Tracing tremor: Neural correlates of essential tremor and its treatment
Buijink, A.W.G.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 10

Summary and discussion
Part I: neural correlates of essential tremor

The findings described in this thesis, provide evidence for perturbed activity within the cerebellar system in ET, in the absence of clear structural cerebellar changes. ET is a highly prevalent and heterogeneous disorder, with no currently known causative genetic mutations. Whether ET can be seen as one single disease entity is progressively questioned. Although the clinical characteristics among ET patients are quite similar, the aetiology might differ significantly, leading to ambiguous results of previous studies on the pathophysiology of ET. Therefore, we included a homogeneous group of familial, propranolol sensitive, ET patients throughout this thesis.

Functional changes in the cerebellar system of essential tremor

In our selected group of propranolol sensitive, familial ET patients, we have identified areas with brain activation which are correlated to tremor intensity. These include specific areas within the bilateral somatomotor regions of the cerebellum. This is in line with our hypothesis that the cerebellum plays an important role in ET. Tremor-related activity was located within two distinct regions, namely in lobules V-VI and in lobule VIII of the right cerebellum, ipsilateral to the correlated tremor intensity of the right hand, using a combination of EMG and fMRI. 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 (Chapter 3). These results expand on earlier findings that the bilateral cerebellum is involved in ET. To observe how these ET-related brain activations arise, and possibly give rise to tremor, we have subsequently studied the cerebello-thalamo-cortical network, or ‘tremor’ network, using effective and functional connectivity analyses in Chapter 4. Using an effective connectivity analysis called Dynamic Causal Modelling (DCM), we have explored how observed brain activations are generated by estimating the effective connectivity between and within specified regions of interest. Using tremor variation measured during scanning as an input, the effective connectivity analysis demonstrated an excitatory effect on several regions within the motor system. This effect was present for the extrinsic cerebellar outflow tracts to the thalamus (entailing the dentate nucleus), and intrinsic cerebellar and thalamic activity (Figure 1). Furthermore, a functional connectivity analysis was employed, investigating the functional connections between cerebellar and
cortical motor regions. Decreased functional connectivity between the motor cerebellum and the primary motor cortex was observed in essential tremor compared to controls during a motor task (Figure 2). A functional ‘disconnection’ between the cerebellum and cerebral cortex has been found previously in ET. Furthermore, increased functional connectivity between the motor cerebellum and the thalamus was observed, correlated with increasing tremor severity in ET patients.

*Figure 1. Influence of tremor variation on regions within the motor network using Dynamic Causal Modelling.* Graphical representation of the significant estimated connectivity parameters resulting from Bayesian Model Averaging in essential tremor. Coupling parameter strength is depicted in red (excitatory effect) and blue (inhibitory effect). M1 = left primary motor cortex; SMA = left supplementary motor area; PMC = left premotor cortex; Thal = left thalamus; CB V = right cerebellar lobule V; CB VIII = right cerebellar lobule VIII. Adapted from Figure 3, Chapter 4 (Buijink et al. *Brain* 2015).

Our findings of tremor-related activity located in the cerebellar cortex (Chapter 3) and hyperactive cerebellar outflow tracts (Chapter 4) can be placed in a broader framework of pathological changes within the cerebellar system (Figure 2). GABAergic cerebellar dysfunction and pathological Purkinje cell changes have been suggested by previous studies. Purkinje cells form the sole output channel from the cerebellar cortex, and lead to the deep cerebellar nuclei, including the dentate nucleus. With their GABAergic synapses, Purkinje cells strongly regulate the intrinsic activity of the dentate nucleus. Electrophysiology in vitro data indicate that neurons within the dentate nucleus possess
Neurotransmission dysfunction and possible neurodegeneration within the cerebellar cortex lead to altered GABAergic output. This causes disinhibition of the dentate nucleus, altering its pacemaker-like activity. Consequently, pathological activity is passed onward towards the thalamus through dentate nucleus afferents, entraining the cerebello-thalamo-cortical network. Due to altered cerebellar output, normal motor activity is disturbed.

