Chapter 6

Discussion
6.1 DISCUSSION

In this thesis, two neurological disorders with motor symptoms were studied. First, the focus was on essential tremor (ET), a movement disorder with too much movement, with the aim to increase knowledge on its pathophysiology. In the second part of this thesis, we focused on functional neurological paresis (FNP), a functional disorder with motor symptoms in the category of lack of movement. The objective of the second part was to examine the potential value of rTMS as a therapeutic tool in FNP. Changes in brain activity as a result of this intervention were measured with fMRI.

6.2 ESSENTIAL TREMOR

ET has been commonly seen as a single syndrome and has been used as a diagnosis for tremor not otherwise specified. Variability in response to treatment, to alcohol, the presence of an intentional component, family history, and age at onset are all features that indicate that ET may actually be a family of diseases, rather than a single entity.1 The variable results of neuroimaging studies2 suggest that different neuronal circuits may be involved in subtypes of ET. Another explanation for the variable imaging results may be the different methods that have been used. The overall objective of the studies in chapters 2, 3 and 4 of this thesis was therefore to identify the brain network involved in tremor generation in a homogeneous group of ET patients. To this end we selected a group of definite ET patients with a disease duration of >5 years, an age at onset < 65 years and a positive response to propranolol. Furthermore, we applied a combination of EMG and fMRI to directly link the tremor to brain activations. In the next paragraphs we will discuss the results of our imaging studies in ET.

6.2.1 PATHOPHYSIOLOGY

In our studies we found that the involvement of the cerebellum is common across the different tasks and analyses in ET patients. Previous studies focusing on the pathophysiology of ET also pointed to an important role of the cerebellum. By simultaneously recording EMG and fMRI, we were able to identify specific cerebellar areas in ET patients that showed activation correlating with variability in tremor intensity over time. In chapter 2, these areas were located bilaterally in the cerebellum: in the left lobules V and VI and in the right lobules V, VI, VIlia and b. fMRI has not often been used to study ET along with a motor task. In one earlier fMRI study,3 using the same motor task of raising the right arm to a posture position, bilateral activation of the cerebellar hemispheres was reported. These bilateral activations in the cerebellum were more diffusely located than the specific areas we identified. We propose that this is due to the different techniques used. By adding EMG to the fMRI measurement, we were able to distinguish tremor-related activations from movement-related activations during
the motor task, allowing a more precise localization of activations in the cerebellum correlating with tremor intensity. The cerebellar areas we identified are located in the somatomotor regions of the cerebellum. There is increasing appreciation of the complex role of the cerebellum in motor (and nonmotor) functions of the entire nervous system. The somatomotor regions of the cerebellum are located in the lateral zone of the cerebellum, also known as neo-cerebellum. This part of the cerebellum is reciprocally connected with the cerebral cortex; it receives input from the cerebral cortex via the pontine nuclei and sends output to the red nuclei and the ventrolateral thalamus. The thalamus, in turn, is connected to the motor areas of the premotor and the primary motor cortices. These parts of the cerebellum thus form an important node in the motor network related to tremor.

