The role of dopamine and serotonin in cervical dystonia
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Citation for published version (APA):
Zoons, E. (2018). The role of dopamine and serotonin in cervical dystonia

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GENERAL DISCUSSION
In this thesis, we investigated the roles of dopamine and serotonin in the brain in the pathophysiology of cervical dystonia (CD). CD is a debilitating movement disorder that is often accompanied by non-motor symptoms like depressive symptoms and anxiety. Thus far, the best treatment for CD is local injections with botulinum neurotoxin (BoNT). This treatment mainly focuses on the motor symptoms (dystonia and jerks) and pain. When we know more about the pathophysiological mechanism underlying motor and non-motor symptoms of dystonia, and more specific the role of neurotransmitters such as dopamine and serotonin we might be able to find a pharmacological therapy that treats all symptoms of CD.

Neuroimaging has been used to investigate brain regions hypothesized to be involved in the pathophysiology of focal dystonia, including CD. In the past, most studies investigating patients with CD or other types of focal dystonia have studied one particular area using a single imaging technique. This approach was either because the authors were interested in a single area or because the used technique was limited to a certain region. We give a general overview of different neuroimaging techniques used and describe why focal dystonia is considered a network disorder with suggested abnormalities mainly in the basal ganglia, thalamus, cerebellum and cortical areas (chapter 2). The basal ganglia are hypothesized to play the most important role in this network.

Dopamine, a neurotransmitter which is abundantly present in the basal ganglia, has been considered to be one of the most important neurotransmitters involved in the pathophysiology of dystonia. Also, dopamine is involved both in motor as well as non-motor functioning. We therefore, performed a study investigating tracer binding to both the presynaptic dopamine transporter (DAT) and postsynaptic dopamine D$_{2/3}$ receptor (D2/3R) in the striatum of patients with CD. In CD, there is an increasing attention over the last years for non-motor symptoms, especially psychiatric symptoms like depression and anxiety. The current opinion is that these non-motor symptoms are part of the phenotype of CD. We therefore, not only correlated the DAT and D2/3R tracer binding to motor symptoms, but also to psychiatric, mainly depressive symptoms (chapter 3).

It is known from the literature that depression and anxiety are related to alterations of the serotonergic system. Furthermore, it is known that the dopaminergic and serotonergic systems are closely related. We performed the first SPECT study investigating the presynaptic serotonin transporter (SERT) in the midbrain of patients with CD (chapter 4), again in relation to motor and psychiatric symptoms. In addition we performed an intervention study with escitalopram, a selective serotonin reuptake inhibitor (SSRI). Thus far, the most used treatment for CD is local injections with BoNT. However, this
treatment has its limitations and, especially in patients with CD and jerks or tremor of the head, it has proven to be difficult to achieve satisfactory results. We aimed to restore the hypothesized imbalance between serotonin and dopamine. In this trial the clinical effect on motor symptoms (dystonia and jerks) as well as on non-motor symptoms (depression and anxiety) was investigated (chapter 5). In a separate article we reported the effects of escitalopram and placebo on SERT, DAT and D2/3R binding (chapter 6).

In the remainder of this chapter the results of these articles will be discussed and placed in a broader perspective. Hereafter, some suggestions for future research will be given.

