Diagnosis and imaging of essential and other tremors

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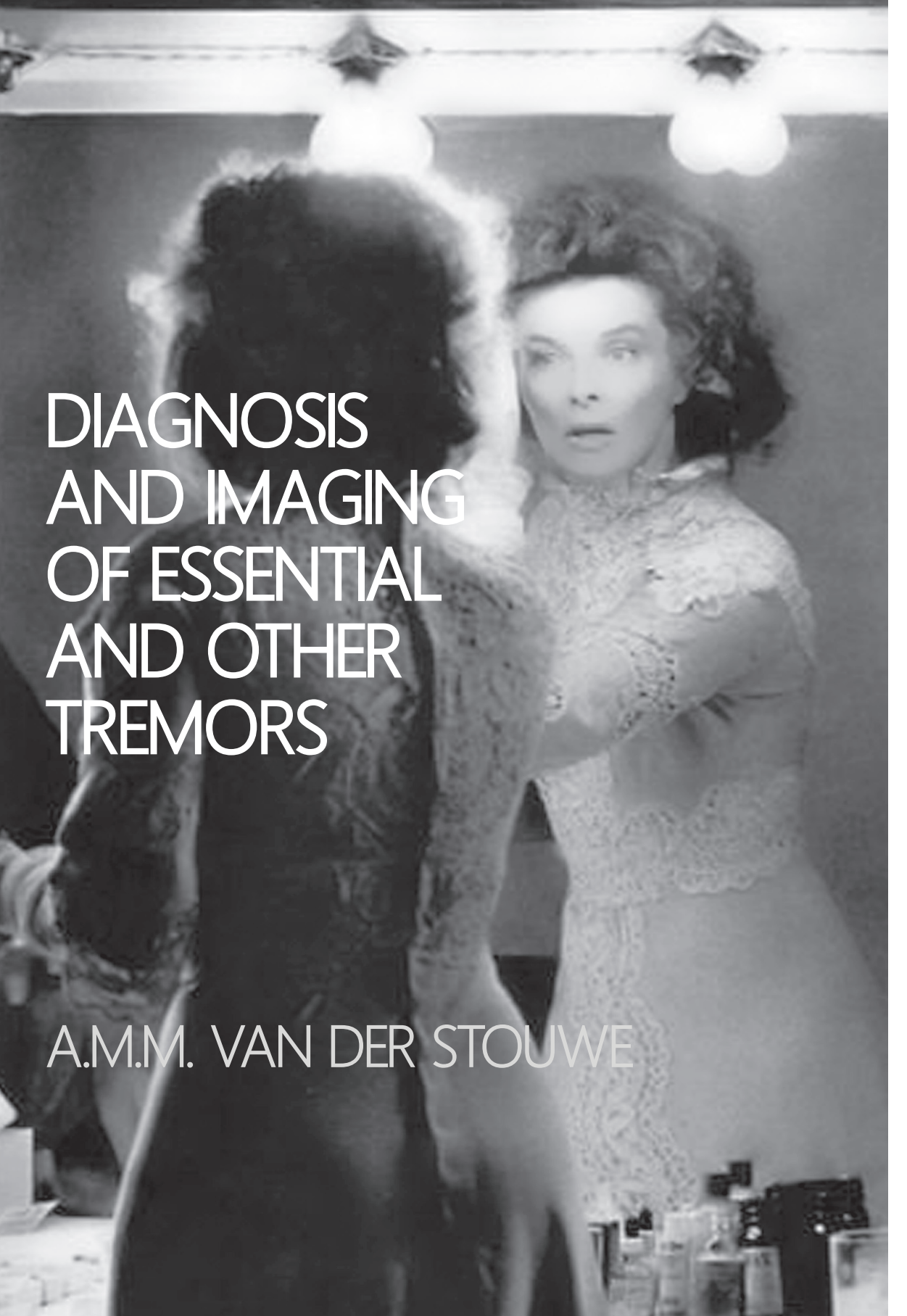

DIAGNOSIS AND IMAGING OF ESSENTIAL AND OTHER TREMORS

A.M.M. VAN DER STOUWE

COVER

The lady on the cover is Katharine Hepburn, possibly the most famous person known to have had essential tremor. In this picture, we see her in the dressing room on the set of the 1962 film adaptation of Long Day's Journey Into Night, a play by Eugene O'Neill. Nobel laureate O'Neill had a progressive tremor himself, making it impossible for him to write in the last ten years of his life. Long Day's Journey Into Night was one of his last works, and although he had given written instructions not to perform the largely autobiographical play until twenty-five years after his death in 1953, it was performed to great critical acclaim and won the Pulitzer Prize in 1957. Her role in the film adaptation won Katharine Hepburn her ninth Oscar nomination, demonstrating that her tremor did not stop her from having professional success.

A.M.M. van der Stouwe
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CHAPTER 1

INTRODUCTION

This is a thesis on tremor, a movement disorder in the category of 'too much movement', which is called hyperkinesia. In this thesis, two aims are addressed: first, to improve on diagnosis of tremor, and second, to investigate the pathophysiology of one tremor disorder, essential tremor, by means of functional neuroimaging.

BACKGROUND: TYPES OF TREMOR

Tremor is the most common movement disorder in adults (1). Most patients presenting with trembling of the upper extremities have either got tremor as a symptom of Parkinson's disease (PT), essential tremor (ET), enhanced physiological tremor (EPT) or functional tremor (FT).

The type of tremor that is best known to the general public is PT, and many tremor patients are initially worried that they may have Parkinson's disease (PD). Typically, PT starts of as a unilateral, 'pill-rolling' rest tremor (2). It is the presenting symptom of 70% of PD patients (3, 4). Clinically, presence of the other cardinal symptoms (rigidity, disturbances in balance and most importantly bradykinesia (5)) increases the likelihood of a diagnosis of PD.

By contrast, ET is a tremor disorder without additional neurological symptoms. It has a worldwide prevalence of 0.9%, increasing to 4.6% in the population older than 65 (1). ET is a bilateral tremor occurring during posturing and movement of the affected limbs (6). Intention tremor, an increase of tremor amplitude towards the end of a goal-directed movement, is described in about half of ET patients (7, 8). Apart from the arms and hands, the head, voice, and less frequently jaw and legs can also be affected. About half of ET patients report a positive family history (9). Moreover, 50% of patients report a beneficial effect of moderate consumption of alcohol on their tremor (10).

Another bilateral tremor is EPT. This tremor closely resembles the tremor every human being experiences from time to time (11, 12), be it after too much coffee, when hungry, after strenuous physical exercise, during a job interview or a PhD defence. EPT is an 'exaggerated', more constant form of physiological tremor. It is usually mild, and distal. A relationship with the circumstances described above may point to the diagnosis. Similar to ET, EPT may be familial.

FT completes the list of tremors that consultation is most commonly sought for. It was recently argued to define FT on the basis of its clinical appearance as a tremor that is significantly altered by distraction or nonphysiological manoeuvres (including a strong placebo response) and which is clinically incongruent with tremor known to be caused by neurological disease (13). FT can be characterized by sudden onset, which is atypical in tremor disorders apart from post-stroke (14). On examination, most patients' tremor has a combined yet fluctuating presence at rest, during posture, and during action, together with positive findings that are described in more detail later on (**Chapter 2**) (15). Apart from the hands and arms, tremor can occur in any body part, including the legs, head or palate (15).

Briefly, other more rare tremor disorders that are encountered are dystonic tremor (of the arms and possibly head, together with dystonic posturing (16)), cerebellar tremor (usually as a consequence of multiple sclerosis (17)) and Holmes tremor (resulting from mid brain stroke (18)).

CLINICAL DIAGNOSIS OF TREMOR

Distinguishing one type of tremor from another is important, because of the consequences for prognosis and treatment. Prognosis can range from generally mild, monosymptomatic and non-progressive in disorders such as EPT, all the way to complicated, progressive and life-shortening in a

disease such as PD. In terms of management, treatment options differ for different tremors, and range from no medication, to dopamine and trihexyfenidyl or beta-blockers and anti-epileptics drugs, to deep brain stimulation (19, 20)(Table 1.1).

In getting to the diagnosis, history taking and clinical examination by a neurologist are of primary importance (21). However, accurate clinical diagnosis can be challenged by the fact that not all patients have a classic presentation. In contrast to the typical presentations described above, action tremor can also occur in PT, ET patients may have rest tremor, EPT may be a serious handicap, FT can be fairly consistent. Finally, stress may exaggerate any neurological disorder. The phenomenology of tremor is complex, involving a broad variety of signs and symptoms. Over the years, large steps have been made in describing the phenomenology of different tremors (22-26), with some tremor disorders presenting with presumably typical signs. However, a lot of the work has described groups in isolation, or either comparing only specific or small groups. In **Chapter 2**, we will describe our work in determining sensitivity and specificity of five 'typical' tremor phenomena in a large and diverse tremor population.

CLINICAL NEUROPHYSIOLOGICAL DIAGNOSIS OF TREMOR

A neurologist can request clinical neurophysiology testing to help establish a diagnosis in more difficult cases. Polymyography, usually combined with accelerometry, assesses tremor frequency and amplitude in a multitude of postures (rest vs action) and during different tasks (for instance, finger-to-nose manoeuvres). The application of clinical neurophysiolocal techniques in tremor diagnosis differs greatly between centres, and is of course dependent on availability but also on a culturally defined attitude towards more (Germany, The Netherlands) or less (United Kingdom) clinical neurophysiology testing. Polymyography objectifies the presence and consistency of tremor characteristics under various circumstances, and is informative of tremor frequency. These tests are usually of great value (27, 28), however, interpretation of results is not always straightforward. For example, tremor frequencies overlap between different tremor disorders (29), and, as mentioned previously, the sensitivity and specificity of certain 'typical' tremor phenomena are generally poorly known. In **Chapter 3**, we describe our work aiming to add to the diagnostic power of routine polymyography, by investigating the potential additional diagnostic value of two advanced EMG measures: intermuscular coherence and cumulant analysis.

ESSENTIAL TREMOR: A MUCH-DEBATED PHENOTYPE

There have been challenges in defining ET. Traditionally, ET has been used and misused as a 'container' diagnosis, gathering together all types of tremor patients that did not fit any particular diagnosis. In 1998, the Movement Disorders Society published their first consensus statement on tremor (30). This was followed, in the year 2000, by the criteria for ET by the Tremor Investigation Group: widely used nowadays, and generally known as the TRIG criteria (6). Core criteria are bilateral tremor of the hands and forearms, with no other neurological signs (except a cogwheel phenomenon and head tremor). Secondary criteria are supportive of a diagnosis of ET, and include duration >3 years, a positive family history and beneficial effect of alcohol. They increase the likelihood of a diagnosis of ET, but are not required. The core criterion of 'no other neurological signs' has evolved over time, with recent reports on gait ataxia (31-33), limb ataxia (34, 35), eye movement abnormalities (36, 37), dystonia

TABLE 1.1 BASIC CHARACTERISTICS AND TREATMENT OPTIONS FOR DIFFERENT TREMOR TYPES

Type of tremor	Frequen -cy (Hz)	Rest	Posture	Goal-directed	Pharmacological treatment options	Stereotactic treatment options
ET	4 to 11	-	++	+	Propranolol, primidone, (level A recommendation), topiramate, atenolol, sotalol, gabapentin, alprazolam (level B)	Thalamotomy and thalamic DBS (VIM, VL, STA) (level C)
PT	5 to 10	++	+-	+-	Pharmacological treatment options for PD effect tremor < bradykinesia and rigidity (level A)	Lesioning or DBS of STN, GPI, VIM, VL (level C)
EPT	7 to 12	-	++	+	Similar to ET (level A-C)	-
FT	4 to 10	+	+	+	Explanation, physiorehabilitation, psychological treatment	-
DT	4 to 10	-	++	+	Trihexyphenidyl, propranolol (level C)	-
CT	2 to 6	-	+	++	Carbamazepine, propranolol, primidone, isoniazid, ondansetron, 4-aminopyride, botulinum toxin (level U)	Lesioning or DBS of VIM (level C)
HT	2 to 5	++	+	++	Levodopa, clonazepam, clozapine, levetiracetam (level U)	Thalamic DBS (level C)

ET: essential tremor, PT: Parkinsonian tremor, EPT: enhanced physiological tremor, FT: functional tremor. DBS: deep brain stimulation, VIM: ventral intermedius (thalamus), VL: ventrolateral (thalamus), STA: subthalamic area, STN: subthalamic nucleus, GPI: globus pallidus interna. Levels refer to the strength of the recommendation based on the quality of the currently published studies. A: established as effective, level B: probably effective, level C: possibly effective, level U: data inadequate or clniflicting, treatment is unproven with current knowledge.

(38), and non-motor symptoms (39) in patients that are otherwise diagnosed as ET.

Application of the TRIG criteria reduces the chance of mislabelling patients as ET significantly. This is important, not only for patients' prognosis and management, but also for research purposes. Being uncritical about including 'ET patients' into any scientific study complicates finding commonalities in such a group, independent of investigating phenomenology, pathology, brain activation or genes. Indeed, lack of a secure diagnosis

has been proposed as a serious limitation for advancement in all four mentioned areas of ET research (40, 41).
Since the publication of the TRIG criteria, it has been suggested to distinguish 'hereditary ET'; patients fulfilling the TRIG criteria and with a positive family history, 'sporadic ET'; fulfilling TRIG criteria, but without a family history, and 'senile ET'; fulfilling TRIG criteria, but with an age-at-onset later than 65 (40). The reason for this distinction is the fact that the penetrance for hereditary ET appears almost complete by age 65, which would make new-onset tremor at old-age more likely to be due to common age-associated neurodegenerative diseases, rather than to specific ET. Although not all currently apply this distinction, we chose to include only hereditary and sporadic ET patients into the ET study that is described in **Chapters 4-7**, resulting in a well-defined group.

ESSENTIAL TREMOR: ASSESSMENT OF TREMOR SEVERITY

ET is often called a benign disorder, but moderate and advanced stages can be physically and socially disabling (42-44). To assess tremor severity, the Fahn-Tolosa-Marin Tremor Rating Scale (TRS) is a well-known and widely used tool in clinical trials (45, 46). The TRS includes both clinician-based ratings in parts A and B, and a patient-based activities-of-daily-life questionnaire in part C. The latter interview takes some time to conduct, and is not always used in clinical trials, nor is it always replaced by another patient-based measure of tremor severity such as subjective visual analogue scale scoring. Intuitively, one would suspect clinician-based and patient-based measures to correlate well; however, because these relations have never been directly investigated. Therefore, the supposition that objective clinical improvement correlates with patients' appraisal of improvement remains unsubstantiated.

ESSENTIAL TREMOR: UNCLEAR PATHOPHYSIOLOGY

Regarding the pathophysiology of ET, three hypotheses exist that are nonexclusive, but may rather work in unison. In all of them, the cerebellum or cerebellothalamocortical circuit plays an important role (see (47) for a review).

The neurodegeneration hypothesis

The hypothesis that has been under debate for the longest time is the hypothesis that ET is a neurodegenerative disease (48). Clinically, the fact that ET is progressive and associated with age supports this hypothesis (49). Moreover, in some studies (50), ET is associated with an increased risk of developing PD or Alzheimer's disease: both neurodegenerative diseases. Three research groups have performed pathology studies, with conflicting results: most of them, but not all, suggesting degeneration of the cerebellum. Currently, the largest pathology study that was done by Louis et al compared 33 ET patients to 21 healthy participants (51). Cerebellar Purkinje cell loss was found in ET patients, with loss of 25% of cells, and 24% of patients had Lewy bodies in the locus coeruleus. Later on, this same research group showed Purkinje axonal swelling (called torpedoes) in ET cerebellums, but not in healthy subjects (52). A second group found evidence of cerebellar degeneration in 29% of patients, but did not replicate the finding of Lewy body disease in the locus coeruleus (53). Finally, a third group reported no Purkinje cell loss nor Lewy bodies in ET, albeit in a smaller sample (54). The controversial results in neuropathology may be explained by patient selection biases and lack of standardization of methods across these studies. Structural brain imaging has also come up with conflicting results: some studies showing decreased cerebellar white-matter integrity and cerebellar atrophy, while others showed no

abnormalities in brain structure (55). Overall, there is heterogeneous evidence mainly for cerebellar neurodegeneration.

The gamma-aminobutyric acid hypothesis

A second hypothesis that has been put forward is that ET is caused by a disturbance of the gamma-aminobutyric acid (GABA) ergic system. In an overall theory to explain ET pathophysiology, the GABA hypothesis contains multiple steps (56). The first step comprises of cerebellar degeneration with Purkinje cell loss. As a consequence, activity of the GABA system decreases in the deep cerebellar neurons. Therefore, the pacemaker activity of the deep cerebellar neurons is disinhibited. As a result, the rhythmic activity of the thalamus and thalamo-cortical circuit increases, ultimately leading to tremor.
Clinically, the notion of abnormal GABA function is supported by the fact that some GABAergic drugs are beneficial in ET, although not all are (19). Secondly, decreased GABA levels have been found in the cerebro-spinal fluid of ET patients (57). Other evidence comes from recent positron-emision topography (PET) studies: it was found that 11C-flumazenil binding to GABA-receptors was increased in ET patients compared to healthy participants (58), and that binding increased with tremor severity (59). Moreover, in a pathology study, decreased levels of GABA receptors were found in the dentate nucleus of the cerebellum in ET patients compared to PD patients and healthy controls (60). On the whole, studies on GABA all point towards decreased cerebellar GABA, however, the hypothesis concerns relatively few studies and not all results have been duplicated.

The oscillating network hypothesis

Historically, a lot of tremor research focused on finding one driving oscillator facilitating tremor based on the fact that some neurons have oscillating properties at an independent frequency, including in the deep cerebellar nuclei (61-64). The idea of a single oscillator has been challenged by several inconsistent findings, for instance that lesions at several locations in the cerebellothalamocortical circuit can relieve tremor (65), and deep brain stimulation of multiple clusters of the ventrolateral and ventral intermediate nucleus of the thalamus and subthalamic area can alleviate ET (35, 66, 67). As a result, current research is focused on identifying a network of oscillators, taking into account the connectivity and interactions between different parts of the cerebellothalamocortical circuit (68). Good examples of this approach are the recent EEG/MEG/thalamic microelectrode-EMG coherence studies, which support involvement of a large part of the physiological central motor circuit in ET (69-71). Moreover, it was found that although both voluntary and pathological tremor arise from the cerebellothalamocortical circuit, bithalamocortical interactions are only found in pathological tremors (72).

Note that while the in the GABA hypothesis cerebellar neurodegeneration is taken as the starting point of its notion (56), in the oscillating network hypothesis it remains open whether the emerging picture of neurodegeneration reflects primary degeneration or secondary changes (68). The strongest advocates of the neurodegeneration hypothesis see the structural changes as primary (73). To add to the clarification of ET pathophysiology, we set up a neuroimaging study in ET patients that is described in **Chapters 4-6**. We felt that functional magnetic resonance imaging (fMRI) had not yet been used to its full potential: therefore, we used the advanced technique of combining fMRI with EMG. I will briefly discuss these techniques in the following section.

TECHNIQUES EMPLOYED IN THIS THESIS: EMG AND FMRI

Two major techniques are used in this thesis. The first one is electromyography (EMG), a technique that is used to measure electrical activity in skeletal muscles: either by placing an electrode intramuscularly (needle EMG) or on the skin above the muscle (surface EMG). The most basic use of EMG is to determine whether a muscle is active. In tremor research, one of the applications of EMG is to quantify the extent of tremor (28), especially fluctuations within one recording. In **Chapter 4**, we employed EMG in this manner: by quantifying the EMG signal, we were able to express changes in tremor intensity over time, which we could then correlate with changes in brain activity over time. This way, we were able to link tremor activity directly to brain activity.

Brain activity was measured with the second major technique used in this thesis, which is fMRI. Clinically, MRI is widely used to investigate human anatomy and pathology in vivo. MRI makes use of the differences in magnetic properties between various types of tissues in the human body to create an anatomical image. Functional MRI adds mapping of regional brain activity to anatomical (structural) MRI, by applying this principle to hemoglobin. When a brain area becomes more active, there is a local increase in blood that is rich in oxyhemoglobin, containing more oxygen than can be used by the brain tissue. This haemodynamic response results in a local increase of the proportion of oxyhemoglobin versus deoxyhemoglobin. The difference in magnetic properties of deoxygenated and oxygenated blood enables the detection of local increases in brain activity. The signal thus obtained is called the Blood Oxygenation Level Dependent (BOLD) signal. Local changes in the BOLD signal can be analysed statically. For example, it is common to compare brain activation in all voxels during a certain task to activation during rest; first for individual participants, then in a group of participants. Finally, statistics can then be used to compare brain activation between different groups.

In this thesis, we employed several fMRI analysis designs and techniques, such as task-related and 'EMG'-related (**Chapter 4**), event-related (**Chapter 5**), and connectivity analysis (**Chapter 6**), all to study changes in brain activations in ET patients.

AIMS

We address two aims in this thesis. In the first part, we aim to improve on the diagnosis of tremor. In **Chapter 2**, we do this by examining the sensitivity and specificity of several presumably 'typical' tremor characteristics. In **Chapter 3**, we examine the potential value of intermuscular coherence and cumulant analysis as additional diagnostic measures in the clinical neurophysiological assessment of tremor. In the second part of the thesis, we aim to investigate the pathophysiology of ET by means of functional neuroimaging. In **Chapter 4**, we take the approach of correlating fluctuations in tremor severity during scanning with brain activity in ET patients performing a postural task. In **Chapter 5**, we perform effective and functional connectivity analysis in the same ET population. In **Chapter 6**, we compare brain activity related to goal-directed movement between ET patients and healthy participants. We investigate to what extent clinician-based and patient-based measurements of tremor severity correlate in **Chapter 7**.

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CHAPTER 2

HOW TYPICAL ARE 'TYPICAL' TREMOR CHARACTERISTICS?

SENSITIVITY AND SPECIFICITY OF FIVE TREMOR PHENOMENA

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ABSTRACT

BACKGROUND: Distinguishing between different tremor disorders can be challenging. Some tremor disorders are thought to have typical tremor characteristics: the current study aims to provide sensitivity and specificity for five 'typical' tremor phenomena.

METHODS: Retrospectively, we examined 210 tremor patients referred for electrophysiological recordings in the period January 2008-January 2014. The final clinical diagnosis was used as gold standard. The first step was to determine whether patients met the neurophysiological criteria for their type of tremor. Once established, we focused on 'typical' characteristics: tremor frequency decrease upon loading (enhanced physiological tremor (EPT)), amplitude increase upon loading, distractibility and entrainment (functional tremor (FT)), and intention tremor (essential tremor (ET)). The prevalence of these phenomena in the 'typical' group was compared with the whole group.

RESULTS: Most patients (87%) concurred with all core clinical neurophysiological criteria for their tremor type. We found a frequency decrease upon loading to be a specific (95%), but not sensitive (42%) test for EPT. Distractibility and entrainment both scored high on sensitivity (92%, 91%) and specificity (94%, 91%) in FT, whereas a tremor amplitude increase was specific (92%), but not sensitive (22%). Intention tremor was a specific finding in ET (85%), but not a sensitive test (45%). Combination of characteristics improved sensitivity.

DISCUSSION: In this study, we retrospectively determined sensitivity and specificity for five 'typical' tremor characteristics. The characteristics proved specific, but few were sensitive. These data on tremor phenomenology will help practicing neurologists to better distinguish between different tremor disorders.

INTRODUCTION

Although tremors are the most common movement disorders, distinguishing between different tremor disorders can be challenging (1, 2). The phenomenology of tremor is complex, involving a broad variety of signs and symptoms. Some tremor disorders seem to have a typical tremor characteristic that points to the diagnosis, but if sensitivity and specificity of these presumed hallmarks are unknown, their significance remains uncertain. In the present study we establish how well the clinical tremor diagnosis met the clinical neurophysiological criteria. Furthermore, we aim to provide sensitivity and specificity numbers for five 'typical' tremor characteristics.

Firstly, a frequency decrease after loading or weighing of the tremulous hand is found in enhanced physiological tremor (EPT). This phenomenon has been long known (3) and is also reported in normal subjects (4). The frequency shift is thought to appear because EPT is considered to be caused partly by mechanical reflex oscillation. This oscillation is dependent of the hand's resonant frequency and therefore changes with increased inertial loading (5). The frequencies of tremor disorders that are considered to be generated by a central oscillator are supposed to be invariable upon loading (6). However, no studies on the sensitivity and specificity of this phenomenon exist.

Secondly, we aim to investigate three phenomena that seem typical of functional tremor (FT): an amplitude increase after loading of the tremulous hand (7), entrainment (7-9) and distractibility (10, 11). These characteristics have been described in previous small studies, and are considered to be positive symptoms for the diagnosis of FT. On the other hand, it is known from clinical experience that these features occasionally occur in 'organic' tremor patients, which raises the question how specific these characteristics really are (12).

Lastly, intention tremor, which is tremor increasing during goal-directed movement, is known to occur in essential tremor (ET) (13), but is atypical in most other tremors. A recent study reported intention tremor in 28% of ET patients versus only 4% of Parkinson's disease patients (14). We would like to extend these numbers to the general tremor population.

In this study, we retrospectively determine sensitivity and specificity for typical tremor phenomena, to extend the available data on clinical tremor phenomenology and aid clinicians in their neurological examinations and diagnostic process.

METHODS

SUBJECTS

We searched the database of the department of Clinical Neurophysiology of the University Medical Center Groningen, a tertiary referral centre, for patients who had undergone a polymyography as part of the diagnostic work-up for upper limb tremor. All subjects had to be >18 years old. The search started at January 1st, 2014, and continued until the three groups of which we intended to test specific tremor characteristics (EPT/ET/FT) each contained 50 subjects (January, 2008). Patients with other tremor diagnoses were also included to attain a diverse general tremor population as a control group.

CLINICAL DIAGNOSIS

As a starting point, we took the most recent clinical diagnosis by the attending neurologist as the gold standard: the final diagnosis after polymyography and possibly imaging or laboratory testing. Patients were not in-

cluded if the neurologist had considerable doubt about the diagnosis: in case of a current impossibility to differentiate between two disorders. Another exclusion criterion was lack of a final clinical diagnosis, if correspondence was unavailable. For each subject, we recorded from their clinical records: age, sex, primary diagnosis pre-polymyography, and the final clinical diagnosis.

CLINICAL NEUROPHYSIOLOGY TESTING

In our centre's tremor-specific polymyography recording, tremor is assessed during rest, posture and specific tasks. All our data

TABLE 2.1. CLINICAL NEUROPHYSIOLOGICAL GUIDELINE.