In chapter 3 we conducted an additional study with ET patients executing a goal-directed pointing task. This task was used to provoke intention tremor, allowing to study brain areas involved in the intentional component in ET. Our results resembled the results of chapter 2. Again, we found that activations in the cerebellum, in the somatomotor areas, correlated with variability in tremor intensity. The specific locations of these cerebellar activations accurately fit with a previous study on functional connectivity of the cerebral motor hand region and regions of the cerebellum. In chapters 2 and 3 increased activations were not only found in the cerebellum, but also in a more widespread cerebello-thalamo-cortical network. This network was more activated during intentional tremor compared to postural tremor in ET patients. The findings described in this thesis, together with evidence from previous neuropathology studies and studies using PET or fMRI, point to a key role of the cerebellum in the pathophysiology of ET. With its reciprocal connections with the cortex and via the thalamus, the cerebellum may be a node that plays a major role in the cerebello-thalamo-cortical network in ET. In chapter 4 a functional and effective connectivity study in ET patients further supports these findings. In the effective connectivity analysis, tremor variability during the motor task had an excitatory effect on both the extrinsic connection from cerebellar lobule V to the thalamus, and on the intrinsic activities of cerebellar lobule V and the thalamus. A decrease in functional connectivity between cortical and cerebellar motor regions in ET was also revealed. This lower functional connectivity was related to higher clinical tremor severity. We also found increased functional connectivity between right cerebellar lobules I–IV and the left thalamus, correlating with higher clinical tremor severity. This increase in functional connectivity is especially interesting in conjunction with the increased activation of the cerebello-thalamo-cortical network when comparing intentional tremor with postural tremor in chapter 3, as intentional tremor is sometimes seen as a more severe form of ET, with higher clinical tremor severity.

Signs of neurodegeneration such as Purkinje cell loss and torpedoes have been reported particularly in the cerebellar cortex in ET. In this light, the increased connectivity and activations of the cerebellum in ET may seem counter-intuitive. However, considering that
affected cells are deficient and disorganized, they are likely to be less efficient. Inefficiency could then lead to increased activations correlating with tremor intensity.

In conclusion, by including a homogeneous group of ET patients and combining EMG and fMRI, we provided further evidence that the cerebellum plays a major role in ET.

6.2.2 FEATURES OF ET
In ET, it has been difficult to study specific symptoms and pathophysiology, as the clinical definition of ET has been inconsistent over the years. ET has long been used as a diagnosis for all types of tremor that did not fit another tremor diagnosis. This resulted in a heterogeneous group of patients with a variable set of symptoms being included in ET studies. In chapters 2 and 3, we aimed to study two different specific features of ET: the postural and intentional component of ET. These two features of tremor can actually both be present in individual patients. In our studies we tried to distinguish brain networks involved in the intentional component and in the postural component of ET. We found that during manifestation of both tremor types the same cerebello-thalamo-cortical network was active. However, in intention tremor this brain network was more active and included a larger portion of the brainstem compared to postural tremor. This more extensive abnormal network suggests that intention tremor is a more impaired feature of ET than the presence of postural tremor (only).

6.2.3 OTHER CONSIDERATIONS REGARDING OUR STUDIES OF ET
6.2.3.1 INTERPRETATION OF FMRI RESULTS
fMRI has not been widely used to study the pathophysiology of ET; postmortem and neurophysiological studies have been more common. In the studies of ET in this thesis we added EMG to fMRI, to record tremor intensity variability over time, allowing us to directly link tremor to brain activity. To our knowledge, and surprise, no other studies using combined EMG-fMRI have been performed in ET, even though this technique is very well suited to capture movement in hyperkinetic movement disorders. One reason might be that, although fMRI has been a popular technique for studying brain function for over 15 years, the analysis and interpretation of the results are still rather difficult and ambiguous. When interpreting the results in the light of pathophysiology it is impossible to decide whether the identified brain network is a causal or a compensational network, as fMRI is a correlational technique. We found that the cerebello-thalamo-cortical network and in particular the cerebellum is involved in ET, but whether this involvement is reactive or causative remains unclear.

6.2.3.2 IDENTIFYING SUBTYPES
In our studies of ET pathophysiology we selected patients using strict inclusion criteria to include a homogeneous group of definite ET patients. Although ET is a common movement disorder, it was difficult to include definite ET patients that fulfilled all TRIG criteria. This demonstrated that ET was also used as a diagnosis for tremor not otherwise specified, in the
population that we considered for inclusion. Furthermore, by using additional criteria, we aimed to identify a specific subtype of ET. Variability in response to treatment and to alcohol, the presence of different features of tremor, family history, and age at onset are all thought to be factors identifying different ‘members’ of the ET family.\textsuperscript{1,15} Our additional criteria incorporated age at onset, response to propranolol and the presence of intention tremor to study specific brain networks involved in postural and intentional tremor in ET. The criteria that we selected are only one of the many possible sets of criteria that could be used to distinguish subtypes of ET and selecting another subset of criteria could have changed our results.

