In this thesis, an attempt was made to gain better insight into the pathophysiology of visual hallucinations (VH) and dementia in Parkinson’s disease (PD) and dementia with Lewy bodies (DLB) by identifying neuropsychological, neuroimaging and neuropathological determinants of these symptoms. We formulated the following specific research questions:

1. Are VH in PD associated with a specific cognitive profile?
2. Are VH in PD associated with changes in functional interactions between brain regions?
3. Are VH in PD associated with changes in structural connections between the cholinergic neurons in the basal forebrain and the rest of the brain?
4. Is there a difference in the pattern or severity of the neuropathological cholinergic deficits between PD(D) and DLB that may (partly) underlie the different clinical phenotypes of these diseases?
5. Is there a difference in the neuropathological load of proteins other than α-synuclein, in particular amyloid-β, between PD(D) and DLB in the brain that may (partly) underlie the different clinical phenotypes of these diseases?

In this chapter, we will first discuss our findings regarding the first three research questions, which concern a better understanding of the pathophysiology of VH in PD. Thereafter, we will summarize our conclusions with regard to the neuropathological differences underlying the clinical heterogeneity of PD(D) and DLB, thereby focusing on research questions 4 and 5. We will formulate the potential clinical implications of our results and outline future perspectives. At the end of this chapter we will recapitulate all our conclusions.

**Visual hallucinations in PD: do we have a clear picture?**

**Visual hallucinations in PD: indicative of a malignant subtype?**

In the study described in Chapter 2, we addressed our first research question by exploring cognitive disturbances in non-demented PD patients with (PD+VH) and without VH (PD–VH). Attention and verbal memory were more impaired in PD+VH compared to PD–VH patients, whereas performance on tests of other cognitive functions, i.e. executive functions, visual perception and language, was comparable between the two groups. In addition, hallucinating PD patients displayed more severe symptoms of anxiety,
depression and disturbed sleep, compared to PD–VH patients.

The identification of a specific cognitive profile in PD+VH patients may aid in the identification of a distinct neuropathological subtype underlying both cognitive impairment and VH in PD. The ‘dual syndrome hypothesis’ of cognitive impairment in PD is very interesting in this respect.\textsuperscript{1} According to this hypothesis, a dopaminergic deficit in fronto-striatal circuits underlies executive dysfunction, while widespread cholinergic denervation causes memory and ‘posterior cortical’, e.g. visuospatial, deficits. Patients with a cognitive phenotype dominated by posterior cortical dysfunction are believed to progress more rapidly to dementia, that is, convert to Parkinson’s disease dementia (PDD), while cognitive function in patients with predominant executive dysfunction typically remains stable.\textsuperscript{2,3}

Verbal learning and attention are two cognitive functions that are believed to involve the cholinergic system.\textsuperscript{4,5} Thus, non-demented PD+VH patients – in whom memory and attention were more affected in our study – might have a more severe cholinergic deficit compared to PD–VH patients. According to the dual syndrome hypothesis, PD+VH patients would therefore be more likely to develop dementia. This is in line with a previous longitudinal study, in which VH at baseline were a significant predictor of conversion to PDD 8 years later.\textsuperscript{6} Possibly, more severe cholinergic denervation contributes to both VH as well as specific cognitive impairments in PD patients, suggesting that patients exhibiting these symptoms may thus represent a so-called ‘malignant’ PD subtype. The worse severity of other non-motor symptoms, such as symptoms of anxiety, depression and disturbed sleep, in the PD+VH group may be an aspect of this malignant subtype as well.