	Criteria for electrophysiological diagnosis at our hospital	**Prevalence***
EPT	Core criteria: • Unstable tremor frequency: change >1 Hz upon change of posture or loading • Predominantly distal tremor Supportive criterion: • High frequency (>7 Hz)	 90% 78% 78%
ET	Core criteria: • Bilateral tremor during posture/action • Stable tremor frequency: <2 Hz variation throughout registration Supportive criterion: • Intention tremor	96% 94% 42%
FT	Core criterion: • Unstable tremor frequency: >1 Hz variation or temporal tremor suppression upon change of posture, mental distraction or entrainment Supportive criteria: • Increase in tremor amplitude upon loading	 94% 22%
PT	Core criteria: • Tremor at rest • Stable tremor frequency: <2 Hz variation throughout registration Supportive criteria: • Increase in tremor amplitude during mental tasks • Frequency between 4 and 7 Hz	 95% 100% 39% 95%
DT	Core criteria: • Signs of dystonia, co-contraction between agonists and antagonists, overflow Supportive criteria: • Irregular tremor • Proximal tremor • Influence of sensory stimuli	 33% 50% n.a. n.a.
CT	Core criteria: • Tremor predominantly during action • Intention tremor	 100% 100%
HT	Core criteria: • Tremor present at rest, posture and action • Low frequency (<4Hz) Supportive criterion: • Intention tremor	 100% 100% 100%

EPT: enhanced physiological tremor, ET: essential tremor, FT: functional tremor, PT: Parkinsonian tremor, DT: dystonic tremor, CT: cerebellar tremor, HT: Holmes tremor. *: prevalence in the study group with a corresponding final clinical diagnosis; for group information see Results section and Table 2, n.a.: not available, these criteria were not consistently reported.

is derived from reports of these standardized electrophysiological recordings, written by two experienced clinical neurophysiologists (JWE, JvdH). They based their reports on continuous recordings of accelerometry, EMG, and video. EMG was recorded with Ag/AgCl surface electrodes placed over wrist and elbow flexors and extensors. Accelerometers were placed on the dorsal side of both hands. All frequency analyses were based on accelerometry. Data was recorded using BrainRT software (OSG BVBA, Rumst, Belgium).

In Table 2.1 we have summarized the criteria used in our clinic for the clinical neurophysiological diagnosis (15-17). For each group, we calculated how many patients met these criteria.

To assess the influence of polymyography on diagnosis, we compared the clinical pre-polymyography diagnosis, the neurophysiological diagnosis derived from polymyography, and the final clinical post-polymyography diagnosis to determine how the outcome of the neurophysiological testing affected the diagnosis. In case of a change in diagnosis, we noted the nature of the conversion.

'TYPICAL' TREMOR PHENOMENA

We will describe the five specific tremor characteristics of which we aimed to test sensitivity and specificity in more detail. These are routinely assessed: results could be derived from the clinical neurophysiology reports.

Loading of the arm was realized by attaching one or two 500 g weights, depending on the patient's strength, to the patient's wrist. We recorded whether there was a decrease of tremor frequency (>1Hz) upon loading, and/or an increase of tremor amplitude compared to the unloaded condition, as reported by the neurophysiologist.

Entrainment was investigated while the most-affected hand was held in the position that evoked maximal tremor. Patients were instructed to imitate tapping motions with their least-affected hand at the same speed as the laboratory technician, who would vary the frequency between ±1-4 Hz. A positive entrainment test result was scored in case of a notable tremor frequency shift (decrease>1Hz) of the contralateral hand, or temporary tremor suppression.

Distractibility was assessed formally with hands held in the position that evoked maximal tremor. Patients were instructed to serially subtract seven from a hundred out loud (100, 93, 86, etc.). Moreover, distractibility was investigated informally during conversation and instruction of tasks. We chose to combine these assessments because it is our impression that not all patients are sufficiently distracted by formal yet simple tasks: assessment during the rest of the consultation is of equal importance. Distractibility was defined as notable frequency shift (decrease>1Hz) or temporary tremor suppression during formal or informal mental distraction.

Intention tremor was assessed with finger-to-nose manoeuvres, where patients were instructed to move the index finger of their outstretched arm to the tip of their nose. If tremor amplitude increased as the patient's finger approached the nose this was scored as a positive test result.

STATISTICAL ANALYSIS

Patient and tremor characteristics were compared between groups using Chi-square tests for gender and Kruskal-Wallis tests for all continuous, not-normally distributed data in SPSS 20 (SPSS, Chicago, IL). In case of differences between groups, post-hoc testing was performed using Mann-Whitney tests. We compared the frequency of positive test results for each tremor characteristic with Fisher's exact tests, and calculated sensitivity and specificity for each test. We considered results significant if $p<0.05$.

To place the phenomena in a broader per-

spective and improve discriminative value, we combined tests (presence of tremor phenomena) with tremor frequency and frequency variability. In case of multiple significantly different tests for one diagnosis versus all others we investigated combinations. Cut-off values for tremor frequency and variability were first estimated based on visual inspection, and we calculated ROC-curves for frequencies between 6.0-7.0 Hz and frequency variability between 1.25-2.0 Hz at 0.25 Hz intervals: the combinations with the largest area under the ROC-curve (AUC), reflecting the highest discriminative value, are reported.

RESULTS

PATIENT CHARACTERISTICS

Two hundred-ten patients were included in this study (Table 2.2). Patients had a diagnosis of EPT (n=50), ET (n=50), FT (n=50), Parkinsonian tremor (PT, n=41), dystonic tremor (n=7), cerebellar tremor (CT, mostly MS-related, n=8) or Holmes or rubral tremor (HT, n=4). Gender distribution did not differ between groups. There was an age difference ($p<0.001$): EPT patients were younger than ET, FT and PT patients (all: $p<0.001$). Moreover, ET patients were older than FT ($p<0.001$) and PT patients ($p=0.006$).

CLINICAL NEUROPHYSIOLOGY

The final clinical diagnosis met with all (87%) or at least one (92%) of our core neurophysiological criteria in most cases. The supportive criteria were met less frequently (see Table 2.1). Median tremor frequency was 8.2 Hz in EPT patients, 5.8 Hz in ET patients, 5.3 Hz in FT patients and 5.4 Hz in PT patients (Table 2.2, Figure 2.1). There was a difference between patient groups ($p<0.001$): tremor frequency was higher in EPT compared to ET, FT and PT (all: $p<0.001$). Frequency variability was different between groups ($p<0.001$): frequency variability was higher in EPT (2.5 Hz) and FT (2.3) compared to ET (1.0) and PT patients (0.9) patients (all: $p<0.001$).

TABLE 2.2. PATIENT CHARACTERISTICS

	N	M/F	Age	Mean frequency	Frequency variability
EPT	50	30/20	44 (38)*	8.2 (2.0)*	2.5 (1.4)*
ET	50	29/21	71 (11)*	5.8 (0.8)	1.0 (0.4)
FT	50	27/23	60 (16)	5.3 (1.4)	2.3 (1.4)*
PT	41	24/17	59 (18)	5.4 (1.3)	0.9 (0.3)
DT	7	3/3	51 (37)	5.7 (4.4)	2.0 (1.3)
CT	8	4/4	43 (13)	5.0 (1.9)	1.0 (0.8)
HT	4	1/3	66 (42)	3.3 (0.6)	0.8 (1.0)

All values except gender are displayed as median (interquartile range). EPT: enhanced physiological tremor, ET: essential tremor, FT: functional tremor, PT: parkinsonian tremor, DT: dystonic tremor, CT: cerebellar tremor, HT: Holmes tremor. M/F: Male/Female. *Significant difference, direct post-hoc comparison between EPT, ET, FT and/or PT (see text).

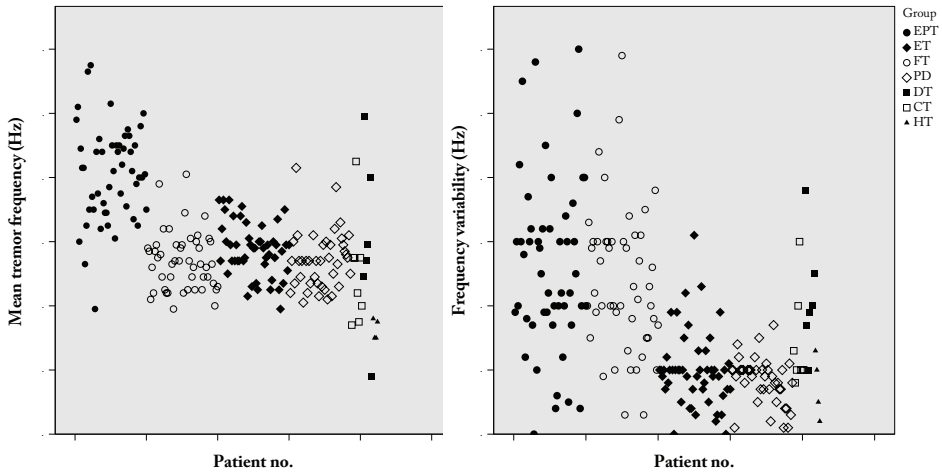

Figure 2.1. Mean tremor frequency (left) and tremor frequency variability (right) in Hz. ET: essential tremor, FT: functional tremor, PT: parkinsonian tremor, DT: dystonic tremor, CT: cerebellar tremor, HT: Holmes tremor.

INFLUENCE OF POLYMYOGRAPHY ON CLINICAL DIAGNOSIS

The diagnosis that topped the differential diagnosis pre-polymyography was confirmed by the polymyography in 70% of all cases. Contrarily, in 22%, the initial diagnosis changed. In those 45 cases, the incorrect pre-polymyography diagnosis was ET (n=21), EPT (n=10), PT (n=8), DT (n=2), tremor due to a structural lesion (n=2), neuropathic tremor (n=1), or myoclonus (n=1). These 45 incorrect diagnoses turned into a final clinical diagnosis, after polymyography and occasionally other testing, of FT (n=18), EPT (n=13), ET (n=8), PT (n=5), and HT (n=1) (Table 2.3). In a small number of patients (5%), the initial pre-polymyography diagnosis did not change, although the conclusion of the polymyography report suggested

TABLE 2.3. CHANGES IN DIAGNOSIS (N=45)

Pre \ Final	**EPT**	**ET**	**PT**	**FT**	**HT**	***Total***
EPT	-	6	0	4	0	*10*
ET	11	-	3	6	1	*21*
PT	2	2	-	4	0	*8*
DT	0	0	1	1	0	*2*
Structural lesion	0	0	0	2	0	*2*
Neuropathic	0	0	1	0	0	*1*
Myoclonus	0	0	0	1	0	*1*
Total	*13*	*8*	*5*	*18*	*1*	

Cases in which there was a difference between pre-polymyography diagnosis ('Pre': rows) and final diagnosis ('Final': columns).

TABLE 2.4. SENSITIVITY AND SPECIFICITY OF FIVE TYPICAL TREMOR CHARACTERISTICS.

	Sensitivity	Specificity
Frequency decrease upon loading in EPT	42%	95%
≥2 positive for EPT: • Frequency decrease upon loading • Frequency > 6 Hz • Frequency variability > 1.75 Hz	84%	94%
Amplitude increase upon loading in FT	22%	92%
Entrainment in FT	91%	91%
Distractibility in FT	94%	92%
≥2 positive for FT: • Entrainment • Distractibility • Frequency variability > 1.75 Hz	100%	93%
Intention tremor in ET	42%	85%

a different diagnosis (in 3/8 cases the final diagnosis was DT, in 2 cases ET, 1 case FT, 1 case EPT, 1 case PD). In 3% of the cases, the clinician added a so-called 'functional component' to the final diagnosis because of 'functional features' in an otherwise organic-considered tremor, for example 'PT with functional component'.

'TYPICAL' TREMOR CHARACTERISTICS

Enhanced physiological tremor

A decrease of tremor frequency upon loading was found in 42% of EPT patients, versus 5% of non-EPT patients (p<0.001). Test sensitivity for EPT was 42%, specificity 95% (Table 2.3). A score of at least 2 out of 3 positive tests from 1) frequency decrease upon loading, 2) tremor frequency >6 Hz, and 3) tremor frequency variability >1.75 Hz, resulted in increased test sensitivity for EPT of 84%, and specificity of 94% (AUC=0.946, p<0.001).

Functional tremor

An increase of tremor amplitude upon loading was seen in 22% of FT patients, versus 8% of non-FT patients (p=0.331). Test sensitivity for FT was 22%, specificity was 92%. Entrainment occurred in 91% of FT patients, versus 9% of all other patients (p<0.001): test sensitivity for FT was 91%, specificity 91%. A decrease of tremor frequency or amplitude upon distraction was seen in 94% of FT patients, versus 8% of all other patients (p<0.001). Test sensitivity for FT was 94%, specificity 92%. A score of ≥2 out of 3 positive tests from 1) entrainment, 2) distractibility, and 3) tremor frequency variability >1.75 Hz, resulted in test sensitivity for FT of 100%, and specificity of 93% (AUC=0.985, p<0.001).

Essential tremor

We found intention tremor in 42% of ET patients, versus 15% of non-ET patients (p=0.000). Test sensitivity for ET was 42%, test specificity 85%. Test specificity was decreased by the occurrence of intention tremor in CT and HT patients: specificity increased to 92% after omission of CT and HT patients.

DISCUSSION

We retrospectively determined sensitivity and specificity for five presumed typical

tremor characteristics, by comparing prevalence of each phenomenon in 50 patients from the relevant tremor disorders versus patients from a diverse, general tremor population.
First, we detected that in 87% of our patients the final clinical diagnosis concurred with all our core clinical neurophysiological criteria. Supportive criteria for different tremor types were met less frequently, underpinning their role as secondary criteria. As some of the used clinical neurophysiological criteria are consensus-based (15), we are pleased to reinforce these parameters here.

The polymyography diagnosis supported the pre-registration clinical tremor diagnosis in the majority of cases, whereas the diagnosis changed in 22%. It is noteworthy to see what changes in diagnosis were made under the influence of the tremor-specific polymyograpy. In nearly half the cases where the diagnosis changed the initial diagnosis was ET. Apparently, we are quick to think of ET, which is fitting with ET's image as an overdiagnosed disorder (1, 2). Another point of interest is that FT was never an incorrect top differential, whereas of the incorrect diagnoses, 18 out of 45 changed into FT. We conclude that in our tertiary referral centre neurologists are conservative in diagnosing tremor as functional. This is understandable, but also dangerous, as a positive, unambiguous diagnosis is key in the treatment of functional disorders (21).

Regarding the 'typical' tremor phenomena, our findings reveal that a frequency decrease upon loading of the tremulous arm is specific for EPT (95%). However, it is not a sensitive test (42%): lack of a change in frequency is therefore not informative, but if the tremor frequency decreases this points to EPT. To our knowledge, this is the first study to report sensitivity and specificity numbers for this test. Sensitivity increases to 84% when the effect of loading is combined with tremor frequency (>6 Hz) and frequency variability (>1.75 Hz). These results suggest that a scoring system of at least 2 positive tests out of 3 for EPT may be diagnostically useful.

Of the phenomena we investigated that are believed to be typical for FT, testing for distractibility was most useful. A noticeable frequency decrease or temporary tremor suppression upon distraction occurred in almost all FT patients, making this a very sensitive feature (94%), while at the same time the phenomenon was specific for FT (92%). Tremor distractibility has been described before in FT (10) and one study reported a sensitivity for mental distraction by means of a simple calculation task ("serial subtractions of 7") of 58.3% (11). We report a much higher sensitivity in the current study, probably because we assessed distractibility both formally with the same calculation task and informally throughout the registration.
The test for entrainment resulted in similar high sensitivity (91%) and specificity (91%), and is therefore also informative. Again, we report higher numbers than previous studies (7, 8) probably because we applied less formal testing: either true entrainment, a noticeable frequency shift, or temporary tremor suppression scored as entrainment. We consider these extended definitions of distractibility and entrainment appropriate because they represent what neurologists want to assess clinically: the influence of mental or motor tasks on the tremor.
Finally, testing for tremor amplitude increase upon loading was the least useful test for FT. Overall, the phenomenon was uncommon, and statistically, it did not occur significantly more often in FT than in other tremor disorders. Test sensitivity was very low (22%), although specificity was high (92%). Although a previous study (7) used a quantified accelerometry measure instead of our visual assessment of video/EMG/accelerometry

recordings, their results for sensitivity and specificity were highly similar: 33% and 92%. In general, we would like to point out that although all FT-tests have a high specificity, none reached 100%. As is known from previous work (7-9,11), 'functional' characteristics can occur in otherwise 'organic' tremor. In this study, we confirm that distractibility, entrainment and an increase of tremor amplitude after loading can all be seen in organic tremor. It is of course possible that an existing organic tremor is worsened by functional tremor. This was sometimes acknowledged by the neurologist, by adding 'plus a functional component' to their final diagnosis.
Overall, a combination of entrainment, distractibility and tremor frequency variability (>1.75 Hz) was most suited to classify FT patients. Scoring ≥2 positive test results out of 3 resulted in a test sensitivity of 100% and specificity of 93%, increasing the feasibility of diagnosing FT on positive findings instead of per exclusionem. This fits well with the current clinical approach of counting the positive rather than the negative symptoms in functional movement disorders (12).

Our data further reveal that intention tremor occurs in two out of five ET patients, which is in accordance with previous studies (13,18). We extended previous work on prevalence of intention tremor in ET versus PT patients (14) to the general tremor population, and found that intention tremor occurs in 15% of non-ET tremor patients. The feature was most common in CT and HT patients, which is to be expected as intention tremor is a sign of cerebellar disease, and in these disorders the cerebellum or cerebellar outflow-tract is affected (19,20). Omission of CT and HT patients increased test specificity to 92%. Therefore, a positive finger-to-nose test is informative in distinguishing ET from EPT, PT, DT, and FT, but not CT and HT.

There are two potential weaknesses that relate to our 'gold standard': the most recent clinical diagnosis. As the clinical diagnosis is partly based on features of which we set out to test sensitivity and specificity, there is a risk of a circular argument: patients are included in the EPT group because their tremor frequency decreases upon loading, and then we investigate loading as a diagnostic test for EPT. To test the extent of this potential problem, we performed a subanalysis on the 70% of patients in whom the primary differential diagnosis was confirmed by the polymyography report, thus excluding changes in diagnosis due to the polymyography-findings. As sensitivity and specificity of the five characteristics hardly changed in this subgroup, we concluded that the diagnosis circular argument does not play a major role in our findings. Note that the final diagnosis did not rely solely on the characteristics we investigated, but also takes into account history taking, examination and imaging. Another weakness is that the clinical diagnosis may not have been correct in all cases. However, as most patients were seen by experienced movement disorders specialists, all underwent a tremor-specific polymyography, and MR- and PET-imaging were performed when indicated, we are confident that the vast majority of cases was assigned to the appropriate group.
Two final limitations that need to be noted are that patients with an inconclusive diagnosis were excluded. Finally, distractibility was investigated both informally and formally. This increases the sensitivity but may also increase bias.

A strength of this study is that characteristics were tested in a general tremor population, and not only in isolated groups such as ET vs PD. This makes it possible to relate the results to the actual clinical setting of a patient presenting with tremor. These data on tremor phenomenology will help practicing

neurologists to better distinguish between different tremor disorders.

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CHAPTER 3

CAN WE DIFFERENTIATE POSTURAL TREMOR USING INTERMUSCULAR COHERENCE AND CUMULANT ANALYSIS?

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ABSTRACT

OBJECTIVE: To investigate the potential value of two advanced EMG measures as additional diagnostic measures in the polymyographic assessment of postural upper-limb tremor.

METHODS: We investigated coherence as a measure of dependency between two EMG signals, and cumulant analysis to reveal patterns of synchronicity in EMG activity in muscle pairs. Eighty datasets were analysed retrospectively, obtained from four groups: essential tremor (ET), Parkinson's disease (PD), enhanced physiological tremor (EPT), and functional tremor (FT). We used strict diagnostic inclusion criteria combining clinical, neurophysiological and imaging information

RESULTS: Intermuscular coherence was highest in the PD group (0.58), intermediate in FT (0.43) and ET (0.40), and weakest in EPT (0.16) ($p=0.002$). EPT patients could be distinguished by low coherence: coherence<0.18 in the wrist + elbow extensors differentiates EPT in this sample with a sensitivity of 86% and specificity of 84%. Cumulant analysis showed predominantly alternating activity between wrist and elbow extensor in ET patients, while a more synchronous pattern was predominant in PD, EPT and FT ($p=0.008$). EMG activity in wrist and elbow flexors tended to be more synchronous in PD ($p=0.059$).

CONCLUSION: Our results suggest that coherence and cumulant analysis may be of additional value in the diagnostic work-up of postural tremor.

SIGNIFICANCE: These additional measures may be helpful in diagnosing difficult tremor cases.

INTRODUCTION

Although tremors are the most common movement disorders, distinguishing one type of postural tremor from another can be challenging (1). History taking and clinical examination by a movement disorders specialist are of primary importance. Additionally, a clinician can request polymyography. Unfortunately, this general work-up of choice does not always lead to a conclusive diagnosis (2).

In the outpatient clinic, accurate diagnosis can be challenged by the fact that not all patients have a classic presentation. Tremors that present as a postural tremor could mainly be essential, enhanced physiological, dystonic, parkinsonian, or functional. However, not all parkinsonian tremors start of as a typical pill-rolling rest tremor, and not all essential tremor is symmetrical, action-induced and with a slight intention component (3). Neither does every essential tremor patient have a positive family history or a positive response to alcohol (4, 5). Organic tremor patients can present with a story that seems 'functional', whereas functional tremor patients might be hard to distract from their symptoms, making their tremor appear organic.

In more difficult cases, a clinician can request polymyography to help establish a diagnosis (6). These tests are usually of great value: for instance, a prospective study by Gironell and colleagues proposed a set of six neurophysiological criteria for essential tremor with very high sensitivity and good specificity for this type of tremor (7). However, this has not been done for all types of tremor, and interpretation is not always straightforward. For example, although tremor frequency can be of help, the typical frequencies of different types of tremor overlap and as a result frequency is not always a distinguishing feature (8). Other tremor characteristics, such as frequency change at loading in enhanced physiological tremor, or entrainment in functional tremor (9) are not present in all patients and sensitivity and specificity are generally poorly known.

In the current study, we sought to add to the diagnostic power of routine polymyography, by investigating the potential additional diagnostic value of two advanced EMG measures: coherence and cumulant analysis. Coherence analysis is a method to detect a common input for the generation of two signals, and is therefore relevant for the study of relationships between the activities of tremulous muscles (10). Coherence is a normalized measure, which takes on a value of 1 in case of absolute dependence, and 0 in case of complete independence between two signals. Applied to two tremulous EMG signals, this implies that high coherence indicates a common drive from a generator mechanism. While coherence analysis provides a measure in the frequency domain, cumulant analysis is informative of the relationship between two signals over time. Applied to two tremulous EMG signals, cumulant analysis can be used to assess the timing relations between EMG bursts in pairs of muscles, in a more objective way than by visual inspection of the EMG signals.

Previous studies have investigated intermuscular coherence (11-13) or muscle activity patterns (11, 14, 15), but generally without direct comparison between commonly encountered tremor types. In this study, we compared four groups of carefully selected patients with essential tremor (ET), parkinsonian tremor (PD), enhanced physiological tremor (EPT) and functional tremor (FT). Our aim was to examine whether intermuscular coherence and cumulant analysis might be of help as additional diagnostic measures in polymyographic assessment of postural tremor.

TABLE 3.1. INCLUSION AND EXCLUSION CRITERIA.

ESSENTIAL TREMOR	PARKINSONIAN TREMOR
Bilateral postural tremor Developed before the age of 65 Present for >3 years Absence of rest tremor, or if present, frequency approx. 1.5 Hz lower than the postural tremor and without tremor latency No other neurological signs/symptoms	Unilateral or asymmetrical tremor Postural tremor + resting tremor Partial or complete suppression during movement FDOPA-PET positive
ENHANCED PHYSIOLOGICAL TREMOR	**FUNCTIONAL TREMOR**
Postural + rest and/or action tremor Positive electrophysiological report: • Frequency variability >1 Hz • High frequency (>7 Hz, not mandatory) • Effect of loading (frequency decrease >1 Hz, not mandatory)	Postural + rest and/or action tremor Positive electrophysiological report: • Frequency variability >1 Hz • Effect of mental distraction
EXCLUSION CRITERIA	
Occurrence of entrainment, in all groups except FT Decrease of tremor on mental distraction, in all groups except FT History of stroke or co-existing neurological disease	

METHODS

SUBJECTS

We analyzed 80 polymyography datasets that were obtained as part of the diagnostic work-up in patients suffering from postural tremor. The patients were equally distributed over the four tremor groups most commonly encountered in our clinic: ET, PD, EPT and FT. We used strict inclusion criteria (Table 3.1), combining clinical, neurophysiological and imaging information, to ensure that the diagnosis was as reliable as possible. Inclusion criteria for ET are in accordance with the TRIG criteria (16) and the neurophysiological criteria proposed by Gironell et al (7).

POLYMYOGRAPHY RECORDINGS

EMG was recorded with Ag/AgCl surface electrodes placed over flexor and extensor muscles in the fore- and upper arm, using palpation and maximal voluntary contractions to identify the muscles. Four muscle pairs were studied; 1) wrist flexor + extensor, 2) elbow flexor + extensor, 3) wrist + elbow extensors, 4) wrist + elbow flexors. We selected parts of the recording where patients sat with their most affected arm stretched out parallel to the ground, with the palm of the hand facing the floor and a neutral (no flexion, no extension) wrist position. Note that the included PD patients had a postural tremor in this specific position, in addition to classic tremor at rest. This group is a relevant and important addition to our study, as action tremor in PD can complicate distinction from other tremor disorders. All selected patients showed tremor bursts in the EMG of at least two of the assessed muscles during this postural task. The selected sections of the EMG signal were extracted using BrainRT software (OSG BVBA, Rumst, Belgium) and exported to ASCII format. The mean duration of the sections was 32.7 s (range: 21-57 s). As classic tremor characteristics, we documented tremor frequency (mean of maximal and minimal tremor frequency throughout the polymyography recording)

and frequency variability (maximal – minimal tremor frequency) for each patient from their clinical neurophysiology reports.

COHERENCE AND CUMULANT ANALYSIS

Data was analyzed in MATLAB R2007a (MathWorks, Natick, MA, USA), using the signal processing toolbox and NeuroSpec 2.0 (www.neurospec.org) to calculate power spectra, coherence, phase and cumulant density for each patient and each of the muscle pairs (17) (Figure 3.1 A-E). Our script is available as supplementary material online.
Sample frequency was 1 kHz. A Butterworth 10 Hz highpass filter was used to remove drift and movement artefacts. Next, the data was full-wave rectified, thereby enhancing the firing rate information of the signal (17). Segments were selected to contain 210 datapoints (1.024 sec). We did not apply smoothing or tapering.
The quality of the EMG signals was judged based on the 95% confidence interval of the individual power spectra for each muscle; signals with a dominant (first) tremor peak smaller than the confidence interval were excluded. Moreover, signals where the amplitude of the dominant tremor peak was smaller than were excluded because this was assumed to reflect poor signal-to-noise ratio. For each patient and muscle pair, we registered the presence or absence of significant coherence (as reflected in at least one coherence value above the confidence limit), and coherence values at the dominant tremor peak were used for statistical analysis.
Cumulant density plots were classified manually for each patient and muscle pair. Plots were classified as 1) a broad positive peak around zero, indicating tremor burst activity that was more in-phase and synchronous, 2) a broad negative peak around zero, indicating tremor burst activity that was more out-of-phase and alternating, and 3) a narrow central peak close to zero, indicating short-term synchronization with tremor bursts consistent with a common presynaptic drive (18-

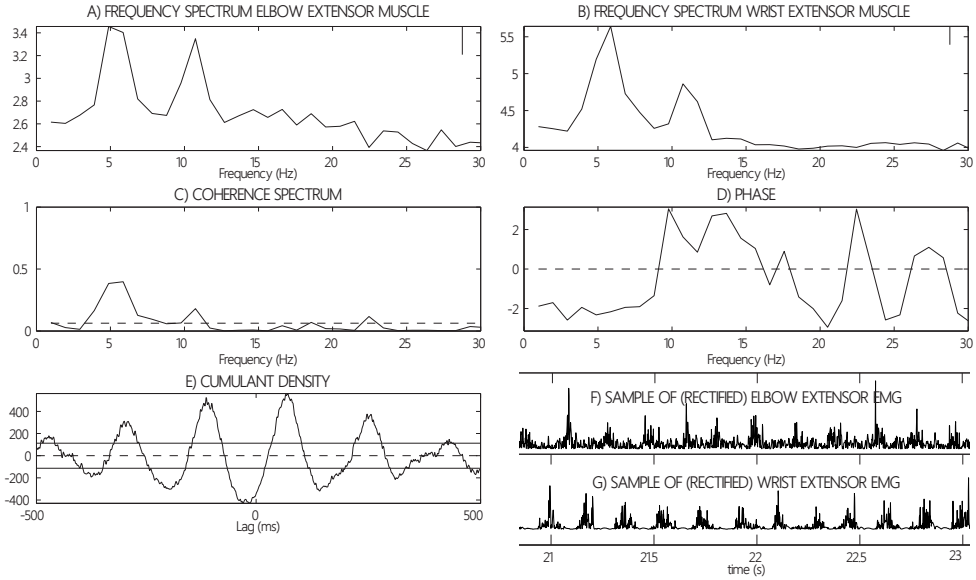

Figure 3.1. Example of output of the coherence and cumulant analysis for an ET patient. Note the appearance of two peaks in the coherence spectrum (C), at the dominant tremor frequency and its first harmonic. The cumulant (E) shows a broad negative peak around zero for muscle pair 3: wrist + elbow extensors and indicates an alternating pattern of muscle activity, as can be verified in the EMG (F and G).