6.2.4 FUTURE DIRECTIONS
The differences in neural network involvement in postural and intentional tremor in our homogeneous group of ET patients suggest that differentiating between features of ET may improve the understanding of different subtypes in ET. It would be interesting to take this even further and define endophenotypes of ET by identifying classes of patients on the basis of clinical features as well as fMRI network activations, using machine learning methods. Possibly, in such groups genetics and pathology may also be more homogeneous. Using the TRIG-criteria\textsuperscript{19} for the clinical features, proposed by clinicians and scientists in the field of ET, seems reasonable. Such a clear definition of ET could then be used in future research to develop a more standardized research approach and include more homogeneous groups of definite ET patients, leading to more consistent study results. Defining subtypes of ET patients using these criteria and including other types of isolated tremor syndromes that do not meet the definition of ET, will likely improve the understanding of pathophysiology in ET.

In addition, prospective studies are needed to determine the changes in oscillations in the cerebello-thalamo-cortical network over time and the role of each node in this network with neuroimaging. This could lead to a better understanding of and distinction between causative or reactive mechanisms leading to changes in brain network activity and the contribution of these brain activations to postural and intentional components in ET. Furthermore, subsets of patients can be better categorized when collecting more information about the way their symptoms emerge or adapt over time.

6.3 FUNCTIONAL NEUROLOGICAL PARESIS

In the second part of this thesis, we focused on FNP. The overall objective of these studies was to evaluate rTMS as an effective treatment for FNP and to investigate the changes in cerebral activation that accompany possible symptom improvement.
6.3.1 RTMS AS A TREATMENT

In chapter 5 we found a significant increase in muscle strength as measured with dynamometry when comparing the effect of rTMS with sham rTMS and concluded that below motor threshold rTMS may have potential as a treatment for improving motor function in FNP. These effects were smaller compared to the effects previously found in studies investigating the effect of rTMS in patients with functional neurological disorder.20,21 This could very likely be explained by the fact that these other studies applied a different approach compared to our study, in two ways. First, they did not exclude additional therapeutic strategies in combination with rTMS. A larger effects in these studies may enhance the interaction between cognitive behavioral therapies and somatic movement execution. Indeed, a combination of this kind was proposed in a three-stage approach for the treatment of functional motor disorder.22 Second, previous studies stimulated above motor threshold resulting in movement of the stimulated limb that could be observed and sensed by the patient. This may be beneficial for providing afferent feedback, important information that can be used in feed-back and feed-forward mechanisms of the motor network in the brain. The reoccurrence of normal brain activation may help patients to subsequently initiate the movements voluntarily. Indeed, Chastan and Parain (2010) showed that a group of patients with FNP recovered partially or completely after a single diagnostic session of TMS applied to the motor cortex opposite from the corresponding paralysis.23

The changes in muscle strength we found did not consistently correlate with the subjectively perceived changes in muscle strength. This suggests that decreased muscle strength itself is not the core symptom of FNP. This may be in line with patients typically reporting that they do not feel in control of initiating and executing movements. It could be recommended to monitor FNP patients and find out whether they are able to use their paralysed limbs better after some time, as some of our patients reported during clinical follow-up.