Previous studies have put great effort in exploring the presence of different clinical PD subtypes by applying cluster analysis techniques.\textsuperscript{3,7,8} In a recent, prospective, longitudinal cohort study, a ‘diffuse/malignant’ PD phenotype was identified, characterized by a more rapid rate of cognitive decline over a follow-up period of 4.5 years.\textsuperscript{7} Although the presence of hallucinations was not included as a variable in this cluster analysis, hallucinations were reported more often in the malignant phenotype, reaching trend level significance in the statistical analysis. Furthermore, patients with the malignant phenotype were more likely to have orthostatic hypotension, mild cognitive impairment and REM sleep behavior disorder (RBD) at baseline, the latter two manifestations being strongly associated with the presence of VH in PD.\textsuperscript{9} The authors emphasized that non-motor symptoms were the most critical determinants of PD subtype and prognosis, and that a non-
motor composite score – which included VH, anxiety, depression, sleep and autonomic disturbances – had the highest odds ratio for disease progression at follow-up.7

The association we found between the presence of VH on the one hand and disturbed sleep and symptoms of anxiety and depression on the other was reported previously.10,11 Lee et al., for example, demonstrated that the odds of experiencing psychotic symptoms (including VH) were approximately five times higher in PD patients with disorders of depression and sleep-wakefulness.11 Possibly, more severe or selective neuropathology and degeneration of cholinergic, noradrenergic, as well as serotonergic nuclei, which are suggested to play a role in the pathophysiology of anxiety and depression12,13, may occur in PD+VH patients, and may also be the underlying neural substrate of the more malignant PD subtype. The recognition of clinical subtypes, and even more importantly, understanding the underlying neurobiological mechanisms of these subtypes may improve prognostic accuracy and, eventually, the development of personalized treatment of PD patients. A promising approach to investigate these underlying neurobiological mechanisms in vivo is the application of modern and non-invasive MRI techniques such as functional MRI (fMRI) and diffusion tensor imaging (DTI) which so far had not been used in studies investigating clinical subtypes in PD.3,7,8 Therefore, in the studies described in Chapters 3 and 4 we applied these techniques to analyze the pathophysiological mechanisms of VH in PD.

Visual hallucinations in PD: loss of functional interactions between brain areas

In Chapter 3 of this thesis, we describe an fMRI study, in which we analyzed differences in functional connectivity between PD+VH and PD–VH patients and healthy controls. In PD patients, irrespective of the presence of VH, functional connectivity of paracentral and occipital brain regions was lower than in controls. In PD+VH patients, nine additional brain regions were less strongly connected to other regions. The additional regions with a loss of functional connectivity included frontal, temporal, occipital and striatal brain areas. We concluded that a disconnection of paracentral and posterior brain regions is not sufficient for VH to occur. The pathophysiology of VH probably requires a more widespread loss of network efficiency.

The loss of functional connectivity of frontal and temporal brain regions, which occurred exclusively in our PD+VH patients, was associated with lower scores on tests of attention, perception and attentional set shifting. This is partly in line with our findings in Chapter 2 and emphasizes the importance
of attentional dysfunction in the pathophysiology of VH in PD. Reduced functional connectivity of brain regions that are part of the visual pathways, i.e. the fusiform gyrus and the inferior occipital gyrus, was also exclusively observed in PD+VH patients. This may suggest that, besides attentional dysfunction, relative impairments of visual processing may contribute to the pathophysiology of VH in PD. Our findings are in accordance with the results of previous fMRI, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) studies, in which reduced activation or altered metabolism was reported for both frontal and temporal cortices\textsuperscript{14-16}, as well as for the visual pathways in PD+VH patients as compared to PD–VH patients.\textsuperscript{15,17-20}

In contrast to the PD+VH patients in the neuropsychological study (Chapter 2), the PD+VH patients in the fMRI study (Chapter 3) scored significantly lower on cognitive tests of visual perception than PD–VH patients. Lower visual perception scores in the PD+VH patients were correlated with decreased functional connectivity. A possible explanation for the discrepancy between the two studies, as far as differences in visual perception test scores between patient groups is concerned, is that patients in the neuropsychological study had very mild cognitive disturbances and visual perception was examined using a single test of visual perception only. Therefore, subtle impairments in visual perception may have been missed.