Tremor could consequently result from a disinhibited dentate nucleus and subsequent pathological entrainment of the cerebello-thalamo-cortical network (Figure 2). The observed increased functional connectivity between the cerebellum and the thalamus, correlated with increasing tremor severity, supports this. Our findings of excitatory activity within the cerebellar outflow tracts in the context of tremor variation during the motor task may be explained as a result of loss of GABAergic tone in the
cerebellar system (Figure 2). Whether the cerebellar cortical pathology is secondary to changes in the dentate nucleus, or vice versa, remains controversial.

**Is normal cerebellar motor function impaired in essential tremor?**

In Chapter 4, we have used a rhythmic finger tapping during fMRI scanning to actively engage the cerebellar motor circuitry of ET patients. We found a positive correlation between dentate nucleus activation and clinical tremor severity (Figure 3). Additionally, ET patients showed reduced activations in widespread cerebellar and cortical regions and the inferior olive nucleus compared to healthy controls. A decrease in cerebellar activations has previously been described during a similar finger tapping task in patients with spinocerebellar ataxia. This decrease in activation might be a consequence of limited functional reorganization in spinocerebellar ataxia. Similarly, one would expect that a dysfunctional cerebellar cortex in ET leads to a decrease of ‘healthy’ cerebellar motor activity within these regions. This could consequently lead to increased dentate nucleus activity, as observed in the positive correlation between dentate nucleus activity and clinical tremor severity. Subsequently, this might cause functional changes in areas outside the cerebellum. Again, whether the cerebellar cortical pathology is secondary to changes in the dentate nucleus, or vice versa, is unknown. In Chapter 3, decreased cerebello-cortical functional connectivity with increasing tremor severity was observed during a motor task, supporting the hypothesis that increasing tremor severity proportionally disrupts physiological motor-related activity. The increased dentate nucleus activity associated with tremor severity observed in Chapter 4 matches this hypothesis (Figure 3). 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 ET, and increased 11C-flunazenil binding to GABA-receptors has also been found in the ventrolateral thalamus and lateral premotor cortex in essential tremor. However, in the case of tremor interference, one would possibly expect increased cerebello-cortical coupling due to entrainment of the cerebello-thalamo-cortical network, instead of a decrease in functional connectivity between cortical and cerebellar motor regions (Chapter 3). Alternatively, this entrainment could generate improper output onto the thalamus and consequently disrupt physiological motor-related activity (Chapter 4, Figure 2). Coherence studies using EEG combined with EMG have shown that cortical involvement in tremor is only intermittent, and therefore does not seem to be a crucial player within the tremor network.
Figure 3. Dentate nucleus activation correlated with increasing clinically-assessed tremor severity in ET. Projected on a three-dimensionally rendered dentate nucleus mask (small volume correction, p < 0.05 FWE corrected).

Absence of structural changes in essential tremor

As ET has been hypothesized to be a neurodegenerative disease, with evidence for microscopic cerebellar changes, many structural imaging studies have been performed with the interest to objectify whether macroscopic structural changes are present or absent in ET. Previous studies show a diverse and incongruent picture of cortical and cerebellar changes.50 Another neurological disorder previously associated with Purkinje cell changes and cerebellar atrophy, is FCMTE (familial cortical myoclonic tremor with epilepsy).184,205,206 FCMTE is an autosomal dominant condition characterized by distal tremulous action myoclonus, epilepsy and signs of cortical hyperexcitability.204–206 Clinically, the tremulous movements of FCMTE can be confused with ET.101 Considering the fact that Purkinje cells are hypothesized to be affected in both ET and FCMTE, morphological grey matter changes in specific ET subgroups were investigated and compared with FCMTE patients and healthy controls in Chapter 5. In our large sample of propranolol-sensitive familial ET patients, there was no decrease in cortical or cerebellar grey matter compared to age matched healthy controls. In ET patients with head tremor, we can even report a volume increase in cortical regions, involved in head movement. This also supports our hypothesis that macroscopic atrophy is not a feature of, at least familial, ET. In contrast, in patients with FCMTE, we did report widespread localized cerebellar grey matter reduction, compared to healthy controls and ET patients, underlining the usability of this technique to identify localized atrophy in disorders associated with Purkinje cell changes.

