Multimodal investigations into the pathophysiology of myoclonus-dystonia

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General Discussion
In this final part of the thesis, first the main results of the performed studies on the pathophysiology of Myoclonus-Dystonia (M-D) will be summarized and discussed. Furthermore, issues in the implementation and use of multimodal imaging methods will be presented, including the effects of movements on the electromyography (EMG) signal acquisition in the MRI environment. Finally, suggestions for future research with respect to the pathophysiology of M-D and further development of multimodal methods will be discussed.

8.1 Pathophysiology of M-D

In this thesis, we examined the pathophysiology of M-D using a combination of standard methods and multimodal methods, being electroencephalography (EEG) and electromyography (EMG) with wrist manipulations, diffusion tensor imaging (DTI) with voxel-based morphometry (VBM) and combined EEG with functional magnetic resonance imaging (fMRI). The investigated pathophysiological mechanisms are subdivided into four different categories: loss of inhibition, defects in sensorimotor integration, maladaptive plasticity and myoclonus.

8.1.1 Loss of inhibition

We investigated cortical and sub-cortical loss of inhibition with a response-inhibition task and functional MRI (chapter 2). We found that M-D patients had blood oxygenation level dependent (BOLD) hyperactivity during response inhibition in the insula and the motor cortex, while the performance of the task was normal. Hyperactivation in these regions is likely associated with the hyperactive ‘state’ of the motor system in M-D patients and can be related to a relative increased effort in M-D to function according to the demands of the task; or to reduced inhibition of the cortex.

Even though direct basal ganglia evidence was not found, in a post-hoc analysis altered function in these structures is suggested as increased task errors in M-D patients were associated with thalamic BOLD activation. This is supportive for malfunction of basal ganglia in M-D, since the thalamus acts for motor planning as a relay station between basal ganglia.

Another post-hoc analysis was performed comparing DYT-11 positive versus DYT-11 negative M-D patients. In this comparison, we found BOLD hyperactivity in the cerebellum in DYT-positive M-D. This suggests heterogeneity in pathophysiological pathways in patients with and without a mutation in the DYT-11 gene. In particular, it points to a genetic predisposition of abnormalities in the cerebellum for DYT-11 positive M-D; in essence, a ‘trait’ marker. The pathophysiology associated with the DYT-11 mutation likely affects a greater circuitry including the cerebellum. In conclusion, direct basal ganglia activations were not detected, but the response-inhibition fMRI study possibly showed indirect evidence for the presence of disinhibition in these regions.

8.1.2 Defects in sensorimotor integration

We tested the hypothesis that the hyperactive state of the motor system in M-D patients could be due to a defect in sensorimotor integration; specifically as resonance
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between sensory and motor systems, in chapter 3. In order to do so, we used the common motor drive as a marker for motor output and applied perturbations with a wrist manipulator (WM) as sensory input. We hypothesized that the sensorimotor system is a loop which would behave in such a way that this resonance could be brought ‘off-balance’ by perturbation stimuli which were slightly off-resonance. The WM provided these perturbations to the hand through a handle, while the common motor drive (the marker for dystonia) was monitored using two EMG signals from neck muscles and EMG-EMG coherence analysis.

In four out of six M-D patients, neck dystonia was apparent upon clinical examination and this was associated with a 3-7 Hz peak in the coherence spectrum of the EMGs of two neck muscles. The first main finding was that the perturbations to the wrist did not affect the dystonia common drive in these M-D patients with neck dystonia. This result suggests that dystonia in M-D probably is not due to a resonance within the sensorimotor loop, but possibly finds its origin in an independent generator in the central nervous system (CNS).

The second finding was that the WM induced a common drive of its own in arm and shoulder muscles, which could be detected with EMG-EMG coherence analysis applied to EMG signals of the shoulder; this ‘WM’ common drive was at the exact the same frequency as the perturbations, and is likely a normal muscular response to proprioceptive stimuli [203]. Furthermore, it was detected in all participants without neck dystonia i.e., all six control subjects as well as the two M-D patients without neck dystonia. In the other four M-D patients in which neck dystonia was confirmed by EMG-EMG coherence of the neck muscles, this ‘WM common drive’ was absent. Possibly, neck dystonia in M-D interferes with normal sensorimotor processing of proprioceptive stimuli.

A third finding was that in three of the four M-D patients with neck dystonia, the dystonia common drive was found not only with EMG-EMG coherence using the EMG two neck muscles, but also with EMG-EEG coherence using the EMG of one neck muscle, and the (bipolar) EEG signal near the contra-lateral motor cortex. This may indicate cortical involvement in M-D, which has not been demonstrated in other studies until now.

