Chapter 6

SUMMARY AND GENERAL DISCUSSION
The single photon emission computed tomography (SPECT) tracer $^{123}$I-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane ($^{123}$I-FP-CIT) binds with high affinity to the presynaptic dopamine transporter (DAT), and with modest affinity to the presynaptic serotonin transporter (SERT) (50). The aim of this thesis was to explore the feasibility and usefulness of $^{123}$I-FP-CIT SPECT to assess extrastriatal SERT binding next to striatal DAT binding in patients with different forms of parkinsonism. We did this by 1) establishing the potential of the additional information that $^{123}$I-FP-CIT binding to the SERT in extrastriatal regions can provide to improve the differential diagnosis of Parkinson’s disease (PD), progressive supranuclear palsy (PSP), multiple system atrophy (MSA) with parkinsonian features (MSA-P), MSA with cerebellar features (MSA-C), and dementia with Lewy bodies (DLB), 2) assessing the relationship between $^{123}$I-FP-CIT binding to the extrastriatal SERT and anxiety (35, 138) in PD patients to gain more insight in the pathophysiology of anxiety in PD. We addressed the following specific research questions:

1. Do various forms of parkinsonism (PD vs PSP vs MSA-P vs MSA-C) differ in extrastriatal $^{123}$I-FP-CIT binding, on top of the well-documented loss of striatal $^{123}$I-FP-CIT binding?
2. Is there a difference in striatal and/or extrastriatal $^{123}$I-FP-CIT binding between PD and DLB?
3. Do PD and DLB have reduced extrastriatal $^{123}$I-FP-CIT binding relative to healthy controls?
4. Is extrastriatal $^{123}$I-FP-CIT binding associated with neuropsychiatric symptoms in PD patients, specifically anxiety?

The first three questions are considered together in this discussion from a diagnostic and scientific perspective. Subsequently, questions 2 and 3 are discussed in the light of the α-synucleinopathy spectrum. The clinical implications of the present findings are also considered. Lastly, anxiety as a neuropsychiatric symptom of PD is discussed separately.

**Main findings in chapters 2-4**

In Chapter 2 we demonstrate that PSP and MSA-P patients have significantly more degeneration of terminals in the DAT-rich caudate nucleus and posterior putamen, and in the SERT-rich hypothalamus than PD or MSA-C patients. There were no significant group differences in serotonergic degeneration in other extrastriatal SERT-rich areas. The results for the striatal regions of interest (ROIs) are consistent with the results of prior studies, where nigrostriatal dopaminergic degeneration was more pronounced in PSP and MSA-P compared to PD, with relative sparing of the caudate nucleus in PD (66, 68, 69, 74). In Chapter 3 we report an absence of significant differences between PD and DLB patients in extrastriatal serotonergic degeneration in the thalamus, hippocampus and amygdala. Consistent with prior studies, we observed that the degeneration of pre-synaptic dopaminergic terminals in the posterior putamen is more severe in PD patients than in DLB patients (61, 62). From Chapter 4, it becomes apparent that, in addition to the degeneration of striatal dopaminergic terminals in early-stage PD and DLB patients, there is a significant loss of serotonergic terminals in the hypothalamus of DLB patients compared with healthy controls, but not in the SERT-rich thalamus and hippocampus.
In PD patients, there was no significant serotonergic neurodegeneration in the hypothalamus, thalamus or hippocampus compared with healthy controls.

**The hypothalamus in parkinsonisms: a gradient in neurodegeneration?**

In the present thesis, we describe lower in vivo hypothalamic $^{123}$I-FP-CIT binding, reflecting predominantly lower SERT binding, in MSA-P and PSP as compared with PD, and in DLB compared with healthy controls. Prior neuropathological studies have shown hypothalamic abnormalities in each of these disorders (38, 169-171). The presence of any differences between these disorders with respect to the degree of hypothalamic serotonergic terminal degeneration has not been studied in vivo before. DLB, MSA-P and PSP are disorders that generally progress more rapidly than PD (172, 173), and taking together the results described in Chapters 2 and 4, $^{123}$I-FP-CIT binding in the hypothalamus seems to be lower in patients with one of the more rapidly progressive neurodegenerative disorders than in patients suffering from PD.