Since Lewy body (LB) pathology eventually spreads to all brain regions in PD, one might assume that local cortical neuropathology is the underlying cause of the observed loss of functional connectivity in PD+VH patients. However, the fact that paracentral and occipital brain regions are disconnected in all PD patients, while LB pathology in these regions – even in the final pathological stage of PD\textsuperscript{21} – is usually limited, argues against this assumption. Alternatively, disrupted projections, either cortico-cortical projections or afferent projections from subcortical brain regions, may lead to a loss of functional connectivity of specific brain regions in PD+VH patients. Considering the hypocholinergic hypothesis of VH in PD, denervation of the cholinergic projections arising in the nucleus basalis of Meynert (NBM) – the primary source of acetylcholine in the brain – may be particularly relevant in this respect.\textsuperscript{22,23}

**Visual hallucinations in PD: cholinergic denervation**

To find further evidence for a role of disrupted projections from the NBM in the pathophysiology of VH in PD, we performed a diffusion tensor imaging (DTI) study, which is described in Chapter 4. In this study, the NBM was used as a seed region and DTI metrics, i.e. fractional anisotropy (FA) and
mean diffusivity (MD), where compared between groups of PD+VH patients, PD−VH patients and controls. The MD values of the tracts between the NBM and the rest of the brain were significantly different between groups, i.e. highest in PD+VH patients and lowest in controls, while FA values were comparable between groups. Post-hoc analysis of tracts between the NBM and specific cortical regions revealed higher MD values in PD+VH compared to PD−VH patients of the tracts connecting the NBM to parietal and occipital brain regions, which was mainly driven by a higher radial diffusivity (RD) and may be indicative of microstructural damage to these tracts.24

The higher MD we observed in cholinergic parietal and occipital tracts of PD+VH patients may reflect the presence of debris and/or changes of the axonal organization as a consequence of neuronal and axonal degeneration. However, it should be noted that the exact underlying substrates of the observed diffusivity changes (i.e. higher MD and RD) have not yet been elucidated. In fact, the interpretation of DTI metrics in neurodegenerative diseases in general, including PD, is still challenging, since the molecular and histopathological substrates of anisotropy and diffusivity in these diseases are not fully understood. Moreover, the degree of myelination, which is an important factor in DTI analysis, of the NBM projections in the healthy human brain is unknown. In animals, most ascending NBM fibers appear to be unmyelinated, although the frontal projections may be finely myelinated.25,26 Thus, changes in MD and RD may not only reflect the presence of debris and axonal reorganization, but also a decrease in the proportion of unmyelinated fibers.27

The projections of the NBM are topographically organized. The NBM can be subdivided into an anterior, intermediate and posterior division, each of which innervates specific cortical brain regions. The anterior division innervates frontal, limbic and medial cortical regions; the intermediate division innervates parietal and occipital regions; and the posterior division innervates the superior temporal gyrus and the temporal pole.27,28 The fact that in our PD+VH patients only the posterior, i.e. parietal and occipital, NBM tracts were affected therefore suggests more severe LB pathology and/or neuronal loss in the intermediate NBM division.

Interestingly, in Alzheimer’s disease (AD), there is evidence to suggest a caudo-rostral pattern of neuronal loss in the NBM, the posterior NBM division being most severely affected.28 Since the posterior division of the NBM innervates the temporal pole and superior temporal gyrus, this would explain the dominant language and memory impairments in AD patients. In PDD, neuropathological reports have revealed a slightly greater neuronal loss in the
intermediate, compared to the anterior, division of the NBM\textsuperscript{28,29}, from which a predominant loss of innervation to parieto-occipital brain areas may be expected, similar to what our DTI data suggest. In an \textit{in vivo} cholinergic tracer study, a fronto-occipital gradient of increasing cholinergic dysfunction was demonstrated in demented PD patients, again emphasizing the idea of more severe posterior cortical cholinergic denervation in PD.\textsuperscript{30} It is important to note, however, that the severity of neuronal loss in the posterior division of the NBM has never been examined in PD(D). Also, the NBM as we delineated it in our DTI study mainly encompasses the intermediate NBM division. Thus, the absence of group differences in the integrity of tracts other than the parietal and occipital tracts, may be partly explained by suboptimal measurements of the tracts to frontal, temporal and limbic brain regions.