In conclusion, our experiments suggest that dystonia in M-D is not due to a resonance within the sensorimotor loop, but is likely to originate from a central generator in the brain; furthermore, this generator possibly interferes with normal sensorimotor processing. The plausibility of a central generator in the brain is supported by mathematical models [170], as well as culture studies of the brain [154;192], and a local field potential study in a heterogeneous group of patients with dystonia [183].

8.1.3 Maladaptive neural plasticity

We have performed two studies to investigate maladaptive neural plasticity in M-D. In the first study (chapter 4), we have investigated whether M-D patients had enlarged grey matter regions in the motor system. No such regions were found. However, we did find a strong correlation between the Burke-Fahn-Marsden dystonia rating scale (BFMDRS) and the grey matter volume in the left and right putamen. This correlation is consistent with the hypothesis that neural plasticity in the putamen is associated with typical dystonia movements.
The absence of significant volumetric differences between M-D patients and control subjects could indicate that plasticity in the putamina is not necessarily associated with the pathophysiology of M-D, but could rather reflect secondary changes due to hyperkinaesia (i.e., dystonia and myoclonus). Such secondary changes have been demonstrated earlier; brain volume may change after a three-month period of motor skill training\[^{57}\].

In the second study that addresses the issue of plasticity (chapter 5), we examined whether changes in white matter macrostructure (enlarged relative volumes) and microstructure (differences in fractional anisotropy and mean diffusion) in M-D could be detected using VBM with DTI. We found changes in white matter in the thalamic and sub-thalamic region, i.e., the upper region of the brainstem which contains the substantia nigra, red nucleus and subthalamic nucleus. A combination of increased white matter volume with reduced mean diffusion in this region indicates changes in axonal membrane properties or denser axonal packing\[^{15}\]. These are consistent with increased traffic between cerebellum and the basal ganglia\[^{27}\], and are also compatible with current studies in M-D showing hyperactivity in the basal ganglia\[^{18,65}\] and changes in the cerebellum\[^{166}\].

In conclusion, we found striatal and sub-thalamic plasticity changes in the brain of M-D patients. These changes are likely associated with excessive use of the motor system. However, the grey matter volumetric changes in the striatum likely are more indirectly related to the pathophysiology of M-D. With respect to the white matter, the volumetric and microstructural findings in the sub-thalamic area and the microstructural findings in the sensorimotor cortex are likely to be linked to the pathophysiology of M-D.

### 8.1.4 Myoclonus

In order to detect which brain areas in M-D showed increased activity with myoclonus, in a selected patient group BOLD activation was examined using combined EMG-fMRI in chapter 7. In order to do so, several technical issues relating to movement inside the scanner, and the recording of electromyography during scanning, had to be overcome (see chapter 6).

The main finding of this study was that myoclonus was associated with BOLD activation in the contralateral putamen, contralateral subthalamic region, and the bilateral supplementary motor areas. Contralateral activation of both the subthalamic region and the putamen is supportive for a 'generator' role of these regions. Furthermore, virus tracer studies have shown that the contralateral subthalamic region is associated with the communication between the cerebellum and the basal ganglia\[^{27}\]. Therefore, these results suggest that myoclonus in M-D could be related to a (mis-) communication between basal ganglia and cerebellum. The cerebellum has been shown to have high expressions of epsilon-sarcoglycan (SGCE) in healthy subjects\[^{166}\]. In addition, even though dystonia is not the same as myoclonus, in dystonia there is growing evidence that pathophysiological changes can also occur in the cerebellum\[^{140}\]. The BOLD activation in the bilateral supplementary motor areas that we found are probably a secondary effect; in M-D, the cortex lacks the (for dystonia typical) hyperexcitability\[^{125,205,111,187}\], and also there was no BOLD activation in the primary motor cortex related to the presence of myoclonus. In conclusion, myoclonus in M-D is probably initiated in sub-cortical regions of the brain; the striatum and the
sub-thalamic area of the brainstem play an important role in this process.

8.1.5 Conclusions

Our imaging studies suggest that the subthalamic region is involved in the pathophysiology of M-D. Firstly, during our go-no go study (see chapter 2), BOLD activation was found in the ventral lateral nucleus of the thalamus which lies close to the subthalamic region. This activation was associated with task errors, which in turn may be associated with dystonia hampering normal control of the hands. Secondly, with the DTI-VBM study (see chapter 5), white matter changes were detected in this subthalamic region which are consistent with changes in membrane properties or denser axonal packing. Thirdly, with the EMG-fMRI study (see chapter 7), BOLD-activation was found exactly in the subthalamic region.

