Chapter 9

Summary, general discussion and future perspective

This chapter reviews the main findings of the structural and functional neuroimaging studies on Myoclonus-Dystonia (M-D) described in the present thesis, places the results into a broader perspective of other performed research in this field and gives suggestions for future research.

Summary

M-D is an autosomal dominantly inherited hyperkinetic movement disorder, frequently caused by mutations in the epsilon sarcoglycan (*SGCE*) gene.[1,2] This mutation is designated DYT-11, classified under the hereditary dystonias.[1] An interesting genetic phenomenon in M-D is that of maternal imprinting, meaning that only the paternal *SGCE* allele is expressed.[2] This implies that only subjects inheriting the mutation from their father can exhibit clinical symptoms of M-D.

The goal of this thesis was to investigate existing theories on the pathophysiology and maternal imprinting mode of inheritance of (Myoclonus)-Dystonia and thereby contributing to our understanding of dystonia in general and M-D specifically. Because of the monogenic nature of Myoclonus-Dystonia (M-D), this disease constitutes an excellent human model to study these mechanisms.

Different theories exist on the pathophysiology of dystonia. The first functional theory is based on neuronal models of dystonia suggesting excessive motor cortex excitation due to hyperactivity of the direct putamino-pallidal pathway.[3,4] Another theory proposes abnormalities in sensorimotor integration, while yet another theory consists of a relatively new idea: that these involuntary movements could lead to (micro)-anatomic changes through cortical plasticity.[5] Finally, over the last few years there is growing evidence that besides the basal ganglia, the cerebellum plays a role in dystonia as well.[6] These theories are not mutually exlcusive and none of these theories are conclusive as yet.

Several structural and functional imaging techniques are available to investigate these hypotheses; voxel based morphometry (VBM) and diffusion tensor imaging (DTI) can be used to detect anatomical changes in gray and white matter thereby investigating neuronal plasticity. Imaging of the striatal Dopamine 2 receptor (D2R) using [123-I]IBZM SPECT allows for visualization of the D2R in vivo and can be used to investigate the hypothesis of an overactive putamino-pallidal pathway.

Functional MRI detects changes in blood flow, based on the different magnetic properties of oxyhemoglobin and deoxyhemoglobin and can be used to image sensorimotor structures during a motor task, thereby investigating patterns of sensorimotor integration.

Functional imaging

Functional magnetic resonance imaging (fMRI) was used in a group of paternally inherited, clinically affected mutation carriers. Subsequently, we were able to investigate the effect of maternal imprinting mechanism in an fMRI study in asymptomatic or only very mildly affected maternally inherited mutation carriers.

fMRI was used to investigate abnormal responsiveness of sensorimotor brain structures in genetically confirmed, paternally inherited and clinically affected M-D patients and a matched control group **(chapter 2)**. This technique was used in a classic boxcar design involving a fingertapping motor task. Significant hyperresponsiveness in contralateral inferior parietal cortical areas, ipsilateral premotor and primary somatosensory cortex, and ipsilateral cerebellum were observed during the motor task compared to healthy controls. The cortical activation patterns in *SGCE* mutation carriers during this motor task are consistent with a disorganised sensorimotor integration in cerebello-thalamo-cortical pathways in paternally inherited M-D patients. A study in another inherited dystonia syndrome (DYT-1) using [18-F]Fluorodeoxyglucose (FDG) positron emission tomography (PET) found increased glucose metabolism in the same areas: putamen, globus pallidus, cerebellum and supplementary motor area (SMA) of DYT-1 mutation carriers.[7-9] All clinically affected DYT-1 and DYT-6 mutation carriers showed additional metabolic increases in the pre-SMA and parietal association regions in an additional study, suggesting a distinct metabolic pattern related to penetrance.[10] These results also support the hypothesis of altered sensorimotor integration in dystonia.