The exact localization of LB pathology and/or neuronal loss within the NBM in PD is of particular relevance to the emergent technique of deep brain stimulation (DBS) of the NBM as a treatment for AD and PD(D).\textsuperscript{31-33} Pilot data on the effect of NBM-DBS on cognitive function in AD are promising, showing stable or improved cognition in four out of six AD patients.\textsuperscript{32} In addition, a case report of NBM-DBS treatment in a PDD patient (tips of the electrodes in the intermediate portion of the NBM) mentions a relevant improvement of cognitive function.\textsuperscript{34} At the time of writing this thesis, two randomized controlled clinical trials (one in the Netherlands and one in the USA) testing NBM-DBS in PD patients with mild cognitive impairment are underway. Stereotactic gene delivery of trophic factors within the NBM is another treatment option targeting the cholinergic deficit that is currently under investigation in AD.\textsuperscript{35} Knowledge of the exact distribution of neuro-pathological changes within the NBM in PD patients with and without VH and cognitive impairment would greatly help to determine the optimal target for stereotactic treatment.

**Visual hallucinations in PD: theoretical models revisited**

Multiple integrative, theoretical models have been postulated to explain VH in PD. A disturbance of cholinergic neurotransmission is an important contributing factor in the majority of these models.\textsuperscript{36} In particular, the ‘Activation, Input, Modulation (AIM)’ model, the ‘Perception and Attention Deficit (PAD)’ model, and just recently, the model of ‘dysfunction of attentional control networks’ emphasize a cholinergic deficit as a causative factor in the pathophysiology of VH in PD.\textsuperscript{37-39}

According to the AIM model, dysfunction of cholinergic brain stem centers leads to disturbed REM sleep and abnormal vigilance, which facilitate the occurrence of VH in PD, for example by REM sleep intrusions into wake-
fulness. In addition, decreased levels of cortical acetylcholine are assumed to cause aberrant functioning of associative frontal and occipital cortices, leading to a disturbance of perception and modulation of visual input. The PAD model also emphasizes disturbed perception as a major contributing factor to the occurrence of VH. More specifically, in this model an imbalance between attentional and perceptual processes (i.e. “top-down” and “bottom-up” processes) is believed to trigger VH in PD. According to the model, disturbances of attention and visual perception synergistically lead to the activation of contextually expected but incorrect ‘proto-objects’. Proto-objects are abstract object representations that compete to reach awareness by drawing attention towards themselves. VH occur when an incorrect proto-object is drawn into the attentional focus due to impairments in attentional binding and/or limited sensory activation. Along this line of reasoning, the severity of hallucinatory phenomena might be determined by the degree of difference between correct and incorrect proto-objects. For example, visual illusions, passage or presence hallucinations may be caused by a subtle difference between correct and incorrect proto-objects, while complex and threatening VH – occurring later in the disease – may result from a large dissimilarity.

In the model of ‘dysfunction of attentional control networks’, complex VH reflect dysfunction between and within brain networks involved in attention, leading to an inappropriate interpretation of ambiguous percepts. In this model, VH result from an overreliance on the ventral attention network (VAN) and the default mode network (DMN), which is caused by a dysfunction of the dorsal attention network (DAN). The DAN, which focuses attention on externally driven percepts, may be abnormally dormant in PD+VH patients, hence the overreliance on the VAN and DMN, networks that are normally involved in the rapid shift of attention towards salient stimuli and the retrieval and manipulation of episodic memories and semantic knowledge. Since the cholinergic system is crucial for a normal level of selective attention, also in this model a cholinergic deficit is implicated in the pathophysiology of VH.

The results of the studies described in Chapters 2 and 4 provide further evidence for a cholinergic deficit as a major contributory factor to the pathophysiology of VH in PD, and are therefore in line with the above-described theoretical models. In particular, the attentional deficit we observed in PD+VH patients fits well with the PAD model and the model of dysfunction of attentional control networks. Furthermore, cholinergic denervation of parietal and occipital brain regions (chapter 4) is an element of both models, as well as the AIM model.
The widespread loss of functional connectivity we observed in PD patients with visual hallucinations (Chapter 3) appears also in line with the theoretical models described above. For example, the frontal and temporal brain regions that were less well connected in the PD+VH patients are important for intact attentional processes as implicated in the PAD model and in the model of ‘dysfunction of attentional control networks’. According to the latter model, the DAN is abnormally dormant, an assumption that appears to be supported by our observation that the superior frontal gyrus and striatum – crucial hubs in this network – are exclusively disconnected in PD+VH patients. Moreover, our finding of weaker functional connectivity of brain regions that are part of the ventral visual stream, i.e. the fusiform and inferior occipital gyrus, seems to confirm the disturbance of “bottom-up” visual processing that is an element of the PAD model.