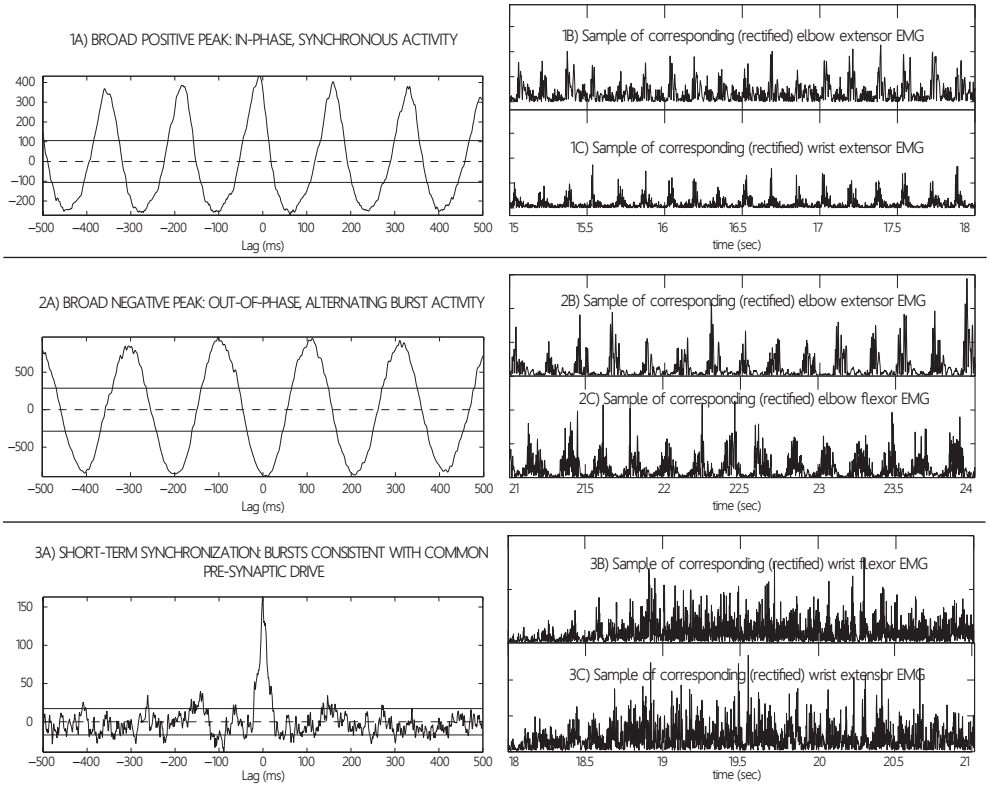

Figure 3.2. Examples of the three types of cumulant density plots; a broad positive peak around zero (1A), a broad negative peak around zero (2A), and a narrow peak around zero indicating short-term synchronization (3A), with their corresponding EMG signals (1-3B&C).

20)(see Figure 3.2 for typical examples). We chose cumulant density as a measure over phase, because it is the direct counterpart of coherence, in the time domain.

In some data sets, cumulant analysis did not reveal any significant correlation structure between EMG bursts. In addition, when data was contaminated by EMG cross-talk as identified by the combination of a sharp narrow central peak (<10 ms) in the cumulant, broadband coherence (>0.5 above 30 Hz) and flat phase (21, 22), the related data sets were excluded from further analysis.

STATISTICAL ANALYSIS

Patient characteristics including classic tremor characteristics, were compared between groups using Chi-square tests for categorical data, one-way ANOVAs for normally distributed data and Kruskal-Wallis tests for non-normally distributed data in SPSS 20 (SPSS, Chicago, IL). Normality of distributions was tested using the Shapiro-Wilk test. In case of significant effects, post-hoc testing was performed using Games-Howell-corrections because of unequal variances (post-ANOVA) or Mann-Whitney tests (post-Kruskal-Wallis), with Bonferroni correction for multiple comparisons.

The occurrence of significant coherence (in a binary fashion) was analyzed for each muscle pair and compared between groups using Fisher's exact tests in SPSS.

Actual coherence values were Fisher-Z transformed prior to statistical analysis. We analyzed intermuscular coherence by means

of a linear mixed effects model with muscle pair and tremor group as factors, instead of using a repeated-measures ANOVA, because mixed-effects models are more robust to missing data (23). Analysis was performed with the lmer and pvals.fnc functions in the lme4 library, as well as the pamer.fnc function in the LMERConvenienceFunction library for the statistical software R (www.r-project.org, version 3.0.1).
Statistical analysis of the cumulant density classifications was done by means of Fisher's exact tests.

RESULTS

PATIENT CHARACTERISTICS

No difference in gender distribution was detected between groups (Table 3.2). There was a difference in age across groups (p=0.003); ET patients were older than PD (p=0.01), EPT (p=0.008) and FT patients (p=0.005).

CLASSIC EMG TREMOR CHARACTERISTICS

Mean tremor frequency was 5.54 Hz in ET patients, 5.54 Hz in PD patients, 7.84 Hz in EPT patients and 5.42 Hz in FT patients (Table 3.2). There was a difference between patient groups (p=0.001): tremor frequency was higher in the EPT group (p=0.000). Frequency variability, defined as maximal tremor frequency – minimal tremor frequency throughout the polymyography, was on average 1.0 Hz in ET patients, 0.8 Hz in PD patients, 2.3 Hz in EPT patients and 2.1 Hz in FT patients. There was a significant difference between groups (p=0.000); post-hoc analyses revealed that frequency variability was higher in EPT and FT than in ET and PD patients (p=0.000). We found no difference in tremor amplitude on EMG (defined as mean absolute EMG of the lower extensor in μV) between groups (p=0.104).

ADVANCED EMG MEASURES: COHERENCE & CUMULANT

Overall, significant intermuscular coherence was found in 98% of PD measurements, 97% of FT measurements, 80% of ET measurements, and 72% of EPT measurements. Statistically, a significant difference between groups was found in the occurrence of significant coherence for muscle pair 3: wrist + elbow extensors. For this muscle pair, intermuscular coherence occurred in all PD patients, 94% of FT patients, 70% of ET patients, and only in 50% of EPT patients (p=0.001).
Median intermuscular coherence was 0.58 (IQR 0.37) in the PD group, 0.43 (0.34) in the FT group, 0.40 (0.32) in the ET group, and 0.16 (0.14) in the EPT group (Figure 3.3). Coherence differed between groups (p=0.002). Coherence was higher in PD patients than in ET patients (p=0.049), as well as in EPT patients (p<0.001). Moreover, coherence was higher in ET patients compared to EPT patients (p=0.041). Finally, coher-

TABLE 3.2. PATIENT CHARACTERISTICS.

	M/F	Age	Mean freq.	Freq. var.	Amplitude
ET	11/9	69 (10)*†	5.54 (0.93)	1.0 (0.7)*	33.4 (34.2)*
PD	9/10	54 (10)*	5.54 (0.70)	0.8 (0.9)*	32.3 (38.1)*
EPT	13/6	43 (36)*	7.84 (1.36)†	2.3 (2.4)*†	25.2 (47.8)*
FT	11/10	57 (18)*	5.42 (0.56)	2.1 (2.0)*†	49.1 (59.1)*

ET: essential tremor, PD: parkinsonian tremor, EPT: enhanced physiological tremor, FT: functional tremor. M/F: male/female. Mean frequency in Hz, frequency variability in Hz, amplitude in μV. Mean (standard deviation). *Median (interquartile range). †Significant difference at $p<0.05$.

ence was larger in FT patients than in EPT patients (p=0.004). To summarize: the relationship between groups for intermuscular coherence was PD > ET > EPT, FT > EPT.

As coherence was highest in PD patients, and lowest in EPT patients, we investigated cut-off values for coherence measures in these tremor types, using ROC-curves. Unfortunately, we did not find useful (i.e. with a sensitivity and specificity > 80%) cut-off values for PD. For EPT, we were able to establish two useful cut-off values. First, in the muscle pair wrist flexor + extensor, a coherence value below 0.35 distinguishes EPT from the other tremors with a sensitivity of 89% and a specificity of 80% (positive predictive value 0.53, negative predictive value 0.97, odds ratio 40). Secondly, in the muscle pair wrist + elbow extensors, a coherence value below 0.18 differentiates EPT with a sensitivity of 86% and a specificity of 84% (positive predictive value 0.60, negative predictive value 0.96, odds ratio 32).
Moreover, we directly compared ET versus EPT, as this is a clinically relevant comparison; distinction is frequently asked for in polymyography requests and can be difficult. Cut-off values were useful in the same two muscle pairs. In the muscle pair wrist flexor + extensor, a coherence value above 0.27 indicates ET with a sensitivity of 91% and a specificity of 78% (positive predictive value 0.88, negative predictive value 0.83, odds ratio 35), and in the muscle pair wrist + elbow extensors a coherence value above 0.18 differentiates ET from EPT with a sensitivity of 71% and a specificity of 86% (positive predictive value 0.71, negative predictive value 0.86, odds ratio 45).

In the cumulant analysis, results differed between groups for muscle pair 3: wrist + elbow extensors. In ET patients, the cumulant for this muscle pair showed more broad negative peaks, indicating more alternating activity. Contrarily, PD, FT and EPT showed more broad positive peaks, signifying more synchronous activity (p=0.008, test sensitivity: 91%, specificity 64%, positive predictive value 0.5, negative predictive value 0.95, odds ratio 6). Moreover, results trended towards significance in muscle pair 4: wrist + elbow flexors in all PD patients showed broad positive peaks, whereas a broad negative peak was found in about half of the other tremors (p=0.059, test sensitivity: 100%, specificity 43%, positive predictive value 0.41, negative predictive value 1.0; odds ratio incalculable). There were no differences between groups for muscles pairs 1 (p=0.379), or 2 (p=0.327).

DISCUSSION

In this study, our aim was to examine the potential value of intermuscular coherence and cumulant analysis as additional diagnostic measures in the polymyographic assessment of postural tremor.

Firstly, differences in coherence were found between patient groups. PD patients scored highest (median coherence 0.58), EPT patients lowest (0.16) and FT and ET patients scored intermediate (0.43 and 0.40 respectively). We determined cut-off values that may be diagnostically useful for the comparison of EPT versus other tremors, and EPT versus ET specifically. Moreover, absence of significant coherence was also found significantly more often in EPT patients. These features may be of additional clinical value, most of all for diagnosis of EPT.

With regard to what is known about tremor pathophysiology, our coherence results are in broad agreement with expectations. As high coherence between different muscles at the tremor frequency indicates a common drive and generator mechanism (10) we expected high coherence in PD, which we indeed found. ET is also considered to be of cen-

tral origin (11) and our results also support this. In FT, coherence has only been studied between limbs so far: coherence between two tremulous limbs seems to be a sign of functional origin as it occurs in about half of FT patients but rarely in tremor of organic origin (9, 11, 12). The strong intermuscular, intralimb coherence we found in the current study is a new finding, and points towards a highly organized common drive most likely of central origin in FT.
The strong coherence in PD, ET and FT is in contrast with the weak coherence found in EPT. EPT is regarded as a composite of 1) a peripheral mechanical reflex oscillation, which is dependent of the hand's resonant frequency and therefore changes with increased inertial loading, and 2) a centrally driven component in the 7-14 Hz range (10). Under normal circumstances, coherence does not occur as a result of mechanical reflex oscillation; this only happens under high load conditions (24). The low coherence in our EPT patients corresponds well with a relatively strong role for the peripheral mechanism in EPT, compared to other types of tremor.

Cumulant analysis, the second advanced EMG measure we investigated, resulted in typical characteristics for ET and for PD. First, EMG activity in the wrist and elbow extensors was almost exclusively predominantly alternating in ET patients, whereas a more synchronous, co-contracting pattern was predominant in other tremors. This feature of ET has not been reported previously; it is informative on the phenomenology of ET, regarding the muscular expression of this movement disorder. The high sensitivity of this measure (91%) means that the finding of a synchronous instead of an alternating pattern of muscle activity between wrist and elbow extensors in a patient decreases the likelihood of a postural tremor being ET. Secondly, a trend was found regarding the relation between muscle activity in the wrist and elbow flexors: EMG burst activity was more synchronous between these muscle groups in all PD patients, but a more alternating burst activity pattern was found in about half of the other tremors (sensitivity 100%, specificity 43%). This feature was described before in a group of PD patients that showed synchronous muscle activity patterns for the wrist and elbow flexors, and wrist and elbow extensors, as well (11). Those patients were performing a slightly different postural task, where they sat with the hand out-stretched and the arm supported by an armrest. Our findings are in accord with this previous study, and additionally the present study extends these results by comparing muscle activity patterns between tremor groups.

A limitation of this study is that the population studied was a carefully selected cohort. Diagnosis in all patients was maximally reliable. This is ideal for an initial study, but sensitivity and specificity should ultimately be tested prospectively in an unselected group of patients with postural tremor, before the definite diagnostic value of the advanced EMG measures can be determined. A second limitation is that we used EMG power as an approximation for tremor amplitude: the optimal approach would be to derive tremor amplitude from accelerometry. However, in this paper, the focus lies on EMG analysis.

For those who are interested in implementing coherence and cumulant analysis in their neurophysiology clinic the Matlab script that we used is provided as supplementary material on the journal's website. It can be used freely, together with the NeuroSpec library (open source, www.neurospec.org, (17))

In this carefully selected cohort, we investigated whether intermuscular coherence and cumulant analysis might be of help as

additional diagnostic measures in polymyographic assessment of postural tremor. We have shown that intermuscular coherence differs between tremor groups, and were able to identify EPT patients with high specificity and sensitivity by low coherence in our study population. Cumulant analysis helped to distinguish ET or PD from other tremors. We conclude that coherence and cumulant analysis may be of additional value in the diagnostic work-up of postural tremor.

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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

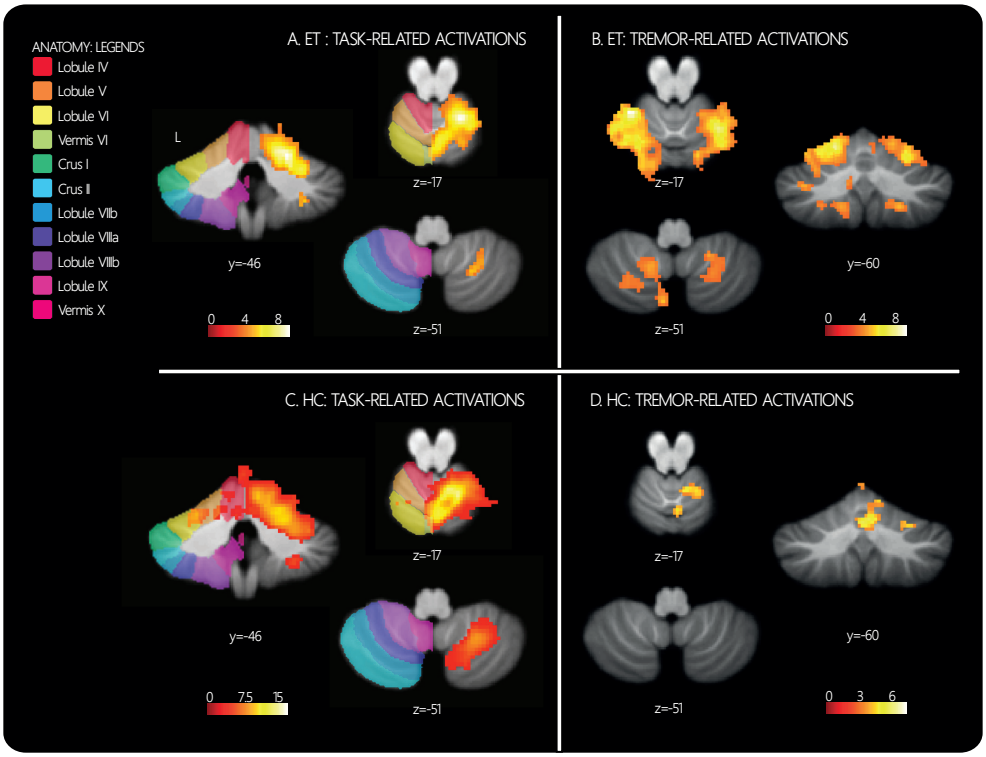

Figure 3.2. Increased cerebellar activations in essential tremor patients related to the within group comparisons for the task contrast, p<0.05 (FWE corrected, extent k = 10) (A: task-related activations), and activations related to the within group comparisons for the r-EMG contrast, p<0.001 (uncorrected, 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-

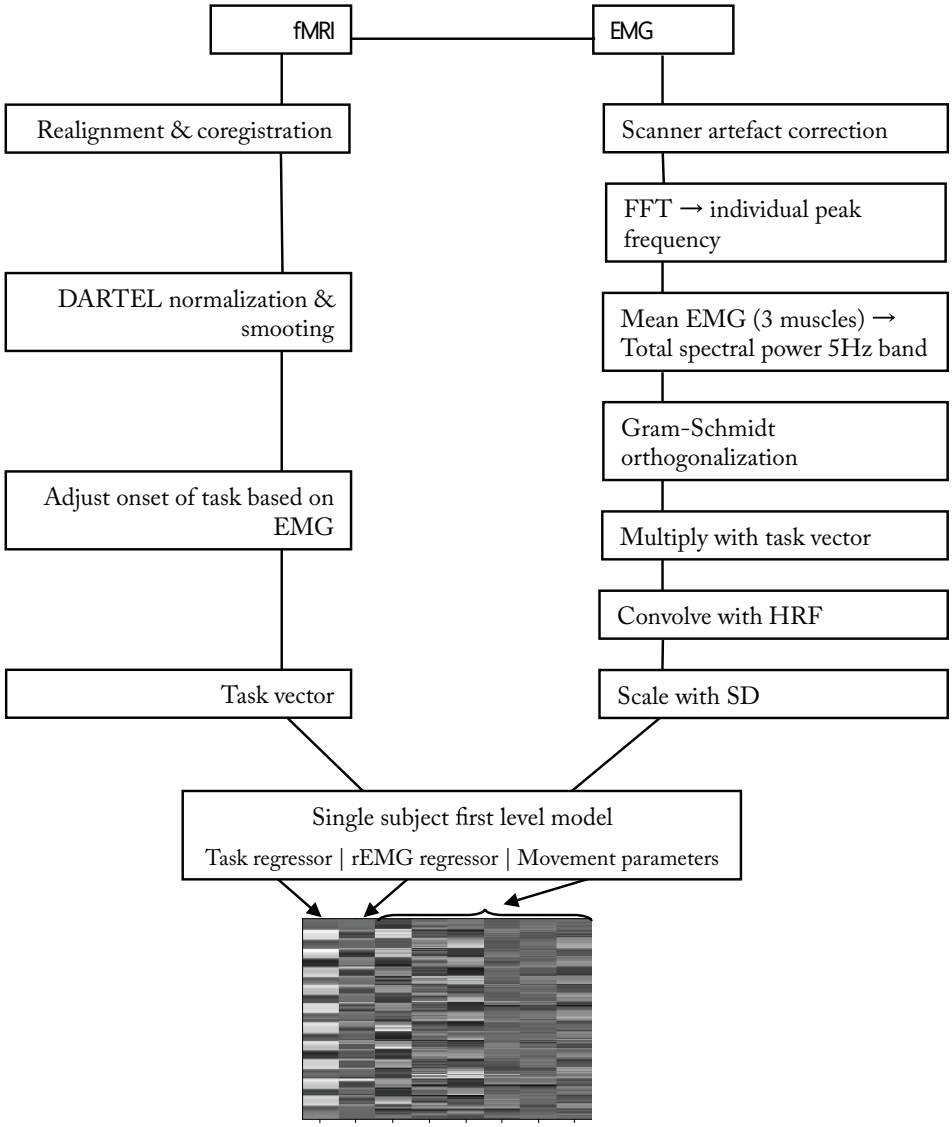

Figure S1. Flowchart of the EMG-fMRI Analysis. fMRI: functional magnetic resonance imaging; EMG: electromyography; DARTEL: Diffeomorphic Anatomical Registration using Exponentiated Lie algebra; FFT: fast Fourier transform; HRF: Haemodynamic response function; SD standard deviation.

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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CHAPTER 5

MOTOR NETWORK DISRUPTION IN ESSENTIAL TREMOR: A FUNCTIONAL AND EFFECTIVE CONNECTIVITY STUDY

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ABSTRACT

BACKGROUND: Although involvement of the cerebello-thalamo-cortical network, has often been suggested in essential tremor, the source of oscillatory activity remains largely unknown. To elucidate mechanisms of tremor generation, it is of crucial importance to study the dynamics within the cerebello-thalamo-cortical network. Using a combination of electromyography and functional Magnetic Resonance Imaging, it is possible to record the peripheral manifestation of tremor simultaneously with brain activity related to tremor generation. Our first aim is to study the intrinsic activity of regions within the cerebello-thalamo-cortical network using Dynamic Causal Modelling to estimate effective connectivity driven by the concurrently recorded tremor signal. Our second aim is to objectify how the functional integrity of the cerebello-thalamo-cortical network is affected in essential tremor.

METHODS: We investigated the functional connectivity between cerebellar and cortical motor regions showing activations during a motor task. Twenty-two essential tremor patients and 22 healthy controls were analysed. For the effective connectivity analysis, a network of tremor-signal related regions was constructed, consisting of the left primary motor cortex, premotor cortex, supplementary motor area, left thalamus, and right cerebellar motor regions lobule V and lobule VIII. A measure of variation in tremor severity over time, derived from the electromyogram, was included as modulatory input on intrinsic connections and on the extrinsic cerebello-thalamic connections, giving a total of 128 models. Bayesian Model Selection and Random effects Bayesian Model Averaging were used. Separate seed-based functional connectivity analyses for the left primary motor cortex, left supplementary motor area and right cerebellar lobules IV, V, VI and VIII were performed.

RESULTS: We report two novel findings that support an important role for the cerebellar system in the pathophysiology of essential tremor. First, in the effective connectivity analysis, tremor variation during the motor task has an excitatory effect on both the extrinsic connection from cerebellar lobule V to the thalamus, and the intrinsic activity of cerebellar lobule V and thalamus. Second, the functional integrity of the motor network is affected in essential tremor, with a decrease in functional connectivity between cortical and cerebellar motor regions. This decrease in functional connectivity, related to the motor task, correlates with an increase in clinical tremor severity. Interestingly, increased functional connectivity between right cerebellar lobules I-IV and the left thalamus correlates with an increase in clinical tremor severity.

CONCLUSION: Our findings suggest that cerebello-dentato-thalamic activity and cerebello-cortical connectivity is disturbed in essential tremor, supporting previous evidence of functional cerebellar changes in essential tremor.

INTRODUCTION

Essential tremor is one of the most common neurological disorders, and is characterised by a progressive postural and kinetic tremor (1). Evidence of alleviation of tremor following thalamic deep brain stimulation, and after stroke anywhere in the cerebello-thalamo-cortical network, prompted the hypothesis of essential tremor as an 'oscillating network' disorder (2, 3). Evidence is accumulating that the cerebellum plays an important role in the pathophysiology of essential tremor (4-6). An important supportive feature is the positive effect of alcohol on essential tremor (7). Furthermore, emerging clinical features such as ataxic gait (8, 9, 10), eye movement abnormalities (11-13) and intention tremor (14, 15) all point to cerebellar changes in essential tremor. Whether these abnormalities relate to structural or functional cerebellar changes is under debate. Pathology studies show an incongruent picture, but provide evidence for neurodegeneration of the cerebellum (16). There is evidence for morphometric changes and possibly loss of Purkinje cells (17-20). Moreover, changes in the dentate nucleus have been established, with decreased numbers of GABA receptors reported in essential tremor cases (21). On the other hand, imaging studies show a striking lack of convincing structural involvement, but do provide evidence for functional abnormalities of the cerebellum (see (22) for a review).

Although the notional involvement of the cerebello-thalamo-cortical network, and of the cerebellum in particular, is becoming increasingly evident, the source of oscillatory activity in essential tremor remains largely unknown (23, 24). To elucidate the mechanisms of tremor generation it is of crucial importance to study network dynamics within the cerebello-thalamo-cortical network. Using a combination of EMG and functional MRI (EMG-fMRI), we can record the peripheral manifestation of tremor simultaneously with brain activity related to tremor generation. Previous studies by our group and others have proven that EMG-fMRI allows identification of brain areas involved in the generation of tremor (25-28). In a recent EMG-fMRI study, we have demonstrated tremor-related increases in activations in specific somatomotor regions of the bilateral cerebellum in essential tremor, as described in Chapter 4. In the current, complementary study, we study effective and functional connectivity within the tremor network, incorporating information from the concurrently recorded EMG signals to provide better insight into changes within the cerebello-thalamo-cortical network in essential tremor. While functional connectivity describes simple correlations between spatially segregated neuronal events, effective connectivity tries to estimate the underlying, direct, causal connections, which is of crucial importance in the investigation of the underlying biological network (29).