When our fMRI study protocol was used comparing a group of eight maternally inherited *SGCE* mutation carriers (of whom four were clinically slightly affected) to eight paternally inherited mutation carriers and a matched control group **(chapter 3),** the main finding is that maternally inherited mutation carriers show similar abnormalities in the abovementioned sensorimotor areas, although to a lesser extent. Functional changes in neuroimaging studies in other inherited forms of dystonia, eg, DYT-1, have been described in unaffected gene mutation

carriers.[10] However, the mode of inheritance in these other monogenetic forms of dystonia is autosomally dominant with reduced penetrance but without the maternal imprinting phenomenon. Our results suggest biased gene expression based on parent of origin rather than a strictly dichotomous maternal imprinting mechanism, consistent with clinical observations.[2,11]

[123-I]IBZM SPECT was used in a cohort of 15 M-D patients and in three of these patients after they had undergone deep brain stimulation (DBS) of the globus pallidus internus (GPi).
In the study regarding the striatal dopamine D2 receptor using [123-I]IBZM SPECT in genetically confirmed M-D patients and a matched control group **(chapter 4),** multivariate analysis with corrections for age and smoking showed significantly lower specific striatal to occipital IBZM uptake ratios (SORs) both in the left and right striatum in clinically affected patients and also in all DYT-11 mutation carriers compared to control subjects. Unfortunately, we had insufficient data to statistically analyse the non-manifesting carriers separately. Similar results were found in studies of DYT-1 and DYT-6 mutation carriers, using a slightly different method of imaging the D2 receptor, [11-C]Raclopride PET. These studies found significant reductions in striatal D2 receptor availability in asymptomatic DYT-1 mutation carriers[12] as well as symptomatic DYT-1 and DYT-6 mutation carriers[12], implying increased endogeneous striatal dopamine regardless of clinical penetrance. These findings are consistent with the theory of reduced dopamine D2 receptor availability in dystonia, possibly due to increased endogenous dopamine, and consequently, competitive D2R occupancy.

In a subsequent study we used the same technique to investigate the effects of deep brain stimulation of the globus pallidus internus (GPi-DBS) in three patients from the previous study, with two patients who had not undergone DBS as controls **(chapter 5).** Clinically, the GPi-DBS showed striking beneficial effects on myoclonus and dystonia symptom severity measured with validated clinical rating scales, consistent with similar recent studies of GPi-DBS in M-D.[13,14] D2R binding however, did not differ before and after GPi-DBS. This lack of change is consistent with studies regarding STN-DBS in Parkinson's disease,[15] but not consistent with the GPi-DBS study describing normalization (decrease) of D2R binding potential in Parkinson's disease.[16] In our two re-scanned control subjects, D2R binding seems to have lowered further. These findings confirm that GPi-DBS has beneficial effects on motor symptoms in M-D and suggest that GPi-DBS might stabilize D2R binding.

Structural imaging

We used voxel based morphometry (VBM) to quantify macrostructural changes in gray matter volumes and white matter volumes, and to relate these changes to disease severity measured by clinical rating scales for myoclonus and dystonia.

In the VBM study concerning gray matter (GM) changes in genetically confirmed M-D patients and a matched control group **(chapter 6)**, dystonia severity in mutation carriers was strongly correlated with increased gray matter volume in bilateral putamina. This is of interest as the putamina are thought to play a major role in the pathogenesis of dystonia. Neuronal models of dystonia postulate hyperactivity of the direct putamino-pallidal pathway with reduced inhibitory output of the internal segment of the globus pallidus (GPi), with subsequently increased thalamic input to the (pre) motor cortex, resulting in excessive motor cortex excitation.[3;4] More specifically for M-D: *SGCE* knockout mice, aside from displaying a clinical phenotype resembling M-D, showed higher concentrations of striatal dopamine and its metabolites.[17]

In other VBM studies in inherited and non-inherited dystonia the putamina also showed changes; in primary torsion dystonia (DYT-1) and idiopathic cervical dystonia patients an increase in bilateral putaminal GM has been found in the idiopathic patient group and in asymptomatic DYT-1 mutation carriers, but not in manifesting DYT-1 carriers, possibly suggesting some sort of compensatory mechanism.[18] Two morphometric studies regarding blepharospasm also found GM increases in both putamina.[19,20] In these non-hereditary dystonia patient groups, there were also other areas altered: two morphometric MRI studies in cervical dystonia reported an increase in GM volume predominantly in the GPi[21,22], whereas a third study found an increase in the caudate nuclei, thalamus and right cerebellum.[23] To summarize, we found a clear indication of putaminal involvement in M-D, which may be specific for M-D. Different pathophysiological mechanisms causing the different types of dystonia might account for the heterogenous results in the literature.

