VIIIa and b (T>3.61, p<0.001 uncorrected, see Table 4.4 and Figure 4.1CA). ET patients had no increased activations compared to healthy

TABLE 4.4. RESULTS FOR SUIT-ANALYSIS, CEREBELLUM

CONTRAST	VOXELS (K)	AREA	R/L	T-VALUE	X[a]	Y[a]	Z[a]
Task-related[1]							
ET patients	1579	Lobule V	R	8.57	20	-46	-21
	sc	Lobule V	R	7.80	4	-62	-23
	sc	Lobule VI	R	6.80	28	-48	-29
	22	Lobule IX	L	4.83	-4	-50	-29
	62	Lobule VIIIa	R	5.23	28	-48	-47
	sc	Lobule VIIIa	R	4.80	22	-60	-51
Healthy participants	5187	Vermis VI	R	17.69	6	-66	-27
	sc	Lobule V	R	13.45	16	-54	-17
	31	Lobule VIIIa	L	5.47	-28	-40	-43
	22	Lobule VIIIa	L	5.60	-30	-54	-53
	43	Lobule VIIIb	L	4.62	-12	-48	-57
ET patients> Healthy participants		No significant results					
Healthy participants> ET patients	448	Lobule V	R	6.74	18	-52	-25
	sc	Vermis VI	R	5.38	4	-66	-21
	sc	Lobule V	R	4.44	10	-54	-13
Tremor related[2]							
ET patients	1903	Lobule V	L	8.48	-26	-46	-15
	sc	Lobule VI	L	7.67	-20	-62	-11
	sc	Brainstem	L	6.99	-8	-36	-35
	1071	Lobule VI	R	7.23	26	-54	-17
	sc	Lobule VI	R	6.41	12	-68	-9
	sc	Lobule VI	R	6.28	28	-48	-23
	602	Crus II	R	5.30	4	-76	-37
	sc	Lobule IX	L	5.27	-16	-50	-47
	sc	Lobule VIIb	L	4.92	-6	-76	-51
	113	Lobule VIIIa	R	4.77	22	-60	-49
	sc	Lobule VIIIb	R	4,52	20	-50	-51
	76	Brainstem	M	5.45	8	-16	3
	sc	Brainstem	M	4.99	8	-26	-7
	sc	Brainstem	M	3.78	4	-34	1
	303	Brainstem	M	5.21	-10	-28	-5
	sc	Brainstem	M	4.82	-20	-28	-5
	sc	Brainstem	M	4.52	-12	-24	3
Healthy participants	75	Lobule V	R	6.29	2	-74	-7

TABLE 4.4. (CONTINUED)

CONTRAST	VOXELS (K)	AREA	SIDE	T-VALUE	X[a]	Y[a]	Z[a]
	sc	Lobule V	R	4.81	0	-62	-1
	415	Lobule V	R	8.32	4	-62	-21
	sc	Lobule V	R	5.27	16	-54	-21
	sc	Lobule V	R	5.17	12	-56	-11
ET patients> Healthy participants	22	Lobule V	L	3.73	-16	-54	-9
	44	Lobule VIIIb	L	4.16	-16	-52	-47
	65	Brainstem	M	4.21	-8	-28	-11
	sc	Brainstem	M	3.72	-6	-18	-5
	42	Brainstem	M	4.22	-8	-34	-27
Healthy participants> ET patients	10	Crus II	R	3.81	42	-76	-47

ET: essential tremor; sc: same cluster; R: right; L: left; M: midline; [a]: MNI [1] p<0.05 (FWE corrected, extend threshold k=10 voxels). [2] p<0.001 (uncorrected, extend threshold k=20 voxels)

controls in the cerebellum. Healthy controls showed increased activations in the right lobules V, and VI and in Vermis VI compared to ET patients (both T>3.32, p<0.001 uncorrected, see Table 4.4).

TREMOR-RELATED ACTIVATIONS, CEREBELLUM (SUIT ANALYSIS)

Tremor-related activations in the cerebellum were found in left lobules V, VI,VIIb and IX, right lobules V, VI, VIIIa and b and in the brainstem in ET patients (T>3.58, p<0.001 uncorrected, see Table 4.4 and Figure 4.1B). Healthy controls showed mimicked tremor-related activations in the right cerebellum in lobule V (T>3.61, p<0.001 uncorrected, see Table 4.4 and Figure 4.1D). Increased activations were detected in ET patients when compared with healthy controls in the brainstem and left lobules V and VIIIb. Healthy controls showed increased activation compared to ET patients in the right crus II (T=3.32, p<0.001 uncorrected, see Table 4.4).