In Chapters 3 and 4, we observed that activity of cerebellar outflow tracts is perturbed in ET. In this context, it is interesting to study microstructural damage of cerebellar white
matter using DTI. In Chapter 6, we have compared cerebellar white matter composition between a group of FCMTE patients, healthy controls and ET patients. We have observed decreased cerebellar fractional anisotropy in the cerebellar white matter of FCMTE patients compared to ET patients and healthy controls, suggesting structural changes within these structures in FCMTE, and not in ET. This provides additional support for our hypothesis that the cerebello-dentato-thalamic system is functionally, but not structurally, disrupted in ET. Both Chapters 5 and 6 support the hypothesis that atrophy is not a hallmark of ET.

Anatomical location of changes within the cerebellum

The cerebellum can be divided in several ways, based on anatomical and physiological features. Commonly the cerebellum is divided into an anterior-posterior direction in the anterior, posterior and flocculonodular lobes. 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. Both the anterior and posterior cerebellum are involved in motor control. However, although both involved in motor control, they probably exhibit different functions. An interesting old example is the autosomal recessive ‘leaner’ mutation in mice, which results in severe ataxia accompanied by Purkinje and Golgi cell loss. However, this loss is much more apparent in the anterior lobe, whereas other parts of the cerebellum are relatively spared. This example illustrates that such a subdivision would be interesting to study in ET. Yet to our knowledge, the anterior and posterior cerebellum, are not discussed separately in previous ET research. Throughout this thesis we have tried to separate tremor-related effects related to the anterior and to the posterior cerebellum. Both the anterior and posterior cerebellum showed activations related to tremor. However, excitatory intrinsic activity related to tremor was present within the anterior cerebellum, whereas the posterior cerebellum exhibited inhibitory intrinsic activity related to tremor. For future studies, especially pathology studies, it might be useful to separate the involvement of the anterior and posterior cerebellum in ET.

Part II: neural correlates of treatment of essential tremor

In Chapters 7 and 8, different aspects of ET treatment have been investigated. Insights into the mechanism of action of these treatments shed light on the underlying pathophysiology. ET probably emerges from oscillations within the cerebello-thalamocortical network, ultimately manifesting peripherally in tremor. Both propranolol (Chapter 7) and thalamotomy (Chapter 8) intervene at different levels within the tremor...
network. For both treatments, tremor often does not completely resolve,\(^9\) suggesting that the underlying network oscillations are still intact.

**How does propranolol affect tremor?**

The tremorolytic mechanism of action of propranolol in essential tremor is unknown. It has been postulated that propranolol alleviates tremor by altering the sensitivity of muscle spindles.\(^{11}\) By applying continuous perturbations, inducing stretch reflexes, it is possible to characterize motor behaviour and determine the effect of propranolol on motor control.\(^{51}\) Using a combination of system identification and neuromuscular modelling, muscular and reflexive contributions can be separated, and specific effects of propranolol on different parts of the motor system dissociated.\(^{51,52}\) We have demonstrated an effect of propranolol on several modelled reflex parameters in patients with ET (Chapter 7). In the propranolol condition, patients exhibited increased muscle elasticity during active muscle contraction and a decrease of reflexive feedback. Furthermore, there is a trend towards a lower maximum voluntary contraction in the propranolol condition. Our results suggest that there is an increase of tonic muscle activity and a decrease of reflexive activity. We suggest that propranolol affects the tremor network at several locations within the central nervous system, with an important role of Renshaw cells within the spinal cord (Figure 4, next page). Increased inhibition of Ia interneurons by Renshaw cells leads to decreased inhibition of antagonistic motor neurons and a less effective reflex response. Furthermore, Renshaw cells recurrently inhibit agonistic motor neurons, further decreasing the effectiveness of the reflex response. Renshaw cells themselves are not known to possess beta-receptors. However, it has been known for a long time that locus coeruleus activity suppresses Renshaw cell discharges.\(^{263}\) Beta-agonists have been shown to increase the firing rate of the locus coeruleus and is known to possess adrenergic receptors.\(^{13,264–266}\) We therefore hypothesize that increased activity of Renshaw cells through beta-blockade leads to partial attenuation of tremor at the spinal cord level.