**Neuroimaging: pathophysiologic changes in the brain in focal dystonia**

Using neuroimaging tools in focal dystonia, including CD, abnormalities have been found in different brain areas: basal ganglia, thalamus, cerebellum and cortical areas, mainly sensorimotor cortex. We wrote an extensive review summarizing the found abnormalities. In this review, studies using different MRI, PET and SPECT methods were included. Using voxel-based morphometry (VBM), an MRI technique that is commonly used to measure the volume of brain regions, a 10% increased volume of the putamen was found in patients with several types of focal dystonia compared to controls. Other reported abnormalities using VBM were an increased volume of the sensorimotor cortex, thalamus and cerebellum, although these were less consistent than the putaminal increase. Using diffusion tensor imaging (DTI), abnormalities were found in the white matter tracks connecting the basal ganglia, sensorimotor cortex and cerebellum. The most consistent finding in DTI studies was increased cellularity in the basal ganglia of patients with focal dystonia. One main disadvantage of both VBM and DTI is that they are static investigations. In comparison, one of the most used dynamic imaging techniques to study patients with focal dystonia is functional MRI (fMRI), which reflects brain activity. Using fMRI, brain activity can be compared between patients and controls as well as between performing a task and rest session. In dystonia, fMRI has mainly been used in patients with writer’s cramp and related forms of task-specific hand dystonia, as they can perform tasks in the MRI scanner that will evoke dystonia. During the tasks that evoke dystonia, an increase in activation of the primary sensory and motor cortex, accessory motor cortices, basal ganglia, thalamus and cerebellum was seen. Tasks that do not evoke dystonia lead to a decrease in activation of these same areas. It is interesting that a decrease in activation of these brain areas was also found in patients with CD, while performing and imagining hand movements. This suggests a common pathophysiologic mechanism between different forms of focal dystonia. Similar results in brain activity were found using PET and glucose ([18F]fluorodeoxyglucose; [18F]FDG) or blood flow [15H2O] tracers. There are different theories about what causes the
abnormalities found with fMRI in the brains of patients with focal dystonia. One theory is that there is disturbed sensorimotor integration, which means that sensory and motor information needed to initiate voluntary movements is combined in a faulty manner, leading to dystonic movements. An interesting finding in fMRI and [18F]FDG PET studies to support this theory, which has also been confirmed using magnetoencephalography (MEG), is altered representation of the hand and different digits in hand dystonia and of the lips in embouchure dystonia. This is of interest since many patients with focal dystonia have a sensory trick, where a slight touch of the affected body part leads to temporarily diminishing of dystonia. However, such a finding can also be explained by the theory of reduced surround inhibition. The hypothesis is that the initiation of a voluntary movement leads to activation of a cortical area that is larger than needed, which in turn leads to activation of too many muscles and dystonic movements. A larger activated area on the motor cortex has been found using fMRI in patients with writer’s cramp. As mentioned above, found abnormalities in the basal ganglia, thalamus, cerebellum and cortical areas are comparable between different types of focal dystonia and it is very likely that an abnormality somewhere in this network causes focal dystonia by inducing neuroplastic changes in the other brain regions that will lead to excessive muscle activation. Dysfunction of the dopaminergic system has been implicated in the origin of abnormal plasticity in cortico-striatal transmission, which is why we studied this neurotransmitter in more detail.

The role of dopamine in CD
In most molecular imaging studies, including ours, the non-displaceable binding potential (BP_{ND}) is used as main outcome measure. This BP_{ND} can be calculated using a region of interest (ROI) and reference region as \([\text{activity in ROI} – \text{activity in reference region}]/\text{activity in reference region}\), and this ratio represents the binding potential (BP_{ND}). In our study, we found a trend towards lower D2/3R BP_{ND} and normal DAT BP_{ND} in patients with CD compared to controls. However, the most novel finding was the strong correlation between depressive symptoms and a lower BP_{ND} to both D2/3R as well as DAT, while there was no such correlation for severity of dystonia. The trend towards lower D2/3R BP_{ND} in CD patients disappeared when we corrected for depressive symptoms.

Dystonia has for long been considered a hyperdopaminergic disorder. There is compelling evidence for a role of the basal ganglia (see above) in the pathophysiology of dystonia and dopamine as one of the most important neurotransmitters in the basal ganglia. Dystonia has often been described as a side effect of neuroleptic drugs, which mainly block D2Rs. Patients with dopa-responsive dystonia have a genetic defect in
the synthesis of dopamine and clinically have dystonia that responds well to levodopa.\textsuperscript{11} In the past several PET and SPECT studies have been performed to investigate the dopaminergic system in patients with different types of dystonia. Overall, most studies found decreased binding of tracers to striatal D2/3R, although increased D2/3R has also been reported. In the past no abnormalities in tracer binding to the striatal DAT have been found. In most studies the presence of a possible correlation between binding and severity of dystonia was not tested and when a possible correlation was investigated, it was not detected.\textsuperscript{12-15} A correlation between tracer binding and non-motor symptoms has not been investigated thus far.

