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Motor and non-motor symptoms in cervical dystonia

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CHAPTER 8

General Discussion

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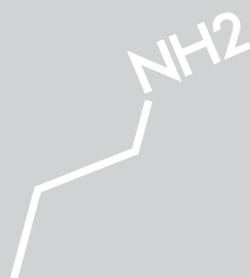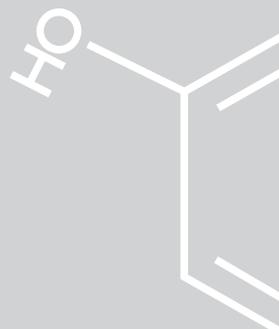
GENERAL DISCUSSION

Cervical dystonia (CD) is a hyperkinetic movement disorder which affects daily living of many patients. In the Netherlands, the prevalence of cervical dystonia (CD) is estimated at 8000 patients. This thesis focuses on two important aspects of CD, namely its phenotype, including motor and non-motor symptoms (NMS), and the pathophysiology of CD, focusing at the role of serotonin and the relative cerebral blood flow.

We first systematically investigated the frequency and severity of NMS in CD patients and matched controls, including psychiatric co-morbidity, fatigue, excessive daytime sleepiness and sleep disturbances (Chapter 2 and 3). Moreover, we examined the influence of both motor and NMS on HR-QoL. We also explored an extended dystonia NMS questionnaire, as a first step towards a validated NMS questionnaire suitable for the use in daily practice (Chapter 4). With this questionnaire, we not only assessed which NMS are highly prevalent, but also which NMS are experienced as most tedious.

In the second part of th is thesis, we explored the role of serotonin in motor and NMS in patients with several forms of dystonia, described in a systematic review (Chapter 5). Based on frequently described low levels of the breakdown product of serotonin and the influence of several serotonergic drugs on dystonia, we have tried to explain how the serotonergic system could influence dystonia as an extrapyramidal movement disorder.

Then, in the last part of this thesis, we set a first step in investigating the role of serotonin in CD patients and matched controls using *in vivo* techniques. This was performed with [¹¹C]DASB positron emission tomography (PET) imaging, examining serotonin transporter (SERT) binding as important presynaptic serotonergic marker (Chapter 6). Moreover, this technique revealed also information about the relative cerebral blood flow, which is discussed in Chapter 7.

Our main findings will be discussed in the following paragraphs. After this discussion, I will present some ideas for future research directions.

Psychiatric co-morbidity

In the first part of this thesis, we used a case-control design to investigate the frequency and severity of several NMS and their influence on HR-QoL. In Chapter 2, we first investigated psychiatric co-morbidity in 50 CD patients and 50 matched controls. We demonstrated that 64% of CD patients fulfilled the criteria of a psychiatric disorder in the present and/or past, which was significantly higher than in the control group (28%). Also in our study, most of the psychiatric illnesses consisted of depression and anxiety. Of the 32 patients who fulfilled the criteria of a psychiatric disorder according to the DSM-IV criteria, 20 patients (62.5%) retrospectively suffered from one or more psychiatric disorders before the onset of motor symptoms. This finding suggests that psychiatric

disorders are more likely part of the phenotype of dystonia, instead of a secondary phenomenon in response to the burden of motor symptoms. Moreover, we could not detect a relation between the severity of motor symptoms and the frequency and severity of psychiatric disorders, which also suggests a primary mechanism of NMS. Since a few years, the origin of psychiatric comorbidity in dystonia has been the subject of a continuous debate. Recently, Zurowski et al. summarized the arguments in favor of either a primary or secondary cause [1]. Again, the onset of psychiatric co-morbidity before the onset of motor symptoms was one of the strongest arguments that psychiatric disorders are intrinsic to the neurobiology of dystonia. Another argument included the 1:1 ratio of men to women with dystonia and psychiatric co-morbidity, while in the general population women are more frequently diagnosed with psychiatric disorders (ratio men to women: 1:2). Also, the missense relation between motor severity and psychiatric co-morbidity, which was confirmed in our study, and the higher ratio of psychiatric disorders in dystonia patients compared with other visible and disabling disorders argues for a primary cause. An argument in favor of a secondary cause is the high correlation of pain with psychiatric co-morbidity in previous studies. In our study, pain appeared to be related to disability and anxiety symptoms, but not to other psychiatric co-morbidities like depression. Until now, only retrospective studies examined psychiatric co-morbidity, a design that is not suitable to draw firm conclusions about a primary or secondary cause of psychiatric co-morbidity in dystonia. Also in our study, we noticed that diagnosing psychiatric disorders that happened in the past had some drawbacks. Retrospectively assessing psychiatric disorders is difficult, as subjects often did not remember their exact symptoms. Finally, to distinguish between a primary or secondary cause, prospective studies are warranted.