To further examine this apparent gradient, we performed a post-hoc analysis of covariance in the PSP, MSA-P and MSA-C patients from Chapter 2 and the PD and DLB patients and healthy controls from Chapter 4. All patients and controls were serotonin reuptake inhibitors (SRIs)-free. We observed significant group differences in hypothalamic $^{123}$I-FP-CIT binding, after correction for both age and disease duration ($F(5,64)=4.277$, $P=0.002$; $\omega^2=0.19$). The results of this post-hoc analysis confirm the presence of a gradient in which PD and MSA-C patients have levels of hypothalamic $^{123}$I-FP-CIT binding that are closer to the binding levels in healthy controls than the patients with either DLB, MSA-P, or PSP (Figure 1).

![Figure 1](image_url)

*Figure 1.* Mean hypothalamic $^{123}$I-FP-CIT binding ratios in different parkinsonisms and healthy controls. The error bars represent the standard deviation. *P*-values shown are significant differences in a comparison of HC with each of the other disorders. HC, healthy controls; MSA-C, multiple system atrophy with cerebellar features; PD, Parkinson’s disease; DLB, dementia with Lewy bodies; MSA-P, MSA with parkinsonian features; PSP, progressive supranuclear palsy.
It is tempting to speculate that this gradient of hypothalamic serotonergic degeneration reflects the differences in disease progression between these disorders. Clinical studies have shown a longer mean duration from disease onset to death in PD than in PSP and MSA-P/MSA-C, as well as a more detrimental disease course in the atypical forms of parkinsonism (172-174). Unfortunately, neuropathological post-mortem studies that directly compare the different hypothalamic serotonergic subnuclei, or projections from the raphe nuclei to the hypothalamus in PD, MSA-P/MSA-C and PSP have not been performed yet. This type of comparison is warranted to be able to draw a more definitive conclusion regarding the nature of the hypothalamic gradient.

Patients with DLB and MSA-P generally experience more autonomic symptoms than PD patients (123, 175), which might be due to more severe loss of serotonergic neurons in the hypothalamus. The hypothalamus regulates autonomic and endocrine functions and sleep to maintain homeostasis (176). These functions are disrupted when connections of the hypothalamus are disturbed: recently published data show that early-stage PD patients who have more severe autonomic symptoms have disrupted functional connectivity between the hypothalamus, thalamus and striatum (177). The gradient that we observed in the loss of hypothalamic serotonergic integrity roughly reflects the severity of autonomic symptoms in PD, DLB and MSA (123). It is therefore a small, yet speculative, step to connect the hypothalamic differences in 123I-FP-CIT binding found in the α-synucleinopathies in Chapters 2 and 4 to differences in prevalence and severity of autonomic symptoms. In PSP, autonomic symptoms are less commonly observed (178), but the disease progresses rapidly, which might be a reason for the observed lower hypothalamic 123I-FP-CIT binding (174). Indeed, in Chapter 2, we observed more autonomic symptoms in MSA patients than in PD patients, but PD and PSP did not differ. However, we did not have healthy controls and DLB patient in this comparison. This issue thus needs further research.

Another issue to address in future research is that DAT-expressing neurons are also sparsely present in the hypothalamus (54). Although 123I-FP-CIT binding in the hypothalamus preferentially represents binding to the SERT (85, 179), we cannot completely rule out that some of the hypothalamic 123I-FP-CIT binding represents binding to the DAT. This could form a challenge to the theoretical basis of Chapters 2 and 4. It may therefore be relevant to evaluate in future studies whether the loss of 123I-FP-CIT binding in the hypothalamus indeed primarily reflects loss of SERT binding, by performing studies using a selective SERT tracer, such as 11C-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (11C-DASB). Furthermore, the limited resolution of SPECT does not allow for an accurate distinction between the various subnuclei of the hypothalamus (180). This problem may be resolved partly with a super-resolution track-density imaging technique, developed for thalamic subnuclei, which combines 7 tesla magnetic resonance imaging (MRI) scanning with high resolution research positron emission tomography (PET) and results in a superior spatial resolution (181-183).
The hypothalamus in parkinsonisms: clinical application in differential diagnosis?