In conclusion, we have demonstrated that VH in PD are associated with a specific, supposedly ‘cholinergic mediated’ cognitive profile, a widespread decrease of functional connectivity between brain regions, and a loss of integrity of the projections connecting the NBM to the posterior cortices. In doing so, we provide further evidence for an important contribution of non-dopaminergic neurotransmitter deficiencies, in particular a cholinergic deficit, to the pathophysiology of VH in PD, and possibly also to the associated cognitive impairments.

**Visual hallucinations in PD: future perspectives**

In-depth phenotyping of PD patients integrating clinical and neuropsychological information, as well as imaging data (e.g. fMRI and DTI), may lead to an earlier identification of patients with a malignant clinical subtype, characterized by a faster disease progression, and will ultimately contribute to the development of tailored therapeutic regimens in PD patients. Clinical phenotyping should not only include a detailed registration of VH (e.g. using the Scale for the Assessment of Positive Symptoms) but also of other non-motor symptoms, including cognitive impairment, sleep and affective disturbances.

Considering that our results support the idea of a cholinergic deficit contributing to VH, and possibly the associated cognitive impairments, specific therapeutic strategies should be investigated further. As mentioned above, NBM-DBS treatment for mild cognitive impairment in PD is currently under investigation in randomized clinical trials and may prove valuable in suppressing VH as well. The selective loss of NBM projections to parietal and occipital brain regions in PD+VH patients, as described in Chapter 4, suggests a selective loss of neurons in the intermediate NBM. Confirmation of
the alleged selective neuronal loss requires detailed neuropathological studies of the NBM – including cell counts in all three NBM subdivisions – in PD patients with and without VH and/or cognitive impairment.

Future neuropathological studies should also address the degree of myelination of NBM projections to the cerebral cortex in healthy controls, as well as the pathological changes in these fiber tracts in PD patients. Combining anatomical and pathological information on the NBM and its cortical projections with corresponding post-mortem high-field DTI images may help us understand the changes in DTI metrics of these projections in vivo. This, in turn, could lead to a more detailed in vivo assessment of NBM tract integrity and determination of the exact origin of the loss of tract integrity – e.g. cell loss in a specific NBM subdivision – in relation to specific clinical symptoms (such as VH) of individual PD patients. Ultimately, this may lead to optimal target localization for NBM-DBS treatment in individual patients.

Since DBS treatment is an invasive therapy many patients may not be suitable candidates and/or may not be motivated for this treatment option. For these patients acetylcholine enhancing medication, such as cholinesterase inhibitors, is an alternative option. In previous studies, cholinesterase inhibitors improved cognitive function in PD patients. Moreover, the use of rivastigmine was associated with reduced VH in PD in a case-control study and a relatively small observational open-label study. The observations in these studies still await confirmation in larger randomized, placebo-controlled clinical trials. At the time of writing this thesis, we are conducting a large randomized placebo-controlled trial investigating rivastigmine treatment in PD patients with minor VH (CHEVAL study). Valuable additions to consider in future RCT’s investigating the effect of acetylcholine enhancing medication on VH in PD are detailed neuropsychological examinations and DTI imaging to evaluate NBM tract integrity before the start of treatment. In this way, specific cognitive impairments (i.e. deficits in attention and verbal memory) and/or evidence of cholinergic denervation on DTI images can be identified as predictors of treatment response.