6.3.2 CEREBRAL ACTIVATIONS

A second finding that may reflect the idea that muscle strength is not the core symptom in FNP, is the fact that we did not find any correlations between the changes in muscle strength and changes in brain network activations on group level as measured by fMRI (chapter 5). We expected to find an association between changes in, particularly, parietal activation and changes in muscle strength. Abnormal parietal activations have been associated with the neural process underlying (a lack of) self-agency. Self-agency is the individual’s perception that an action is the consequence of his or her own intention. This sense of self-agency is considered intact when one meets two conditions: perceiving ownership over an action and the action matching the intention.24 If at least one of these conditions is not met, a mismatch is detected and an action without the sense of agency is evoked. In patients with FNP, the lack of movement is often perceived as involuntary; patients reported that they were no longer in control of their motor functions.25 A lack of self-agency is therefore proposed to be a core symptom of FNP, instead of the decreased muscle strength itself.25-27 The parietal cortex seems to play an important role
in the mismatch detection\textsuperscript{24,28} and a decrease in activation of the parietal cortex has frequently been found in patients with functional neurological disorders.\textsuperscript{25,26,29} In the study described in chapter 5, we investigated the individual differences in brain activations before and after rTMS treatment. We found large variability in brain activation patterns between patients, with increased parietal activation after treatment in some, but not all patients. This large variability in brain activations between individuals, can explain why no significant differences were found on group level within this group of patients. Our patient group was diverse, with a great variability in age at onset of the disorder and a disease duration that varied between 4 weeks and 25 years. In previous studies inconsistent results have also been reported and were suggested to be due to different paradigms used, variability in clinical presentation and the different control groups used. With our fMRI study, we were not able to identify changes in specific networks or areas within a network that could be associated with rTMS treatment in this group of patients with FNP. Therefore, we could not support or reject the theory of a lack of self-agency in FNP.

Overall, rTMS might be a useful tool in the treatment of FNP. Our placebo controlled study showed that stimulating below motor threshold can improve the muscle strength in patients with FNP. Yet, the protocol we used might not be the optimal paradigm from a clinical point of view, as other studies showed that stimulating above motor threshold and for a shorter period can be even more effective.

6.3.3 OTHER CONSIDERATIONS REGARDING OUR STUDIES OF FNP

6.3.3.1 RTMS STIMULATION LOCATION
Changes in activation of the primary motor cortex have not consistently been found in neuroimaging studies of FNP. rTMS of the primary motor cortex may therefore not be the ideal/optimal brain area of choice. Considering the theory of impaired self-agency in patients with FNP and the decrease in activity of the parietal cortex that accompanies this theory, the parietal cortex may be a valuable alternative stimulation location to investigate. Using rTMS as a therapeutic invention in FNP is still in its infancy, and more research into replicating the effect in larger samples and choosing the most optimal stimulation paradigm is required.

6.3.3.2 SELECTING PATIENTS
The diagnosis of FNP comprises a patient group with large variability in clinical presentation. The patients with FNP included in our study all met the minimum criteria of having FNP in at least one hand. The additional symptoms varied widely between patients; in some patients other parts of the body were affected or sensory symptoms were present. Furthermore, the sample size of the study was small. It was difficult to include patients that were willing to spend two times two weeks in the hospital. Plausibly, given the variability in clinical background and activations across patients, the sample size in the present study was too small to reach significant results on group level for the fMRI analysis.
6.3.4 FUTURE DIRECTIONS

Different stimulation paradigms should be investigated to find the optimal clinical paradigm for rTMS as an intervention in FNP. The results of other studies using shorter stimulation periods and above motor threshold stimulation, are promising in that respect. The exact physiological mechanism behind the improvement in symptoms after such a short intervention is still unknown, however. Future studies of rTMS as a treatment should therefore focus on identifying this mechanism to understand the brain networks involved in FNP. Using fMRI to investigate brain networks involved in motor tasks before and after rTMS stimulation, as we did in our study, can enhance the understanding. A further improvement could be to image the brain before and immediately after a rTMS treatment session to capture the acute changes in brain networks better. Additionally, larger groups of patients can be included in such a study as it is less time-consuming and less burdening for the patient. Furthermore, more standardized and objective techniques to measure symptom improvement across studies are needed, including both physician-rated measures and patient-rated measures. Adding EMG or accelerometry measurements to fMRI to control for movement or even differentiate between phases of movement may further improve the understanding of brain networks involved in FNP.