Diffusion tensor imaging (DTI) was used to assess microstructural changes in white matter in M-D patients. These techniques were used to study the neuronal plasticity theory, which states that the altered function of affected brain areas could lead to (micro)-anatomic changes in these areas. The white matter study

using VBM and DTI in genetically confirmed M-D patients and a matched control group **(chapter 7)** showed the main abnormality in the brainstem area, especially the red nucleus. VBM showed a significant increase in white matter volume and DTI a decreased mean diffusivity and increased fractional anisotropy (FA). DTI also showed decreased mean diffusivity in the white matter close to cortical sensorimotor areas. This area of abnormal connectivity in the sub-thalamic region of the brainstem was also seen in a small group of primary torsion dystonia (DYT-1 and DYT-6) patients using DTI; however, in contrast to our study, a reduction in FA was reported.[24] Possibly, the more prominent myoclonus in M-D could account for this. Another reason might be that increased plasticity in M-D causes more directional axonal growth, resulting in an increase of FA, where axonal growth in DYT-1 and DYT-6 might have been more divergent. DTI studies in other types of dystonia do not show a consistent pattern of affected brain areas. Again, different pathophysiological mechanisms causing the different types of dystonia might account for this.

In general, two plausible explanations can be put forward for the phenomenon of altered gray and white matter volumes in dystonia. First, these increases in gray matter could be primary and hence underlie the involuntary movements through altered neuronal activity patterns. Alternatively, this increase could be secondary to excessive involuntary movement, causing reactive changes in gray matter due to neuronal plasticity. Physiological neuronal plasticity has been demonstrated in healthy subjects learning a complex motor skill (i.e. juggling) and has been shown to be reversible.[25] Aberrant plasticity of the sensorimotor circuitry is considered to be an integral part of the pathophysiology of dystonia[26] and has been clearly demonstrated in patients with focal hand dystonia[27], although it is as yet unclear whether these changes might also be reversible. Either way, these results provide additional evidence of involvement of the cerebral structures involved in motor function in the pathophysiology of dystonia. This is in accordance with increased cerebello-thalamic connectivity and could be caused by neuroplasticity. Whether these changes are the cause of the clinical symptoms or an effect remains to be elucidated. To clarify this issue, it would be interesting to study larger groups of non manifesting gene mutation carriers to discern whether similar changes can be found in this group.

Finally, **chapter 8** discusses the clinical similarities between M-D and another inherited movement disorder: Spinocerebellar ataxia type 14 (SCA-14). Both can present with a combination of trunk tremor, multifocal myoclonus and axial

dystonia as predominant clinical features. We suggest that in patients with this M-D phenotype without a mutation in the DYT-11 gene, SCA14 should be considered. Of special interest is the fact that the cerebellar involvement is one of the key features of the spinocerebellar ataxias and recent studies illustrate an important involvement of the cerebellum in (myoclonus)-dystonia as well.[6]

General discussion and future perspective

Little is known about the pathophysiology of M-D and the function of the *SGCE* protein. The studies in this thesis used neuroimaging techniques to investigate the three major theories on the pathophysiology of (hereditary) dystonia: hyperactivity of the direct putamino-pallidal pathway, defective sensorimotor integration and neuronal plasticity. As mentioned earlier, these theories are not mutually exclusive.

Our structural imaging studies using VBM of grey and white matter to study macrostructural changes in M-D, as well as our DTI study investigating microstructural changes support the idea of neuronal plasticity, especially in bilateral putamina and cerebello-thalamic pathways.

Our functional imaging studies using fMRI support the idea of defective sensorimotor integration and confirm cerebellar involvement in M-D. Furthermore, the maternal imprinting mechanism is shown to be more likely based on biased gene expression based on parent of origin rather than a strictly dichotomous phenomenon. Finally, the hypothesis of hyperactivity of the direct putamino-pallidal pathway is supported by our D2R imaging study.