DISCUSSION

Using a combination of EMG and fMRI we identified specific, explicable areas in the bilateral somatomotor regions of the cerebellum associated with tremor. The technique employed here has been used successfully before in a small sample of ET patients, in patients with cortical myoclonic tremor and in Parkinson's tremor (23-25). By carefully selecting patients with a clear diagnosis of ET, we aimed to identify brain areas correlating specifically with ET. To our knowledge this is the first controlled EMG-fMRI study investigating a large homogeneous group of ET patients.

TASK-RELATED BRAIN ACTIVATIONS

The participants performed a unilateral (right) arm extension task and, not to our surprise, the classical motor network was activated in both patients and healthy controls. These motor network activations were stronger in healthy participants compared to patients, probably because the tremor simulating movement made by the healthy

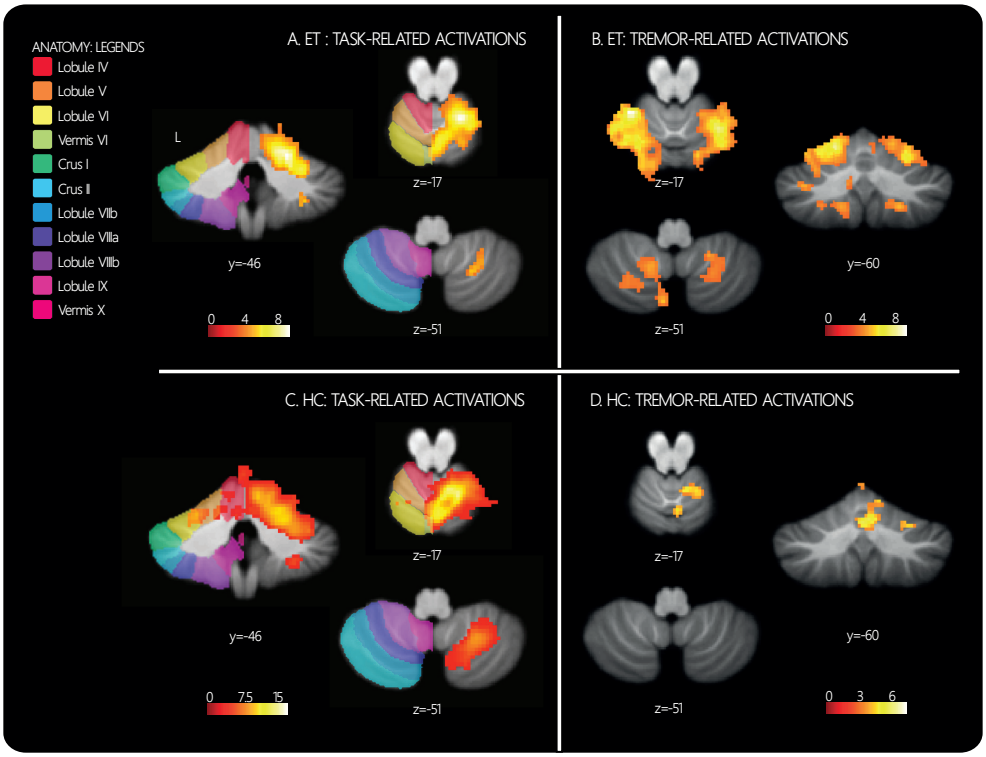

Figure 3.2. Increased cerebellar activations in essential tremor patients related to the within group comparisons for the task contrast, p<0.05 (FWE corrected, extent k = 10) (A: task-related activations), and activations related to the within group comparisons for the r-EMG contrast, p<0.001 (uncorrected, extent k = 20) (B: tremor-related activations). Results are projected on the SUIT-template (ref). The color coded bars at the bottom of the figure indicate SPM T-map intensities. The z-coordinates indicate the position of the transversal planes relative to the anterior commisure-posterior commisure plane. L: left hemisphere, R: right hemisphere.

controls was deliberate and had an observed larger amplitude than the trembling in ET patients.

TREMOR-RELATED BRAIN ACTIVATIONS

In ET patients, tremor-related activations were found bilaterally in the cerebellum: in left and right lobules VI and V, and additionally in right lobules VIIIa and b, and in the brainstem. These results expand on earlier findings that the bilateral cerebellum is involved in ET (13,26). Indeed, with our EMG-fMRI approach, we discovered specific, well-defined areas within the cerebellum, thus adding detailed information to the more diffuse localisations that have previously been described.