Based on the findings described in Chapter 3 of this thesis, another potential noninvasive treatment to consider for VH in PD is repetitive transcranial magnetic stimulation (rTMS). By means of rTMS, cortical excitability of specific target areas can be changed, even persisting beyond the stimulation session itself. The results of a recent study demonstrated that rTMS of the motor cortex can improve motor function in PD patients. Similarly, stimulation of specific brain regions known to have reduced functional connectivity in PD+VH patients could hypothetically restore functional
connectivity and lead to a decrease in VH. Based on our findings, potential target regions for rTMS in PD+VH patients include the superior frontal gyrus, the inferior occipital gyrus, as well as specific temporal brain regions (fusiform gyrus, superior temporal gyrus, middle temporal gyrus and middle temporal pole). A much related option is multifocal rTMS, i.e. stimulating multiple brain regions simultaneously. However, since the neurophysiology of multifocal rTMS is at present unknown cancellation of effects may occur just as well as summation. As a first step, selective stimulation of a single specific hub region that is crucially involved in the pathophysiology of VH in PD – e.g. the superior frontal gyrus – may be a more attractive option than multifocal stimulation.

**PD(D) AND DLB: FINDING THE DIFFERENCES**

**Consistent PPN degeneration in PD(D), but not in DLB patients**

In Chapter 5 we describe a study investigating neuropathological changes and neuronal loss in the compact part of the pedunculopontine nucleus (PPNC) in PD(D) and DLB patients. In PD(D) patients the number of PPNC neurons was reduced by 39% and 41% compared to DLB patients and controls, respectively. Furthermore, the load of extracellular α-synuclein pathology was higher in PD(D) compared to DLB patients, while Aβ pathology was more pronounced in DLB patients. The burden of neurofibrillary (NFT) tangle pathology was comparable between the disease groups. In the DLB patients PPNC cell counts were quite variable and not significantly different from controls. We concluded that degeneration of the PPN – the main brainstem cholinergic output structure of the human brain – is consistent and severe in PD(D), but more variable in DLB patients.

Since all PD(D) patients in our study experienced VH, it was not possible to compare PPNC cell loss and neuropathological changes between PD+VH and PD–VH patients. However, it is noteworthy that all DLB patients in our study experienced VH, yet the number of cells in the PPNC in these patients, though variable, was not significantly different from controls. Thus, the assumption that PPN degeneration contributes to the development of VH – by decreased cholinergic projections to the thalamus and thereby disturbed arousal and REM sleep (as suggested in the AIM model for VH in PD) – may be true for PD(D) patients, but not for (all) DLB patients. By contrast, in both PD(D) and DLB, the presence of a cortical cholinergic deficit is firmly established. Apparently, degeneration of the NBM is a pathological feature of both diseases, while consistent and severe degeneration of the PPN occurs primarily in PD(D) patients.
In a previous structural imaging study, we observed that PPN atrophy was more pronounced in PD(D) patients with VH than in DLB patients with VH, although this difference did not survive multiple comparison correction in the statistical analysis.\textsuperscript{51} In a neurochemical study, loss of choline acetyltransferase activity in the thalamus, representing PPN degeneration, was found in PDD but not in DLB patients.\textsuperscript{52} These observations and the results described in Chapter 5 together support the abovementioned hypothesis of differences in the origin of the cholinergic deficits between PD(D) and DLB patients. That is, in PD(D) patients PPN as well as NBM degeneration occurs, while in DLB patients NBM degeneration takes place in all patients, yet PPN degeneration appears to be variable.

Although the variability in the number of neurons in the PPN\textsubscript{C} of DLB patients may be due to methodological issues or human biological variability, alternatively, neuropathological changes and/or neuronal loss in the PPN\textsubscript{C} may be associated with specific symptoms other than VH. These symptoms may include gait and posture abnormalities and REM sleep behavior disorder (RBD), as previously suggested.\textsuperscript{50,52,53} Unfortunately, we cannot draw any firm conclusions regarding the association between cholinergic PPN\textsubscript{C} cell loss and postural instability or RBD in our patients due to a lack of standardized clinical information and the limited sample size of our study. So far, only one previous study compared brainstem cholinergic cell loss in Lewy body disease patients (either PD(D) or DLB) with and without probable RBD.\textsuperscript{54} Although no group differences were found, these results should be interpreted with some caution as this study had a limited sample size and no distinction was made between PD(D) and DLB.