Can we improve diagnostic accuracy by combining the information on extrastriatal $^{123}$I-FP-CIT binding and striatal $^{123}$I-FP-CIT binding in the same diagnostic workup? In other words:

*Would the combined information from SERT- and DAT-binding within a single $^{123}$I-FP-CIT SPECT scan collectively increase the diagnostic accuracy for PD, DLB, MSA-P, MSA-C and/or PSP?*

To answer this question, different aspects that define an imaging biomarker need to be considered: **accuracy** is how close the measured value is to the true value. With a high accuracy one will have a better approximation of the real values than with a low accuracy. **Sensitivity** is the number of positive findings in a population that are truly positive, for example the number of tests of patients with a disease that actually give a positive result. **Specificity** is the number of negative results that are truly negative, for example the number of patients without a disease that have a negative result in a test. A high sensitivity and specificity are characteristics of a good diagnostic test. **Reproducibility** is the extent to which repeated measurements of the same object of interest give the same results. A high reproducibility will give the same result in multiple measurements. These aspects are discussed below for the differential diagnosis of parkinsonisms using $^{123}$I-FP-CIT SPECT scanning.

1) **Accuracy:** $^{123}$I-FP-CIT is a radiotracer with multiple binding targets. This becomes evident when calculating binding ratios: SERT-rich ROIs in our data have binding ratios that are on average much lower than binding ratios in the DAT-rich ROIs, which is mainly caused by the lower affinity of the radiotracer for the SERT compared to the DAT. Additionally, $^{123}$I-FP-CIT SPECT has a limited resolution with voxels of approximately 4 mm$^2$, depending on scanner type, number of camera heads and types of collimation, which can lead to bleeding of signal emitted from a DAT-rich ROI into a SERT-rich ROI when DAT and SERT-rich ROIs are adjacent to each other. The hypothalamus, for example, is such an area: it is small and adjacent to DAT-rich areas. Nevertheless $^{123}$I-FP-CIT binding in the hypothalamus has been validated previously as reflecting binding to the SERT (85).

2) **Sensitivity and specificity:** In an ideal world, a cut-off point for binding ratios in an $^{123}$I-FP-CIT SPECT scan would tell us with great certainty whether an individual patient suffers from, for example, PD. In other words, we need high sensitivity and a high specificity. When looking at our data in Chapter 2, we found group differences in $^{123}$I-FP-CIT binding ratios between PD on the one hand, and PSP and MSA-P on the other hand in both the striatum and in the hypothalamus. We analysed our data post hoc to determine the discriminatory value of combining information from striatal and extrastriatal ROIs by constructing receiver operator curves. After adding the hypothalamic binding ratios to the striatal binding ratios, we observed in the group comparison between PD ($n=25$) and PSP/MSA-P ($n=23$) patients an increase of the area under the curve (AUC, representing sensitivity and specificity) from 0.90 to 0.93 (*Figure 2*).

This change, however, was not statistically significant when comparing the AUCs according to DeLong *et al.* (184) [change: 0.0348, standard error: 0.0264, 95% confidence interval: -0.0170 to 0.0865, $P=0.19$]. Moreover, to be able to combine all ROIs, a standardised score had to be
calculated for each patient, which does not reflect actual binding ratios. Nevertheless, since our post-hoc analysis suggests that combining striatal and extrastriatal binding from a single $^{123}$I-FP-CIT SPECT scan may increase the AUC, the analysis should be repeated in a larger population to ascertain whether combining striatal DAT and extrastriatal SERT binding data may indeed improve diagnostic accuracy. To obtain values that can be used in clinical practice, multiple well-defined ROIs may need to be combined to find the best match of striatal and extrastriatal ROIs.