6.4 GENERAL CONSIDERATIONS CONCERNING FMRI

What the studies in this thesis have in common is the use of fMRI in disorders with motor symptoms. The advances in MRI hardware and superior spatial resolution of higher field-strengths, as well as the advances in the software and analysis methods were responsible for the growth in use of fMRI in clinical research in the last decades. Despite these rapid developments, some remarks can be made regarding the clinical applicability of the studies in this thesis.

We analyzed fMRI results on group level. The motivation for reporting results on group level is to identify universal processes of human brain function. This builds on the assumption that such a universal mechanism exists and that it is possible to derive general features of this universal mechanism that are shared among the patient group. By averaging over subjects the noise-related part of the signal is diminished.\textsuperscript{30} There are a few issues with these assumptions and general approach. First, within a (patient) population, the spatial and temporal dynamics of brain function vary between individuals. When assuming a universal mechanism, deviations from this mechanism are then described as noise, even though they can be ‘real’ within individuals. Second, in mixed- and random effects group analysis, the overlapping activations shared across subjects are seen as the ‘true’ brain activation, while brain activation that is only shared by a small subset of the group is considered noise. When only considering group level results, one might thus miss other ‘true’ brain activations that are shared in a subset of patients and might give clues on other disease mechanisms.
In the fMRI studies of ET, as well as in the fMRI study of FNP, we also took a close look at the results on individual level. Our ET patients showed little variability in their patterns of brain activations, but the effect size of the activated patterns differed between participants. The FNP patients on the other hand, showed considerable variability in activated brain areas. Therefore we decided to show the results on individual level for the latter group of patients, as it might be more informative than showing group level result only.

Another issue when using fMRI as a research tool, is the number of choices that have to be made during the preprocessing and analysis phases. The typical fMRI analysis workflow contains a large number of steps to be made, each requiring choices about how to set parameters and which method to use. In 2012, researchers applied 6912 analysis workflows to a single data set and quantified the variability in resulting statistical maps. This approach revealed a large degree of variability in some brain regions across the different workflows, while other regions were relatively consistent. These choices every researcher has to make, can lead to substantial inflation of type I error rates. It is therefore important that all the steps and choices that have been made in the analysis process are described in detail, to provide a clear and transparent picture.

One of these choices concerns motion artifact correction. This is of interest for our studies, because we used motor tasks while scanning. Particularly during motor tasks, head motion may be correlated with the task; especially at the beginning and end of tasks, a greater risk of head motion is present. In addition, patients and elderly participants tend to move more than younger healthy participants. Even small amounts of head movement can produce motion-induced signal changes that may persist for tens of seconds after a motion. This can lead to an increase in the prevalence of false activations and therefore to an increase in the presence of type I errors. In our fMRI studies, we tried to take these potential problems into account by including the movement parameters derived from realignment corrections and by adding two extra global signal regressors in the connectivity study (chapter 4). These types of retrospective motion correction are currently the most used methods in the majority of fMRI studies as they are robust, sequence independent and have minimal impact on the fMRI study setup. However, recent developments show that prospective motion correction appears to be more effective, particularly in cases of substantial motion. Still, more research into these techniques is needed to improve their robustness.

6.5 CONCLUSIONS

To conclude, in the first part of this thesis we added to the discussion on the pathophysiology of ET. With the use of EMG-fMRI, we showed that increased cerebellar activations are related to increased tremor severity in postural and intention tremor and that postural tremor is related to disturbances in cerebellar connectivity. In the second part of this thesis, we established that
rTMS can be a potential therapeutic application for patients with FNP and we added to the debate on the deficit in initiation and cognitive planning of a movement in the motor network. In general, longitudinal and prospective studies, together with developments in neuroimaging techniques, can lead to better understanding and diagnosis of disorders with motor symptoms, such as those studied in this thesis.
REFERENCES

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