The involvement of hyperactive putamina, as implicated by both the lower striatal D2 receptor binding and the association of dystonia severity with putaminal volume is consistent with findings in DYT-1 dystonia, suggesting at least a partially shared pathophysiology of inherited forms of dystonia.[7-9,18] The cerebellar involvement, as shown with fMRI is not only consistent with DYT-1 dystonia[8], but strongly supported by the recent discovery of a brain specific isoform of *SGCE* with particularly high cerebellar expression.[6]

However, several important questions remain unanswered: first of all, it remains unclear whether the structural and functional changes found in the sensorimotor structures of M-D patients constitute part of the cause of the clinical phenotype, or are an effect. In an ideal world without technical or practical constraints, I would recommend comparing large groups of untreated and effectively treated patients to resolve this question, assuming that in effectively treated patients, these changes would revert back to a physiological state due to neuronal plasticity. However, since the only currently apparently effective treatment is DBS, this prevents MRI investigations after surgery. Perhaps the only course of action that could resolve this question is post-mortem examination of the brains of treated and untreated patients.

A second important question is the influence of myoclonus on our results. Several strategies could be implemented to resolve this, the most apparent one being to test larger groups of clinically heterogeneous myoclonic M-D patients with and without dystonia. A more elegant way would perhaps be to use event related combined EMG-fMRI using the recorded myoclonic bursts as a regressor.

A third question is whether the changes we found are genotype- or phenotype specific. Two approaches could be used to solve this problem; the most practical solution would be to investigate subject groups with the phenotype but without the mutation, because approximately 50% of patients displaying the clinical phenotype of M-D are without a mutation in the *SGCE*-gene. Another approach to resolve this question could be testing patient groups with the mutation but without the phenotype, i.e. larger groups of maternally inherited *SGCE* mutation carriers.

To summarize, the studies performed in this thesis provide further evidence for structural and functional abnormalities in the sensorimotor system, particularly the putamina and the cerebellum. All three mentioned pathophysiological models, i.e. sensorimotor integration, hyperactive putamina and neuroplasticity are supported by these results.

I propose an integrated model for the pathophysiology in M-D in which the SGCE mutation leads to altered structural development and thereby function of the above mentioned putamino-pallidal and associated cerebello-thalamic pathways. This in turn leads to hyperactivity of these pathways causing excessive excitation

of cortical motor structures eliciting the involuntary movements. This continuous hyperexcitation might then lead to altered sensorimotor inregration and even secondary structural changes due to neuronal plasticity.

The maternal imprinting mechanism seems less dichotomous and more based on biased gene expression based on parent of origin than was previously assumed. Deep brain stimulation of the GPi has a spectacular effect on clinical symptoms of myoclonus and dystonia, and may mitigate further decline of D2R availability.