As stated above, in dystonia most studies found decreased tracer binding to D2/3R, but it is unclear whether this effect is caused by decreased expression of either D2R or D3R or by increased levels of synaptic dopamine. \textsuperscript{\[123I\]}IBZM is the most used SPECT tracer to study D2/3R, but it is known that \textsuperscript{\[123I\]}IBZM binding can be displaced by endogenous dopamine and this tracer binds to both D2R and D3R with the same affinity while D3R are greatly outnumbered in the striatum by D2R.\textsuperscript{16} D3R was always thought to be predominantly present in the ventral striatum, related to the limbic system. Recently it has however been demonstrated that there is a substantial amount of D3R in the dorsal striatum, related to the motor system.\textsuperscript{9} One study used PET and the highly selective D2R tracer \textsuperscript{\[18F\]}NMB in patients with hand and cranial dystonia and found no difference in D2R BP\textsubscript{ND} between patients and controls.\textsuperscript{17} Even though this is only one observation and results were not corrected for presence or severity of depression, it may suggest that the decreased D2/3R BP\textsubscript{ND} found in most studies is actually a decreased D3R BP\textsubscript{ND} caused either by loss of D3R or by competition between increased levels of synaptic dopamine and the radiotracer. D3R has been proven to play an important role in the pathophysiology of other basal ganglia disorders, including Parkinson’s disease (PD), as well as in major depression. In drug-naïve patients with PD it is known that there is mainly downregulation of D3R and not of D2R.\textsuperscript{18} In patients with major depression, an upregulation of D3R was found in the nucleus accumbens of patients after successful treatment with either tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase-B inhibitors (MAO-B inhibitors) and electroconvulsive therapy.\textsuperscript{19-21} Selective D3R tracers have been developed and tested in primates, but no studies in patients with dystonia have been performed thus far.\textsuperscript{9} \textsuperscript{\[\textsuperscript{11C}\]}PHNO is a PET tracer that has been used in human studies before and that is more D3-selective than other available D2/3R tracers. Especially in structures outside the striatum, such as the hypothalamus, substantia nigra, globus pallidus and thalamus, this tracer almost exclusively binds to D3R.\textsuperscript{22} It would be interesting to conduct a study using this tracer in patients with CD. Given the size of some of this regions, for example the substantia nigra, this should be a study with MRI co-registration.
As stated above, CD is associated with depressive symptoms and these symptoms often precede the motor symptoms. Furthermore, in some genetic forms of dystonia such as DYT1, family members with the DYT1 mutation but without dystonia, have an increased prevalence of major depression. Depressive symptoms are now considered part of the dystonia phenotype. Major depression has for long been hypothesized to be a serotonergic disorder, but the past years there is increasing evidence that a decrease in synaptic dopamine also plays a key role in its pathophysiology. Reduced levels of homovanillic acid (HVA), which is the main metabolite of dopamine, have been found in the cerebrospinal fluid (CSF) of patients with major depression. This could indicate that in CD there are already changes to the dopamine system causing depressive symptoms, before the first motor symptoms occur. These changes to the dopaminergic system in CD could resemble the changes found in patients with major depression. Molecular imaging studies in patients with major depression can help understand the (early) changes in the dopaminergic system of patients with CD.

Studies towards dopamine receptors in patients with major depression have been limited. Overall, no significant difference has been found in striatal D2/3R BPND with either tracer. Over the years, both PET and SPECT studies have evaluated DAT binding in patients with depression using different tracers and results have been conflicting, likely due to differences in tracer characteristics and patient selection, as well as comparing treated to drug-naive patients. In patients with major depression and high levels of anhedonia there is more consistency about a decrease in striatal DAT availability. The current hypothesis in depression is that there is altered DAT availability, either increased or decreased, in the striatum which impairs limbic input to the cortex. At first, this seems to contradict with results found in dystonia, where synaptic concentrations of dopamine are hypothesized to be increased. However, it is known that alterations to the dopaminergic system are far more complicated than just simple overall increases or decreases in the concentration of dopamine. Regional differences in different parts of the striatum can lead to different outcomes. Neurons originating in the medial part of the ventral tegmental area project to the ventral striatum and nucleus accumbens, which are mainly related to the reward system. This system is likely to play an important role in depression. Neurons originating in the lateral part of the substantia nigra project primarily to the dorsomedial striatum, which is more related to the motor system. Therefore if there is increased activation of the dorsal striatal dopamine neurons and decreased activation of the ventral striatal dopamine neurons a patient can suffer from dystonia and depressive symptoms. A similar finding was described in patients with PD, where depression is also a common symptom. In an [123I]FP-CIT SPECT study it was shown that in patients with early PD depressive symptoms were related to DAT loss in the caudate nucleus, while motor symptoms were related to DAT loss in the putamen.
The hypothesis is that the nigrostriatal dopaminergic projections to the putamen degenerate at a different rate compared to the dopaminergic projections from the ventral tegmental area to the caudate nucleus.\textsuperscript{31}