Fatigue and sleep disturbances
The frequency and severity of the NMS fatigue, excessive daytime sleepiness and sleep disturbances were examined in chapter 3. One of the problems when examining fatigue and sleep-related measures is the high correlation with psychiatric co-morbidity and the overlap between questionnaires examining fatigue, sleep and psychiatric disorders. Therefore, it is important to systematically investigate all those aspects, in order to be able to distinguish between a primary NMS or a secondary phenomenon in reaction to or related with psychiatric co-morbidity. In previous studies, methodological limitations regarding this systematic approach were noted. Another issue in previous research is that fatigue was often not defined, while its meaning can be interpreted in many ways. Two major domains of fatigue include the perception of fatigue, influenced by homeostatic factors and psychological factors, and performance fatigability, which depends on both peripheral and central factors [2]. In our study, we used a quantitative questionnaire examining the level of perceived fatigue.

In our cohort of CD patients, fatigue scores were increased independently from psychiatric co-morbidity, while excessive daytime sleepiness and impaired sleep quality were highly associated with depression and anxiety. Motor severity, including both dystonic posturing,

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jerks and tremor, did not explain variation in fatigue, excessive daytime sleepiness and sleep quality scores, which again suggest a primary mechanism of these NMS.

Interpreting fatigue as primary NMS, intrinsic to the neurobiology of dystonia, might shed new light on the underlying pathophysiology. In Parkinson's disease, an abnormal perception of fatigue has been related to altered basal ganglia function [3], regions that are also related to the dystonia pathophysiology [4,5]. Moreover, disturbances in the domains perception of fatigue and fatigability often occur together. This means that also specific cortical and subcortical networks involved in fatigability, which encompass the motor cortices, thalamus and again the basal ganglia [2], might be involved in the dystonia pathophysiology. These dysfunctional networks and structures involving the basal ganglia point towards a possible role of neurotransmitters. Central disturbances in the neurotransmitters serotonin and dopamine are described in both fatigue and dystonia [2,7,8]. The serotonergic hypothesis of central fatigue includes that serotonergic activity increases during prolonged exercise, resulting in lethargy and loss of drive [9]. In general, serotonergic agonists induce a dose-dependent reduction in exercise capacity while serotonergic antagonists enhances this capacity [9]. Relating to motor symptoms, an increased serotonergic state also increases the excitability of motoneurons and thereby facilitates the generation of action potentials [10]. In an animal model of dystonia, injections with serotonin in the facial nucleus induced focal dystonia of the eyelids [11], also supporting the hypothesis that regional increased levels of serotonin might form a shared pathophysiological pathway of motor and NMS in dystonia. All together, these findings reinforce the hypothesis of a primary mechanism, intrinsic to dystonia, being responsible for the perception of fatigue in CD patients. In these mechanisms, serotonergic networks might well be involved. I'll come back to this hypothesis in the part on our study of serotonin involvement.