References

1. Zimprich AF, Grabowski MF, Asmus FF, Naumann MF, Berg DF, Bertram MF et al. Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. Nature Genetics 2001; 29:66-69.
2. Grabowski M, Zimprich A, Lorenz-Depiereux B, Kalscheuer V, Asmus F, Gasser T et al. The epsilon-sarcoglycan gene (SGCE), mutated in myoclonus-dystonia syndrome, is maternally imprinted. Eur J Hum Genet 2003; 11:138-144.
3. Berardelli A, Rothwell JC, Hallett M, Thompson PD, Manfredi M, Marsden CD. The pathophysiology of primary dystonia. Brain 1998; 121:1195-1212.
4. Vitek JL. Pathophysiology of dystonia: A neuronal model. Mov Disord 2002; 17(S3):S49-S62.
5. Vidailhet M, Grabli D, Roze E. Pathophysiology of dystonia. Current Opinion in Neurology 2009; 22:406-413.
6. Ritz K, van Schaik BD, Jakobs ME, van Kampen AH, Aronica E, Tijssen MA et al. SGCE isoform characterization and expression in human brain: implications for myoclonus-dystonia pathogenesis. Eur J Hum Genet 2011; 19:438-444.
7. Eidelberg D, Moeller JR, Ishikawa T, Dhawan V, Spetsieris P, Przedborski S et al. The metabolic topography of idiopathic torsion dystonia. Brain 1995; 118:1473-1484.
8. Eidelberg D, Moeller JR, Antonini A, Kazumata K, Nakamura T, Dhawan V et al. Functional brain networks in DYT1 dystonia. Ann Neurol 1998; 44:303-312.
9. Trost M, Carbon M, Edwards C, Ma Y, Raymond D, Mentis MJ et al. Primary dystonia: Is abnormal functional brain architecture linked to genotype? Ann Neurol 2002; 52:853-856.
10. Carbon M, Su S, Dhawan V, Raymond D, Bressman S, Eidelberg D. Regional metabolism in primary torsion dystonia: Effects of penetrance and genotype. Neurology 2004; 62:1384-1390.
11. Foncke EMJ, Gerrits MCF, van Ruissen F, Baas F, Hedrich K, Tijssen CC et al. Distal myoclonus and late onset in a large Dutch family with myoclonus-dystonia. Neurology 2006; 67:1677-1680.
12. Asanuma K, Ma Y, Okulski J, Dhawan V, Chaly T, Carbon M et al. Decreased striatal D2 receptor binding in non-manifesting carriers of the DYT1 dystonia mutation. Neurology 2005; 64:347-349.
13. Azoulay-Zyss J, Roze E, Welter ML, Navarro S, Yelnik J, Clot F et al. Bilateral Deep Brain Stimulation of the Pallidum for Myoclonus-Dystonia Due to epsilon Sarcoglycan Mutations: A Pilot Study. Arch Neurol 2011; 68:94-98.
14. Gruber D, Kuhn AA, Schoenecker T, Kivi A, Trottenberg T, Hoffmann KT et al. Pallidal and thalamic deep brain stimulation in myoclonus-dystonia. Mov Disord 2010; 25:1733-1743.
15. Hesse S, Strecker K, Winkler D, Luthardt J, Scherfler C, Reupert A et al. Effects of subthalamic nucleus stimulation on striatal dopaminergic transmission in patients with Parkinsons disease within one-year follow-up. Journal of Neurology 2008; 255:1059-1066.
16. Nakajima T, Nimura T, Yamaguchi K, Ando T, Itoh M, Yoshimoto T et al. The impact of stereotactic pallidal surgery on the dopamine D2 receptor in Parkinson disease: a positron emission tomography study. J Neurosurg 2003; 98:57-63.
17. Yokoi F, Dang MT, Li J, Li Y. Myoclonus, Motor Deficits, Alterations in Emotional Responses and Monoamine Metabolism in epsilon Sarcoglycan Deficient Mice. Journal of Biochemistry 2006; 140:141-146.

18. Draganski B, Schneider SA, Fiorio M, Kl÷ppel S, Gambarin M, Tinazzi M et al. Genotype-phenotype interactions in primary dystonias revealed by differential changes in brain structure. NeuroImage 2009; 47:1141-1147.
19. Black KJ, Ongur D, Perlmutter JS. Putamen volume in idiopathic focal dystonia. Neurology 1998; 51:819-824.
20. Etgen T, Muhlau M, Gaser C, Sander D. Bilateral grey-matter increase in the putamen in primary blepharospasm. Journal of Neurology, Neurosurgery & Psychiatry 2006; 77:1017-1020.
21. Draganski B, Thun-Hohenstein C, Bogdahn U, Winkler J, May A. "Motor circuit" gray matter changes in idiopathic cervical dystonia. Neurology 2003; 61:1228-1231.
22. Egger K, Mueller J, Schocke M, Brenneis C, Rinnerthaler M, Seppi K et al. Voxel based morphometry reveals specific gray matter changes in primary dystonia. Mov Disord 2007; 22:1538-1542.
23. Obermann M, Yaldizli O, De Greiff A, Lachenmayer ML, Buhl AR, Tumczak F et al. Morphometric changes of sensorimotor structures in focal dystonia. Mov Disord 2007; 22:1117-1123.
24. Carbon M, Kingsley PB, Tang C, Bressman S, Eidelberg D. Microstructural white matter changes in primary torsion dystonia. Mov Disord 2008; 23:234-239.
25. Draganski B, Gaser C, Busch V, Schuierer G, Bogdahn U, May A. Neuroplasticity: Changes in grey matter induced by training. Nature 2004; 427:311-312.
26. Hallett M. Neurophysiology of dystonia: The role of inhibition. Neurobiology of Disease 2011; 42:177-184.
27. Quartarone A, Siebner HR, Rothwell JC. Task-specific hand dystonia: can too much plasticity be bad for you? Trends in Neurosciences 2006; 29:192-199.