In conclusion, in our study abnormalities in striatal DAT and D2/3R binding were mainly related to depressive symptoms. The presence of depressive symptoms before motor symptoms in patients with dystonia and correlation of major depression with alterations in the dopaminergic system, could indicate that in dystonia alterations of the dopaminergic system precede motor symptoms, although this has not been investigated thus far. This would be an interesting topic for research in the future. Differences in DAT binding could variate between different subregions of the striatum that relate either to the limbic or to the motor system, as has been shown in PD. With studies using PET and selective tracers for DAT, such as $[^{11}\text{C}]\text{PE2I}$, it will be possible to investigate smaller striatal subregions in the future, especially when co-registration with MRI is performed.\textsuperscript{32} Furthermore, results from a singular study using a selective D2R tracer in hand dystonia and from studies in patients with PD and major depression indicate a more prominent role for the D3R compared to D2R. A study with a D3R-selective tracer, such as $[^{11}\text{C}]\text{PHNO}$ in CD is warranted.

**The role of serotonin in CD**

The increased recognition of non-motor symptoms, such as depression, anxiety and sleep disorders in CD, stimulated interest in the role of the serotonergic system in the pathophysiology of dystonia. We performed the first SPECT study investigating the SERT in midbrain/diencephalon using $[^{123}\text{I}]\text{FP-CIT SPECT}$. We did not find a significant difference in SERT binding between CD patients and controls, but we did find a trend towards lower SERT binding in CD patients with depressive symptoms versus CD patients without these symptoms. There are other reasons to hypothesize that serotonin plays an important role in the pathophysiology of CD besides the frequently occurring depressive symptoms and anxiety. Many case reports of drug-induced dystonia after the use of SSRIs have been published, and decreased levels of serotonin metabolites have been found in the CSF of patients with dopa-responsive and focal dystonia.\textsuperscript{33} Furthermore, jerks/myoclonus and tremor are a frequent symptom in dystonia, especially in CD.\textsuperscript{34} Myoclonus has been hypothesized to be related to abnormalities in the serotonin system. Many case reports have been published of myoclonus as a side effect of serotonergic drugs. In posthypoxic myoclonus, it is known that there is a decrease in serotonin metabolites in the CSF and this type of myoclonus can respond well to serotonergic drugs.\textsuperscript{35} The hypothesis in posthypoxic myoclonus is that there is loss of serotonergic neurons at the level of the inferior olive which causes olivary neurons
to fire in a rhythmic manner possibly causing myoclonus. Even though myoclonus as a separate movement disorder has a very broad differential diagnosis and it is unlikely that pathophysiology in all cases is the same, this may indicate that the serotonin system plays an important role in some subtypes of myoclonus.

Even though we did not find significant differences in SERT BP_{ND} in patients with CD in relation to motor symptoms, this does not rule out serotonin as a contributor to the pathophysiology of dystonia. First, in our analyses we used a region of interest (ROI) that contained multiple midbrain and diencephalic structures including the raphe nuclei. Because of the limited spatial resolution, and relatively low BP_{ND} in this region when using $^{[123]}$FP-CIT SPECT, it is not possible to study these structures separately. In this regard, it is of interest that in a recent study, using the selective SERT tracer $^{[11]}$C]DASB and PET, the binding in the dorsal raphe nucleus correlated significantly with motor performance in patients with CD. In contrast to this, in patients with major depression the ratio between SERT binding in the medial raphe nucleus and its output areas including the habenula, amygdala-hippocampus complex and subgenual cingulate cortex seems to be related to pathophysiology and expected treatment response. A small change in the dorsal or medial nuclei can be relevant but remains undetected using $^{[123]}$FP-CIT SPECT.