Our first aim is to study intrinsic activity of regions within the cerebello-thalamo-cortical network by using an effective connectivity analysis called Dynamic Causal Modelling (DCM). DCM explores how observed brain activations are generated by estimating the effective connectivity between and within specified regions of interest (30). For instance, DCM has been shown to be able to identify the correct neural driver behind epileptic seizures by including the occurrence of spike-and-wave-discharges obtained from concurrently recorded EEG signals into the model (31). We hypothesise that internal cerebellar feedback is altered in essential tremor. The cerebellum is thought to have multiple somatotopic representations (32). However, until now these have not been studied nor discussed separately in essential tremor. Hence, we will look specifically at intrinsic feedback changes within the anterior motor regions, composed of cerebellar lobules I to V, and posterior motor regions,

mainly composed of cerebellar lobule VIII, of the cerebellum (32).
Our second aim is to objectify how the functional integrity of the cerebello-thalamocortical network is affected by any cerebellar changes in essential tremor, by means of a functional connectivity analysis, investigating the functional connections between cerebellar and cortical motor regions using a seed-based correlation approach (32, 26). As suggested in a previous study, due to altered cerebellar functioning, we expect to find consequential alterations to functional connectivity between cerebellar and cortical motor regions in essential tremor (33).
Advancing insights strongly suggest that essential tremor patients form a widely heterogeneous group, possibly giving rise to conflicting results between essential tremor studies (34). In this study, we have defined a homogeneous group of essential tremor patients, with a clear diagnosis according to the criteria defined by the Tremor Investigation Group (35) and a positive effect of propranolol, a drug with level A evidence for treatment of essential tremor (36).

METHODS

PARTICIPANTS

In total, forty patients and twenty-two healthy controls were included. This study was conducted in two academic hospitals in the Netherlands: the Academic Medical Center in Amsterdam and the University Medical Center Groningen. Patients with a definite diagnosis of essential tremor according to criteria defined by the Tremor Investigation Group were selected if they fulfilled the following criteria (35): bilateral upper limb tremor, an age at onset <65 years, and a disease duration >5 years. Furthermore, patients had to be right handed and report a positive effect of propranolol on the tremor. Healthy controls, matched for age, gender and handedness, were selected. Exclusion criteria were: a score <26 on the Mini Mental State Examination, neurological disorders (for patients: other than essential tremor), age < 18 years, the use of medication affecting the central nervous system and MR-related contra-indications. Tremor severity was assessed off medication by an experienced movement disorders neurologist (JDS) using the Fahn-Tolosa-Marin Tremor Rating Scale (TRS) parts A and B (37). Medication was discontinued at least 3 days before the study. Item A on the TRS represents tremor severity of the arms in rest, posture and during action. Item B represents clinical assessment of tremor severity during tremor-inducing task performance. Finally, tremor severity was assessed using a visual analogue scale (VAS). The study was approved by the local medical ethical committees and conducted according to the Declaration of Helsinki (Seoul, 2008). All participants gave written informed consent.

FUNCTIONAL MRI TASK

An fMRI scan was performed, while EMG was recorded simultaneously, off-medication. Participants executed a motor task in which they were instructed to alternate 21 periods of 30 seconds rest with 20 periods of 30 seconds performing the task. Before scanning, subjects were first carefully instructed about the motor task and then practised it outside the scanner to ascertain correct task performance. ET patients performed right hand and arm extension, the aim being to induce action tremor. Healthy controls were instructed to mimic a tremor during all task blocks by extending the right arm and performing self-paced wrist flexion-extension. Since essential tremor is known to aggravate during mental tasks, an additional silent reading task was presented during half of all action blocks, with the aim to evoke more variation in tremor amplitude (38). During the other half of action blocks, a visual task

instruction "stretch out your arm" was presented during scanning, which elicited tremor as well. All instructions were presented using slides projected onto a screen located outside the scanner bore and visible by way of a mirror. Correct task performance was assessed by visual inspection during scanning.

DATA ACQUISITION AND PRE-PROCESSING

For full details of fMRI and EMG acquisition and pre-processing see supplementary material. Images were acquired using a Philips 3T Magnetic Resonance (MR) scanner at both sites. 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. EMG was recorded simultaneously (BrainProducts GmbH, Munich, Germany (UMCG) and MicroMed, Italy (AMC)) from five right arm muscles. EMG data were corrected for MR artefacts using the MR-artefact correction algorithms (Imaging Artefact Reduction method (39); UMCG data) embedded in the BrainVision Analyzer software (BrainProducts GmbH, Munich, Germany) and FARM (fMRI artefact reduction for motion (40); AMC data). FMRI data was analysed using SPM12 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK; http://www.fil.ion.ucl.ac.uk/spm, v6225, DCM version 12), and included standard pre-processing (supplementary material). Inspection of the EMG was used to correct the block design regressor for actual on- and offsets of the motor task. For each subject, scan-by-scan EMG power was calculated in a 5-Hz band around the peak tremor frequency. Finally, this EMG 'tremor' vector was orthogonalised with respect to the block regressor, scaled to the maximum value per subject to ensure that the variance was similar between subjects, convolved with the canonical hemodynamic response function and used as a regressor (residual-EMG) in the General Linear Model (27). As motion-related and other non-neuronal signal changes are effectively reduced by global signal regression, tissue-based signals and their first derivative were also used as nuisance regressors and were calculated as the average signal across all voxels within the whole-brain mask (41). Each single-subject first-level model thus consisted of two block regressors for the motor task, a residual-EMG regressor, six movement regressors and two global signal regressors. For the functional connectivity analysis, the residual-EMG regressor was excluded from the first-level models since the objective of this analysis was to primarily look at the integrity of the motor network without concurrently assessing tremor severity. Brain activations during motor task execution and tremor-related (EMG-based) activations are reported in more detail in Chapter 4. In short, motor task-related activations were found in the well-known upper-limb motor network, i.e. both for essential tremor patients and healthy controls in motor, premotor and supplementary motor areas. In essential tremor patients, we found tremor-related (EMG-based) activations in the left primary motor cortex, supplementary motor area, premotor cortex and thalamus, and bilaterally in the cerebellum: in left lobules VI and V, and in right lobules V, VI, and VIII, and in the brainstem. Ipsilateral cerebellar activity was related to mimicked-tremor in healthy participants. Tremor based activations are used in the effective connectivity analysis; motor-task-based activations are used in the functional connectivity analysis. Finally, the amount of head movement during scanning was estimated by calculating the summed Euclidean distance between the first and last scan per individual subject for translation (i.e. x, y and z direction) and rotation (i.e. pitch, roll, yaw) separately, and compared between patients and healthy controls using two-sample two-tailed t-tests (26).

EFFECTIVE CONNECTIVITY - DYNAMIC CAUSAL MODELLING

DCM models how neural activity within a network of brain regions is driven by external perturbations that result from experimentally controlled manipulations (30). These perturbations are described by means of external inputs u that can enter the model in one of two ways (30). First, they can elicit responses through direct influences on specific regions and can be described as "driving" inputs or "stimulus-bound perturbations". An example would be the command to stretch out your arm. Second, they can change the strength of coupling among or within regions, and can be described as "modulatory" inputs or "contextual perturbations". For example fluctuations in tremor severity over time could change the intrinsic activity within regions of the cerebello-thalamo-cortical network. An important concept in DCM is that regions contain self-inhibitory properties, mediated by self-connections ("intrinsic" or within-region connections), preventing runaway outbursts of neural activity. The left primary motor cortex (M1), left premotor cortex (PMC), left supplementary motor area (SMA), left ventral lateral nucleus of the thalamus, right cerebellar lobule V/VI and right cerebellar lobule VIII were included in our models since these regions have been associated with tremor previously using fMRI (42, 33) and showed tremor-related (EMG-based) activations in the patient group, as mentioned previously (Chapter 4). Regions were defined for each patient individually, based on activations associated with the residual-EMG regressor, and centred at the location of the local maxima with a 4 mm radius, within 10 mm of the group maximum (MNI coordinates: M1 x -36 y -22 z 61; PMC x -28 y -22 z 54; SMA x -2 y -14 z 55; thalamus x -12 y -24 z -1 ;cerebellar lobule V/VI x 34 y -50 z -25; cerebellar lobule VIII x 21 y -52 z -56). We assumed full endogenous connectivity between regions, with the exemption of connections between cerebellar regions and the thalamus (only unidirectional from cerebellum to thalamus) and between cortical and cerebellar regions (only unidirectional from cortical to cerebellar regions) based on neuronal tracing studies in macaque monkeys (Fig. 1) (43), leaving 28 endogenous connections. We furthermore assumed a direct effect of the motor task on the activity of all premotor regions (left SMA, left PMC) (44, 45). The task regressor was divided into two separate regressors to compare the direct effects of the motor task and the motor plus silent reading task to each other.

The residual EMG regressor, which represents variations in tremor amplitude over time, was included as a modulatory input on the intrinsic connections of all regions (Fig. 2A). In this manner, the residual EMG regressor functions as a modifier of the 'state' a region is in depending on the intensity of tremor. Since the dentate nucleus is an important region within the tremor network, but not included as a node in our network, additional interest was focused on the cerebello-thalamic connections. These connections represent the net effect of the cerebello-dentatal output onto the thalamus. Therefore, modulatory input of tremor onto the cerebello-thalamic connections was added to the model space (Fig. 1). This gave a total of 27 = 128 models. Figure 1 gives an overview of the DCM framework for this study; a list of models and their modulatory inputs is provided in the supplementary material. Models were compared using Bayesian model selection (BMS) on group level (46-47). Subsequently, a post hoc BMS family analysis was used to evaluate the exceedance probabilities of a modulatory effect on each region or connection. The exceedance probability (Φ) corresponds to the belief that a model or family is more likely than any other, given the data from all subjects (46).

We then used random effects Bayesian mod-

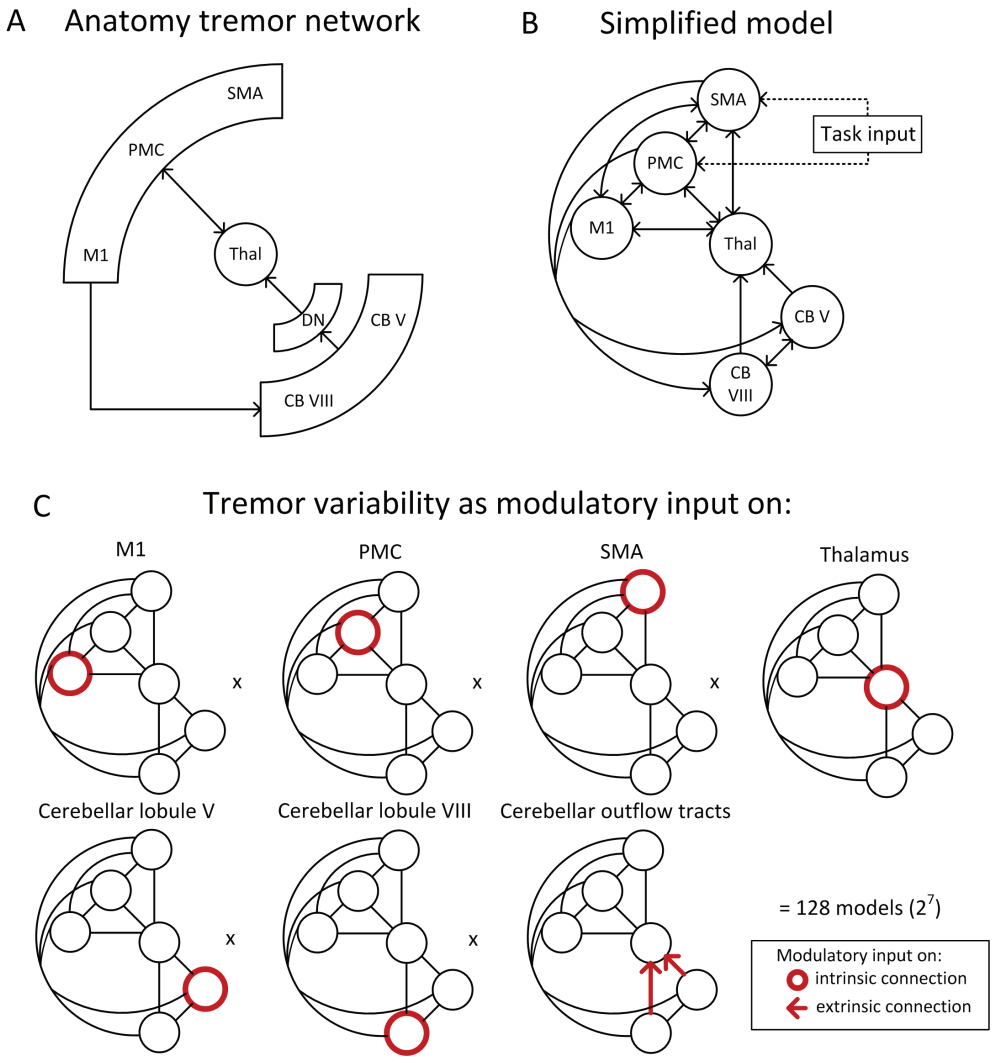

Figure 5.1. Overview of the model space A. Anatomy of the tremor network as derived from (Helmich et al., 2013). B. Simplified model derived from the anatomical tremor network to be used for the DCM analysis. Task input (the command to stretch out the arm) entered the model on SMA and PMC. C. The residual EMG regressor, or 'tremor variability', entered the model as modulatory input, affecting the intrinsic connections within regions and affecting the extrinsic cerebello-thalamic connections. 128 models in total were set up. M1 = left primary motor cortex; SMA = left supplementary motor area; PMC = left premotor cortex; CB lob V = right cerebellar lobule V; CB lob VIII = right cerebellar lobule VIII.

el averaging (BMA) on the winning halve of model space, in which parameter estimates are weighted by the model evidence to compare resulting coupling parameters (47,48). This method is convenient when many models are compared and when there is no obvious winning model. The posterior densities of the parameters are calculated across subjects and across the winning halve of models. More weight is given to the models with the highest posterior probability according to Bayes' rule (46). The resulting coupling parameters represent connection strengths (30). The posterior distributions are calcu-

TABLE 5.1. PATIENTS' CHARACTERISTICS.

	Age	Gender	Tremor frequency (Hz)	TRS A+B	Duration (years)	Family history	Propranolol use (mg)	VAS-score off medication	Alcohol sensitivity
1	21	Male	10	8	11	+	40	5.4	+
2	22	Male	7	6	10	-	20	5.2	+
3	27	Male	7.5	16	27	-	160	8.7	+
4	30	Female	8	7	15	+	20	2.9	?
5	35	Male	8	11	28	+	80	7.8	?
6	46	Male	7.5	10	41	+	80	4.4	+
7	47	Male	7	10	32	+	40	6.0	+
8	48	Female	7	27	38	+	120	5.4	-
9	53	Female	7.5	22	25	+	30	7.8	+
10	62	Female	8.5	5	57	+	100	8.5	?
11	63	Male	7	11	20	+	40	3.4	+
12	63	Female	7.5	21	24	+	80	7.4	+
13	64	Male	6.5	7	52	+	20	4.0	+
14	65	Female	7.5	4	5	+	80	2.7	?
15	69	Male	7.5	8	29	+	40	9.2	-
16	73	Female	5	21	55	+	80	2.6	+
17	74	Male	9	23	24	-	80	6.6	?
18	80	Female	6	29	20	+	80	6.9	+
Excluded from the *effective* connectivity analysis:									
19	32	Female	7	10	29	+	40	6.0	?
20	53	Male	8	15	37	+	50	8.6	+
21	57	Female	7	17	40	+	10	4.0	?
22	72	Male	6	31	62	+	320	9.2	+
Median (range)	59.5 (21-80)	M: 12 F: 10	7.5 (5-10)	11 (4-31)	30.5 (5-62)	+: 19 -: 3	65 (20-320)	6.0 (2.6 – 9.2)	+: 13 -: 2 ?: 7

Four patients were excluded for the *effective* connectivity analysis due to absent significant tremor-related activations at an uncorrected threshold of $p < 0.001$. VAS: Visual Analogue Scale, range 0-10. TRS: tremor rating scale (off medication). TRS A + B scores were assessed while off medication. + = positive; - = negative; ? = unknown.

lated using a Gibbs sampling approach by drawing samples from a multinomial distribution of posterior beliefs for the included models (46). Subsequently, posterior means and standard deviations of parameters were obtained and tested for significance using one-sample two-tailed t-tests. Because we tested forty parameters of interest (28 endogenous, 8 modulatory and 4 task inputs) we have adjusted the significance threshold

using the Bonferroni method (α = 1-(1-α)1/40) = 0.001282). Positive coupling parameters suggest a facilitation of neural activity, whereas negative coupling parameters can be interpreted as inhibition of neural activity. Coupling parameters are reported in Hz, reflecting the amount of activity that 'flows' from one region to another per second. For the effective connectivity analysis, we chose to include only essential tremor patients and not to include a group comparison as the two "tasks" performed by both groups (mimicking tremor vs. real tremor) are qualitatively different.

FUNCTIONAL CONNECTIVITY – SEED BASED CORRELATION ANALYSIS

To assess the functional integrity of the motor network in essential tremor, we performed separate seed-based functional connectivity analyses between six areas showing the strongest response relating to the motor task in essential tremor patients and healthy controls: left M1, left SMA and right cerebellar hemisphere lobules IV, V, VI and VIII (supplementary material). We chose to look at activations related to the motor task because this allowed us to compare essential tremor patients to healthy controls, and because functional coupling between cerebellar and cortical motor regions is most specific during motor tasks (42). Time-courses of all regions were obtained by extracting the first eigenvariates with SPM12, adjusted for effects of interest, for significant voxels using a threshold of P < 0.001 (uncorrected) (32, 29, 26). Regions were defined for each subject, individually centred at the location of the local maxima with a 4 mm radius, within 10 mm of the group maximum (MNI coordinates: M1 x -28, y -28, z 53; SMA x -2, y -8, z 57; cerebellar lobule I-IV x 4 y -64 z -21, cerebellar lobule V x 14 y -50 z -19, cerebellar lobule VI x 22 y -50 z -25, cerebellar lobule VIII x 24 y -58 z -49). For each subject and each region, we then entered this time-course as a regressor in a multiple regression analysis together with the task regressor and nuisance regressors. The task regressor was added to exclude activations related to the motor task. For the second-level between group comparisons, nonparametric permutation tests were performed; this is preferred over parametric methods as this does not require that the data is normally distributed (49)(Statistical non-Parametric Mapping 13b, http://www.sph.umich.edu/ni-stat/SnPM/ (50), 10,000 permutations). Contrasts were built to test (i) for significant between group differences in functional connectivity, and (ii) for significant correlations of functional connectivity within the patient group with clinically assessed tremor severity (TRS A+B), subjectively assessed tremor severity (VAS) and disease duration. Correlations between objective (i.e. TRS A+B) and subjective (i.e. VAS) measures of tremor severity are known to be limited (51; Chapter 7). We expect TRS A+B to give the best representation of tremor amplitude, whereas VAS scores entail several entities such as tremor severity, psychological and social factors (51; Chapter 7). A cluster-wise inference was used (P < 0.05 (FWE corrected), cluster-forming threshold P < 0.001). To test specifically for changes in cerebellar-cortical correlations, seed-based correlations were masked with either the whole cerebellum (for the M1 and SMA seed) (52) or a cerebral motor mask including left M1, left PMC, left SMA and left thalamus (for the cerebellar seeds) (52). The probabilistic atlas of the cerebellar cortex and the AAL toolbox were used to define anatomical locations of activations (52, 53).

RESULTS

PARTICIPANTS

Eighteen patients and one healthy control were excluded for further analysis. Reasons

TABLE 5.2. LOCAL MAXIMA OF GROUP DIFFERENCES IN CEREBELLO-CORTICAL FUNCTIONAL CONNECTIVITY

REGION	VOXELS (K)	R/L	T-VALUE	X	Y	Z
Controls > ET - left M1 seed						
Cerebellar lobule V	86	R	4.68	14	-50	-13
Cerebellar lobule V	sc	R	3.95	22	-46	-23
Cerebellar lobules VI	sc	R	3.93	18	-54	-21
Cerebellar lobules VI	43	R	4.36	30	-64	-27
Controls > ET - left SMA seed						
Cerebellar vermis VI	29	R	4.49	2	-62	-27
Cerebellar lobules VI	128	R	4.41	24	-50	-25
Cerebellar lobule V	sc	R	4.24	16	-46	-25
Cerebellar lobule V	102	R	4.40	12	-48	-13
Cerebellar lobule V	sc	R	4.03	12	-60	-13
Cerebellar lobules VI	38	L	4.35	-12	-74	-25
Controls > ET - right cerebellar lobule IV seed						
Primary motor cortex	85	L	6.46	-32	-22	49
Supplementary motor area	24	L	4.33	-2	2	59
Controls > ET - right cerebellar lobule V seed						
Supplementary motor area	26	L	4.89	0	4	61
Primary motor cortex	34	L	4.68	-32	22	45
Controls > ET - right cerebellar lobule VI seed						
Primary motor cortex	49	L	5.59	-34	-24	49
Supplementary motor area	72	L	4.92	-2	2	61
Supplementary motor area	sc	L	3.81	0	4	51
Primary motor cortex	29	L	4.58	-44	-4	49
Controls > ET - right cerebellar lobule VIII seed						
Supplementary motor area	27	L	4.02	-2	2	59
Supplementary motor area	sc	L	3.68	0	10	55
Primary motor cortex	23	L	3.80	-50	-5	51
Primary motor cortex		L	3.67	-44	-2	53

Stereotactic coordinates of local maxima of cerebello-cortical functional connectivity in essential tremor patients compared to controls ($p > 0.05$, FWE corrected, cluster-defining threshold of $p < 0.001$), coordinates in MNI space. ET: essential tremor, SMA: supplementary motor cortex, sc: same cluster.

for exclusion of datasets were sudden excessive head movements during scanning causing striping artefacts (one patient, one healthy control), insufficient tremor during fMRI data collection (16 patients) or failure of equipment during scanning (one patient).

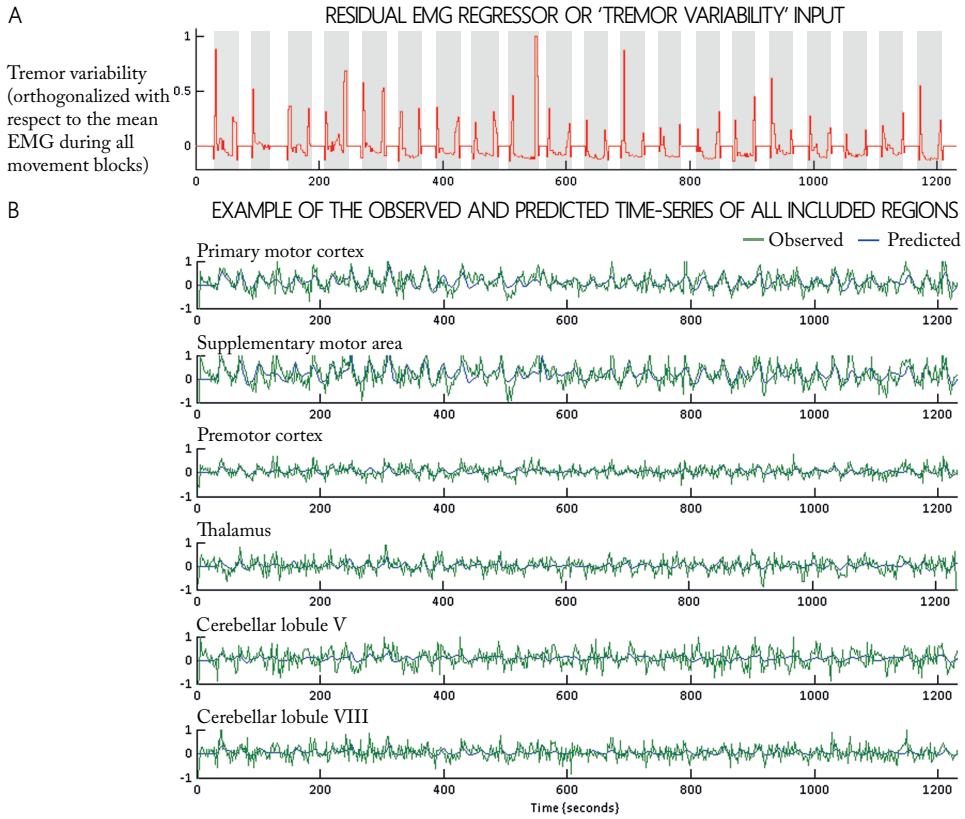

Figure 5.2. Example of the included residual EMG regressor and observed and predicted BOLD time-courses based on DCM. (A) Scaled residual EMG regressor or 'tremor variability' input displayed as a function of time, representing changes in EMG power over scans of one subject. Grey bars represent the motor task during which subjects had to stretch out their arm. (B) Example of model fit of the same subject; observed and predicted BOLD time-courses of all regions included in the model based on the DCM estimation. Green = observed, blue = predicted.

Healthy controls (14 male) had a median age of 56.5 years (range 20-72). For the effective connectivity analysis, four additional patients were excluded because they did not show significant tremor-related activations at an uncorrected threshold of $p < 0.001$, a prerequisite for the DCM analysis, thus 18 patients were included in the effective connectivity analysis. See table 1 for a full overview of included essential tremor patients. Included patients and healthy controls exhibited similar amounts of head movement during scanning (mean translation parameters; patients: 2.64 mm (SD 1.36), healthy controls: mean translation parameters 2.68 mm (SD 0.97), $t[42] = 0.2720$, $p = 0.92$ & mean rotation parameters patients 0.056 degrees (SD 0.03), healthy controls 0.052 degrees (SD 0.03), $t[42] = 0.43$, $p = 0.67$).

EFFECTIVE CONNECTIVITY - BAYESIAN MODEL SELECTION

Figure 2B gives an example of observed and predicted BOLD time-courses of one subject, based on the DCM estimation. Model 124 showed the highest posterior exceedance probability ($\Phi = 0.0128$), but is closely followed by several other models. Based on the

TABLE 5.3. LOCAL MAXIMA OF CEREBELLO-CORTICAL FUNCTIONAL CONNECTIVITY CORRELATED WITH TREMOR SEVERITY

REGION	VOXELS (K)	R/L	T-VALUE	X	Y	Z
ET correlated *negatively* with TRS A+B - left M1 seed						
Cerebellar lobule crus II	645	L	5.98	-28	-78	-51
Cerebellar lobule VIIb	sc	L	5.29	-6	-74	-39
Cerebellar lobule crus II	sc	R	5.09	4	-80	-35
Cerebellar vermis VI	38	M	4.51	0	-70	-23
Cerebellar lobule VI	sc	R	3.66	10	-72	-29
Cerebellar lobule VI	34	R	4.38	18	-56	-27
ET correlated *negatively* with TRS A+B - right cerebellar lobule VIII seed						
Primary motor cortex	41	L	5.37	-6	-22	73
Primary motor cortex	sc	L	4.66	-4	-14	71
ET correlated *positively* with TRS A+B - right cerebellar vermis seed						
Thalamus	23	L	5.40	-10	-20	11
Thalamus	sc	L	3.78	-10	-28	9

Stereotactic coordinates of local maxima of cerebello-cortical functional connectivity in essential tremor patients correlated with tremor severity ($p > 0.05$, FWE corrected, cluster-defining threshold of $p < 0.001$), coordinates in MNI space. M1: primary motor cortex, ET: essential tremor, TRS: tremor rating scale, sc: same cluster.

BMS there was no obvious winning model (Fig. 3A). The post-hoc family analysis, where models are grouped by the presence of modulatory effects on the six tremor regions and cerebello-dentato-thalamic pathway, showed quite convincingly that modulatory input on the cerebello-thalamic connections ($\Phi > 99$) was more likely than no input on the cerebello-thalamic connections (Fig. 3B). The thalamus ($\Phi = 0.74$), cerebellar lobule V ($\Phi = 0.71$), SMA ($\Phi = 0.74$) and PMC ($\Phi = 0.63$) were also more likely to be modulated by tremor variation (Fig. 3B). The primary motor cortex ($\Phi = 0.52$) and cerebellar lobule VIII ($\Phi = 0.45$) showed no clear preference for models with or without modulatory input of tremor variation.