**Figure 2.** Receiver operator curves of PD patients versus MSA-P/PSP patients (“PD yes or no”); with SRI-free patients. **A:** combined binding ratios of bilateral caudate nucleus and posterior putamen. **B:** combined binding ratios of bilateral caudate nucleus and posterior putamen with added binding ratios of the hypothalamus. A non-statistically significant increase of the area under the curve is observed. PD, Parkinson’s disease; MSA-P, multiple system atrophy with parkinsonian features; PSP, progressive supranuclear palsy; AUC, area under the curve.

3) Reproducibility: There is much individual variance in $^{123}$I-FP-CIT binding ratios. Studies have shown that age, gender, disease duration, cognitive status, the existence of several DAT and SERT polymorphisms and the use of medication may influence striatal and/or extrastriatal $^{123}$I-FP-CIT binding ratios (106, 107, 117, 119, 185, 186). In addition, external factors also play a role in this variation, for example scanner type, time between injection and scanning, and even the season may influence striatal and extrastriatal $^{123}$I-FP-CIT binding (187-190). An example of a group of drugs known to have an effect on $^{123}$I-FP-CIT binding ratios are the SRIs, which bind to the SERT to prevent reuptake and prolong post-synaptic signalling. SRIs are commonly used in patients with parkinsonisms, potentially influencing the reliability of the $^{123}$I-FP-CIT scans (53). Since SRIs block the SERT, baseline (i.e. non-occupied) SERT information in SRI users is by definition not available. Neither do we know what the effects of acute and chronic use of SRIs are on SERT expression, and consequently on extrastriatal (and possibly also striatal) $^{123}$I-FP-CIT binding ratios. We also do not know at what rate possible changes in SERT expression in parkin-
sonian patients occur. Additionally, it is unknown how long these drugs should be suspended to stop its competition with \(^{123}\text{I}-\text{FP-CIT}\) binding to the SERT. In Chapter 2, we therefore chose to analyse extrastriatal brain areas only in patients that were SRI-free, which of course makes it harder to generalise the results to a broader patient population, since SRIs are used on a regular basis by patients with parkinsonism.

Keeping this in mind, it is too soon to definitively conclude that extrastriatal \(^{123}\text{I}-\text{FP-CIT}\) binding can improve diagnostic accuracy of parkinsonisms. The results from Chapters 2 and 3 first need corroboration. A logical next step would be to use a larger group of patients and see whether test-retests give similar extrastriatal results. Patient groups should be scanned on different occasions and using different scanners in a multi-centre setting to optimally reflect clinical practice. Set up as prospective studies, future studies should use unified symptom scales that are validated for the use in parkinsonisms, also including the non-motor symptoms, such as autonomic and neuropsychiatric symptoms.

Machine learning is an emerging technique that is already widely applied in imaging research. The technique can be used to identify predictive patterns in scans (possibly enriched with clinical measures) in one population and verify these in a second population. By means of this technique it is also possible to, for example, combine MRI data with data derived from \(^{123}\text{I}-\text{FP-CIT}\) SPECT scans to increase the amount of information that is used for the learning algorithm.

Another option to examine both striatal DAT and hypothalamic SERT binding, is to perform two separate scans using SERT and DAT selective tracers (see Box 1 below), for example the SERT-selective tracer \(^{11}\text{C}-\text{DASB}\) and the DAT-specific tracer \(^{11}\text{C}-\text{N-(3-iodoprop-2E-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane}\) (\(^{11}\text{C}-\text{PE2I}\)). These two tracers are used in PET scanning, which has a better resolution than clinical SPECT scanners and allows a better localisation of ROIs. Another advantage of using of two specific tracers is that SERT and DAT binding can be assessed in the same ROIs, and thereby obtain more information. Three obvious disadvantages of this approach are the increased amount of radiation exposure for the patient, the necessity to perform two scans on different days, and the fact that these tracers are currently too costly to use in routine clinical practice. This would therefore only be a viable option for routine clinical care when large cost-reductions can be achieved.