NMS questionnaire
As described in chapter 2 and 3, psychiatric co-morbidity, fatigue and sleep disturbances are highly prevalent in CD patients. However, the NMS spectrum of dystonia also includes other (less prevalent) symptoms. While examining psychiatric co-morbidity in the first part of the study, patients spontaneously mentioned problems with for example concentration or sexual activities, both NMS that have previously not been systematically examined. In Chapter 4, we explored a NMS questionnaire for dystonia. For this purpose, we used an extended version of the NMS rating list for CD patients as proposed by Klingelhoefer et al., who in turn composed a list distracted from the NMS Questionnaire validated in Parkinson's disease [12]. Our list proved a valuable exploration of the frequency of NMS. Up to 95% of the included patients experienced NMS, with a median number of NMS of 6.5 (range 0-13, maximum 15). Moreover, it provided information on which motor and NMS were experienced as most burdensome, rather than only highly prevalent. This revealed that patients were bothered the most by tremor/jerks, pain, sleep disturbances, daily-life limitations and fatigue. The results of this study highlighted the impact of NMS

and emphasized the importance of a validated NMS-questionnaire in dystonia. Similar as developed for Parkinson's disease, this would ideally exists of both a NMS questionnaire that can be used by the patients themselves and a NMS rating scale for professionals. The questionnaire for patients would then focus on the frequency of different NMS, and the more elaborate rating scale used by professionals would include a quantitative scoring system for both the frequency and severity of NMS.

Influence on health-related quality of life

In all our studies, we additionally examined the influence of motor and NMS on HR-QoL. It appeared that only NMS influenced HR-QoL, while motor symptoms did not influence any of the HR-QoL domains. This was first shown in the paper about psychiatric co-morbidity, and confirmed in the paper about fatigue and sleep disturbances that took into account the influence of both psychiatric co-morbidity, pain, fatigue and sleep-disturbances. The multiple linear regression analysis revealed that fatigue had a significant negative influence on the domain physical functioning, on the domain mental health and on the domain pain. Excessive daytime sleepiness had a significant negative influence on the domain mental health and on the domain vitality. The severity of depression, anxiety and pain was also negatively associated with the domains physical functioning, social functioning, role limitation emotional, vitality and pain. Sleep disturbances were not associated with any of the HR-QoL domains. Motor symptoms were not included in the multiple regression analysis, because they were not associated with HR-QoL in the univariate analysis.

Our findings emphasize the need for more personalized medicine. Until now, the treatment of CD patients usually focuses on improvement of the motor symptoms, with periodic botulinum toxin injections in the affected muscles. However, regarding HR-QoL as important outcome measure of treatment, NMS should receive more attention. The significant influence of several NMS on all aspects of HR-QoL highlights the need for systematic screening of these symptoms in the daily practice. Future studies are needed to investigate whether targeted treatment of NMS could improve HR-QoL in CD patients.

Is serotonin a shared pathophysiological pathway of motor and NMS?

In the second part of this thesis, we performed a systematic review of the literature concerning the role of serotonin in both motor and NMS in patients with several forms of dystonia (Chapter 5). Serotonin is an important neurotransmitter involved in structures and circuitries that are currently hypothesized to play a key role in the dystonia pathophysiology, such as the basal ganglia, the basal ganglia-thalamo-cortical circuit and the cerebello-thalamo cortical circuit [13]. Moreover, its role in the NMS that are highly prevalent in CD, like psychiatric co-morbidity, pain and sleep disturbances, is well established [14,15]. Our review revealed that disturbances of the serotonergic system were found in both inherited, acquired and idiopathic dystonias and that they might be related to both motor severity and NMS. One of the most consistent findings comprised

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a decreased level of 5-hydroxyindolacetic acid (5-HIAA), the breakdown product of serotonin, in cerebrospinal fluid, suggestive of either an altered serotonergic turnover or synthesis. As expected, this was mainly found in dopa-responsive dystonias, in which several gene mutations directly influence the metabolism of serotonin. However, changes in 5-HIAA levels were also detected in other dystonia subtypes like CD.

Another important finding comprised the effect of serotonergic acting drugs, either eliciting or improving dystonia, probably depending on several factors such as the specific target on the serotonergic system, the underlying dystonia etiology, the age when initiated and the duration of treatment.

The NMS related to serotonergic dysfunction mainly included psychiatric co-morbidity and sleep disturbances, although they were often not systematically examined and therefore likely underestimated.