EFFECTIVE CONNECTIVITY - BAYESIAN MODEL AVERAGING

Modulatory inputs on the six intrinsic and two extrinsic, cerebello-dentato-thalamic, connections were extracted. Modulatory input of tremor variation exhibited a significant excitatory influence on the intrinsic thalamic (mean 1.26, SD 0.42, $p < 0.0000$) and cerebellar lobule V (mean 0.32, SD 0.32, $p = 0.0006$) connections, and on the extrinsic connection from cerebellar lobule V to the thalamus (mean 0.82, SD 0.89, $p = 0.00128$). Modulatory input of tremor variation exhibited a significant inhibitory influence on M1 (mean -0.30, SD 0.25, $p < 0.0000$), SMA (mean -0.91, SD 0.27, $p < 0.0000$), PMC (mean -0.55, SD 0.29, $p < 0.0000$) and cerebellar lobule VIII (mean -0.28, SD 0.27, $p = 0.0003$). Results are summarized in Figure 3. There was a significant driving force of task on SMA and PMC (see supplementary material for full details of coupling parameters). Furthermore, there was a difference in driving force on the SMA between the motor

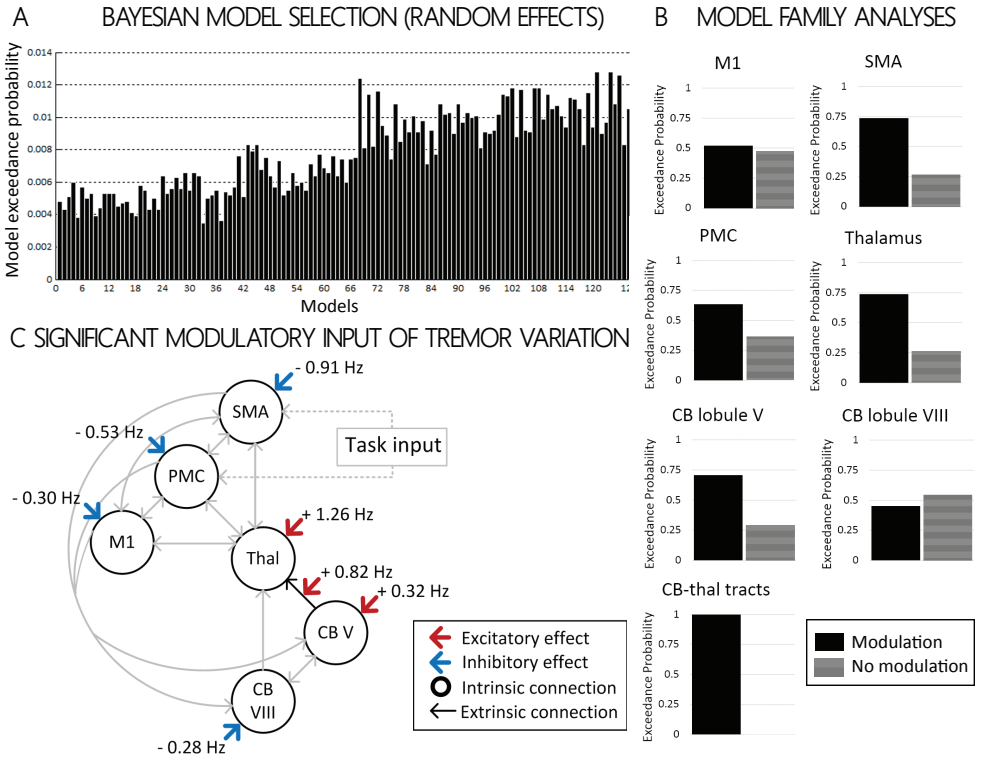

Figure 5.3. Results of Bayesian model selection and Bayesian model averaging in essential tremor patients. (A) Exceedance probabilities of all 128 models. Models 1-64 have no modulatory input on the cerebello-thalamic connections, models 65-128 have modulatory input on the cerebello-thalamic connections. (B) Post hoc family analysis identified a preference for models with a modulatory effect on the cerebellar-thalamic connections (Φ > 99%) (C) Graphical representation of the significant estimated connectivity parameters resulting from Bayesian Model Averaging in essential tremor. For clarity reasons only modulatory influences are depicted. Coupling parameter strength is depicted in red (excitatory effect) and blue (inhibitory effect). Significant modulatory input is depicted in Hz. M1 = left primary motor cortex; SMA = left supplementary motor area; PMC = left premotor cortex; Thal = left thalamus; CB lob V = right cerebellar lobule V; CB lob VIII = right cerebellar lobule VIII. For full coupling parameter details see supplementary material.

task with reading versus without reading ($t[34] = 10.79, p < 0.0000$). There was no difference in driving force between tasks on the PMC ($t[34] = 0.13, p = 0.39$).

FUNCTIONAL CONNECTIVITY RESULTS IN ESSENTIAL TREMOR AND HEALTHY CONTROLS

In essential tremor patients, the M1 and SMA seeds showed reduced functional connectivity with right cerebellar lobules V and VI compared to healthy controls (Fig. 4A, Table 2). Right cerebellar lobules I-IV, V, VI and VIII seeds all showed reduced functional connectivity with M1 and SMA compared to healthy controls (Table 2).

For the M1 seed, functional connectivity with right cerebellar lobules VI, crus II, vermis VI and lobule VIII, and left cerebellar lobule VIIb, crus II and lobule VIII, correlated negatively with tremor severity (TRS A+B). For the cerebellar lobule VIII seed, functional connectivity with the primary motor cortex correlated negatively with tremor severity (TRS A+B) (Fig. 4B, Table 3). M1 and cerebellar lobule VIII thus show a reciprocally

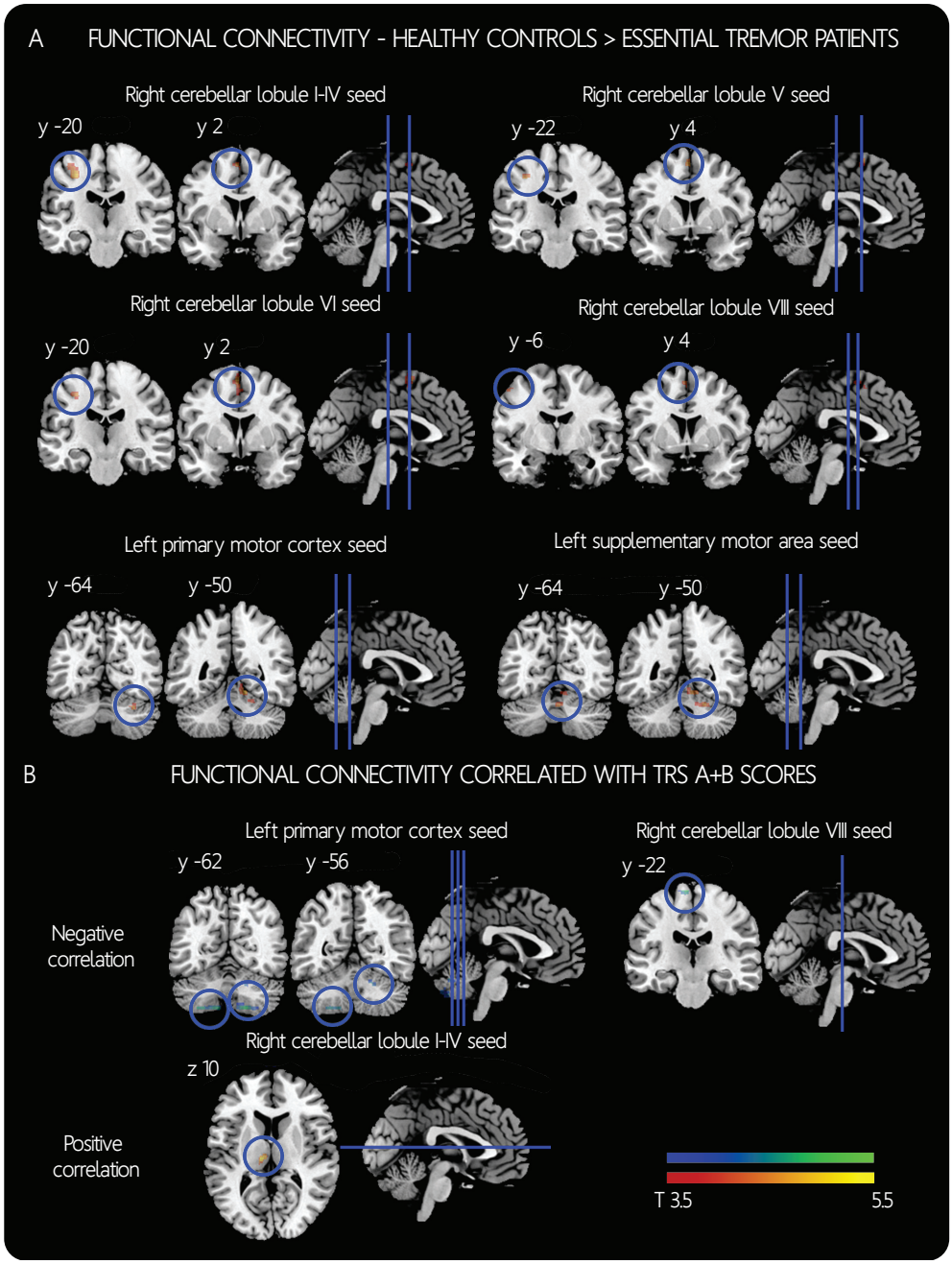

Figure 5.4. Decreased cerebellar-cortical functional connectivity in essential tremor. (A) between group differences illustrating areas of decreased connectivity in essential tremor patients compared to healthy controls for the M1, SMA, cerebellar lobule I-IV, V, VI and VIII seeds. (B) Correlation between connectivity and TRS A+B scores for the M1, cerebellar lobule I-IV and VIII seed. Results are projected on the ch2better-template using MRIcroN. Cluster-wise inference is used ($p < 0.05$ FWE corrected, cluster-defining threshold of $p < 0.001$).

observed functional disconnection correlated to increasing tremor severity. For the right cerebellar lobule I-IV seed, functional connectivity with the left thalamus correlated positively with tremor severity (TRS A+B) (Fig. 4B, Table 3). None of the seed regions' functional connectivities correlated with VAS scores or disease duration.

DISCUSSION

This study provides two novel findings that support an important role for the cerebellum, the thalamus, and the cerebello-dentato-thalamic tracts in the pathophysiology of essential tremor. First, the effective connectivity analysis demonstrated a significant excitatory modulating effect of tremor variation on the extrinsic cerebello-dentato-thalamic connection and on intrinsic thalamic and cerebellar lobule V activity. Furthermore, we have replicated and expanded findings of decreased cerebello-cortical functional connectivity, related to a motor task, between the motor cerebellum and cortical motor areas in essential tremor patients compared to controls (33). More importantly, decreased functional coupling between the primary motor cortex and posterior cerebellum was associated with an increase in clinically assessed tremor severity during the motor task. Additionally, an increase in clinically assessed tremor severity was associated with increased functional connectivity between cerebellar lobule I-IV and the motor thalamus in patients with essential tremor.

ALTERED CEREBELLAR OUTPUT

Our findings advocate that modulatory tremor input is associated with activity within the cerebello-dentato-thalamic network. During the motor task, inducing action tremor, all included motor regions exhibited self-inhibiting properties. When incorporating tremor variation during the motor task, intrinsic inhibitory activity of the cortical motor regions and cerebellar lobule VIII increased. However, tremor modulation exhibited an excitatory modulating effect on the cerebello-dentato-thalamic tract, leading from cerebellar lobule V to the thalamus, and intrinsic cerebellar lobule V and thalamic activity. Our results do not give a direct answer as to whether this excitation would give rise to tremor. It is important to note that this excitation does not directly represent a neurophysiological correlate, but is modelled based on the fMRI and EMG signals. Our results do indicate that cerebello-dentato-thalamic activity is perturbed in essential tremor, which can be placed in a broader framework of evidence regarding the pathophysiology of essential tremor. Previously, GABAergic neurotransmission dysfunction within the cerebellum has been observed, with increased 11C-flunazenil binding to GABA-receptors in the cerebellar cortex, increasing with tremor severity (54). Pathology studies also show evidence for cerebellar changes, with Purkinje cell loss and axonal swelling (19, 20, 55), and simultaneous remodelling of the cerebellar cortex (17, 18, 56). Purkinje cells form the sole output channel from the cerebellar cortex, and lead to the deep cerebellar nuclei, including the dentate nucleus. GABAergic Purkinje cell synapses constitute the majority of all synapses in the dentate nucleus, with their action strongly regulating the intrinsic activity of the dentate nucleus (57). Besides pathological changes in the cerebellar cortex, altered dentate nucleus function has been postulated in essential tremor (58, 4, 21). Whether the cerebellar cortical pathology is secondary to changes in the dentate nucleus, or vice versa, remains controversial. Altered 11C-flunazenil binding to GABA-receptors (58) and a decrease in the number of GABA receptors in the dentate nucleus in essential tremor patients (21) both suggest abnormal functionality of GABA receptors within the dentate nucleus. Electrophysiology data indicate that neurons within

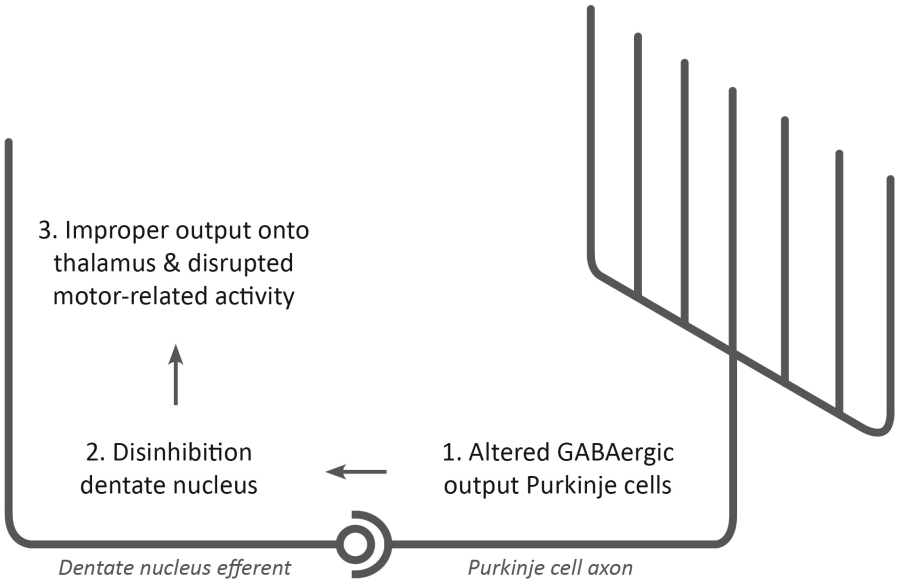

Figure 5.5 Hypothetical chain of pathological events inducing tremor. Firstly, neurodegeneration and neurotransmission dysfunction within the cerebellar cortex lead to altered GABAergic cerebellar cortical output. Secondly, this causes disinhibition of the dentate nucleus, altering its pacemaker-like activity. Consequently and thirdly, pathological activity is passed onward towards the thalamus through dentate nucleus efferents, disrupting physiological motor-related connectivity within the cortex.

the dentate nucleus possess a pacemaker-like activity, with the ability to generate spontaneous inhibitory postsynaptic potentials, that can be increased or decreased depending on GABAergic Purkinje cell input (59). Tremor could consequently result from a disinhibited dentate nucleus and subsequent pathological entrainment of the cerebello-thalamo-cortical network (60). This may be explained as a result of loss of GABAergic tone in the cerebellar system (Fig. 5). A recent fMRI study using a finger-tapping task showed increased activity of the dentate nucleus with increasing clinical tremor severity, in line with this hypothesis (4).

FUNCTIONAL INTEGRITY OF THE MOTOR NETWORK

Essential tremor patients demonstrate decreased functional coupling between cerebellar motor areas and cortical motor areas compared to controls during a motor task. Furthermore, a decrease in functional coupling between the primary motor cortex and posterior cerebellum is correlated with an increase in tremor severity. Two recent fMRI studies employing a motor task showed decreased activity of cerebellar motor regions related to a motor task in essential tremor (4, 33). Increased functional coupling between cerebellar lobule I-IV and the thalamus is correlated with an increase in tremor severity. It is possible that the previously mentioned altered cerebellar output gives rise to changes in cerebello-cortical connectivity. The positive correlation between tremor severity and functional coupling between cerebellar lobule I-IV and the thalamus in essential tremor patients, together with the excitatory effect of tremor modulation on the cerebellum, cerebellar outflow tracts and

the thalamus during the motor task as observed in the effective connectivity analysis, support the idea of pathological entrainment within the cerebellar-thalamic system. In the case of tremor interference, and tremor oscillations throughout the motor network, one would also expect increased cerebello-cortical coupling due to entrainment of the cerebello-thalamo-cortical network. However, an EEG-EMG coherence study has shown that cortical involvement in tremor is only intermittent, and therefore does not seem to be a crucial player within the tremor network (61). Alternatively, perturbed cerebellar output could generate improper thalamic activity and consequently disrupt physiological motor-related connectivity with the motor cortex (59, 60). Our results support the hypothesis that increasing tremor severity proportionally disrupts cerebello-cortical connectivity. Moreover, continuous increased input from the dentate nucleus via the thalamus could cause amplification of inhibitory mechanisms within the cerebral cortex. Inhibitory circuits within the motor cortex are reported to be aberrant and less modifiable in essential tremor (62). In addition, increased 11C-flunazenil binding to GABA-receptors has also been found in the ventrolateral thalamus and lateral premotor cortex in essential tremor (58).

DIFFERENTIAL INVOLVEMENT OF THE ANTERIOR AND POSTERIOR CEREBELLUM IN ESSENTIAL TREMOR

The anterior cerebellum is formed by lobules I to V/VI, and is divided by the primary fissure from the posterior cerebellum, formed by lobules VI/VII to X (63, 64). Interestingly, to our knowledge, the anterior and posterior cerebellum, although both involved in motor control, are not discussed separately in essential tremor research, even though the physiological, developmental and genetic properties of each are quite different (63-65). Our functional and effective connectivity results suggest that both the anterior and posterior cerebellum are involved in essential tremor. There is however a discrepancy in reduced functional connectivity between M1 and the posterior cerebellum associated with increasing tremor severity, and an apparent lack of this reduced functional connectivity between M1 and regions within the anterior cerebellum. On the other hand, an excitatory modulatory effect of tremor was observed in cerebellar lobule V (anterior cerebellum) and on the connections between cerebellar lobule V and the thalamus. We currently have no clear explanation for this observed difference. Although this discrepancy could be due to insufficient sample size, for future pathology studies, it would be of interest to divorce the involvements of the anterior and posterior cerebellum by assessing them separately.

METHODOLOGICAL CONSIDERATIONS

A known and persistent problem with fMRI studies is their limited temporal resolution. This makes the identification of a tremor generator challenging. However, it is a useful technique for studying properties of regions within the cerebello-thalamo-cortical network, especially when combined with EMG recordings. This is the first time EMG signals were incorporated in a DCM analysis. It needs to be stressed that the residual EMG regressor is not the EMG signal as recorded from the muscle. It is a reflection of the waxing and waning EMG signal with respect to the task, i.e., the involuntary movements, and does not necessarily say something about clinical severity. Further studies employing electrophysiological techniques may be required to provide deeper insights into the synaptic mechanisms involved. Although our results appear robust, they will need to be replicated in the future. For this study, the parameters characterizing the cerebello-thalamic connections were chosen as indirect

measures to assess the possible involvement of the dentate nucleus and the cerebello-dentato-thalamic tract in essential tremor. These connections represent the net effect of the cerebello-dentato-thalamic tracts. No tremor-related activity was observed in the dentate nucleus in individual subjects, possibly due to the high iron-content of the dentate nucleus and resulting low signal-to-noise ratio of its BOLD signal (66). To be able to include the dentate nucleus in future models, studies with a higher spatial and temporal resolution are warranted to reproduce our observed excitatory effect on the cerebello-dentato-thalamic pathway.

A common difficulty in functional imaging studies lies in selecting a suitable task for healthy controls that corresponds well with the patients' task. For this study, a mimicked tremor was chosen. Consequently, the two groups were actually performing a qualitatively different task. These tasks were chosen to allow optimal distinction of brain networks for the functional connectivity analysis, involved in involuntary tremor as opposed to compensation or afferent feedback by deliberate, mimicked tremor movements. However, due to this qualitative difference, for the effective connectivity analysis the patient group was not compared with a healthy control group. Future studies could circumvent this problem by employing other techniques such as enforcing passive wrist oscillations as an additional control condition, as has been used previously by Bucher and colleagues (42). One could then additionally assess whether there are differences within the tremor circuitry in excitatory and inhibitory connections between patients and healthy controls.

Finally, as mentioned in the methods section, a silent reading task was offered during half of the task blocks, which may have influenced activity within the motor network and could therefore have affected our effective connectivity results. There was a significant difference in driving effect of the two tasks on the SMA and not on the PMC, as observed in the effective connectivity analysis. However, the motor task with silent reading had merely an additional excitatory effect compared to the motor task in which only the command to stretch the right arm was given. We expect that this will not have affected the final conclusions of the effective connectivity analysis.

In conclusion, our findings suggest that cerebello-dentato-thalamic activity and cerebellar-cortical connectivity are perturbed in essential tremor, supporting previous evidence of cerebellar pathology in essential tremor. This perturbed cerebello-dentato-thalamic activity could subsequently affect the rest of the cerebello-thalamo-cortical network, leading to tremor on the one hand and possibly less effective physiological output on the other hand. Investigating effective connectivity changes in essential tremor represents a new avenue of study that may shed light on its underlying pathophysiology.

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Model no.	Cerebellar of	SMA	PMC	Thalamus	CB V	CB VIII	M1	Model no.	Cerebellar of	SMA	PMC	Thalamus	CB V	CB VIII	M1	Model no.	Cerebellar 0f	SMA	PMC	Thalamus	CB V	CB VIII	M1
1	0	0	0	0	0	0	0	**45**	0	1	0	1	1	0	0	**89**	1	0	1	1	0	0	0
2	0	0	0	0	0	0	1	**46**	0	1	0	1	1	0	1	**90**	1	0	1	1	0	0	1
3	0	0	0	0	0	1	0	**47**	0	1	0	1	1	1	0	**91**	1	0	1	1	0	1	0
4	0	0	0	0	0	1	1	**48**	0	1	0	1	1	1	1	**92**	1	0	1	1	0	1	1
5	0	0	0	0	1	0	0	**49**	0	1	1	0	0	0	0	**93**	1	0	1	1	1	0	0
6	0	0	0	0	1	0	1	**50**	0	1	1	0	0	0	1	**94**	1	0	1	1	1	0	1
7	0	0	0	0	1	1	0	**51**	0	1	1	0	0	1	0	**95**	1	0	1	1	1	1	0
8	0	0	0	0	1	1	1	**52**	0	1	1	0	0	1	1	**96**	1	0	1	1	1	1	1
9	0	0	0	1	0	0	0	**53**	0	1	1	0	1	0	0	**97**	1	1	0	0	0	0	0
10	0	0	0	1	0	0	1	**54**	0	1	1	0	1	0	1	**98**	1	1	0	0	0	0	1
11	0	0	0	1	0	1	0	**55**	0	1	1	0	1	1	0	**99**	1	1	0	0	0	1	0
12	0	0	0	1	0	1	1	**56**	0	1	1	0	1	1	1	**100**	1	1	0	0	0	1	1
13	0	0	0	1	1	0	0	**57**	0	1	1	1	0	0	0	**101**	1	1	0	0	1	0	0
14	0	0	0	1	1	0	1	**58**	0	1	1	1	0	0	1	**102**	1	1	0	0	1	0	1
15	0	0	0	1	1	1	0	**59**	0	1	1	1	0	1	0	**103**	1	1	0	0	1	1	0
16	0	0	0	1	1	1	1	**60**	0	1	1	1	0	1	1	**104**	1	1	0	0	1	1	1
17	0	0	1	0	0	0	0	**61**	0	1	1	1	1	0	0	**105**	1	1	0	1	0	0	0
18	0	0	1	0	0	0	1	**62**	0	1	1	1	1	0	1	**106**	1	1	0	1	0	0	1
19	0	0	1	0	0	1	0	**63**	0	1	1	1	1	1	0	**107**	1	1	0	1	0	1	0
20	0	0	1	0	0	1	1	**64**	0	1	1	1	1	1	1	**108**	1	1	0	1	0	1	1
21	0	0	1	0	1	0	0	**65**	1	0	0	0	0	0	0	**109**	1	1	0	1	1	0	0
22	0	0	1	0	1	0	1	**66**	1	0	0	0	0	0	1	**110**	1	1	0	1	1	0	1
23	0	0	1	0	1	1	0	**67**	1	0	0	0	0	1	0	**111**	1	1	0	1	1	1	0
24	0	0	1	0	1	1	1	**68**	1	0	0	0	0	1	1	**112**	1	1	0	1	1	1	1
25	0	0	1	1	0	0	0	**69**	1	0	0	0	1	0	0	**113**	1	1	1	0	0	0	0
26	0	0	1	1	0	0	1	**70**	1	0	0	0	1	0	1	**114**	1	1	1	0	0	0	1
27	0	0	1	1	0	1	0	**71**	1	0	0	0	1	1	0	**115**	1	1	1	0	0	1	0
28	0	0	1	1	0	1	1	**72**	1	0	0	0	1	1	1	**116**	1	1	1	0	0	1	1
29	0	0	1	1	1	0	0	**73**	1	0	0	1	0	0	0	**117**	1	1	1	0	1	0	0
30	0	0	1	1	1	0	1	**74**	1	0	0	1	0	0	1	**118**	1	1	1	0	1	0	1
31	0	0	1	1	1	1	0	**75**	1	0	0	1	0	1	0	**119**	1	1	1	0	1	1	0
32	0	0	1	1	1	1	1	**76**	1	0	0	1	0	1	1	**120**	1	1	1	0	1	1	1
33	0	1	0	0	0	0	0	**77**	1	0	0	1	1	0	0	**121**	1	1	1	1	0	0	0
34	0	1	0	0	0	0	1	**78**	1	0	0	1	1	0	1	**122**	1	1	1	1	0	0	1
35	0	1	0	0	0	1	0	**79**	1	0	0	1	1	1	0	**123**	1	1	1	1	0	1	0
36	0	1	0	0	0	1	1	**80**	1	0	0	1	1	1	1	**124**	1	1	1	1	0	1	1
37	0	1	0	0	1	0	0	**81**	1	0	1	0	0	0	0	**125**	1	1	1	1	1	0	0
38	0	1	0	0	1	0	1	**82**	1	0	1	0	0	0	1	**126**	1	1	1	1	1	0	1
39	0	1	0	0	1	1	0	**83**	1	0	1	0	0	1	0	**127**	1	1	1	1	1	1	0
40	0	1	0	0	1	1	1	**84**	1	0	1	0	0	1	1	**128**	1	1	1	1	1	1	1
41	0	1	0	1	0	0	0	**85**	1	0	1	0	1	0	0								
42	0	1	0	1	0	0	1	**86**	1	0	1	0	1	0	1								
43	0	1	0	1	0	1	0	**87**	1	0	1	0	1	1	0								
44	0	1	0	1	0	1	1	**88**	1	0	1	0	1	1	1								

SUPPLEMENTARY TABLE 5.1. LIST OF MODELS AND MODULATORY INPUTS

SUPPLEMENTARY MATERIALS

1. List of models and modulatory inputs
2. Full details DCM coupling parameters based on Bayesian Model Averaging
3. One sample T-test – motor task conjunction analysis of essential tremor patients and healthy controls
4. One sample T-test – functional connectivity maps per seed region, conjunction analysis of essential tremor patients and healthy controls
5. Supplementary references

1. LIST OF MODELS AND MODULATORY INPUTS

(See previous page.)