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<td>(^{123}\text{I-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane})</td>
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<td>(^{11}\text{C}-\text{DASB})</td>
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PD and DLB as part of a spectrum of α-synucleinopathies

PD and DLB are both characterised by the presence of Lewy bodies, and their clinical phenotypes show considerable overlap. Therefore, it has been proposed to consider them as a single disease with phenotypes that can be placed on a spectrum (191). Consequently, in the recently proposed new clinical diagnostic criteria for PD (192), and the diagnostic criteria for prodromal PD (193), DLB as a separate entity has been abolished. Nevertheless, PD and DLB do differ in the way the brains of patients are affected. With similar disease duration, there are differences in the load and distribution of α-synuclein pathology, in neurochemical pathology and in striatal $^{123}$I-FP-CIT binding (59-62).

In the dorsal and median raphe nuclei of DLB patients a loss of serotonergic neurons has been observed (63), and evidence of lower midbrain $^{123}$I-FP-CIT binding to the SERT has been found in DLB compared with PD (64), both suggesting degeneration of the serotonergic system in DLB. Since α-synuclein pathology is present in the raphe nuclei in Braak stage II of PD, this suggests early-stage involvement of serotonergic neurons in PD as well (194). In this thesis we observed no difference in serotonergic neurodegeneration between PD and DLB in the thalamus, hippocampus and amygdala. However, in the hypothalamus we found less serotonergic terminals in DLB patients than in healthy controls, and that difference was not present between PD patients and healthy controls. From this, one might construe that DLB patients have more severe degeneration of serotonergic neurons in the hypothalamus than patients with PD. However, the PD patients in Chapter 4 had a relatively shorter disease duration than the DLB patients. A negative effect of disease duration on hypothalamic SERT has been reported in PD patients (125). Therefore, from our data it cannot be concluded that PD and DLB have a different pattern of serotonergic neurodegeneration. The observed difference may simply reflect the difference in disease duration between the two patient groups. This would support the growing consensus that PD and DLB are part of a disease spectrum instead of representing two different disease entities.

Main findings in chapter 5

In Chapter 5 we describe an association between $^{123}$I-FP-CIT binding and anxiety symptoms in PD patients. We found a negative association between the severity of anxiety and thalamic $^{123}$I-FP-CIT binding, but only in PD patients that were not using dopaminergic replacement therapy (DRT). In patients on DRT the association was absent.
Anxiety, $^{123}$I-FP-CIT and serotonin in parkinsonisms

Anxiety in PD is a non-motor symptom with an exacerbating effect on other symptoms such as the motor symptoms, but it also has a detrimental effect on quality of life in itself (131-133, 195). Anxiety can present as a symptom, but can also be a part of a more formalised diagnosis like generalised anxiety disorder, social phobia or panic disorder (196), each with their different aetiology. Deficits in the neurotransmitters dopamine, serotonin and acetylcholine are assumed to play a role (197). A general mechanism that induces anxiety in PD is therefore not easily identified. The lack of understanding of the underlying processes makes treatment of anxiety difficult. Moreover, anxiety is often underdiagnosed in PD (198, 199).

An association between anxiety and a serotonergic deficit in the thalamus, similar to what we describe in Chapter 5 for PD patients, was previously reported in patients without a neurodegenerative disorder (147, 155). The thalamus consists of multiple nuclei that each have numerous connections throughout the brain. The limited resolution of $^{123}$I-FP-CIT SPECT scans precludes identifying which specific thalamic nuclei are involved in the serotonergic deficit in PD, which leaves us to speculate on which other parts of the brain are influenced by this deficit. As discussed above for the $^{123}$I-FP-CIT binding in the hypothalamus, a study using a SERT-selective PET tracer might shed more light on this interesting topic. Nevertheless, the evidence presented in this thesis combined with the result obtained in the abovementioned patient groups without neurodegeneration (147, 155) strongly suggests that a serotonergic deficit in the thalamus is associated with anxiety.