The PPN is part of the mesencephalic locomotor region, which plays an important role in gait control by activating spinal cord networks inducing gait. In animal models of PD, dysfunction of the PPN has been associated with abnormal gait and postural instability.\textsuperscript{53,55,56} Future neuropathological studies with larger sample sizes and standardized clinical data on the presence or absence of VH, postural instability and RBD, are essential to shed more light on the association between PPN degeneration and these symptoms in PD(D) and DLB. Combining the results of such neuropathological studies with in vivo, high-resolution DTI analysis of fiber tracts connecting the PPN to the thalamus and basal ganglia may lead to improved patient selection for PPN-DBS, which is a promising treatment in PD patients with gait disturbances including freezing and postural instability.\textsuperscript{57}

In this thesis, we demonstrated more severe α-synuclein pathology in the PPN\textsubscript{C} of PD(D) compared to DLB patients, while Aβ pathology was more pro-
nounced in DLB patients. These findings, as well as those in other neuropathological reports comparing α-synuclein and co-existing AD pathology in PD(D) and DLB patients,58-62 inspired us to study Aβ pathology in other brain regions as well. We hypothesized that differences in the load and distribution of Aβ pathology between PD(D) and DLB might explain the clinical differences with regard to the presence and timing of dementia.

**Extensive amyloid-β pathology in DLB, less in PDD, and least in PD patients**

In **Chapter 6** we describe the results of a quantitative postmortem study comparing Aβ pathology in 133 patients fulfilling the clinical diagnostic criteria for PD, PDD or DLB.63-65 Overall, the prevalence and severity of Aβ pathology was highest in DLB, less in PDD and least in PD patients. β-Amyloidosis in the medial temporal lobe was most advanced in DLB patients. Aβ pathology was more prevalent in limbic and striatal regions, and more severe in these and additional neocortical areas in DLB compared to PDD patients. In PDD compared to PD patients, Aβ pathology was more frequently observed in the temporal cortex, and Aβ load was higher in multiple cortical regions and striatum. We concluded that the extent and load of Aβ pathology may contribute to cognitive dysfunction in PDD and the early-stage severe dementia of DLB patients.

Although PDD and DLB share numerous clinical symptoms, there are important clinical differences as well. First of all, the onset of dementia relative to the onset of parkinsonian motor symptoms is earlier in DLB than in PDD. This difference is presently used in a rather arbitrary way by defining that for a diagnosis of DLB the onset of dementia and parkinsonism both have to occur within the same year, while for a diagnosis of PDD the interval between the onset of motor symptoms and the subsequent development of dementia should be at least one year.63,64 The results described in Chapter 6 of this thesis show that the distribution pattern of Aβ pathology differs between PDD and DLB, while the distribution of α-synuclein pathology and the presence of significant NFT pathology is similar in both groups. Thus, the presence of Aβ pathology appears to be an important determinant of the timing of dementia in these disease entities, i.e. early in DLB and late in PDD.

Interestingly, a shorter disease duration in our DLB patients correlated with more advanced β-amyloidosis in the medial temporal lobe, which supports the theory that a more rapid rate of progression of Aβ pathology may accelerate clinical disease progression. Moreover, in a recent CSF study comparing AD biomarkers between PDD and DLB patients, low CSF Aβ levels were more often reported in DLB than in PDD patients, and were associated with a
shorter disease duration. We did not observe any correlation between the time of onset of dementia in PD patients and the severity of Aβ patholgy, but this may have been due to the retrospective character of our study. Information on the exact onset of dementia in the PD patients may have been imprecise. Histopathological studies of brains collected from prospectively followed cohorts, incorporating detailed and standardized clinical evaluations including cognitive assessments, as well as thorough quantitative measurements of Aβ, α-synuclein and tau pathology, are warranted to shed more light on the relationship between the rate of development of disease-related pathology and clinical cognitive profiles.