Considering all studies in this thesis, the pathophysiology of M-D is likely related to a dysfunction in the communication between basal ganglia, thalamus and cerebellum[140]. This is in contrast to more conventional theories underlying dystonia which primarily point to the basal ganglia as a source for the disorder[211;29;149]. Re-appraisal of pathological, clinical and imaging evidence in other types of dystonia have indicated that there most likely are different pathophysiological mechanisms underlying different types of dystonia, and that cerebellar abnormalities have been found more often than appreciated[189;140]. In this sense, DYT-11 M-D is one distinct type of dystonia which is characterized by dysfunction in basal ganglia, sub-thalamic and cerebellar regions. Cortical abnormalities may play a secondary role; as the motor pathway to the musculature goes through the motor neurons in the cortex, and (returning) sensory information is also processed there.

8.2 Multimodal methods

In this work, the information from multiple modalities added information and possibilities to the examination of M-D compared to separate measurements. Three combinations were used in this thesis: (1) combined electromyography (EMG) and electroencephalography (EEG) with proprioceptive manipulations of the wrist; (2) diffusion tensor imaging (DTI) with voxel-based morphometry (VBM); and (3) EMG with functional magnetic resonance imaging (fMRI). Especially in the imaging experiments, movements of the patient inside the MRI scanner may profoundly disturb scanning results and therefore these studies require special attention.

8.2.1 Combined perturbations with EEG/EMG

The use of an external wrist manipulator (WM) in conjunction with standard EEG and EMG measures, allows manipulation of stimuli better defined over time. One finding, that would indicate increased sensitivity of this method, is that in the wrist manipulator study (chapter 3), significant cortico-muscular (EEG-EMG) coherence at frequencies of the dystonic drive was detected. In previous studies in the same patient group, such EEG-EMG coherence was searched for but not found[68].

To investigate the sensorimotor system in M-D, specific force and position manipulations with the WM were used. The frequency of these WM manipulations was
designed such that it coincided with the frequency-peaks in the coherence spectrum of the common drive in mobile dystonia. Furthermore, in the applied WM perturbations each frequency contained an equal amount of power. In this way, the sensitivity for detecting the perturbations in the EMG, using EMG-EMG coherence analysis, was greatly enhanced.

8.2.2 Combined VBM with DTI

Combined use of DTI and VBM (chapter 5) yields the possibility to investigate the white matter, and provides advantages compared to VBM alone since it allows for both macrostructural (volumetric) and microstructural (FA and MeanD) investigation in M-D.

To perform voxel-wise comparison of two different imaging modalities (DTI and VBM) requires images of both modalities in exactly the same orientation and voxel dimensions. For this purpose the more advanced DARTEL normalization method has been used\[^{11;10}\]. Judging from a study in which 14 different registration algorithms were compared, DARTEL registration produced better results than the standard registration used for fMRI, especially in sub-cortical areas\[^{104}\]. We created a workflow in which the parameters for normalizing the VBM data were also used to normalize the DTI data. To this end, we used the property that FA brain images co-register particularly well to segmented white matter maps at the single-subject level. The only brain region where DTI data and VBM data were dissimilar was the anterior part of the corpus callosum. In this region, the sharp transition in magnetic susceptibility (i.e., the transition between air in the nasal cavity and (fore)brain tissue introduces a localized distortion of the raw DTI images (DTI imaging is more susceptible in this regard than structural (T1) imaging). However, since the anterior part of the corpus callosum is relatively small compared to the rest of the brain, this did not impede co-registration.

8.2.3 Combined EMG with fMRI

Combined EMG with fMRI (chapters 6 and 7) was the biggest challenge of these series of studies and enabled us to investigate the possible neural substrate of myoclonus. Therefore, we had to overcome difficulties with the EMG signal recorded in the MRI scanner.