2. FULL DETAILS DCM COUPLING PARAMETERS BASED ON BAYESIAN MODEL AVERAGING

The posterior densities of the parameters are calculated across subjects and across the winning half of models. More weight is given to the models with the highest posterior probability according to Bayes' rule (1). The resulting coupling parameters represent connection strengths (2). The posterior distributions are calculated using a Gibbs sampling approach by drawing samples from a multinomial distribution of posterior beliefs for the included models (1). Subsequently, posterior means and standard deviations of parameters were obtained and tested for significance using two-tailed t-test. Because we tested forty parameters of interest (28 endogenous, 8 modulatory and 4 task inputs) we have adjusted the significance threshold using the Bonferroni method (α = 1-(1- α)1/40) = 0.001282). Positive coupling parameters suggest a facilitation of neural activity, whereas negative coupling parameters can be interpreted as inhibition of neural activity. The coupling parameter unit is Hertz (Hz), reflecting the amount of activity that flows from one region into another per second.

SUPPLEMENTARY TABLE 5.2. DCM A-MATRIX – ENDOGENOUS CONNECTIVITY BASED ON BAYESIAN MODEL AVERAGING

PARAMETER ESTIMATE:	MEAN	SD	T-VALUE	P-VALUE
M1 à M1	-0.45	0.04	51.4	<0.001
M1 à SMA	-0.13	0.05	10.5	<0.001
M1 à PMC	-0.03	0.04	3.5	0.003
M1 à Thal	-0.15	0.04	16.1	<0.001
M1 à CB V	-0.13	0.03	16.3	<0.001
M1 à CBVIII	-0.13	0.03	15.4	<0.001
SMA à SMA	-0.43	0.03	53.8	<0.001
SMA à M1	0.29	0.03	40.7	<0.001
SMA à PMC	0.14	0.04	14.3	<0.001
SMAàThal	0.25	0.03	29.9	<0.001
SMA à CB V	0.26	0.04	30.7	<0.001
SMA à CB VIII	0.19	0.03	29.2	<0.001
PMC à PMC	-0.47	0.04	53.5	<0.001
PMC à M1	0.09	0.04	9.2	<0.001
PMC à SMA	0.08	0.05	7.1	<0.001
PMC à Thal	0.08	0.05	7.0	<0.001
PMC à CBV	0.11	0.05	10.0	<0.001
PMC à CB VIII	0.15	0.04	14.9	<0.001
Thal à Thal	-0.62	0.03	75.4	<0.001
Thal à M1	0.13	0.05	12.1	<0.001
Thal à SMA	0.50	0.08	25.0	<0.001
Thal à PMC	0.31	0.07	19.3	<0.001
CBV à CBV	-0.42	0.03	50.1	<0.001
CBV à Thal	-0.06	0.04	6.2	<0.001
CB V à CB VIII	0.04	0.05	3.0	0.009
CB VIII à CB VIII	-0.43	0.04	46.5	<0.001
CB VIII à Thal	-0.05	0.05	3.8	0.002
CB VIII à CB V	0.07	0.05	6.1	<0.001

Statistical significance determined by one sample two-tailed *t*-test. Full endogenous connectivity was assumed with the exemption of connections between cerebellar regions and the thalamus (only unidirectional from cerebellum to thalamus) and between cortical and cerebellar regions (only unidirectional from cortical to cerebellar regions) based on neuronal tracing studies in macaque monkeys (3), leaving 28 connections. M1: primary motor cortex, SMA: supplementary motor area, PMC: premotor cortex. Thal: thalamus, CB: cerebellar lobule.

SUPPLEMENTARY TABLE 5.3. DCM B-MATRIX – MODULATORY TREMOR VARIATION INPUT

MODULATORY INPUT ON:	MEAN	SD	T-VALUE	P-VALUE
Primary motor cortex (intrinsic)	-0.30	0.25	5.1	<0.001
Supplementary motor area (intrinsic)	-0.91	0.27	14.3	<0.001
Premotor cortex (intrinsic)	-0.53	0.29	7.7	<0.001
Thalamus (intrinsic)	1.26	0.42	12.6	<0.001
Cerebellar lobule V (intrinsic)	0.31	0.32	4.2	<0.001
Cerebellar lobule VIII (intrinsic)	-0.28	0.27	4.5	<0.001
Cerebellar lobule V to thalamus (extrinsic)	0.82	0.89	3.9	0.001
Cerebellar lobule VIII to thalamus (extrinsic)	0.60	1.38	1.8	0.08

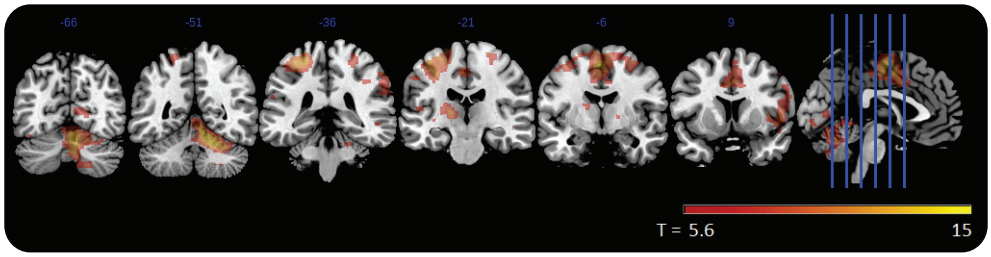
Posterior means and standard deviations of the estimated modulatory effect of tremor variation on all regions and cerebello-dentato-thalamic tracts. Tested for significance using two-tailed *t*-tests. Bonferroni-corrected significant *p*-values in bold ($p > 0.00128$). Results are summarized graphically in Figure 3 within the main text.

SUPPLEMENTARY TABLE 5.4. DCM C-MATRIX – DIRECT (TASK) INPUT

PARAMETER ESTIMATE:	MEAN	SD	T-VALUE	P-VALUE
Task input SMA (with silent reading task)	0.10	0.01	43.3	<0.001
Task input SMA (without silent reading task)	0.07	0.01	33.4	<0.001
Task input PMC (with silent reading task)	0.04	0.01	18.6	<0.001
Task input PMC (without silent reading task)	0.05	0.01	22.5	<0.001

Mean influence of task input. statistical significance determined by one sample two-tailed *t*-test. Bonferroni-corrected significant parameters in bold ($p > 0.00128$). SMA = supplementary motor area. PMC = premotor cortex.

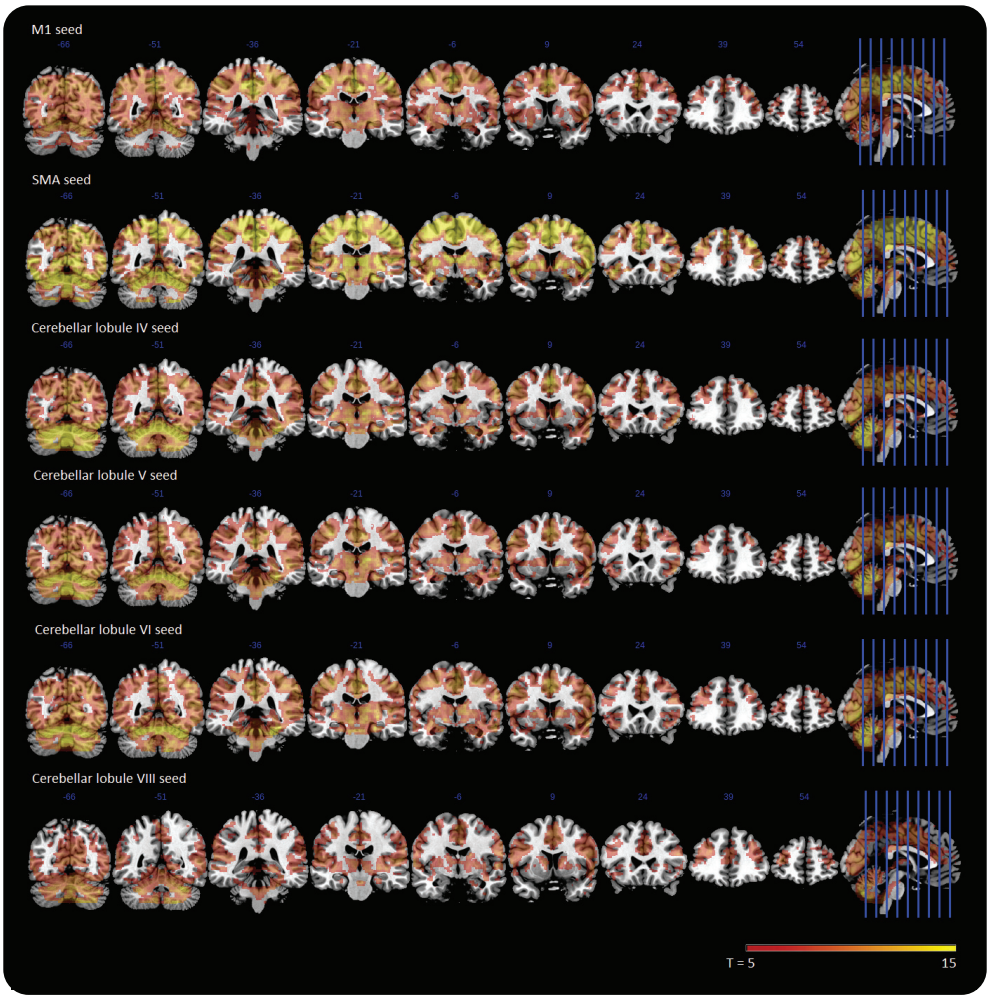
3. ONE SAMPLE T-TEST – MOTOR TASK CONJUNCTION ANALYSIS OF ESSENTIAL TREMOR PATIENTS AND HEALTHY CONTROLS



REGION	VOXELS	R/L	T-VALUE	X	Y	Z
Cerebellar lobule IV	3841	Right	14.9	4	54	-21
Cerebellar lobule V		Right	14.1	14	-50	-19
Cerebellar lobule VI		Right	13.9	22	-50	-25
Primary motor cortex	5048	Left	14.5	-28	-28	53
Supplementary motor area		Left	14.0	-2	-8	57
Primary motor cortex		Left	13.5	-36	-32	61

Task-related activity - results of a one sample T-test – conjunction analysis of essential tremor patients and healthy controls. The six most significant peak-voxels are listed. Cerebellar lobule VIII is located within the most significant cluster with peak-region cerebellar lobule IV.

4. ONE SAMPLE T-TEST – FUNCTIONAL CONNECTIVITY MAPS PER SEED REGION, CONJUNCTION ANALYSIS OF ESSENTIAL TREMOR PATIENTS AND HEALTHY CONTROLS



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CHAPTER 6

INCREASED CEREBELLAR ACTIVATIONS DURING GOAL-DIRECTED MOVEMENT IN ESSENTIAL TREMOR: AN FMRI STUDY

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ABSTRACT

INTRODUCTION: Abnormalities in goal-directed movement are an important symptom of essential tremor (ET), and are clinically related to cerebellar disease. The current study aims to examine how these movements are associated with abnormal brain activity in ET patients.

METHODS: Nineteen ET patients and seventeen healthy participants performed a goal-directed movement task using an MR-compatible wrist device. Spatial coordinates of participants' movements were recorded during scanning and used to calculate reaction times and variability in movement paths. The target stimuli that prompted participants to move were incorporated in an event-related fMRI design, to correlate the goal-directed movements with cerebral activity. Disease severity, disease duration, intention tremor (assessed clinically with finger-to-nose manoeuvres) were used as clinical covariates in separate analyses.

RESULTS: ET patients were affected mildly (off-medication median Tremor Rating Scale score 22 (range 13-45)). We found no significant difference in reaction time or variability in movement paths between patients and healthy participants. We found increased activations in cerebellar vermis 4/5/6 in ET patients (T=5.11, coordinates in MNI space x=3, y=-63, z=-5), when comparing back-to-centre movements versus baseline. There was no correlation between brain activation during the goal-directed movement task and any of the clinical covariates in ET patients.

DISCUSSION: We report increased cerebellar activation in mildly affected ET patients during goal-directed movements, in the absence of differences in task performance compared to healthy participants. These results extend on earlier reports of increased cerebellar activation during postural tasks, and lend further support to the notion of underlying cerebellar abnormalities in ET.

INTRODUCTION

Essential tremor (ET) is the most common tremor disorder, and is characterized by bilateral action tremor of the hands and forearms (1, 2). The tremor occurs during action and posturing, and apart from the hands and arms, the voice, head and more infrequently jaw, tongue, legs and feet can also be affected (3). ET often runs in families, and half of the patients claim their tremor is reduced by the intake of one or two glasses of alcohol.

A feature that is common in ET, but atypical in most other tremor disorders, is intention tremor: tremor worsening during goal-directed movement (4-6)(see also Chapter 2). Intention tremor is considered to be a sign of cerebellar disease (4), and apart from ET, it is also seen in multiple sclerosis (7) and Holmes (rubral) tremor (8): diseases that affect the cerebellum or cerebellar outflow-tract. Therefore, the occurrence of intention tremor in ET fits well with the emerging pathophysiological view of ET as a cerebellar disease (9, 10). Likewise, in support of the cerebellar hypothesis, there is increasing acknowledgement of ataxia in ET. Several studies have shown signs of mild ataxia in ET patients, such as mild gait ataxia (11-13), hypermetria (4, 14), and drawing ataxia (15). These clinical signs and symptoms lend further support to the notion of underlying cerebellar abnormalities.

Neuroimaging studies also provide evidence for cerebellar involvement in ET (16). Two published motor fMRI-studies both demonstrated abnormal cerebellar activity in ET (17)(see also Chapter 4). However, the movement tasks investigated in these studies did not extend beyond a comparison between posturing of the tremulous arm versus rest. As abnormalities in intentional movement are an important clinical symptom of ET, the current study aims to examine how goal-directed movement is associated with abnormal brain activity in the cerebellum and other brain regions in ET patients. Goal-directed movements can be executed and measured in the MRI scanner by employing a centre-out steptracking task and an MR-compatible wrist device (18). Recently, attention has been paid to the effect of deep brain stimulation (DBS) on reach-to-grasp movement in severely affected ET patients (19, 20). DBS of the subthalamic area reduced ataxia especially, returning upon suprathreshold stimulation, leading the authors to hypothesize on the roles of different cerebellar networks in goal-directed movement in ET. We set out to extend on this work by using a goal-directed movement task suitable for fMRI-scanning, thus enabling a link between behavioral and cerebral or cerebellar abnormalities.

METHODS

PARTICIPANTS

The Medical Ethics Committee of the University Medical Center Groningen approved the neuroimaging study of which this study is a subpart. Participants took part after providing informed consent in accordance with the declaration of Helsinki (21). We included patients who had a definite diagnosis of ET according to the TRIG criteria (1): all patients had bilateral upper limb action tremor in the absence of other neurological signs, and disease duration had to be >5 years. Additionally, age at onset was required to be <65 years, thereby excluding late-onset 'senile' ET, which may have a different pathophysiology (2). Patients who took medication for their tremor quit their medication minimally three days before testing. Patients and healthy, age- and gender-matched participants were all right-handed as assessed by the Annett Handedness scale (22). All participants had to score >25 on the Mini Mental State Examination (23) to ensure proper understanding of the task. Exclusion criteria were neurological comor-

bidity (for patients: other than ET), the use of medication affecting the central nervous system, and MR-related contra-indications such as claustrophobia, ferromagnetic implants or pregnancy.

CLINICAL ASSESSMENT OF TREMOR

Tremor was assessed off-medication using the Fahn-Tolosa-Marin Tremor Rating Scale (TRS) (24) parts A, (assessment of tremor amplitude during standardized postures and movements), B (tremor-inducing tasks), and C (a structured interview about limitations in daily life due to tremor). The examination was recorded on video and supplemented with the corresponding spiral drawings. An experienced movement disorders specialist (Dr. J.D. Speelman, AMC) scored TRS parts A and B based on this material. Part C was assessed by vd Stouwe for all patients. Subjective tremor severity was documented using 10 cm visual analogue scales (VAS) ranging from 0: 'no tremor at all' to 10: 'worst tremor imaginable', on which patients marked their scores. Intention tremor was assessed clinically by scoring presence or absence of intention tremor during finger-to-nose manoeuvres, and this score was used as a clinical covariate in the fMRI analysis later on. Scoring of intention tremor was done clinically because the rapid wrist movements within the employed step-tracking task are too brief to detect any tremor, making it impossible to assess intention tremor during task performance.

EXPERIMENTAL SET-UP

Participants performed the task with the right hand, using an MR-compatible manipulandum: a joystick-like device that can rotate in two planes allowing all combinations of wrist flexion-extension and ulnar-radial deviation (Figure 6.1A-C). To provide visual feedback on task performance, angular displacement was measured in both (X and Y) planes by potentiometers mounted in-line with the axes of the manipulandum rings and displayed as a cursor (a 5 x 5 mm square) following digitization using a Power 1401 analog-to-digital converter controlled using Spike 2 (Cambridge Electronic Design (CED), Cambridge, UK). Data were recorded simultaneously at a sampling rate of 100 Hz. During scanning, performance was visually monitored on a second computer in the MR control room.

STEP-TRACKING TASK

Participants were asked to place their cursor in a centre box (3 x 1.5 cm open rectangle) at the start of the experiment. After two seconds, the centre box disappeared and a target stimulus (3 x 1.5 cm open rectangle)

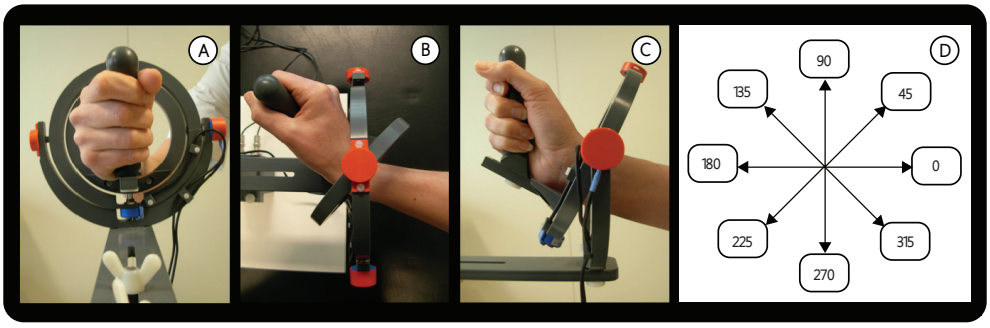

Figure 6.1. MR-compatible wrist device is shown with A) hand held in neutral position, B) wrist extension and C) radial deviation. D) Schematic overview of movement task consisting of 8 different out-of-centre movements. Out-of-centre stimuli appear randomly, and are always followed by a back-to-centre movement.

appeared at one of eight possible positions (Figure 6.1D). Participants were instructed to move towards the target as quickly as possible, and subsequently hold the cursor in the target box until it disappeared (three seconds after appearance), after which they returned to the reappeared centre box. Each entire (outward-inward) step-track trial lasted 5 seconds. After every 10 step-track trials, there was a short break of 4 seconds. One step-track block consisted of 40 out-of-centre and 40 back-to-centre stimuli, 5 for each of the 8 different directions presented in fixed randomised order (randomised but in the same order for every participant). The time intervals between appearances of the stimuli were randomised (jitter: 0.8 s +/- 0.4 s). The entire task consisted of four blocks, totalling 320 stimuli, with 30 seconds of rest in-between blocks.

ANALYSIS OF REACTION TIME

Kinematic data was further analysed using Matlab (Matlab R2013, Mathworks, Natrick, USA). In-house developed software was used to determine reaction time (RT) for each movement in a semi-automatic way. Movement onset was defined as the first time point where the derivative of the smoothed distance vector exceeded 0.02. Initial automatic definitions were checked manually and corrected, if necessary. When no apparent movement was present in the traces, the corresponding stimulus was not used as an event in the fMRI-analysis. Mean reaction time per movement direction per participant was calculated and exported to be analysed in SPSS.

ANALYSIS OF VARIABILITY IN MOVEMENT PATHS

As differences in task execution between groups may induce differences in BOLD activation, we tested for differences in variability in movement execution by applying an approach similar to an analysis employed in earlier studies (19, 20). Since in healthy people recurrent movements are characterized by low variability in movement execution, the deviation from a participants' own average movement trajectory while performing the goal-directed movement task was chosen as a measure to quantify movement execution variability. To do so, we first computed the mean movement trajectory for each participant per type of movement, resulting in 8 mean out-of-centre movement trajectories, and 8 mean back-to-centre movement trajectories. Subsequently, we calculated the distance participants deviated from their personal mean trajectory per trial, and derived the mean deviation for each of the 16 types of movements. Note that this assessment of movement paths may capture ataxic performance, referring to abnormal, uncoordinated movement, but not to hypermetria (overshoot) per se.

STATISTICS

Baseline participant characteristics were compared between groups using Chi-square tests for categorical data (gender), and Kruskal-Wallis tests for non-normally distributed data (age) in SPSS 22 (IBM, Chicago, IL). Normality of distributions was tested using the Shapiro-Wilk test. When log transformation resulted in a normal distribution, mixed design ANOVAs were employed to assess whether RT and variability in movement execution differed between groups.

MRI CHARACTERISTICS

fMRI data acquisition was performed using a 3 Tesla Magnetic Resonance System (Philips, Best, The Netherlands) with a 32-channel head coil. 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, a matrix size of 256 x 256 mm and isotropic voxel size of 1 x 1 x 1 mm were obtained.

FMRI ANALYSIS

fMRI data was analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.uck.ac.uk/spm). Pre-processing consisted of realignment to correct for individual participant movement, and coregistration to align all functional data to each participant's anatomical scan. A group-specific anatomic template was created (for patients and healthy participants together) with DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (25)) to take age-related changes in anatomy into account, and achieve a more precise inter-subject alignment. Individual functional data were normalized and smoothed using the DARTEL template and a 4-mm full-width half maximum (FWHM) Gaussian kernel. We chose a 4 mm kernel because we were particularly interested in cerebellar (i.e. subcortical) activations, where the effect of a larger 8 mm kernel can be unfavourable (26). To reduce movement artefacts, the six movement parameters derived from realignment corrections were entered as covariates in each participant's analysis. The stimulus onsets derived from the Spike log-file were added to the design as events. This was done separately for out-of-centre and back-to-centre stimuli, because we speculated that differences might exist between these two types of movement, due to the predictable location of the back-to-centre stimuli. Each single-participant first-level model thus consisted of a regressor representing out-of-centre events, a regressor representing back-to-centre events, and the six movement regressors. Subsequently, second level within- and between group comparisons were made on whole brain level. Contrasts were initially thresholded at a voxel level of $p<0.001$, uncorrected, applying a cluster size of 20 voxels. Activations were then considered significant at a cluster corrected p-level <0.05, FWE-corrected.

To test whether brain activations correlated with clinical characteristics in our patient group, we employed Statistical non-Parametric Mapping (version 13b, http://www.sph.umich.edu/ni-stat/SnPM/, 10.000 permutations). We built separate contrasts with presence of intention tremor (scored clinically as either absent or present), disease duration and disease severity (total TRS score) as covariates. Cluster-wise inference was used ($p<0.05$ FWE-corrected, cluster-forming threshold $p<0.05$ FWE-corrected).

RESULTS

PARTICIPANT CHARACTERISTICS

Nineteen ET patients and seventeen healthy participants participated in this study (see Table 6.1 for patient characteristics). Gender and age distribution did not differ between groups ($p=1.000$ and $p=0.733$, respectively). ET patients had a mean age of 55 years, healthy participants of 56 years. Mean disease duration was 31 years. 89% of patients had a positive family history for tremor, whereas 53% reported a decrease of tremor upon alcohol intake. Sixteen patients were treated with propranolol, with a median dose of 60 mg daily, ranging from 10 tot 160 mgs. One patient was treated with primidon, and two patients did not take any medication for their tremor. Clinically, disease severity varied from mild to severe, with a median TRS score of 22 ranging from 13 to 45 off-medication. Patient-perceived tremor severity, measured with visual analogue scales, also varied from mild to severe with off-medi-

TABLE 6.1. PATIENT CHARACTERISTICS

No.	Age	Gender	Age at onset	Disease duration	Family history	Response to alcohol	Medication	Dose (mg)	VAS (off)	TRS (off)	IT	Ataxia (cm)
1	21	M	10	11	+	+	Propranolol	40	5.4	35	-	2.37
2	27	M	0	27	-	+	Propranolol	160	8.7	22	+	1.25
3	30	F	15	15	+	?	Propranolol	20	2.9	17	-	1.72
4	32	F	3	29	+	+	Propranolol	40	6.0	22	-	1.66
5	35	M	7	28	+	?	Propranolol	80	7.8	17	+	1.30
6	46	M	5	41	+	+	Propranolol	80	4.4	19	+	1.10
7	50	M	35	15	+	+	Propranolol	80	6.8	22	+	1.45
8	53	M	15	38	+	+	Propranolol	20	7.2	14	-	1.07
9	57	M	18	39	+	?	Propranolol	10	2.8	25	-	2.21
10	57	F	22	35	+	?	Propranolol	10	4.0	23	+	1.29
11	62	M	22	40	+	?	None	-	9.4	22	-	1.23
12	64	M	12	52	+	+	Propranolol	20	4.0	17	-	1.00
13	65	M	30	35	+	+	Propranolol	40	4.4	20	-	1.28
14	69	M	40	29	+	-	Propranolol	40	9.2	45	-	1.48
15	70	F	30	40	+	?	Propranolol	80	2.1	13	-	1.81
16	74	M	50	24	-	?	Propranolol	80	6.6	32	+	2.92
17	74	M	20	54	+	+	Primidon	125	2.7	15	+	1.28
18	77	M	50	27	+	?	Propranolol	80	5.4	23	+	1.25
19	80	F	60	20	+	+	None	-	9.2	42	+	2.19
Group	55 ±18	15:5	23 ±17	33 ±12	17:2	10: 1:8	17:2:1	40 iqr 60	5.7 ±2.4	22 iqr 8	9: 10	1.3 iqr 0.55

VAS: visual analogue scale, scored off-medication (range 0-10), TRS: Fahn-Tolosa-Marin Tremor Rating Scale, scored off-medication (parts A-C, range 0-88), IT: intention tremor, iqr: interquartile range. Ataxia: mean value in cm across all movements. Group values: mean ± sd, unless otherwise indicated.

cation scores ranging from 2.1 to 9.4 on a 10-point scale. None of the patients showed tremor at rest, whereas 9 out of 19 patients had intention tremor.