We identified two distinct tremor related activations in lobules V-VI and in lobule VIII of the right cerebellum, ipsilateral to the right hand and thus particularly implicated in left-hemisphere functions. This particular cerebellar location indeed accurately fits with a previous study on functional connectivity of the cerebral motor hand region which revealed somatomotor regions of the cerebellum (27). In this study representation in the cerebellum was cross-lateralised and had a double representation, with a strong primary somatomotor representation in lobules V and VI, and a slightly weaker secondary

representation in lobule VIIIb. However, in addition to the cerebellar activity ipsilateral to the tremulous hand, activations were also observed in the contralateral cerebellar hemisphere at the same locations as in the ipsilateral cerebellar hemisphere. Activations were evident in the left somatomotor areas, lobules V and VI, and at a lower threshold we found activations in the left lobule VIII as well. Thus, we found increased activations in specific somatomotor areas of the bilateral cerebellum. We like to point out, in this respect, that these activations were specifically tremor- rather than movement related, as the brain activation in these areas covaried with tremor intensity over time independently of movement task performance. In healthy controls activations covarying with simulated tremor intensity were found in the ipsilateral cerebellum, right lobule V. This corresponds to earlier findings in an EMG-fMRI study examining similar motor tasks in healthy participants (20).

Signs of neurodegeneration such as Purkinje cell loss and torpedoes have been reported particularly in the cerebellar cortex in ET (6, 7) with simultaneous remodelling of the cerebellar cortex (28). Also, GABAergic dysfunction within the cerebellum has been observed, with increased 11C-flunazenil binding to GABA-receptors in the cerebellar cortex, increasing with tremor severity (29). In this light, our findings could be explained by hypothesizing that the cerebellar cortex is disorganized, consequently leading to aberrant cerebellar activity. The fact that we found increased instead of decreased cerebellar activations may seem counter-intuitive at first, but could be explained by suggesting that if the affected cells are deficient and disorganized, they are less efficient, and this inefficiency leads to increased activations.

Activation of the right cerebellum is congruent with the right hand and arm extension task and the activated motor cortex in the left hemisphere. Left cerebellar activation points at functional coherence with cortical regions of the right hemisphere, thus opposite to the executive motor cortex for right arm movement. In this respect, it is noteworthy that, at a lower threshold, we indeed found increased activations in the right cerebral cortex in ET patients compared to healthy participants. These activations were located in the anterior parietal and premotor cortex. Together, these areas are known to play a major role in sensorimotor transformations underlying e.g. task-related visuomotor control (30) and the organization of stereotypic movement (31). Increased coupling between left cerebellum and right parietal cortex was recently demonstrated by functional imaging investigating multisensory processing (32), independently of right or left arm involvement. One might therefore speculate that ET patients encounter more difficulties in maintaining a steady raised-arm position, which is imaginable because of their tremor, and that the increased activations in the functionally coherent areas of left cerebellar and right anterior parietal and premotor cortex reflect increased higher-order somatosensory processing implicated in motor tuning during posture maintenance.

METHODOLOGICAL CONSIDERATIONS

In this study, the use of propranolol was one of the inclusion criteria we applied to define a homogeneous group of ET patients. This is one of the many variables that can be chosen for patient selection. The advantage of choosing this variable is the future option to compare the current propranolol group with other ET patient groups using different medication. This is a first step at an attempt, as far as we know, to differentiate medication-based subtypes of ET.

A common difficulty in fMRI research lies in selecting a suitable task for healthy controls that corresponds well with the patients'

task. In this study, a mimicked tremor was used. Consequently, the two groups were actually performing a different task: we asked the tremor patients to maintain their right arm in a postured position, while the healthy controls had to deliberately move their hand. These tasks were chosen to allow optimal distinction of brain networks involved in involuntary tremor as opposed to compensation or afferent feedback by deliberate, mimicked tremor movements. The mimicked tremor movement overall had a slightly lower peak frequency and had a larger flexion–extension movement of the right wrist compared to the tremor in ET patients. The effect of this behavioural difference can be seen in the task-related activations: the healthy controls showed a more widespread and a higher activation signal in comparison with the ET patients.

CONCLUSIONS

In the current study, we used EMG-fMRI to identify brain activations specifically associated with variations in tremor severity in essential tremor patients. By including a homogeneous patient group we were able to identify specific bilateral areas in the cerebellum involved in essential tremor: lobules V, VI and VIII.