Current MRI artifact removal methods are well suited for retrieving EEG signals below $\sim 40$ Hz by using the property that the MRI artifact consists of slice and volume artifacts and these artifacts are repetitive and therefore removable by a template subtraction algorithm\[^{164}\]. However, these current methods did not work with the EMG data in EMG-fMRI. In our first few pilots, a myoclonic EMG-signal was simply impossible to assess in the artifact-corrected EMG traces, mainly because the slice-artifact changed a lot during movements. Therefore, we had to construct our own MR artifact algorithm that was more optimized for EMG-fMRI. This had two advantages with respect to normal ‘EEG’ methods. First, it worked for the high frequencies that the EMG signal contains ($> 40$ Hz). In addition, our artifact removal algorithm was better in adapting to movements of the subject during MRI scanning. To be more robust against subject movements, we selected only those slice-artifacts that were similar to the slice-artifact that had to be corrected in the template removal algorithm;
volume-artifact correction was avoided altogether. To further improve the artifact-corrected EMG signal quality at higher frequencies (>40 Hz), we used a method that involved ‘phase-shifting’ of slice-artifacts. This combined approach resulted in an artifact-corrected EMG signal in which correction errors were contained to a single slice only and allowed the subjects to move inside the scanner more freely without too much interference from their movements. In chapter 6, we assessed EMG signal and EMG power spectra for different types of movement: dystonic, jerk-like and tremor-like and found the EMG traces outside and inside the scanner to be much more similar than using standard EEG-based artifact correction methods. Using the new artifact correction algorithm, myoclonus could be assessed in the EMG-signal (chapter 7).

In addition, combined EMG-fMRI provides advantages over normal fMRI. One benefit is that the combined method allows to determine the actual motor task onset and offset from the EMG, instead of the instructed onset and offset, which made our (block) BOLD models more accurate\textsuperscript{120}. Another benefit is that in normal fMRI, with the presence of involuntary movements (which can be expected in movement disorders), any difference found in task-related BOLD activation between patients and controls could partly be credited to those involuntary movements instead of intrinsic differences in BOLD response. The addition of EMG as a ‘movement disorder’ regressor in the fMRI analysis, potentially allows one to differentiate between the BOLD activation caused by muscle action itself and the more cognitive components of task execution.

Constructing an EMG regressor for use in fMRI analysis is not straightforward. A unique quantifier-per-scan of the movement disorder is desired, which is unperturbed by confounders, such as (normally occurring) movements, another type of movement disorder, fatigue, (normal) differences in task performance throughout time, or remaining MRI artifacts. Other studies\textsuperscript{207,162} used two different methods to incorporate the movement disorder inside the GLM. Regardless of method, the most important consideration is that it should be specific for the movement disorder investigated. This means that each kind of movement disorder symptom (dystonia, myoclonus, and tremor) should be processed differently, and some kind of quantification should be used as to the quality of how well the regressor quantifies the symptom.

8.3 Future research

8.3.1 Future research Ū M-D

Regardless of the progress made, the primary underling disorder of M-D still needs further investigations. To bring pathophysiological research in movement disorders, such as M-D, to a next level, genetically homogeneous groups must be formed. Without a proven common genetic mutation, it can be very likely that any group of patients represents more than one pathophysiological disorder. Further finetuning in phenootyping, such as grouping them by type or severity of dystonia would enable us to subdivide into symptom-specific and disease-specific markers.

Another suggestion for future research in M-D is to follow subjects from early childhood into adolescence. Symptoms in M-D start during early childhood and develop mostly during puberty and early adolescence; hence most pathophysiological
changes would occur during this period. Such a longitudinal study could shed more light on which findings are more related to a ‘cause’ and which to an ‘effect’ in M-D.

It would be of great interest to investigate, in addition to myoclonus, the dystonia in M-D with EMG-fMRI and to demonstrate whether the clinical distinction between myoclonus and dystonia would also translate into BOLD activation differences.

### 8.3.2 Future research - Methods

Improvements of the methods should be sought in the area of higher resolution of structural imaging. One of the ideas is that instead of MRI images having a dimensionless metric, additional measurements could be performed to measure (for each voxel) physical properties such as T1 and T2 relaxation time or magnetization transfer\[^{93;215}\]. This enables to search for new biomarkers and a much better segmentation of the brainstem than is currently possible\[^{94}\]. Furthermore, improvements for functional imaging could be sought in higher resolution and faster scanning. In addition, the shape of the BOLD response is not well known in sub-cortical structures\[^{5}\] and it would be worthwhile to determine its shape better. This would improve event-related studies, like the EMG-fMRI study on myoclonus. For any MRI of the brain, the use of higher field strengths (i.e., 7 Tesla) enables to visualize the regions of interest with superior temporal and spatial resolutions.

### 8.4 Conclusion

The use, implementation and trial-and-error of multimodal imaging techniques are interesting on its own whereas the MRI artifact correction procedures almost is a field in itself, but it should always be driven by a combination of both clinical knowledge and technical knowledge in a multidisciplinary team. To overcome current neurophysiological problems different approaches are required; both the in depth signal analysis mindset of the physicist as well as the more clinically purpose driven mindset of the physician. This cooperation is challenging for both parties, as they need to work and understand each other. The clinician needs to have a better technical understanding and the physicist needs to learn more about medical science.