TASK PERFORMANCE

Mean RT was 410 (sd 68) ms in ET patients, and 398 (sd 73) ms in healthy participants. There was no difference between groups (p=0.596). Regarding variability in movement paths, during out-of-centre movements, the median distance to mean movement trajectory was 1.4 cm (interquartile range (IQR) 0.39) in ET patients, and 1.4 cm (IQR 0.36) in healthy participants. For back-to-centre movements, median distance

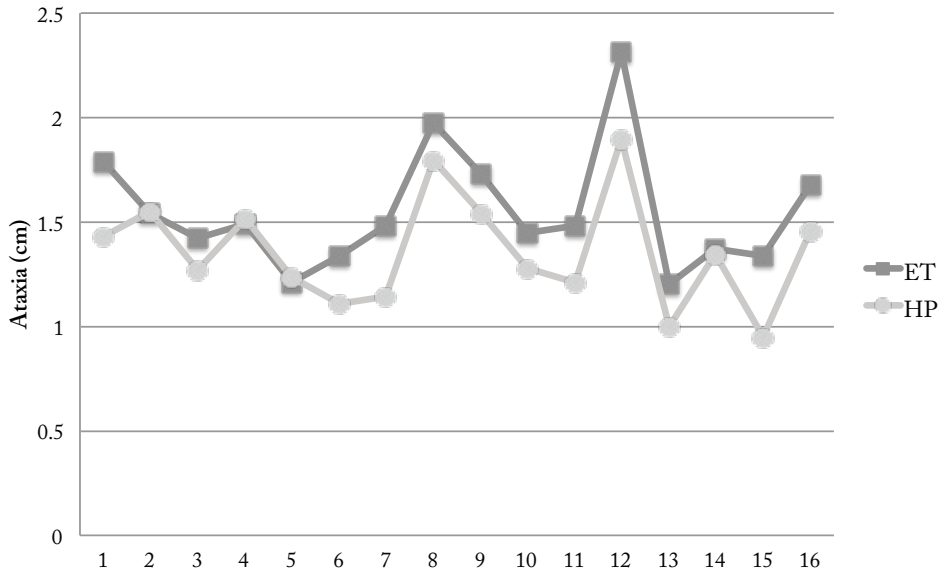

Figure 6.2. Mean variability in movement paths, defined as mean deviation from participants' mean movement trajectory in cm, is depicted for each movement direction. Numbers 1-8 indicate out-of-centre movements; numbers 9-16 indicate back-to-centre movements. ET: essential tremor, HP: healthy participants.

to mean movement trajectory was 1.3 cm (interquartile range (IQR) 0.63) in ET patients, and 1.3 cm (IQR 0.66) in healthy participants. There were no differences between groups (p=0.189)(Figure 6.2).

WITHIN-GROUP FMRI RESULTS

All fMRI results can be found in Table 6.2. For the contrast 'all movements versus baseline', we found cerebral activations in healthy participants in the left premotor cortex, motor cortex, somatosensory cortex and subcentral gyrus. Cerebellar activations were found in the right lobules 4, 5 and 6, and left lobule 6. In ET patients, we found cerebral and cerebellar activations for the contrast 'all movements versus base-line' in the same areas as in the healthy participants, except for the subcentral gyrus and left cerebellar lobule 6.

To check whether differences between the two types of movement exist, we compared brain activations related to out-of-centre movements versus back-to-centre movements directly. In healthy participants, we found increased activations in the left dorsolateral prefrontal cortex, parietal cortex, somatosensory cortex, and anterior cingulate cortex, and in the right secondary visual cortex (V2). For the same contrast ('out-of-centre movement > back-to-centre movement') in ET patients, we found increased activations in the left anterior cingulate and frontal eye fields, and in the right visual cortices (V1-V5), posterior cingulate, occipitotemporal cortex and dorsolateral prefrontal cortex. We found no increased activations for either of the groups for the reverse contrast, 'back-to-centre movements versus out-of-centre movements'.

BETWEEN-GROUP FMRI RESULTS

We found no differences between groups for the contrasts 'all movements versus baseline' and 'out-of-centre movements versus baseline'. For the contrast 'back-to-centre movements versus baseline', we found increased activations in the cerebellum in vermis 6 ex-

TABLE 6.2. SIGNIFICANT FMRI RESULTS FOR ET PATIENTS AND HEALTHY PARTICIPANTS

COMPARISON	LOCATION	CLUSTER SIZE (VOXELS)	T-VALUE	X	Y	Z
All movements: Healthy participants	Motor cortex (BA4), extending into somatosensory cortex (BA3)	146	12.6	-34	-18	52
	Premotor cortex (BA6)	1040	9.68	29	-15	58
	Parietal cortex (BA40/BA5)	1799	10.1	33	-37	46
	Fusiform gyrus (BA37)	339	6.23	51	-67	0
	Subcentral gyrus (BA43)	134	6.15	60	-15	36
	Cerebellum: lobule 6, extending into lobules 4 and 5	2157	9.28	21	-51	-21
	Cerebellum: lobule 6	215	7.32	-22	-51	-24
All movements: ET patients	Premotor cortex (BA6), extending into motor cortex (BA4)	7465	10.56	-2	-10	52
	Somatosensory cortex (BA2/3)	38	8.54	-39	-25	49
	Parietal cortex (BA40/BA3)	456	5.55	36	-36	49
	Fusiform gyrus (BA37)	91	5.07	50	-63	-3
	Cerebellum: lobules 4/5	1650	10.97	15	-51	-21
Out-of-centre movements > back-to-centre movements: HP	Parietal cortex (BA40)	469	7.29	-52	-49	43
	Dorsolateral prefrontal cortex (BA9)	232	7.17	-24	27	37
	Somatosensory cortex (BA3)	95	7.08	-52	-12	34
	Anterior cingulate (BA24)	879	7.49	0	21	37
	Secondary visual cortex (BA18)	180	8.21	20	-67	-8
Out-of-centre movements > back-to-centre movements: ET patients	Anterior cingulate (BA32)	118	6.59	-6	18	42
	Motor cortex (BA4)	95	6.04	-54	6	28
	Dorsolateral prefrontal cortex (BA9)	103	5.85	-26	9	55
	Posterior cingulate (BA23)	128	4.44	8	-28	27
	Occipitotemporal cortex (BA37)	99	5.84	42	-63	6
	Parietal cortex (BA48/40/42/22)	117	6.64	51	-48	28
	Primary visual cortex (BA17)	902	10.08	10	-81	16
	Secondary visual cortex (BA18)	951	8.48	12	-75	-6
	Associative visual cortex (BA19)	272	6.07	26	-72	25
Back-to-centre movements: ET > HP	Cerebellum: vermis 6, extending into vermis 4/5.	118	5.11	4	-66	-8

ET: essential tremor. HP: healthy participants. BA: broadmann area. Voxel-peak level was initially set at $p<0.001$, uncorrected, $k = 30$ voxels. Reported activations correspond with the voxel of maximum activation within clusters of significant activation at $p<0.05$, FWE-corrected. X-, y- and z-coordinates refer to MNI (Montreal Neurology Institute and Hospital) space.

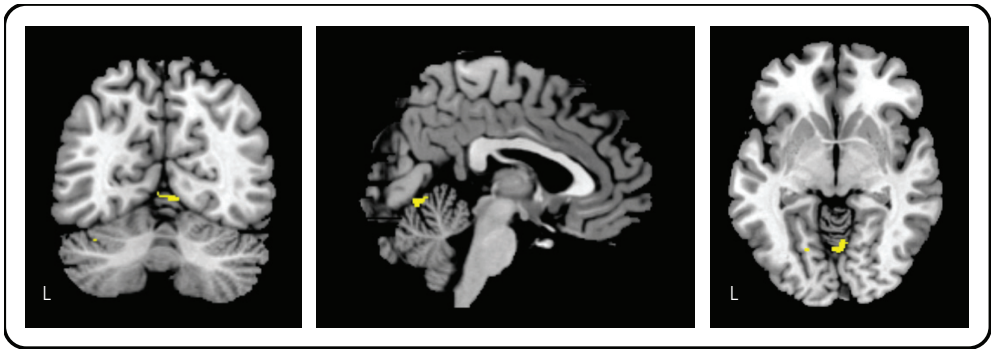

Figure 6.3. Between-group fMRI results: increased activations (SPM T-maps) in ET patients compared to healthy participants in cerebellar vermis lobules 4/5/6 are shown in yellow, for the comparison back-to-centre movements versus base-line. Activations are shown above a clusterwise threshold of $p<0.05$, FWE-corrected. Coordinates of the positions of the three figures are x = 3, y = -63, z = -5 relative to the anterior commissure-posterior commissure plane. L = left hemisphere.

tending into vermis 4 and 5, in ET patients.

CLINICAL COVARIATE FMRI RESULTS

There were no correlations between brain activations in ET patients and presence of intention tremor, disease duration or disease severity.

DISCUSSION

In the current study, we found increased cerebellar activations during goal-directed movement in ET patients compared to healthy participants.

These differences in cerebellar activation were found despite the absence of differences in ataxic performance. We suppose our ET patients performed normally because they were relatively mildly affected: all patients took medication or had no treatment for their tremor, as opposed to the more severely affected cases in other studies in which, for instance, all patients were treated with DBS (19, 20). On that account, we believe our patients represent an earlier disease stage, where patients cannot be distinguished from healthy participants in terms of ataxia. Nonetheless, the fact that we found a difference in brain activation despite the similarities in movement execution makes the cerebral difference even more interesting: the change in brain function may even precede related disease features such as ataxia, and, rather than being related to task performance, may be inherent to ET as a disorder.

In terms of anatomical location, the identified abnormal brain activation is located in a region that is suspected to play a major role in ET pathophysiology (9, 10). At first, it may seem unanticipated that we found increased instead of decreased cerebellar activations, because neurodegeneration, marked by Purkinje cell loss and torpedoes (27, 28), has been reported particularly in the cerebellum including the vermis specifically (29) in ET by some groups. Yet, we may be able to explain our findings by hypothesizing that the cells that are affected in ET are less efficient - being deficient and disorganized – and that this inefficiency may result in increased activations. Another hypothesis would be that the increase in cerebellar activity represents a compensational mechanism for decreased functionality, as our patients performed the task as well as the healthy participants. Moreover, increased cerebellar activations have been reported before dur-

ing simple motor tasks in ET (17)(see also Chapter 4). Why the increased activation is located in the vermal part of lobule 4/5/6 (motor cerebellum) is slightly puzzling, although it has been reported earlier that ET patients have 2-4 times higher torpedo counts in their vermis than healthy subjects (29), indicating that ET involves the spino- as well as the ponto-cerebellum. In the same study, the vermal torpedo count correlated positively with cerebellar hemispheric torpedo count, and with ET cases with head, voice and jaw tremor. Contrarily, in our population, only 1/19 patients had a head tremor, whereas 12/19 patients scored 1/4 points on the TRS' speech item: our patients do not score high on cranial tremor.

It is noteworthy that the difference in brain activation between patients and healthy participants was found specifically for the back-to-centre movements, rather than for out-of-centre or all movements. We speculate this may be because of our task set-up. The peripheral, out-of-centre targets were located quite far from the central target, meaning that participants hardly had to terminate their out-of-centre movement: the movement was terminated because the wrist/device would not allow further outward movement. Contrarily, on the back-to-centre movements, participants had to steer and stop more actively, which might be more demanding as a cerebellar task.
However, when comparing back-to-centre to out-of-centre movements within each group, we did not find any increases in activation, including the cerebellum. For the reverse comparison, out-of-centre versus back-to-centre movements, we found increased activations in the visual cortices, dorsolateral prefrontal cortex, and parietal cortex in both groups. This is probably caused by the fact that the appearance of the next back-to-centre target is predictable (always back to the centre), whereas the up-coming location of the out-of-centre target is unpredictable. The areas where activation was increased can therefore be explained as playing a role in increased visuospatial attention and planning (30-34) for the unpredictable out-of-centre movements.

LIMITATIONS AND STRENGTHS

The fact that our population was in a relatively mild disease stage, where most patients take medication but none would immediately consider deep brain stimulation, can be considered as a limitation to the applicability of our results. It would be interesting to repeat this study in patients in a more advanced disease stage, to see whether the found abnormalities are enhanced.
As mentioned above, it may be considered suboptimal that in our task set-up, we set our out-of-centre targets (too) far from the central target, decreasing the need to accurately terminate movement at these targets. However, this weakness was compensated by the other half of the performed movements, namely the back-to-centre movements, in which there was a need to actively terminate movements.
A strength of our study is the simultaneous measurement of task performance during scanning. By using an MR-compatible device, we were able to study goal-directed movements and their cerebral correlates at the same time.
Moreover, many scientific studies have focused on more severely affected ET patients. Our results can thus be seen as a valuable complimentary effort. The fact that we found cerebellar differences in a less-affected, less-progressed population makes these abnormalities even more distinctive.

CONCLUSION

To conclude, we found increased cerebellar activations related to goal-directed movements in mildly affected ET patients com-

pared to healthy participants, in the absence of differences in movement execution.

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CHAPTER 7

LIMITED CORRELATIONS BETWEEN CLINICIAN-BASED AND PATIENT-BASED MEASURES OF ESSENTIAL TREMOR SEVERITY

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ABSTRACT

INTRODUCTION We investigated the relation between changes in clinician-based and patient-based measures of tremor severity, within the Fahn-Tolosa-Marin Tremor Rating Scale (TRS) and Visual Analogue Scale (VAS) in essential tremor patients.

METHODS: Thirty-seven patients were assessed twice: on- and off-medication. Clinician-based, objective TRS assessments, consisting of part A (postures/movements) and part B (tremor-inducing tasks) were conducted by a blinded assessor using video-tapes. Patients completed TRS part C (limitations in activities of daily life) and indicated subjective tremor severity using VAS.

RESULTS: Patients' total TRS and VAS scores improved on-medication (both $p<0.001$). Mean improvement was 6.3 (sd 5.4) points on the total TRS and 2.3 (sd 2.3) points on the VAS score. Within the TRS, we found moderate correlations between changes in clinician-based TRS-B and patient-based TRS-C scores ($\rho=0.387$, $p=0.011$), but not between changes in clinician-based TRS-A and TRS-C scores ($\rho=0.128$, $p=0.232$). Moreover, changes in subjective VAS scores correlated with changes in total TRS ($\rho=0.422$, $p=0.007$), changes in TRS-C scores ($\rho=0.367$, $p=0.015$) and, more weakly, with changes in TRS-B scores ($\rho=0.281$, $p=0.049$), but again: not with changes in TRS-A scores ($\rho=-0.008$, $p=0.482$).

DISCUSSION: We found no correlation between changes in clinician-based TRS-A, and patient-based measures TRS-C or VAS scores, and a weak correlation between clinician-based TRS-B and VAS scores. The limited correlations between changes in clinician-based and patient-based measures of tremor severity suggest that the different scales measure different aspects of tremor severity and support the additional use of subjective patient-based assessments in clinical practice and clinical trials.

INTRODUCTION

The Fahn-Tolosa-Marin Tremor Rating Scale (TRS)(1) is well known and widely used as a tool to assess tremor severity in clinical trials (2-4). The TRS includes both clinician-based ratings in parts A and B, and patient-based ratings in part C (see Table 7.1). Part A comprises clinical assessment of tremor severity based on observation of tremor amplitude during rest, posture, movement and finger-to-nose manoeuvres. Part B entails clinical assessment of severity during tremor-inducing task performance, including writing, standardized Archimedes spirals, a line-drawing task and a water-pouring task. Together, parts A and B express tremor severity from a clinical point of view: an objective (impartial and unprejudiced) rating. Recently, a Movement Disorders Society task force recommended the use of the Fahn-Tolosa-Marin TRS, although it was expressed that parts A and B have been investigated more thoroughly, and that part C requires additional clinimetric study (5). Part C is patient-based, and consists of a structured interview where patients rate the limitations they experience in daily life due to tremor. This interview is rather time consuming, and is not always used in clinical studies. Therefore, some clinical trials rely quite heavily on the clinician-based parts of the TRS.

Here, we investigate how well neurologist-based, objective assessments of changes in tremor severity (TRS-A and TRS-B) correlate with patient-based, subjective assessments of changes (TRS-C and VAS) upon taking medication. Intuitively, one would suspect these to correlate well; however, these intuitions have not been tested and may be deceiving. Correlations between some parts of the TRS and quality of life have been investigated before (6), but never direct correlations between changes in objectively and subjectively assessed tremor severity.

METHODS

Thirty-seven essential tremor (ET) patients, who were participating in a neuroimaging study in the University Medical Center Groningen and the Academic Medical Center in Amsterdam, were assessed. The Medical Ethical Committees of both sites approved the study. Subjects participated after providing informed consent in accordance with the declaration of Helsinki (7). We included patients who had a definite diagnosis of ET according to the TRIG criteria (8): all had bilateral upper limb action tremor in

TABLE 7.1. DIFFERENT MEASURES OF TREMOR SEVERITY ON/OFF MEDICATION: CLINICIAN-BASED AND PATIENT-BASED

	TRS–A	TRS-B	TRS-C	VAS
TEST DETAILS	Clinician-based assessment of standardized postures and movements	Clinician-based assessment of writing and task performance	Patient-based assessment of limitations in daily life	Patient-based assessment of tremor severity
SCORING RANGE	0-24	0-32	0-32	0-10

TRS: tremor rating scale (Fahn-Tolosa-Marin), VAS: visual analogue scale.

the absence of other neurological signs, and in addition disease duration had to be >5 years. Age at onset was <65 years, thereby excluding late-onset ET, which may have a different pathophysiology (9). The other supportive TRIG criteria, which are a positive family history and positive response to alcohol, were present in most patients but were not required for inclusion. Patients were all right-handed as assessed by the Annett Handedness scale (10). All subjects scored >25 on the Mini Mental State Examination ensuring proper understanding of tasks and questions (11). Exclusion criteria were neurological comorbidity, the use of medication affecting the central nervous system, and, because of the related imaging study, MR-related contra-indications.

Patients were assessed twice: on- and off-medication. Patients had quit their medication minimally three days before off-medication testing. TRS-A and -B were recorded on video, and supplemented with the drawings (two standardized Archimedes spirals, three straight lines, a written standard sentence, signature and date). An experienced movement disorders specialist (Dr. J.D. Speelman, AMC), who was blinded to medication status, determined TRS scores based on this material. AWGB scored TRS-C for all patients from Amsterdam, and AMMS for all patients from Groningen, while they were aware of medication status. Patients indicated VAS scores on each visit by marking a 10 cm line ranging from 'no tremor at all' (0) to 'worst tremor imaginable' (10).

First, differences in tremor severity measures on/off-medication were assessed using paired samples-t tests for normally distributed data, as tested using the Shapiro-Wilk test. Correlations between changes in tremor severity measures were assessed using Pearson's correlation coefficient (ρ). We used one-tailed testing because we hypothesized that larger changes in TRS scores would be related to larger changes in VAS scores. Note that a sample size of 37 patients has a power of 0.8045 to detect correlations of 0.4 for α=0.05 (one-tailed) (12).

RESULTS

Thirty-seven ET patients participated in this study. Patients had a median age of 62 years (interquartile range 21, range 21-80) and a median age at onset of 22 years (interquartile range 34). The mean disease duration was 28 (sd 16) years. 92% of patients had a positive family history for tremor, whereas 43% reported a decrease of tremor upon alcohol-intake. Thirty-five patients were treated with propranolol, with a median dose of 80 mg daily (interquartile range 55), and two patients were treated with primidon. TRS scores varied, with a mean total TRS score of 25 (sd 9) off-medication, improving to a mean TRS score of 19 (sd 9) on-medication (p<0.001). The mean change in TRS score was 6 (sd 5, range from -4 to 20). Patient-perceived tremor severity, as measured with VAS scores, also varied from mild to severe with a mean off-medication score of 6.2 (sd 2), ranging from 2.1 to 9.5 on a 10-point scale. VAS scores improved on-medication to a mean score of 3.9 (sd 2.2, p<0.001). The mean change in VAS score was 2.3 (sd 2.3, range from -2.0 to 7.5).

Correlations are depicted in Figure 7.1. We found moderate correlations within the TRS between changes in clinician-based TRS-B and patient-based TRS-C scores, but not between changes in clinician-based TRS-A and TRS-C scores. Moreover, changes in subjective VAS scores correlated with changes in total TRS scores, changes in TRS-C and (weakly, <0.3) with changes in TRS-B scores, but again: not with changes in TRS-A scores.

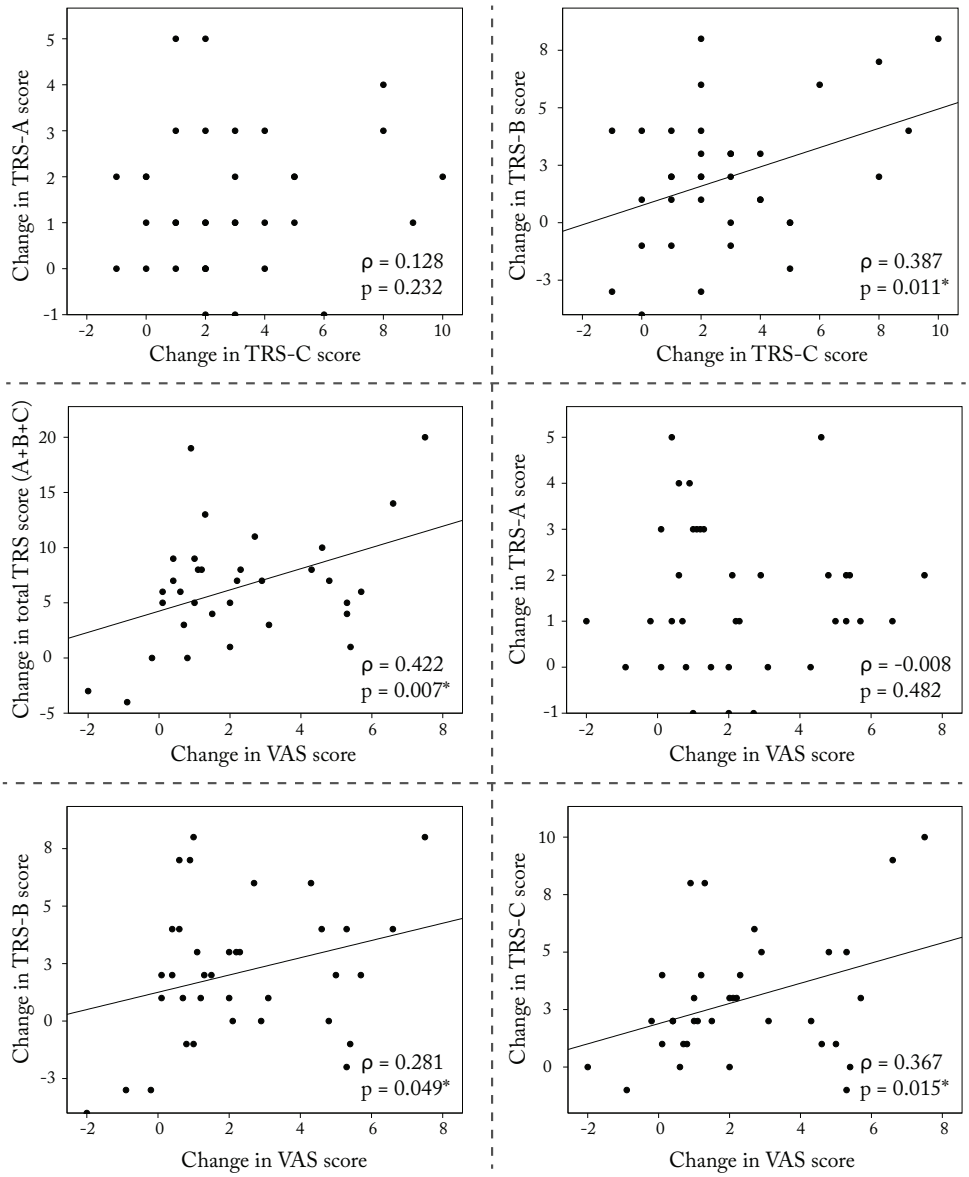

Figure 7.1. Scatterplots of the correlations between changes in clinician-based and patient-based measures of tremor severity. TRS: tremor rating scale (Fahn-Tolosa-Marin), VAS: visual analogue scale, ρ: Pearson's correlation coefficient, *: p<0.05. Linear regression lines are provided for significant correlations.

DISCUSSION

Overall, there is a moderate correlation between changes in tremor severity as rated in the combined three parts of the Fahn-Tolosa-Marin Tremor Rating Scale and subjectively experienced changes in tremor severity as expressed in VAS scoring.

However, when zooming in on our results, we find correlations between objective and

subjective measures of improvement in tremor severity to be limited. When using the TRS and VAS as measures for changes on/off-medication, we found no correlation between changes in part A and C scores *within* the TRS, or between changes in TRS-A and VAS scores. This indicates that patients appraise medication-related changes in their tremor quite differently than clinicians when performing standard tests including posture and finger to nose manoeuvres. This may be because TRS-A is known to be a crude measure (13), and because patients may base their impression of changes in tremor severity more heavily on abilities in daily life, rather than simply on tremor amplitude during standardized postures/movements. It is necessary to consider this result for clinical trials where tremor severity scores are used as outcome measures: our results show that a patient-based measure such as TRS-C or VAS adds important information on medication-related changes in tremor severity, in addition to clinician-based measures.

TRS-A and TRS-B include those tests that are typically done by most neurologists in the examination room to assess tremor. Our results suggest that although assessment of tremor during different postures/movements is key to tremor diagnosis (14), it is useful to recognize that changes in this assessment do not relate to patient-perceived improvement.

Regarding TRS-C, it is interesting to interpret our findings in relation to the scale that is recommended for quality-of-life assessment (QUEST)(5). QUEST was made specifically for ET (15), and assesses slightly different aspects of tremor impact than TRS-C: quality of life versus limitations in activities of daily life. QUEST was found to correlate with TRS-A/B in single measurements (6, 16), however, whether these correlations remain when assessing *changes in tremor severity* needs to be established. Some QUEST-subparts were also found to correlate with a subjective tremor severity measure that is comparable to VAS scoring (15, 17), similar to the correlation we found between TRS-C and VAS. Whether QUEST and TRS-C correlate is also unknown: TRS-C was not assessed in the mentioned studies because of the overlap between both measures. Overall, TRS-C and QUEST seem to share some characteristics, but it is impossible to say which scale is more useful as they have never been compared directly.

As a limitation, we would like to note that although TRS-A and –B were rated blindly; TRS-C and VAS scoring were performed while assessors were not blind to medication status. We cannot verify whether this induced bias. An optimal way to control for any bias would be to use a placebo-controlled, double-blind design. A strength of this study is that the same subscales were assessed by the same rater in each patient, avoiding the problem of inter-rater variability.

To summarize, we found no correlation between clinician-based TRS-A and patient-based assessments of change of tremor severity on/off-medication, and a weak correlation between changes in TRS-C and VAS scores. These findings carry implications for the use of patient-based assessments, in clinical practice and particularly in clinical studies: our results underline the importance of using subjective patient-based assessments alongside objective assessments.

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CHAPTER 8

DISCUSSION

In this thesis, we addressed two aims: to improve on diagnosis of tremor, and to investigate the pathophysiology of essential tremor by means of functional neuroimaging. In the following paragraphs, important findings related to these aims will be discussed. Next, I will share some general considerations connected to this work. Moreover, I will indicate future directions for follow-up research, and provide general conclusions.