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SUPPLEMENTARY MATERIALS

1. Study set-up
2. EMG-fMRI acquisition
3. EMG-fMRI analysis
4. SUIT analysis
5. Head motion

1. STUDY SET-UP

Patients quit their medication for a minimum of three days before participating in the study. Tremor was assessed off medication using the Fahn-Tolosa-Marin Tremor Rating Scale (TRS) and a visual analog scale (VAS). The TRS is composed of three parts. Part A consists of assessment of tremor amplitude during rest, posture, movement and finger-to-nose manoeuvres. Part B consists of tremor-inducing tasks, including writing, two standardized Archimedes spirals, a line-drawing task and a water pouring task. In part C the patients rate the limitations they experience in daily life due to tremor. Parts A and B were performed and videotaped for both hands, separately. An experienced movement disorders specialist (J.D.S.) blindly determined TRS scores for part A and B. The range of the total TRS (part A, B and C) is 0-88. The VAS subjectively rated tremor severity, with patients marking a 10 cm line ranging from 0 to 10, 0 meaning no tremor at all and 10 meaning intolerable tremor. In all patients, propranolol was washed in again at the end of the study, according to a personalized schedule.

2. EMG-FMRI ACQUISITION

EMG was recorded simultaneously with BrainProducts GmbH, Munich, Germany (UMCG) and MicroMed, Italy (AMC) while scanning. EMG was recorded from five right arm muscles:
extensor carpi ulnaris, flexor carpi radialis, extensor carpi radialis longus, flexor carpi ulnaris and first dorsal interosseus. To verify the absence of left arm movement during the tasks, EMG was recorded from three left arm muscles as well: extensor carpi ulnaris, flexor carpi radialis and first dorsal interosseus. Pairs of sintered silver/silver-chloride MR-compatible surface EMG electrodes were placed bilaterally above the mentioned muscles. A ground electrode was placed on the left wrist joint. Further EMG recording procedures were similar to the methodology developed in our previous studies.2, 3

3. EMG-FMRI ANALYSIS

EMG data were corrected for echo planar imaging artefacts using the MR correction algorithms embedded in Brain Vision Analyser (Imaging Artefact Reduction method4; UMCG data) and FARM (fMRI artefact reduction for motion5; AMC data). After correction, data was further analyzed in Matlab (Matlab R2007a, Mathworks, Natrick, USA) using custom-made scripts. For each segment of 2 seconds, corresponding to one scan, the frequency spectrum was calculated using the default fast Fourier transform in Matlab (FFT). The individual frequency at the dominant tremor peak (tremor, mimicked-tremor) was determined for each patient and healthy control by visual inspection of the segments. Patients without a clear and regular tremor frequency in the EMG during the task segments were excluded from further analysis. Total spectral power in a 5Hz symmetrical band around the individual (mimicked) tremor peak frequency was exported for each segment and each right arm muscle, resulting in five vectors of the length of the number of scans/segments. The vectors of the three muscles with the highest total power were averaged. This procedure resulted in an EMG power vector with one entry for every scan. Next, this vector was orthogonalised with respect to the motor task using Gram-Schmidt orthogonalisation, to subtract the information that is already pre-