**What are the long term structural effects outside the thalamus after thalamotomy?**

Deep brain stimulation and thalamotomy are both used in alleviating tremor, and act at a more central level, disrupting the tremor network in the thalamus. It has been suggested that the effect of treatment of deep brain stimulation decreases in some patients over time, in contrast to patients treated with thalamotomy.\(^{16}\) Using DTI in Chapter 8, we report that the tracts leading from the cerebellum to the thalamus, running through the superior cerebellar peduncles, are affected after thalamotomy, which is reflected in decreased
fractional anisotropy and increased mean diffusivity values in the superior cerebellar peduncle on the side leading to the lesioned thalamus. This suggests that long-term structural changes after thalamotomy are not limited to the thalamus, but are more widespread, possibly due to retrograde “dying-back” of axons leading to the lesioned thalamus. As mentioned previously, tremor symptoms often seem to progress again over time in a considerable number of patients after deep brain stimulation, contrary to thalamotomy. In Chapter 3, we have observed hyperactive cerebello-dentato-thalamic tracts in the context of varying tremor severity. Combining this with the finding of structural changes within these tracts after thalamotomy, one could hypothesize that the additional lesioning effect of the cerebello-dentato-thalamic tracts after thalamotomy plays a significant role in why disease progression is less severe over time in patients after thalamotomy versus deep brain stimulation.

Figure 4. Simplified neuromusculoskeletal model explaining the possible effect of propranolol on Renshaw cells. Renshaw cells, normally inhibited by activity of the adrenergic locus coeruleus, increase motor neuron inhibition, possibly through decreased locus coeruleus activity. Subsequently, increased inhibition of the la interneuron by Renshaw cells decreases inhibition of the antagonistic motor neuron, and as a result the effectiveness of the reflex response is reduced (reduced activation of the agonistic motorneuron and increased activation of the antagonistic motorneuron in response to muscle stretch), possibly leading to dampening of tremor.
Connecting the dots

To summarize the main findings of this thesis, we observed functional involvement of the bilateral cerebellum in ET, together with hyperactive cerebellar outflow tracts, entailing the dentate nucleus and thalamus. In contrast, we observed a striking lack of structural involvement. The structural lesioning effects after thalamotomy observed within the cerebellar outflow tracts further supports the functional involvement of these structures. Connecting these findings to previous ET research, some hypotheses on the pathophysiology of ET might be expanded. ET as a disease entity is plagued by heterogeneous clinical characteristics and consequently heterogeneous scientific results. Some, but not all, pathology studies show evidence for Purkinje cell changes in ET. Also, involvement of the dentate nucleus as a primary structure in the pathophysiology of ET is debated. By carefully selecting familial, propranolol sensitive, ET patients we have found clear functional changes within the cerebellar cortex and dentate nucleus, related to ET, as mentioned previously. From the results within this thesis one can infer that both the cerebellar cortex and dentate nucleus are critical players in the pathophysiology of ET, and our data supports previous hypotheses of GABAergic dysfunction within the dentate nucleus, causing disinhibition of this same nucleus (the so-called ‘GABA-hypothesis’). Another hypothesis regarding the pathophysiology of ET considers ET to be a oscillatory network disorder, with possibly one or more oscillators within the cerebello-thalamo-cortical network. Previous studies have cast doubt on the idea that these oscillations are driven by a single oscillator. The role of the cerebral cortex has been studied before, only intermittently showing cortico-muscular coherent activity related to tremor, suggesting that the cortex is not primarily necessary to cause tremor in ET. From our data, the dentate nucleus arises as a possible oscillator. However, we also observe hyperactivity of the thalamus and cerebellum. In vivo neurophysiological recordings of these structures could draw more definitive conclusions regarding the oscillatory nature of involved structures within the cerebello-dentato-thalamic system.

One structure that remains to be discussed, but which was not a main region of interest in this thesis, is the inferior olive nucleus. The importance of this region for ET was proposed after the discovery that the psychoactive drug harmaline was found to evoke tremor in mice. This is possibly due to enhanced electrical coupling of cerebellar afferents within the inferior olive nucleus through GABA receptor inhibition. Additionally, the inferior olive nucleus is thought to exhibit pacemaker-like activity.
However, there is no evidence from pathology studies that the inferior olive nucleus is affected in ET.\(^3\) We have observed decreased activity in the inferior olive nucleus in ET patients compared to controls during rhythmic finger tapping, which suggests involvement of this nucleus. However, this might also be compensatory to, or a result of, cerebellar dysfunction in general. The EMG-fMRI analysis (Chapter 3) did not show tremor-related activity within the inferior olive nucleus.