Importantly, our systematic review clearly highlighted the complicatedness of the serotonergic system and the drawbacks of conventional analyzing methods. This is not surprising, as the serotonergic system forms the most complex efferent system of the human brain. From the raphe nuclei located in the brain stem, 300.000 serotonergic projections reach to various subcortical (basal ganglia) and cortical regions, as well as downward to spinal neurons. The firing rate of the raphe nuclei is mainly regulated by the synthesis of serotonin from diet derived tryptophan and by 5-HT$_{1A}$ somatodendritic autoreceptors, whereas serotonin synthesis and release in the output areas is regulated by 5-HT$_{1B/D}$ receptors and by re-uptake action of the serotonin transporter. After release, serotonin can bind to at least 18 different pre- and post-synaptic receptors, after which it is either broken down by monoamine oxidase A (MAO-A) in 5-HIAA or melatonin, or taken back into the presynaptic neuron by the serotonin transporter [16]. The serotonin transporter is thus an important regulator of serotonergic activity. Most studies used the level of 5-HIAA in cerebrospinal fluid as marker of serotonergic functioning, but this only reflects one part of the system in a different compartment then directly in the brain itself. Moreover, changes in 5-HIAA levels could indicate disturbances in either the synthesis, release or breakdown, which makes interpretation even harder.

Decreased levels of 5-HIAA in dystonia can be interpreted in several ways. For example, dysfunction of MAO-A may well lead to both increased synaptic levels of serotonin and decreased levels of 5-HIAA. Interestingly, in earlier studies the MAO-A agonist reserpine has shown beneficial effects in dystonic symptoms [17]. Decreased levels of 5-HIAA may also point at a decreased serotonin synthesis, a mechanism that is more likely involved in dopa-responsive dystonias. Ideally, all the aspects of the serotonergic system should be studied simultaneously to be able to draw firm conclusions about the exact pathophysiological mechanisms that can be involved in dystonia.

The same problem holds true for understanding the effect of serotonergic medication. As mentioned, serotonergic agents either elicited or improved dystonia, depending on the specific target on the serotonergic system, the underlying dystonia etiology, the age when initiated and the duration of the treatment. Influencing one part of the serotonergic system induces a cascade of effects, both on the serotonergic system as well as on other neurotransmitter systems. For example SSRIs, frequently prescribed for psychiatric symptoms also in dystonia patients, induces differential short- and long-term effects on the serotonergic system. First, it blocks the serotonin transporters both near the raphe nuclei and the output areas. Only for a short time, this results in an increased amount of synaptic serotonin in the brain. Because increased synaptic serotonin levels near the raphe nuclei causes an increased 5-HT_{1A} autoreceptor activation, the firing rate decreases and the increased synaptic levels of serotonin will return to pretreatment levels. The long-term effect of SSRIs is based on the desensitization of the 5-HT_{1A} autoreceptors, finally increasing the level of serotonin in the output areas [18]. Moreover, as the serotonergic system has mainly an inhibiting effect on the dopaminergic system, SSRIs not only influence the serotonergic system but also other neurotransmitters. This is only one example of the complicatedness of influencing the serotonergic system, where influencing one part of the serotonergic system affects many other sub receptors and also other neurotransmitters. Investigating several parts of the serotonergic system involved in basal ganglia functioning will be important to shed more light on the serotonergic perturbations involved in dystonia. Possibilities to do this lie in animal studies but also in patient studies using *in vivo* imaging, where several tracers of serotonergic systems are available. For this thesis, we have set a first step in this direction. I'll discuss this further in the following sections.

[¹¹C]DASB PET imaging in CD patients

With our [¹¹C]DASB PET brain imaging study, we investigated whether altered SERT binding in the brain contributed to the pathophysiology of CD. Our results indicated that cerebral serotonin indeed contributes to the pathophysiology of both motor symptom severity and several NMS.

First, we demonstrated a significant correlation between increased [¹¹C]DASB binding in the dorsal raphe nucleus (dRN) of CD patients and the severity of the dystonic posturing, pain and sleep disturbances. This correlation was consistent with the observed trend of increased SERT binding in the dRN in CD patients when compared to control subjects. At the hemisphere target regions of serotonin innervation, on the other hand, we found a trend towards a bilateral decrease of SERT binding in the putamen and pallidum in the CD patients.