Furthermore, in our study, we used the non-selective SERT tracer $^{[124]}$FP-CIT because we wanted to measure DAT and SERT BP_{ND} in the same patients in one scanning session. $^{[123]}$FP-CIT has a higher affinity for DAT compared to SERT making it impossible to measure SERT in the striatum (in the striatum the binding is preferentially to DAT) or other brain regions than the midbrain/diencephalon, where there are relatively low concentrations of SERT. This could be relevant as in patients with major depression reduced SERT BP_{ND} in the striatum has been found. It is possible that SERT BP_{ND} in the striatum is also reduced in patients with CD, especially as reduced SERT BP_{ND} has been found in several brain regions, including the midbrain, limbic structures and the basal ganglia in patients with PD and depression. Indeed, a recent SERT PET study showed a trend towards lower striatal SERT binding. Also, with progression of PD a reduction in SERT BP_{ND} was found in the striatum, raphe nuclei and amygdala, possibly reflecting loss of SERT. To learn more about the role of the SERT in the pathophysiology of CD an imaging study would have to be performed with a highly selective PET tracer, such as $^{[1]}$C]DASB, and co-registration with MRI in both depressed and non-depressed CD patients. In addition, results have to be correlated both to severity of motor and non-motor symptoms.
Besides the SERT that we studied, serotonin receptors can also play a role in the pathophysiology of dystonia. In total 14 subtypes of serotonin receptors exist and their locations and functions differ. The most evidence has been gathered regarding the role of the serotonin 1A receptor in the pathophysiology of major depression. The serotonin 1A receptor functions as an autoreceptor on the raphe nuclei neurons that produce serotonin. When this serotonin 1A autoreceptor is activated the firing rate of the raphe nuclei neurons decreases and less serotonin is released. Serotonin 1A receptors are also present postsynaptically in different serotonergic projection areas throughout other parts of the brain, where they mediate a more excitatory effect. In patients with PD-related depression decreased serotonin 1A receptor binding has been found in the right insula, the left hippocampus, the orbitofrontal region and the uncus. The hypothesis is that this decrease in binding reflects loss of serotonin 1A receptor, and this may be the underlying cause for depression in PD. A lower serotonin 1A receptors expression has been confirmed post-mortem in depressed PD patients compared to non-depressed PD patients. It is not surprising that serotonin 1A receptors play a key role in the pathophysiology of depression as this receptor is known to be involved in serotonin release. Even though, dystonia is not considered a neurodegenerative disorder a similar, subtle loss of serotonin receptors, especially of the serotonin 1A receptors, could play a role in the frequent occurrence of depression and anxiety in CD. Consequently, it would be of interest to look into this receptor in future studies in CD.

Dopamine-serotonin imbalance in CD

An imbalance between the amounts of dopamine and serotonin present in the brain is an attractive hypothesis in the pathophysiology of dystonia. A similar imbalance has been demonstrated in PD: PD patients with relatively intact serotonin systems are more likely to develop levodopa-induced dyskinesias (LIDs). Using the data from our SPECT study, we demonstrated a similar feature in CD patients. There was a significant and positive correlation between striatal DAT and midbrain SERT BPND in CD patients with jerks that was absent in CD patients without jerks. This might indicate that CD patients with jerks also have more intact serotonergic nerve terminals and that the balance between the levels of dopamine and serotonin determines whether a CD patient develops jerks or not.