Anxiety is a multi-factorial problem rather than the result of a single dysfunctional neurotransmitter system. Against this background, it is useful to review the treatment options for anxious patients. SRIs, for example, are widely used to ameliorate anxiety (200, 201), yet we do not know whether a serotonergic deficit is a prerequisite for a successful treatment with SRIs. One could imagine that PD patients who are suffering from anxiety but do not have a serotonergic deficit might respond less well to treatment with SRIs. It would therefore be of interest to compare groups of anxious PD patients with and without a serotonergic deficit, to determine whether the first group benefits more from SRIs than the latter. This might open up a window towards a more personalised prescription of medication.

Anxiety is often observed in patients with PSP and MSA as well (202), so it would be of interest to know whether anxiety in these forms of parkinsonism is also associated with a serotonergic deficit in the thalamus. This is a possibility, since we observed in Chapters 2 and 3 that the thalamic serotonergic system is affected similarly in all parkinsonisms we studied. Unfortunately, we were unable to link this with anxiety in these groups of patients, because of the limited availability of anxiety scores from patients with atypical parkinsonisms. As a follow-up study, to gain more insight in anxiety in atypical parkinsonisms, a study similar to the one described in Chapter 5 for PD patients would be useful.
Relevance of serotonin for other neuropsychiatric symptom dimensions in parkinsonisms

Other neuropsychiatric symptoms associated with serotonin that frequently occur in PD and other forms of parkinsonism and have an adverse effect on the quality of life include depression, sleep disturbances and impulse control disorders (ICD) (203).

Depression has a high prevalence in parkinsonisms (204), and often presents already before a clinical diagnosis of a parkinsonism is established (205). It is a symptom that, much like anxiety, has a complex aetiology: degeneration of dopaminergic, noradrenergic, cholinergic and serotonergic systems appears to contribute (197). Depression and anxiety are often highly correlated, something we also observed in Chapter 5. Although much of the neuropsychiatric research in parkinsonisms has focused on depression, its precise mechanisms remain elusive.

Many patients with parkinsonisms suffer from sleep disturbances (206, 207). In addition, up to 80% of individuals with rapid eye movement sleep behaviour disorder (RBD) later go on to develop a parkinsonism (208). The serotonergic system appears to play a role in the circadian rhythm (209), and sleep disturbances in PD have been linked with serotonergic dysfunction, for example in the hypothalamus (210). Further investigations of the serotonergic system may therefore benefit the field of sleep disturbances in the various forms of parkinsonism.

Symptoms of ICD comprise pathological gambling, compulsive eating, hypersexual behavior, and compulsive buying (211). These symptoms can impose a heavy burden on PD patients and their caregivers. They are more prevalent in PD patients that are on dopamine agonists (211), and ICD symptoms seem to coincide with a post-synaptic hypersensitivity for dopamine that might be induced by pre-synaptic dopaminergic degeneration (212). Mutations of the serotonin 2A receptor gene and polymorphisms of the SERT gene are associated with a higher risk of developing ICD in PD patients (213, 214). Moreover, SRIs improve inhibitory control (215) and seem to elicit a favourable response in PD patients with ICD (216). This suggests a role for the serotonergic system on top of the dopaminergic deficit, opening up new possibilities for tailored treatment, for example by screening for ICD risk before DRT is prescribed. From a clinical perspective, it is important to clarify the role of other neurotransmitter systems, including the serotonergic system, because the most effective current treatment for ICD is the adjustment of DRT (lowering the dosage or switching from dopamine agonist to levodopa), which induces the risk of a dopamine agonist withdrawal syndrome and worsening of motor symptoms.