The trend of increased SERT binding in the dRN and decreased SERT binding in the basal ganglia did not reach statistical significance. However, given the medium to large effect size, one may argue that this absence of significance was due to a small sample size. Importantly, the identification of decreased bilateral basal ganglia SERT

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binding resulted from both pre-defined VOI and hypothesis-free voxel-based analysis. Particularly the distinct spatial pattern of the voxel-based results supports our inference that this changed SERT binding indeed represents region-specific serotonergic changes within the striatum of CD patients.

Increased SERT binding in de dRN in CD patients may suggest an increased activity of the serotonergic system. The hypothesis that regional increased levels of serotonin in our patient group might contribute to the dystonia pathophysiology is supported by the literature describing serotonin-induced motor effects, which has also previously been discussed. In both cat and monkey, serotonin injections in the facial nucleus induced focal dystonia of the eyelids [11]. Administering the precursor of serotonin elicits a consistent hyperkinetic motor syndrome including lateral head waving [19]. At the spinal cord level, serotonin increases spinal motor neuron excitability, facilitating the generation of action potentials [10]. Moreover, serotonergic activation caused suppression of sensory information processing [20], which is compatible with the disturbed sensorimotor (afferent) integration known to be an important key stone of the dystonia pathophysiology [4].

To understand the role of serotonin in the pathophysiology of extrapyramidal movement disorders like dystonia, it is important to understand the dynamic balance of the serotonergic system and the complex interaction between serotonin and other neurotransmitters. Besides the important role of SERT, the 5-HT_{1A} receptor also plays a crucial role in motor activity. Previous studies have shown that activation of 5-HT_{1A} receptors ameliorates extrapyramidal and specifically hyperkinetic movement disorders [21]. Regarding the interaction between serotonin and other neurotransmitters, the serotonergic system has mainly an inhibiting effect on the dopaminergic system, of which alterations might further contribute to the dystonia pathophysiology [5]. Furthermore, in the striatum, GABAergic medial spiny neurons receive excitatory glutamatergic input from the motor cortex as well as excitatory innervation of cholinergic interneurons positioned within the striatum (reviewed by Ohno et al. [21]). In contrast to the excitatory influence of the glutamatergic and cholinergic system, the dopaminergic system has an inhibitory influence on these neurons located in the striatum. The serotonergic system modulates extrapyramidal movement disorders caused by disturbances in the delicate balance between the GABAergic, glutamatergic, cholinergic and dopaminergic system. This is mainly regulated by the 5-HT_{1A}, $5\text{-HT}_{2A/2C}$, 5-HT_3 and 5-HT_6 receptors, with either a positive or negative influence on the movement disorder.

In our PET imaging study we studied the serotonin transporter as an important presynaptic marker. However, considering the complexity of the serotonergic system, a combined study of SERT and 5-HT_{1A} autoreceptor binding or other pre- or postsynaptically located receptors would potentially provide more insight in the dynamic balance of serotonergic functioning. Furthermore, it would be interesting to combine information about the role of the serotonergic system and other neurotransmitters like acetylcholine or dopamine.

As recently reviewed by Eskow Jaunarajs et al. [22], the cholinergic system is also an important contributor to the dystonia pathophysiology. In several forms of dystonia, cholinergic acting medication such as trihexyphenydil proved a valuable treatment, especially in children [22]. The cholinergic system influences dopaminergic activity and plays a critical role in the plasticity underlying motor learning, a key feature of the dystonia pathophysiology [4]. Moreover, cholinergic interneurons play a central role in the activity of both the direct and indirect basal ganglia motor pathway, of which an imbalance is also suggested to be part of the dystonia pathophysiology [4].