Finally, some physiological mechanisms behind treatment of ET are investigated in this thesis. Both beta-blockade, using propranolol, and thalamotomy lead to attenuation of tremor, but the underlying network oscillations appear to be left intact.\(^9\) All current forms of treatment only seem to dampen the tremor. Future new insights in the pathophysiology and physiology of treatment of ET will hopefully aid in the development of more effective symptomatic, and even curative, treatment for this disorder.

**Future perspectives**

As mentioned previously, ET is plagued by heterogeneity in clinical characteristics, but also by heterogeneous research results. It is of crucial importance for the international community of ET researchers to combine efforts to tackle relatively ‘essential’ outstanding questions. Although ET is highly prevalent, ET research remains in its infancy. Several key issues will need to be addressed in the future to be able to come closer to curing ET:

- Advancements in MR imaging techniques, including higher spatial and temporal resolution and in vivo measurement of specific neurotransmitters, combined with results from intra-operative thalamic recordings and clinical neurophysiology will provide us with more information on specific changes occurring within the cerebello-thalamo-cortical system, ultimately giving rise to tremor. This will also provide insight into mechanisms by which deep brain stimulation alleviates ET. For these studies, it is crucial to well-define the ET subgroup included in studies.

- ET is a highly heterogeneous disorder. One could even hypothesize that ET as a distinct disease entity does not exist. To circumvent this issue, future studies should explicitly provide full clinical characteristics regarding the included patient sample. ET should be redefined as a syndrome rather than as a disease. Separating the clinical phenotype from possible disease mechanisms is crucial in order to decrease the amount of heterogeneity of the patient groups presently all termed ‘ET’. Subsequently, it would become possible to study separate subgroups within the ET spectrum.

- ET patients often show an autosomal dominant inheritance pattern, but a monogenetic cause of ET has yet to be identified. Developing an animal model, based
on a mutation causative of ET, will be of great importance to improve our understanding of the pathophysiology and treatment of ET, and to be able to stratify inclusion of patients for future pathology and imaging studies, and clinical trials. Furthermore, this will help to answer the question whether tremor in ET is caused by a single oscillator, or by a network of oscillators entraining each other. Extensive and clinically well-defined sample sizes are required for future genetic studies. Establishing international collaborations would help the identification of involved genes for ET.

- There is a great lack of evidence from clinical trials on how to treat ET. Gabapentin is currently used as treatment of second choice, after propranolol treatment, by many neurologists. Yet, only one randomised clinical trial is available on gabapentin monotherapy for ET, in which only 16 patients were included. Even more striking, is the fact that there are no randomised clinical trials investigating the effect of thalamic deep brain stimulation. At present, there are several long-term studies on the effect of thalamic deep brain stimulation for upper limb tremor. However, only in one study raters were blinded. A difficulty for therapeutic, and also pathophysiological, studies is the discrepancy between tremor measures such as amplitude and frequency, and patient disability. A 50% decrease in tremor amplitude might be interpreted to be a significant effect, but the presence of the remaining tremor would still impair the patient who is not able to perform his or her daily living activities. Great progress has to be made within the field of clinimetrics and evidence-based medicine for ET.

**Tracing tremor**

Many traces throughout this thesis lead to the cerebellar system as a paramount structure in the pathophysiology of essential tremor. With the help of advanced clinical neurophysiology and neuroimaging, we set out to unravel some of the many outstanding issues surrounding essential tremor. Some key issues however remain to be solved. The lack of a known genetic mutation and the considerable amount of clinical, and probably etiological, heterogeneity is problematic. The lack of knowledge regarding the aetiology of ET consequently hampers the development of new treatments. By clearly defining clinical characteristics and specific subgroups, combined with the future discovery of genetic mutations and the development of animal models, many more of the mysteries surrounding essential tremor are to be solved.

Amsterdam, July 2015