In our medication trial, we treated patients with CD and jerks with escitalopram, an SSRI, and placebo both for a period of 6 weeks. Unfortunately, we did not find a statistically significant improvement in motor or non-motor symptoms compared to placebo. This was especially surprising as we did find adequate occupancy of SERT. In studies in patients with major depression an occupancy of the SERT of >80% was needed to achieve
a good treatment response. In our study we found an occupancy of approximately 65% but scans were performed 24 hours after the last dosage of medication meaning that both plasma levels as well as occupancy were diminishing. When considering the pharmacodynamics of escitalopram it is very likely that the SERT occupancy was >80% shortly after ingestion of escitalopram. There are a few possible explanations as to why we did not find a treatment response. The most simple explanation is that the treatment period was too short. In most SSRI trials in patients with major depression, patients are treated during 10-24 weeks, since treatment effect is usually more prominent after a longer treatment despite a steady-state blood concentration after 1 week. The hypothesis is that after administration of an SSRI intrasynaptic levels of serotonin in projection areas immediately increase, which induces a decrease in firing rate in the raphe nuclei by activation of inhibiting serotonin 1A autoreceptors and thus downregulation of serotonin release. After several weeks these serotonin 1A receptors desensitize and the firing rate in the raphe nuclei increases leading to an increase in serotonin release and increased intrasynaptic levels of serotonin in projection areas. Only at this point a clinical effect will be measurable, even though adequate plasma levels and SERT occupancy are measureable sooner. It is interesting that in mice it has been shown that the amount of serotonin 1A autoreceptors determines the treatment effect of SSRI. Mice with lower levels of serotonin 1A receptors were more responsive to SSRIs than mice with high levels of serotonin 1A receptors. It is not yet established if a similar mechanism applies to humans as well, but if it does this could also explain the lack of a treatment effect in our patients. If CD is associated with increased levels of serotonin 1A autoreceptors in the raphe nuclei, treatment effect of SSRIs might be limited. In this case a serotonin 1A receptor agonist might be more suitable. The difficulty is that thus far it has not been proven possible to manufacture serotonin 1A receptor agonists that are selective to only the autoreceptors in the raphe nuclei. When activating both the autoreceptors in the raphe nuclei and the postsynaptic serotonin 1A receptors in projection areas, effects may counteract each other.

Another possible explanation is that there is either a relative loss or change in configuration of SERT in CD reducing the efficacy of SSRIs. A similar situation has been demonstrated in patients with PD where SSRIs have been used to treat fatigue and depression. In both cases reduced tracer binding to SERT was demonstrated and for PD-related depression a good clinical response to SSRIs was found. However, when SSRIs were prescribed for PD-related fatigue there was no clinical effect. The hypothesis for the lack of effect is that there was loss of SERTs in the limbic system and striatum. In CD patients, we did not find a loss of SERTs in the midbrain, but we were unable to examine important projection areas such as the striatum and limbic system. A third possible explanation is that SERT in patients with CD is not the proper point of engagement. Again, as stated
above CD with jerks in ways resembles the LIDs patients with PD experience. In PD patients with LIDs a positive effect of SSRIs on the severity or duration of LIDs has never been described. In animal studies a positive effect of SSRIs was found for intravenously administered SSRIs, but timing relative to levodopa administration was crucial and SSRIs diminished the levodopa effect.51 Animal studies, as well as the first human trial, with serotonin 1A and 1B agonists such as eltoprazine however showed promising results with a severe reduction in dyskinesias.52 There are still concerns about the reduction of levodopa effect and phase 4 trials assessing safety have not been published yet. While these results are awaited, more selective compounds are being developed, hopefully limiting the chance on side effects.53 It would be interesting to perform imaging studies directed at the serotonin 1A receptors, especially the autoreceptors in the raphe nuclei, as well as a trial with one of these serotonin agonists in CD patients.

Other considerations in the pathophysiology of CD

- Other neurotransmitter systems

It is far too simple to believe that dopamine and serotonin are the only neurotransmitters involved in the pathophysiology of dystonia, especially since all neurotransmitter systems are related to each other. The past years there is also increasing interest for the role of acetylcholine in the pathophysiology of dystonia. One of the main arguments is that the most often used medication for generalized dystonia is trihexyphenidyl, which is a muscarinic M1 receptor antagonist.54 Considering that trihexyphenidyl is already used in the treatment of dystonia for over 30 years it is surprising that there are no data available on the level of muscarinic receptors in patients with dystonia. There are 5 known subtypes of muscarinic receptors labeled M1 till M5, of which M1 and M4 are most commonly present in the striatum. Both M1 and M4 receptor are present on postsynaptically located striatopallidal and striatonigral projection neurons. Activation of either one of these receptors leads to an increase in acetylcholine, as well as an increase in dopamine.55 In a mouse model of DYT1 dystonia an elevation of striatal cholinergic tone, as well as abnormal coupling between cholinergic and dopaminergic signaling in the striatum was shown. Normally, an increase in synaptic dopamine leads to an increase in activity of cholinergic neurons, which in turn inhibits dopamine release. This mechanism is lost in DYT1 knockout mice. In these mice, using in vitro recordings of striatal cells it was shown that there was an increased long-term potentiation hypothesized to reflect increased cholinergic tone, which is hypothesized to facilitate neuroplastic changes. This long-term potentiation could be reduced by trihexyphenidyl.7 In another animal model for dystonia, the dystonic hamster which is a naturally occurring type of dystonia in hamsters, a positive treatment effect of local striatal injections of the M4 receptor antagonist tropicamide has been shown.55
The results from studies in these DYT1 mice and dystonic hamsters suggest a role for the cholinergic system in the pathophysiology, however these results have not been confirmed in other animal models or in patients with dystonia thus far. In this light, it is interesting that recently a new SPECT tracer, named \(^{123}\text{I} \)iododexetimide, was characterized that binds preferentially to muscarinic M\(_1\) and M\(_4\) receptors.\(^{56}\) It would be interesting to perform a study using this tracer in patients with dystonia.