Besides the significant correlation between dRN SERT binding and motor symptoms, also the NMS pain and sleep disturbances were significantly correlated with increased SERT binding is this region. This finding favors our hypothesis of a shared pathophysiological pathway of motor and NMS in dystonia. A relation between serotonergic activity and sleep disturbances has mainly been described in studies using microdialysis techniques. Altered discharge patters of serotonergic neurons were suggested to be involved in sleep disturbances, either directly by influencing the different serotonergic postsynaptic receptors or by influencing other neurotransmitters [15]. Particularly the REM sleep seems to be controlled by serotonergic activity. Moreover, a relation between both serotonergic activity arising from the dRN, REM sleep and muscle tone have been described in animals. The REM sleep paralysis is mediated by inhibition of motoneurons, during which activity of serotonergic neurons is also suppressed. Moreover, inactivation of the dorsal raphe nucleus in animals caused a condition similar to REM sleep and simultaneously induced muscle paralysis [19], further linking this region to sleep disturbances. Serotonin involvement in pain has also been supported by previous studies. For example, spinal analgesic action is mediated by serotonin release, and selective serotonin reuptake inhibitors induce a central analgesic effect [23].

With our study we could not detect a significant relation between SERT binding and psychiatric co-morbidity, an important NMS on which the hypothesis of a shared pathophysiology was also based. However, not corrected for multiple testing, SERT binding in the right hippocampus appeared to be negatively related with psychiatric co-morbidity. A possible explanation that we could not detect a relation between psychiatric co-morbidity and SERT binding is the variance in psychiatric symptom severity among the subjects, prohibiting significant power in a small group. Another possible influence is the differentiation of the raphe nuclei, divided in such small regions that individual delineation is not possible with the current resolution of PET cameras. Eight different raphe nuclei have been described, while we were only able to manually delineate the dorsal and median raphe nuclei based on regional high uptake and MNI coordinates. Moreover, the nuclei themselves are further divided in several sub regions, based on cytoachitecture and topographically organized projections [24]. For example, the dRN exists of nine sub regions, which even allow a more precise regulation of serotonergic output [24]. Besides the anatomical organization, each of the nuclei are also functionally

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differentiated. For example, the raphe nucleus magnus has been related to pain and the raphe nucleus obscurus and pallidus to motor activity [25]. This differentiation with small sub regions involved could be an explanation why we could not detect a relationship between psychiatric co-morbidity and SERT binding. Also in other studies examining depressive patients inconsistent numbers of SERT binding sites were detected [26,27], which is probably caused by differences in patients characteristics, the underlying pathophysiology, different brain regions studied or different analysis approaches.

Perturbations in relative cerebral blood flow
In addition to SERT binding, the [^{11}C]DASB PET imaging study also revealed information about the relative cerebral blood flow (rCBF) (Chapter 7). Using the SRTM2 model, we calculated the relative tracer delivery to specific brain regions as compared to the reference region. This model has proved to generate a reliable measure of the rCBF [28]. With this study, we found a decreased rCBF symmetrically in the temporal lobe and unilaterally in the thalamus, frontal and orbital gyrus, insula and anterior cingulate. The same pattern of a decreased flow in the temporal lobe has been described in two case reports of patients with myoclonus dystonia, which was explained as a result of psychiatric co-morbidity [29,30]. However, the actual relationship between the decreased blood flow and psychiatric symptoms was not described. In our study, when we examined the relation between the rCBF and NMS in all participants, we found a significant correlation between a decreased flow in the superior temporal gyrus and the NMS depression, anxiety and fatigue. This finding, together with the significant relation between a decreased SERT binding in the hippocampus and psychiatric co-morbidity, attributes to the described functional connectivity changes in a network involving the hippocampus, amygdala and temporal cortex in CD patients [31].

Interestingly, we also found a significant relationship between the severity of jerks/tremor and a decreased flow in the basal ganglia and insula. In Parkinson's disease, the insula plays an important role in disturbances in sensorymotor integration and autonomic and cognitive-affective information processing [32], which are all described in dystonia as well [4]. Moreover, the posterior part of the insula plays an important role in head-motion registration, which highlights a likely role of the insula in CD.

In conclusion, by employing the initial distribution of [^{11}C]DASB as an apparent index of relative rCBF we were able to identify changes in the limbic circuitry. Together with decreases in the spatially adjacent insula, the involvement of these regions point at functional impairment of circuitry serving integration of limbic and sensorimotor information likely associated with CD. Indeed, we found a relationship between a decreased rCBF in the temporal lobe and psychiatric co-morbidity, and a relationship between the basal ganglia and motor symptoms. A challenge for future functional brain imaging in CD research is to identify crucial nodes in cerebral circuitry that may be initially affected and how network dysfunction subsequently expands in the brain.