DIAGNOSING TREMOR CORRECTLY

DISTINGUISHING TREMOR DISORDERS BY TYPICAL TREMOR PHENOMENA

It was a valuable endeavour to determine the sensitivity and specificity of tremor characteristics that are thought to be typical for a certain tremor disorder, as we did in Chapter 2. We demonstrated that the five phenomena that we investigated were indeed specific: a decrease in tremor frequency upon loading of the arm does point to enhanced physiological tremor (EPT), while signs of distraction, entrainment or an increase in tremor amplitude upon loading indicate a diagnosis of functional tremor (FT), and intention tremor suggests essential tremor (ET). Contrarily, it should be noted that not all characteristics were sensitive measures to identify their corresponding tremor disorder. This was the case for the measures related to changes in tremor appearance as a result of loading in both EPT and FT, and intention tremor in ET. Therefore, absence of these phenomena is not informative, and clinicians need to be aware of this fact when looking for these characteristics in the examination room. We would also like to point out that although specificity of all characteristics was high (85-95%) it never reached a hundred percent. An absolute one-to-one translation from phenomenon to tremor diagnosis is thus impossible. This is particularly important regarding FT: the reality is that 'functional' characteristics can occur in tremor that is ultimately diagnosed as 'organic'. This has been reported before (3-6), and illustrates that functional symptoms can occur in organic tremor (or other neurological disease (10)). Although the tremor phenomena have been discussed in separate studies (1-9), we are the first group to establish the prevalence of these phenomena in such a large and diverse tremor population, and we believe the information we presented in Chapter 2 will help clinicians to better distinguish different types of tremor.

CLINICAL NEUROPHYSIOLOGY TESTING FOR TREMOR DIAGNOSIS

The frequency-based phenomena that are mentioned in Chapter 2 and in the previous paragraph are best assessed by means of clinical neurophysiology testing. These tests are generally of great value (11, 12), as we also confirmed in Chapter 2. In Chapter 3, we explored the potential value of intermuscular coherence and cumulant analysis as additional diagnostic measures in the polymyographic assessment of postural tremor. This proved a valuable first exploration: coherence values differed between groups, and we were able to distinguish EPT patients by their low coherence, and we found characteristic muscle activity patterns in ET and PD. Apart from contributing to diagnosis, our results are also input to the discourse on pathophysiology in terms of contributions of central oscillators to tremor in ET, PT and FT, versus a larger role for a peripheral origin of tremor in EPT (13-15). Overall, our results do not directly translate to medical practice as of yet, as this was a retrospective study performed in 4 groups of 20 strictly selected patients. However, our investigation does provide indications for potentially fruitful follow-up studies, for instance in distinguishing ET and EPT.

PATHOPHYSIOLOGY OF ESSENTIAL TREMOR

FUNCTIONAL CEREBELLAR ABNORMALITIES IN ET

In our functional MRI studies, we found increased cerebellar activations in ET patients compared to healthy participants. Firstly, in Chapter 4, we demonstrated that increases in tremor severity over the course of an fMRI session correlate with increases in cerebellar activation in ET patients. We were able to identify specific bilateral areas in the cerebellum: in the left lobules V and VI, and right lobules V, VI, VIIIa and b. An early fMRI study, without simultaneous EMG recording, has reported bilateral cerebellar activation before (16), and our findings reinforce the results in this report as well as go beyond. Rather than diffuse bilateral activations (16), our tremor-related activations are specifically located in the somatomotor regions of the cerebellum (17, 18). It should be noted that these activations were specifically tremor-related, rather than movement related, as we correlated the brain activation in these areas with fluctuations in tremor severity over time independently of the movement task of holding the arm at a raised posture. This demonstrates the additional value of simultaneously recorded EMG, when it is mathematically manipulated to represent fluctuations in tremor independent from the task.

In Chapter 6, we again demonstrated increased cerebellar activations in ET patients compared to healthy participants while performing goal-directed movements. We added this task to investigate the brain activations in relation to the intentional component of essential tremor, in addition to the postural component studied in Chapter 4, to examine whether abnormal brain activation may be found in similar areas. Together, the results from these two complementary fMRI studies lend further support to the notion of underlying cerebellar pathology in ET, fitting with some of the evidence from neuropathology studies (19-22), as well PET imaging (23-25), and early MRI studies (16, 26).

At first, the fact that we found increased rather than decreased cerebellar activations may seem counter-intuitive in the light of the neurodegeneration hypothesis, because signs of neurodegeneration such as Purkinje cell loss and axonal swellings (torpedoes) have been reported in the cerebellar cortex in ET by some groups (19, 20), which seems more compatible with decreased than increased activation. However, we can explain our consistent finding of increased activation in ET by hypothesizing that the affected cells are deficient and disorganized, making them less efficient, and that this inefficiency leads to increased activations.

The idea of cerebellar pathology is further advanced by the results from our connectivity analyses. Regarding effective connectivity, tremor variation during the motor task has an excitatory effect on both the extrinsic connection from cerebellar lobule V to the thalamus, and the instrinsic activitiy of cerebellar lobule V and the thalamus. In addition, we found that functional connectivity between the cortical and cerebellar motor regions. This decrease in functional connectivity correlates with an increase in clinical tremor severity.

Overall, in this thesis, we report evidence of increased cerebellar activations related to fluctuations in postural tremor, increased cerebellar activations during goal-directed movement, excitatory intrinsic cerebellar activity when incorporating tremor variation during the motor task as modulator of intrinsic activity, and decreased functional connectivity between primary motor cortex and cerebellum, which is partly correlated with clinically assessed tremor severity. Combined, these results impart a major role of the cerebellum in ET.

A strength of the imaging studies is that we

measured aspects of task performance during scanning. In Chapter 4, we used EMG to derive tremor severity fluctuations over time, and used this measure in Chapter 5 as well. In Chapter 6, we used kinematic data to determine how participants performed goal-directed movement while being scanned. This way, we could directly correlate behavioural performance to cerebral (or cerebellar) activations, and were able to compare functioning of our patients and healthy participants. This matters for the interpretation of results: differences in brain function may be attributed to difference in performance, rather than interpreted as cerebral changes 'in itself'. In Chapter 6, we found that our patients did not perform different during the goal-directed movement task from their healthy counterparts. We expected a different performance based on current essential tremor studies where mild ataxia has been described. The lack of ataxia in our patients might be due to the limited duration and severity of their disease, although it can also be speculated that our method was not sensitive enough. In previous studies, more advanced patients were included (27, 28)). The fact that we found a difference in brain activation despite these similarities in behaviour makes the cerebellar difference even more interesting. This suggests that this change in brain function may even precede related disease features such as ataxia, and that it may be inherent to ET as a disorder.

GENERAL CONSIDERATIONS

SELECTING THE 'RIGHT' PATIENTS

In this thesis, we have used several different methods for patient inclusion, because to best answer our research questions, different inclusion methods were called for. In Chapter 2, we wanted to test sensitivity and specificity of tremor phenomena in 'the real world', which we felt meant in a varied tremor population, not only in the text book cases where there is zero doubt about diagnosis. Therefore, we put no constraints on inclusion, other than that patients had to have had a tremor-specific polymyography, and based our selection on the final clinical diagnosis made by the neurologist. Contrarily, in Chapter 3, we wanted to explore whether we could differentiate different types of tremor using coherence and cumulant analysis. Because of the explorative nature of this study we ascertained that diagnosis in all patients was maximally reliable, using clinical, neurophysiological and imaging inclusion criteria: ideal for an initial study. In the second part of this thesis, we aimed to select definite ET patients to study pathophysiology with functional imaging. All our patients met the core TRIG criteria (29) and additionally they met at least two supportive criteria: disease duration >5 years, and a positive family history and/or alcohol responsiveness. Moreover, we decided that age at onset had to be <65 years, thereby excluding 'senile' ET, which some consider to have a different pathophysiology (30).

Despite the fact that ET is supposed to be such a common movement disorder (31, 32) it was difficult to include definite ET patients. Despite several methods used to find patients, most patients who contacted us did not meet clinical criteria for ET. This illustrates the fact that, traditionally, the label 'ET' has been used and misused as a 'container' diagnosis for all types of tremor that did not fit any particular diagnosis. This situation has improved over the last two decades with the establishment of successive clinical criteria (29, 33), but has not dissolved entirely.

LIMITATIONS OF FMRI ANALYSIS

It is necessary to consider limitations of fMRI analysis to appreciate fMRI results. Here, I focus on interpretation issues concerning group analysis, because this applies to the work on ET presented in this thesis. An (im-

plicit) assumption in the analysis of groups of participants is the assumption of universality: the idea that spatio-temporal dynamics of brain functions have a high degree of uniformity within a population (34). The most commonly used group-analysis methods treat the overlapping activations shared across subjects within the group as true activation (35). Activations that are not shared, and occur only in one participant or a subset of participants are thus considered as noise. Therefore, the significant supra-threshold results depicted in figures and tables may be incomplete, missing activations that occur in a subset of the group, i.e.: may represent false negatives. Contrarily, it has been shown that if only a part of the population shares a certain activation, but with a very strong effect size, such activation may reach a significant level, and is therefore ascribed to the entire group. As such, this method of group-level inference generates false positive findings, as well (34). In the fMRI studies described in this thesis, we were much aware of these issues. The chance of underlying differences in brain activation between ET patients is even more likely than in a group of healthy participants, given the heterogeneity of the disorder (discussed in more detail later on). As a consequence, we paid ample attention to the single-subject (first level) results in our ET patients, particularly in Chapter 4. Macroscopically, we did not find different patterns of activations in our ET patients, although we did see that the effect sizes of the described activations differed between subjects. Conjunction analysis may be of additional value in future analyses (36), as it allows the description of activation on group level while at the same time providing information about how frequently the activations occurred within the group.

FUTURE DIRECTIONS

FUTURE DIRECTIONS IN RESEARCH AIMED AT TREMOR DIAGNOSIS & PHENOMENOLOGY

In terms of continuing our own research, the retrospective study we describe in Chapter 2 should be repeated in a prospective and blind study. A complication in organizing such a study is that this is not how everyday clinical practice works: the clinical neurophysiologists are not blind to the request that was sent in, and this helps them focus their polymyography report. To achieve a prospective blind study, we would need to change our clinical practice for approximately five years to get to the same number of patients. A multi-centre approach would reduce this period.
A prospective, blinded follow-up coherence and cumulant study should be conducted. Such a prospective study is useful; interesting questions to answer are whether the cut-off values for coherence remain sensitive and specific, particularly in distinguishing EPT, and whether the cumulant density functions we found in ET and PD remain typical.

Related to this topic of tremor diagnosis is the definition of ET that remains problematic. Over the past years, a debate has evolved whether it is possible to define ET as a single disease entity (37, 38), or whether ET is better understood as a family of diseases (39-41). The variation and complexity of signs and symptoms present in ET appears to be larger than was previously believed, with recent attention for age at onset (42), rate of progression (43, 44), alcohol responsiveness (45), head tremor (44, 46), resting tremor (47, 48), intention tremor (7-9), gait ataxia (49-51), limb ataxia (27, 52), eye movement abnormalities (53, 54), dystonia (55), and non-motor symptoms (56). It has been hypothesized that the heterogeneity in ET phenomenology has led to heterogeneic findings, and that the lack of adequately defined ET subtypes detains scientific advancements regarding

disease mechanism(s) and treatment.
As a follow-up to our imaging study, we have recently started to map phenomenology in families related to the patients who participated previously. The aim is to investigate the level of diversity within families, to examine which phenomena are familial, and whether patterns of disease progression can be found within families that may differ between families. Ultimately, such phenotypical characterization could lead to a better definition of disease subtypes, and a potential gateway to improved, more powerful neuroimaging and particularly genetic studies.

FUTURE DIRECTIONS IN NEUROIMAGING RESEARCH OF ESSENTIAL TREMOR

Apart from the suggestions regarding the definition and comparison of ET subtypes, functional neuroimaging will benefit from investigation of different tasks. In relation to our own work, a logical next step is to investigate a task where ET patients raise their left arm instead of their right arm, to investigate lateralisation effects in such a bilateral tremor disorder. Moreover, we are currently investigating a different postural task that may maximize postural tremor. Another suggestion, regarding the investigation of ataxia, is to see what abnormalities in brain activations can be found in more advanced ET patients, during goal-directed movement or other ataxia-related tasks such as diadochokinesis.

CONCLUSIONS

To conclude, we advanced the proper diagnosis of tremor in the clinical and clinical neurophysiological setting, by establishing sensitivity and specificity for typical tremor phenomena and exploring the additional value of intermuscular coherence and cumulant analysis. Secondly, we added to the debate on the pathophysiology of ET, with our results of increased cerebellar activations related to tremor and goal-directed movement, and changes in cerebellar connectivity, which lend important new support to the notion of underlying cerebellar abnormalities in ET.

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NEDERLANDSE SAMENVATTING:
DIAGNOSTIEK & BEELDVORMING VAN ESSENTIËLE EN ANDERE TREMOREN

SUMMARY IN DUTCH

Tremor, het ongewild trillen van de handen, is de meest voorkomende bewegingsstoornis bij volwassenen. De meeste patiënten die zich met een tremor presenteren hebben dit symptoom in het kader van de ziekte van Parkinson (PT), dan wel een essentiële tremor (ET), een versterkt fysiologische (VFT) tremor of een functionele tremor (FT). Het type tremor dat bij het algemene publiek het meest bekend is, is PT: zodoende zijn veel patiënten in eerste instantie bezorgd dat ze aan de ziekte van Parkinson lijden. Het is van belang om de ene vorm van tremor van de andere te onderscheiden, aangezien dit consequenties heeft voor de prognose en de behandeling. De prognose varieert van een relatief milde, mono-symptomatische en niet-progressieve aandoening zoals bij VFT, tot een gecompliceerde, progressieve en levensbekortende aandoening als de ziekte van Parkinson. Ook de behandeling van verschillende tremoren loopt sterk uiteen.

Bij het stellen van de juiste diagnose is de anamnese en het lichamelijk onderzoek door een neuroloog van primair belang. Hierbij moet echter worden opgemerkt dat de klinische diagnose soms lastig kan zijn doordat niet alle patiënten een klassieke presentatie laten zien. De fenomenologie van tremor is complex, en bestaat uit een variëteit aan tekenen en symptomen. Sommige tre morsyndromen zouden één of meer typische kenmerken hebben die op de diagnose zouden wijzen: in **Hoofdstuk 2** beschrijven we het onderzoek naar de sensitiviteit en specificiteit van vijf van deze 'typische' tremorfenomenen. Dit deden we door retrospectief 210 patiënten te onderzoeken die tussen januari 2008 en januari 2014 werden verwezen voor elektrofysiologisch onderzoek ter diagnostiek van hun tremor. Hierbij gebruikten we de uiteindelijke klinische diagnose, na klinisch neurofysiologisch en overig aanvullend onderzoek, als gouden standaard. De vijf fenomenen die werden onderzocht waren het zich voordoen van een afname in tremorfrequentie na het verzwaren van de trillende hand (typisch voor VFT), een toename van de tremor amplitude na verzwaren, het voorkomen van afleidbaarheid en het voorkomen van entrainment (typisch voor FT), en het zich voordoen van een intentietremor (typisch voor ET). De prevalentie van deze feno-menen werd vergeleken tussen de 'typische' groep en de hele groep. Frequentie-afname na verzwaren bij VFT, amplitudo-toename na verzwaren bij FT en het voorkomen van een intentietremor bij ET bleken alle drie fenomenen die specifiek waren voor het bij- behorende tremortype, maar niet erg sensitief. Het voorkomen van afleidbaarheid en entrainment bleken zowel specifiek als sensitief voor FT. Zodoende kunnen we stellen dat met name de áánwezigheid van typische tremorkarakteristieken van grote w aarde is bij het onderscheiden van verschillende tremorsyndromen. Daarnaast is het goed te realiseren dat hoewel voor de fenomenen afleidbaarheid, entrainment en een toename van de tremoramplitude na verzwaren geldt dat ze een hoge specificiteit kennen voor een functionele origine, deze specificiteit niet 100% is: functionele kenmerken kunnen voorkomen bij 'organische' tremorsyndromen. Deze informatie over tremorfenomenologie kan praktiserend neurologen helpen om een betere klinische diagnose te stellen.

In **Hoofdstuk 3** wordt verder ingegaan op de diagnostiek van tremoren. Naast het stellen van de diagnose op basis van anamnese en neurologisch onderzoek kan desgewenst aanvullend onderzoek gedaan worden. Een belangrijke rol is hierbij weggelegd voor klinische neurofysiologisch onderzoek middels oppervlakte-EMG. In Hoofdstuk 3 onderzochten we of twee complexe EMG-maten van toegevoegde waarde zouden

kunnen zijn voor de polymyografische beoordeling van houdingstremor. De onderzochte maten waren intermusculaire coherentie, een maat voor de afhankelijkheid tussen twee signalen, en intermusculaire cumulant-analyse, waarmee iets gezegd kan worden over de patronen van synchroniciteit tussen twee spieren in EMG-activiteit. We maakten een vergelijking tussen groepen patiënten met ET, PT, VFT en FT, de vier tremortypen die in het UMCG het meest gezien worden, en vonden dat de intermusculaire coherentie het hoogste was in de groep patiënten met PT, gemiddeld bij ET en FT, en zwak bij VFT. De VFT-patiënten konden op basis van hun lage coherentiewaarden worden onderscheiden van de andere patienten, met redelijke sensitiviteit en specificiteit. De lage waarden zijn passend voor dit type tremor, aangezien bij VFT een perifere component een relatief grote rol speelt, wat de coherentie van activiteit tussen verschillende spieren verlaagt. Wat de intermusculaire cumulant-analyse betreft werd gevonden dat er in het spierpaar van pols- en elleboog-extensoren in ET-patiënten met name alternerende activiteit werd gevonden, terwijl PT-patiënten vooral een synchroon patroon lieten zien. Deze resultaten suggereren dat coherentie- en cumulant-analyse van toegevoegde waarde kunnen zijn in de diagnostiek van houdingstremor, waarschijnlijk vooral bij patiënten bij wie na onderzoek met conventionele maten onzekerheid blijft bestaan.

Na Hoofdstuk 3 verschuift de focus van dit proefschrift naar de diagnostiek van verschillende soorten tremor, naar een bepaald type tremor, namelijk essentiële tremor (ET). Eerder, onder andere pathologisch onderzoek naar de pathofysiologie van ET suggereert dat er sprake zou zijn van cerebellaire afwijkingen. In **Hoofdstuk 4** gebruikten we een innovatieve techniek om de pathofysiologie van ET in vivo te onderzoeken: we voegden EMG als maat voor fluctuaties in tremor toe aan functionele MRI (EMG-fMRI). Op deze manier waren we in staat tegelijkertijd de perifere manifestatie van tremor als de centrale aansturing te onderzoeken. We bekeken een homogene groep ET-patiënten die voor het onderzoek tijdelijk hun tremormedicatie lieten staan. Liggend in de MRI-scanner voerden patiënten een bewegingstaak uit, waarbij ze hun rechterarm en –hand optilden en extendeerden, waardoor houdingstremor geïnduceerd werd, afgewisseld met rust. Een zelfde aantal op leeftijd en geslacht gematchte gezonde deelnemers voerde een zelfde taak uit, alleen simuleerden zij daarbij het trillen tijdens het optillen van de arm. Gedurende deze taak werd zowel EMG als fMRI opgenomen. De variabiliteit in EMG-power op de individuele tremorfrequentie werd als regressor gebruikt in de fMRI-analyse, daarbij wiskundig onafhankelijk gemaakt van de bewegingstaak zelf, om zo specifieke tremor-gerelateerde hersenactiviteit vast te stellen. We vonden bewegingstaak-gerelateerde hersenactiviteit in het klassieke motornetwerk van de bovenste extremiteit, zowel in ET-patiënten als in gezonde deelnemers. Wat betreft de specifieke tremor-gerelateerde activiteit, die vonden we bij ET-patiënten vooral bilateraal in het cerebellum: in de linker lobuli V en VI, en in de rechter lobuli V, VI en VIIIa en b, en in de hersenstam. Bij de gezonde deelnemers werd alleen activiteit gerelateerd aan de gesimuleerde tremor gevonden in het ipsilaterale cerebellum in lobulus V. Deze resultaten zijn een uitbreiding op eerdere bevindingen van cerebellaire betrokkenheid in ET: we vonden hier specifieke gebieden in de bilaterale somatomotore gebieden van het cerebellum. Onze hypothese is dat de cerebellaire cortex gedesorganiseerd is in ET, en dat dit leidt tot abnormale cerebellaire activiteit.

In **Hoofdstuk 5** breiden we het onderzoek naar de pathofysiologie van ET verder uit: na het EMG-fMRI onderzoek in Hoofdstuk 4, wordt hier ingezoomd op de dynamiek in het cerebello-thalamo-corticale netwerk. Het eerste doel hierbij was om de intrinsieke activiteit van de verschillende gebieden in dit netwerk te onderzoeken, waarbij we 'Dynamic Causal Modelling' (DCM) gebruikten om de door het tremor signaal (zoals gemeten middels EMG) gedreven effectieve connectiviteit te schatten. Het tweede doel was om te bepalen hoe de functionele connectiviteit van het cerebello-thalamo-corticale netwerk is aangedaan in ET. Dezelfde twee groepen uit Hoofdstuk 4 werden hier nader bestudeerd. Voor de effectieve connectiviteitsanalyse werd op basis van de gevonden activiteit in de bewegingstaak een netwerk geconstrueerd, bestaand uit de linker primaire motorcortex, premotorcortex, supplementaire motorcortex, linker thalamus en rechter cerebellaire motor gebieden in lobulus V en lobulus VIII. Dezelfde maat voor tremorvariabiliteit als in Hoofdstuk 4 werd hierbij gebruikt als modulerende input. De resulteerde 128 mogelijke modellen werden middels technieken gebaseerd op Bayesiaanse statistiek onderzocht. We vonden dat tremorvariabiliteit gedurende de bewegingstaak een excitatoir effect heeft op zowel de extrinsieke connectie van cerebellaire lobulus V naar de thalamus, als de intrinsieke activiteit van diezelfde lobulus V en de thalamus. Voor de functionele connectiviteitsanalyse werden separate seed-based analyses gedaan voor de linker primaire motor cortex, supplementaire motorcortex en rechter lobuli IV, V, VI en VIII. Hieruit kwam naar voren dat de functionele integriteit van het motornetwerk aangedaan is in ET, met een afgenomen functionele connectiviteit tussen de corticale en cerebellaire motorgebieden. Deze afname in functionele connectiviteit correleert met de klinisch beoordeelde ernst van de tremor. Daarnaast correleerde een toename van functionele connectiviteit tussen ipsilaterale cerebellaire lobuli I-IV en de contralaterale thalamus ook met de ernst van de tremor. Deze resultaten laten zien dat naast de cerebellaire activiteit, ook de cerebello-corticale connectiviteit verstoord is in ET.

In **Hoofdstuk 6** wordt extra aandacht besteed aan de aansturing van doelgerichte bewegingen in ET. In Hoofdstuk 2 hebben we gevonden dat intentietremor bij 1 op de 3 ET-patiënten voorkomt, en zo zijn er meer bijzonderheden beschreven bij doelgerichte bewegingen in ET. Klinisch worden dergelijke symptomen gerelateerd aan cerebellaire aandoeningen. In Hoofdstuk 6 was het ons doel om te onderzoeken hoe doelgerichte bewegingen geassocieerd zijn met abnormale herseninactiviteit in ET, door patiënten en gezonde deelnemers een doelgerichte-bewegingstaak te laten uitvoeren met een MR-compatibel polsdevice. We registreerden de coördinaten van de bewegingen gedurende het scannen en kon den zo de reactietijden en variabiliteit van de bewegingstrajecten bepalen. De doelstimuli werden vervolgens in de fMRI-analyse gebruikt als 'events', om op die manier doelgerichte bewegingen te correleren met hersenactiviteit. De deelnemende ET-patiënten waren mild aangedaan. We vonden geen verschillen in de uitvoer van de bewegingen tussen de ET-patiënten en de gezonde deelnemers. Desondanks vonden we toch toegenomen hersenactiviteit in cerebellaire vermis 4-6 in ET-patiënten. Deze resultaten bevestigen eerdere bevindingen van toegenomen cerebellaire activiteit gedurende houdingstaken, en ondersteunen de gedachte van onderliggende cerebellaire veranderingen in ET.

In de laatste publicatie van dit proefschrift, **Hoofdstuk 7**, verplaatsen we onze aandacht naar de beleving van de patiënt met ET. ET

wordt geregeld een 'benigne aandoening' genoemd, maar in gemiddelde tot gevorderde ziektestadia kan ET zowel fysiek als sociaal beperkend zijn. De Fahn-Tolosa-Marin Tremor Rating Scale (TRS) is een bekende en veel gebruikte maat om de ernst van ET te meten, bijvoorbeeld in klinische trials naar de effectiviteit van medicatie. De TRS bestaat uit zowel dokter-gebaseerde onderdelen in deel A (houdingen/bewegingen) en B (tremor-inducerende taken), als een patiënt-gebaseerde vragenlijst in deel C (beperkingen in het dagelijks leven). Dit laatste onderdeel is vrij tijdrovend en wordt niet altijd gedaan in klinische trials. Een andere patiënt-gebaseerde manier om de ernst van de tremor te meten is middels een Visueel Analoge Schaal (VAS). Intuïtief valt te verwachten dat dokter-gebaseerde en patiënt-gebaseerde maten goed met elkaar correleren: in Hoofdstuk 7 was het ons doel om te onderzoeken in hoeverre dit inderdaad het geval is. Bij de patiënten die meededen aan het EMG-fMRI-onderzoek beschreven in Hoofdstuk 4-6 werden tweemaal een volledige TRS en een VAS-score afgenomen, éénmaal zonder en éénmaal met medicatie: zo waren we in staat om veranderingen in ernst te meten met verschillende maten. TRS-A en TRS-B werden op video opgenomen en beoordeeld door een neuroloog die geblindeerd was voor medicatiestatus. TRS-C werd door een arts-onderzoeker gescoord. Allereerst stelden we vast dat de totale TRS en de VAS-scores verbeterden bij gebruik van medicatie, zoals verwacht mag worden. We vonden geen correlatie tussen verandering in dokter-gebaseerd onderdeel TRS-A en patiënt-gebaseerde maten TRS-C of VAS-scores, en een zwakke correlatie tussen dokter-gebaseerd onderdeel TRS-B en VAS-scores. Deze beperkte correlaties tussen maten van tremor ernst vanuit het perspectief van de dokter en het perspectief van de patiënt suggereren dat de verschillende schalen verschillende aspecten van tremor ernst meten, waardoor het zeer aan te raden is een subjectieve, patiënt-gebaseerde beoordeling toe te voegen aan de dokter-gebaseerde beoordeling in de klinische praktijk en in klinische trials.

Samenvattend worden in dit proefschrift enerzijds handvatten geboden voor de diagnostiek van verschillende soorten tremor, en wordt er anderzijds bewijs geleverd voor verstoorde, verhoogde cerebellaire activiteit in ET, gerelateerd aan variabiliteit in tremor gedurende een houdingstaak en in het kader van doelgerichte bewegingen, alsmede een verstoring van de connectiviteit in het cerebello-thalamo-corticale netwerk. Deze kennis is aan de ene kant direct van toegepaste waarde in de dagelijkse klinische praktijk en draagt aan de andere kant bij aan het begrip van de pathofysiologie van essentiële tremor.

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Shakespeare.