- **Neuroinflammation**

When considering the results above, it is very likely that abnormalities in neurotransmitter systems play an important role in the pathophysiology of CD. However, thus far it is unknown why patients develop these abnormalities. It could be that neuroinflammatory processes are involved. From clinical experience we know that many patients report a relatively mild car accident prior to first motor symptoms. Although, no cohort studies have investigated if there is an association, this could be important as trauma is related to alterations in the dopaminergic system. Even after seemingly mild traumatic head injury, many patients report symptoms like memory loss, difficulty focusing and depressive symptoms. These symptoms are related to alterations in both the nigrostriatal and mesocorticolimbic dopaminergic pathways. In patients who suffered a mild head trauma and have post-traumatic symptoms like memory loss, increased levels of cytokines in CSF were found, which are a marker of neuroinflammation.\(^{57}\) Similar increases in pro-inflammatory cytokines were also found in patients suffering from major depression.\(^{58}\)\(^{59}\) Using PET, it has been shown that there is microglial activation in the brains of patients with depression.\(^{60}\) The hypothesis is that pro-inflammatory cytokines in serotonin-rich areas, activate indoleamine-2,3-dioxygenase (IDO). IDO catabolizes tryptophan, which is a serotonin precursor. Activation of IDO decreases the amount of tryptophan available to convert to serotonin, and thus indirectly lowers the release of serotonin.\(^{60}\) Besides this indirect effect an increased level of interleukine-1\(\beta\), which is also a pro-inflammatory cytokine, has been shown to decrease the firing rate of serotonergic neurons in the dorsal raphe nucleus directly.\(^{61}\) It is especially interesting that these inflammatory changes are mainly found in patients with stress-related forms of mood disorders and that these stress-related disorders are more common in women than in men, especially during times when estrogen levels are low.\(^{60}\)\(^{62}\) Most forms of focal dystonia, including CD, are also more common in women than in men and the median age at diagnosis is approximately 50 years, which is around the age most women become menopausal.\(^{63}\) A hypothesis in CD and other forms of focal dystonia could be that there is an underlying susceptibility to develop dystonia and that a relative mild (car) accident triggers alterations in the dopaminergic, serotonergic and possibly also
cholinergic pathways a neuroinflammatory response that together lead to neuroplastic changes and thus dystonia. This is however just a theory and no studies regarding neuroinflammation have been conducted in dystonia thus far.

**Future directions for research**

Neuroimaging will hopefully continue to learn us more about the pathophysiology of dystonia. Even though the network between cortex-basal ganglia-thalamus-cerebellum has been identified, it is not known which abnormalities are the cause and which the consequence of dystonia. It would be particularly interesting to perform larger longitudinal imaging studies in families with genetic forms of dystonia, e.g. DYT1 dystonia. DYT1 is an autosomal dominant mutation with limited penetrance: approximately 30% of genetic carriers develop dystonia and the rest are considered asymptomatic carriers even though they do have an increased risk to develop depressive symptoms and anxiety. It would be interesting to perform serial imaging studies in these families at young age and compare the family members that develop dystonia to the family members that do not develop dystonia. Since DYT1 dystonia usually debuts during childhood this could raise some important ethical questions. The past years there have also been several genes discovered in families with focal dystonia, which usually develops after 30 years of age. These families might be more suitable to investigate, especially when PET or SPECT is being used, but the prevalence of these genetic mutation is low as far as we know yet. It could be that there are already subtle changes in the dopaminergic and serotonin systems of asymptomatic carriers that are not yet associated with neuroplastic changes and that studies in these asymptomatic carriers show these changes that might precede motor symptoms.