Taken together, neuropsychiatric symptoms frequently occur in PD patients. In the pathophysiology of each of these symptoms, multiple neurotransmitter systems appear to be involved, including serotonin, noradrenalin, dopamine and acetylcholine. To improve treatment of neuropsychiatric symptoms in PD, we therefore need more studies to further elucidate the pathophysiologic contribution of each of these different neurotransmitter systems.
Limitations of the performed studies

The studies described in this thesis have some limitations. First, they were performed retrospectively. This led to some inconsistencies in the available symptom scales between PD, DLB, PSP and MSA patients, which made it impossible to compare motor and non-motor symptoms between PD and DLB patients, for example. It furthermore precluded an analysis of $^{123}$I-FP-CIT binding in association with symptom profiles of these diseases. Furthermore, because of a lack of MRI brain scans in some of the patients and in all healthy controls, we were unable to use personal MRI information to optimise the identification of ROIs in Chapters 2 and 4. The $^{123}$I-FP-CIT SPECT scanning also came with some limitations: all scans were performed on a single scanner, and although this rules out any inter-scanner variability, it does not reflect daily clinical practice where scanner variability exists between centres. In addition, we only acquired a single scan in each patient, which did not allow us to draw conclusions about the within-subject reproducibility of SERT binding. Other potential limitations are the limited sample size of the studies described in Chapters 2 and 4, and the lack of neuropathological confirmation of the clinical diagnoses in all chapters.

Future prospects of molecular imaging in parkinsonisms

In the rapidly developing field of molecular imaging, ever more tools are available to study the brain and its molecular processes. In addition, MRI scanners with higher resolution have become available for more exact localisation of these processes. The plethora of information that is generated by these techniques needs to be interpreted with care. Reproducibility and translation from group level to the individual level are ongoing points of concern.

The differential diagnosis of parkinsonisms with molecular imaging techniques may benefit from a more holistic approach, where all information that is present in a single scan is used, preferably in combination with detailed information on the clinical phenotype. Machine learning techniques could be implemented to develop algorithms that can predict to what diagnostic group an individual patient belongs based on the results derived from a single scan and the clinical phenotype. This would require a large prospective study in which the best available molecular imaging tracer is applied; MRIs are acquired to construct personalised ROIs and to identify specific features of atypical parkinsonism, for instance the *hummingbird sign* (217); and diagnostic certainty is obtained by post-mortem neuropathological examination.

A prospective design of future studies is essential to improve our insight into the neurobiology of neuropsychiatric symptom profiles in parkinsonisms. In an ideal world, such studies would run for many years in clinically well-defined populations. Progression of symptoms should be monitored by administering symptom scales at multiple time points, and subsequently correlate them with information obtained from MRI and PET scans. Ultimately, neuropathological diagnostic confirmation should be obtained.
General implications/conclusions

Neurodegenerative forms of parkinsonism involve much more than dopaminergic degeneration alone. All of these disorders affect multiple brain areas and many different neurotransmitter systems. By means of the studies in this thesis we tried to shed some light on the serotonergic element of these disorders, and the results convert to the following conclusions:

1. There is a gradient in hypothalamic serotonergic neurodegeneration in parkinsonisms in which PSP and MSA-P patients have the most severe serotonergic neurodegeneration in the hypothalamus, followed by DLB and PD, and MSA-C patients the least. This gradient might also underlie the different autonomic symptom profiles of patients with these diseases. This needs to be confirmed by clinicopathological studies. At this time, there is insufficient evidence to support the use of the hypothalamic serotonergic gradient in the differential diagnosis of parkinsonisms in routine clinical practice.

2. Striatal neurodegeneration characterises both early-stage PD and DLB patients. In the bilateral posterior putamen dopaminergic neurodegeneration in PD patients is more extensive than in DLB patients. There is no significant difference between PD and DLB patients in serotonergic neurodegeneration in the thalamus, hippocampus or amygdala.

3. The severity of anxiety in PD is positively associated with the degree of thalamic serotonergic neurodegeneration. This observation may help to further elucidate the mechanisms underlying anxiety in PD, possibly inspiring future studies to use this association to tailor fit anxiolytic drugs to patients with a specific pattern of neurodegeneration.