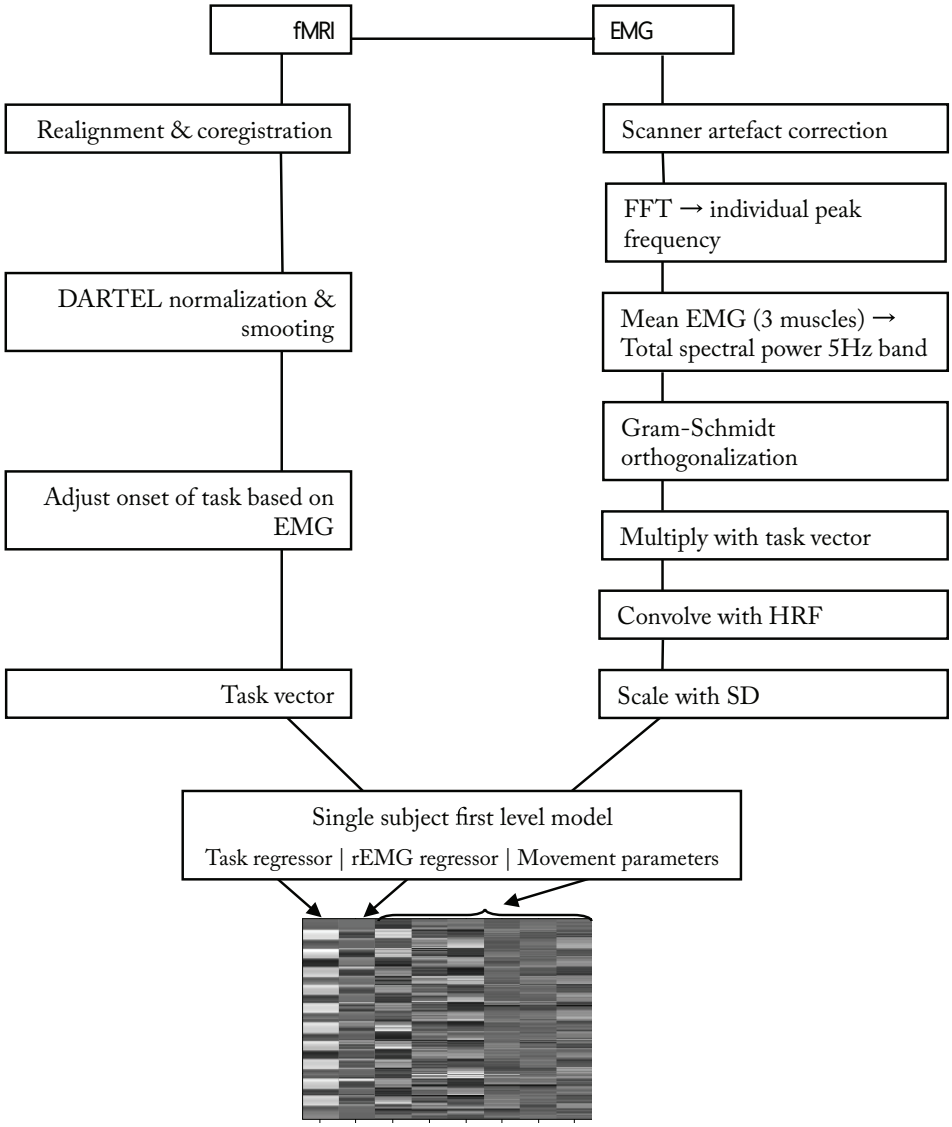

Figure S1. Flowchart of the EMG-fMRI Analysis. fMRI: functional magnetic resonance imaging; EMG: electromyography; DARTEL: Diffeomorphic Anatomical Registration using Exponentiated Lie algebra; FFT: fast Fourier transform; HRF: Haemodynamic response function; SD standard deviation.

sent in the task vector.3 The orthogonalised EMG vector (referred to as residual EMG or r-EMG vector) now provides a measure of additional EMG relative to the mean EMG value across the task. It represents the variation in tremor severity over time, independently of movement task. Subsequently, the r-EMG vector was element-wise multiplied with the task vector to obtain a vector that only has nonzeroes for the r-EMG during task, and zeroes otherwise. Finally, this vector was convolved with the canonical HRF, scaled by its SD and used as a regressor in the fMRI design matrix in addition to the task

regressor. 3 See Figure S1 for a flowchart of the EMG analysis.

4. SUIT ANALYSIS

As we hypothesized cerebellar involvement in ET, we additionally performed an analysis focused on the cerebellum using the Spatially Unbiased Infratentorial Template (SUIT) toolbox.6 This toolbox isolates the cerebellum and creates a mask. The individual T1 image of the cerebellum was normalized to the SUIT template using nonlinear deformations. The contrast images resulting from the first-level whole-brain analysis were masked with the created cerebellum mask, normalized into SUIT atlas space and smoothed with a Gaussian filter of 4-mm FWHM.

5. HEAD MOTIONS DURING SCANNING

Given the tremor, it is plausible that the ET patients made more head movements than healthy controls when executing the motor task. To test this we used the scan-by-scan realignment parameters calculated during fMRI preprocessing. We calculated the total range of head motion for translation (x, y and z direction) and rotation (pitch, roll and yaw) across each session per participant. This showed that head movements during scanning did not differ between ET patients and healthy controls, see Table S1.

TABLE S1. TEST STATISTICS FOR HEAD MOTION.

	ET mean (SD)	HC mean (SD)	t-test results
Translation x (in mm)	0.82 (0.54)	0.85 (0.43)	t(40)=0.001 p=0.87
Translation y (in mm)	1.09 (0.45)	1.09 (0.76)	t(40)=0.27 p=0.97
Translation z (in mm)	2.19 (1.30)	2.20 (0.84)	t(40)=1.26 p=0.99
Rotation - pitch (in degrees)	0.05 (0.03)	0.04 (0.03)	t(40)=1.11 p=0.60
Rotation - roll (in degrees)	0.02 (0.01)	0.02 (0.02)	t(40)=0.08 p=0.96
Rotation - yaw (in degrees)	0.02 (0.01)	0.02 (0.01)	t(40)=0.16 p=0.61

ET: essential tremor; HC: healthy controls

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