Another important question regards the precise role of the dopamine system and more specifically the dopamine D3R. Thus far, PET and SPECT tracers have been used that bind to both D2R and D3R. The D3R may play a larger role in the pathophysiology of dystonia compared to the D2R. For example, D3R appears to be more important than D2R both in depression and PD. It would be interesting to perform a PET study with [³H]WC10, which in animal studies has proven to be a highly selective D3 tracer, or with [¹¹C]PHNO which has been tested in humans and is more D3R selective than other available PET tracers.²⁴ Similar studies can be done with PET and the selective DAT tracer [¹¹C]PE2I. Co-registration with MRI can help to investigate small regions. It might even be possible to perform fMRI studies to identify important areas of the network involved in individual patients (using paradigms that induced dystonic signs), and use these functionally defined ROIs or VOIs instead of anatomic ROIs/VOIs. A similar study has been performed in patients with schizophrenia, in which dopamine release
was examined using functional brain areas of interest. In any future study, we feel it is important to correlate the findings both to motor and non-motor symptoms, since we found that both DAT as well as D2/3R binding correlated with depression in CD patients. Considering the finding of a 10% increase in putaminal volume found in MRI studies using VBM in patients with focal dystonia, results might also have to be corrected for putaminal volume. In molecular imaging studies that do not correct for putaminal volume, this could lead to an underestimation of a possible decreased D2/3R binding.

Furthermore, considering the relation of dopaminergic, serotonergic and cholinergic systems to stress and neuroinflammatory changes it would be interesting to investigate the immune system in patients with CD. This can be done in several manners. Levels of cytokines can be measured both in blood and CSF. To investigate microglial activation, [11C](R)-PK11195 PET can be used. It would again be interesting to perform these studies in patients with focal dystonia and family members that are at risk to develop dystonia, e.g. asymptomatic gene carriers.

The final question concerns the optimal treatment for CD. For the past years, local injections with BoNT were the most used treatment. Even though BoNT has proven to be effective, it is a locally acting neurotoxin that is only treating the motor symptoms of CD. It would be more elegant to find a therapy that is able to alter the underlying neuroplastic changes in the brain. The most logical choice is an agent that restores neurotransmitter levels, more specifically dopamine and serotonin and possibly also acetylcholine, in the brain. This could be a therapy that is effective for all symptoms of dystonia, including the non-motor symptoms. To find something that works it is important that more trials in patients with (cervical) dystonia are conducted.

**Future directions in the care for patients with CD**

As we have shown in our medication trial and was shown previously in many studies, the occurrence of psychiatric symptoms, such as depression and anxiety, is high in CD. Most patients with CD and other types of focal dystonia are seen once every 3 months in the outpatient clinic where they have a 10-20 minute appointment to receive their BoNT injections. During this appointment the main focus is on the motor symptoms, while it is well known that the quality of life is mainly influenced by depression, anxiety and pain. We believe it is time that the treating physicians also pay more attention to psychiatric symptoms in dystonia. If there are severe symptoms of depression and/or anxiety these should be treated, either by medication or by referring a patient to a psychiatrist for therapy. Based on the results of our escitalopram trial, thus far there is no reason to withhold patients treatment with an SSRI for depression or anxiety.
CONCLUSIONS

In conclusion, we found that abnormal tracer binding to striatal dopamine D_{2,3} receptors and dopamine transporters and midbrain/diencephalon serotonin transporters in cervical dystonia is mainly related to depressive symptoms and not to motor symptoms. The correlation between tracer binding to DAT and SERT did relate to motor phenotype: in CD patients with jerks there was a significant, positive correlation between striatal DAT and midbrain SERT BP_{ND} that was absent in CD patients without jerks. It is possible that the relative balance between the amounts of available dopamine and serotonin influences whether a patient develops jerks, but this has to be further evaluated.

Secondly, a 6-week treatment course with escitalopram does not improve motor or non-motor symptoms more than treatment with placebo does, even though there is adequate occupancy of SERT. BoNT injections are currently the best treatment option for focal dystonia, but a treatment with focus on the pathophysiology is needed. A trial with a longer treatment period of an SSRI or medication with another point of engagement is warranted.
REFERENCES


Chapter 7