Future directions

Our research revealed three major knowledge gaps. First, as described in chapter 2 to 4, it appeared that NMS are highly prevalent, but their origin, either primary or secondary, cannot be ascertained based on retrospective studies. Therefore, prospective trials are warranted. Two new developments make prospective trials within reach. First is the discovery of new CD related genes, like the *ANO3*, *GNAL* and *CIZ1* gene [33]. This discovery enables to follow gene carriers over time, which could specify the time course of both motor and NMS. Another possibility is the use of endophenotypes, i.e. to study trait (endophenotype) instead of state (phenotype), also to avoid the misattribution of secondary adaptive changes to pathogenesis [34]. Moreover, this approach facilitates to study the contributing environmental effects that could lead to the development of the phenotype. In CD, the abnormal temporal discrimination threshold may be a reliable endophenotype that could be used to select subjects for prospective trials. It reflects a prolonged interval in which two stimuli may be determined as asynchronous [34]. This endophenotype is found in 50% of unaffected first-degree relatives and therefore a good candidate to use in subject selection in future research.

Second, it appeared that NMS are highly prevalent and significantly influence HR-QoL, but the effect of NMS treatment on HR-QoL is still unknown. For this purpose, a validated dystonia specific NMS questionnaire is indispensable. Such a questionnaire would enable to monitor the effect of motor and NMS treatment on an individual basis and ultimately improve treatment directions.

The third knowledge gap comprised the pathophysiology of both motor and NMS in CD. Although in recent years several aspects of the pathophysiology have been detected, causal mechanism are still not fully understood. Changes at the neurochemical level are an interesting candidate for further research, because structural changes do not provide a diagnostic hallmark in CD. With our study we have made a first step towards a better understanding of the role of serotonin in the pathophysiology of CD, but only one part of this system was investigated. In addition to SERT, it would be interesting to study the role of the 5-HT_{1A} receptor using PET. For this, the most commonly used radioligands are [^{11}C]WAY-100635 [35] and [^{18}F]MPPF [36]. The combination of SERT and 5HT_{1A} receptor imaging, both important regulators of serotonin levels, could lead to a better understanding of the dynamic balance within the serotonergic system in dystonia. Also for MAO-A, the enzyme effective in serotonin breakdown, several tracers have been developed, for example [^{11}C]befloxatone. Moreover, the role of other neurotransmitters remains to be elucidated. Ideally, this should also be performed by using *in vivo* techniques. The tracers [^{18}F]DOPA and [^{11}C]raclopride provide the opportunity to study important presynaptic and postsynaptic aspects of the dopaminergic system, and the recently developed [^{18}F]FEOBV tracer is a good candidate to study presynaptic function of the cholinergic system [37]. Investigation of neurochemical systems that may be involved in dystonia pathophysiology is still in its infancy, and *in vivo* imaging using PET provides a means to expand our knowledge of these mechanisms.

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Conclusions

To conclude, we detected a high frequency of NMS in CD patients, with a high impact on HR-QoL. It appeared that psychiatric co-morbidity and fatigue are most likely primary symptoms and part of the phenotype of CD, while excessive daytime sleepiness and impaired sleep quality were highly related to psychiatric co-morbidity and pain. To improve the diagnostics and treatment of NMS, we proposed and explored a NMS questionnaire as a first step towards a dystonia specific NMS questionnaire. Future studies are warranted to explore the effect of NMS treatment on HR-QoL.

Secondly, we explored the role of serotonin in the pathophysiology of dystonia. It appeared that serotonergic perturbations in the raphe nuclei and in basal ganglia output regions are related to both motor and NMS in CD. Currently, there are no good (pharmaco)therapeutic options for most forms of dystonia or associated non-motor symptoms. Further research using PET imaging or by using selective serotonergic drugs in appropriate models of dystonia is required to establish the role of the serotonergic system in dystonia and to guide us to new therapeutic strategies.

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