Not only the timing of dementia within the disease course, but also the nature of the cognitive impairments in PDD and DLB is somewhat different. In both diseases, executive dysfunction is well-described and probably a consequence of abundant α-synuclein pathology, as well as striatal and frontal Aβ pathology, while memory deficits are more frequent and severe in DLB compared to PDD. Possibly, the more prevalent and severe Aβ pathology in temporal and entorhinal cortices, as observed in DLB patients, may account for the more severe memory impairments in these patients. This is in line with a previous in vivo imaging study, wherein more advanced temporal atrophy was reported in DLB compared to PDD patients and the amount of atrophy correlated with worse memory scores. Although frontal and striatal Aβ pathology was prevalent in both PDD and DLB, the load was higher in DLB patients, which might reflect the earlier onset of more severe executive dysfunction in these patients.

When comparing PD with PDD patients, α-synuclein pathology was more advanced in the PDD cases. In addition, PDD patients had more advanced β-amyloidosis in the medial temporal lobe, as well as higher neuritic plaque scores. Apparently, both AD and α-synuclein pathology contribute to the development of dementia in PD, which is consistent with previous work. Regional measurements displayed more prevalent Aβ pathology in the temporal cortex in PDD patients and a higher load of Aβ pathology in the posterior cortices and striatum. The particular involvement of temporal and posterior cortices in PDD patients is in accordance with the dual syndrome hypothesis of cognitive impairment in PD. According to this hypothesis, specific cognitive impairments associated with dysfunction of temporal and posterior cortices, superimposed on fronto-striatal based deficits, lead to the development of dementia in PD. As mentioned above, more advanced stages of α-synuclein pathology in combination with more widespread and severe Aβ pathology are probably the underlying neuropathological substrates thereof.
In accordance with the results described in Chapter 6, previous PET imaging studies in PDD and DLB patients have demonstrated frequent $\alpha\beta$ positivity in DLB patients and less prevalent $\alpha\beta$ positivity in PDD patients.\textsuperscript{73,74} In PD patients without dementia, $\alpha\beta$ deposition was similar to healthy controls in PET imaging studies, similar to what our neuropathological data suggest.\textsuperscript{75,76} Quantitative molecular imaging studies, as well as CSF studies of $\alpha\beta$ may provide us with in vivo prognostic biomarkers and could, ultimately, be relevant for monitoring treatment effects in future disease-modifying intervention studies targeting $\alpha\beta$. Of note, in most PET imaging studies patients are classified as either $\alpha\beta$ positive or negative depending on the level of $\alpha\beta$ deposition being above or below certain thresholds established for AD. In the setting of an $\alpha$-synucleinopathy, however, lower $\alpha\beta$ levels may be clinically relevant, as a result of a so-called ‘amplifier’ effect on cognition caused by the presence of multiple neuropathological processes.\textsuperscript{73} Therefore, lower $\alpha\beta$ thresholds should probably be used for PDD patients.

In our study, we have not examined regional tau pathology in a quantitative manner, so we are unable to rule out that tau pathology is another contributory factor to the clinical differences between PD(D) and DLB. A quantitative postmortem study of tau pathology is a recommended topic for future research, in particular since PET imaging of tau is now underway and appears to be promising.\textsuperscript{77} As described in Chapter 6, pathological scores of $\alpha$-synuclein, $\alpha\beta$ and NFT were positively correlated with each other in PD(D) and DLB patients, which may suggest synergistic effects and/or common pathophysiological mechanisms of the different types of pathology. Future studies investigating the interaction of different pathologies using in vitro, as well as in vivo measurements can provide essential basic pathophysiological insights regarding the process of neurodegeneration in PD(D) and DLB, and in this way provide clues for the right development of future intervention studies targeting different types of pathology.

**PDD and DLB: two of a kind?**

Whether PDD and DLB really represent two distinct nosological entities or exist along the spectrum of a single disease – i.e. Lewy body dementia – is the objective of an ongoing scientific debate.\textsuperscript{58,78–82} Arguments in favor of defining PDD and DLB as clinical subtypes of a single disease include the extensive overlap of clinical features and the lack of a single symptom that definitely distinguishes between them. Parkinsonism and dementia are core features of both diseases, and neuropsychiatric symptoms and autonomic dysfunction are common in both PDD and DLB.\textsuperscript{63,64} Moreover, symptomatic treatment, i.e. dopaminergic treatment for motor impairment and treatment with cholinesterase inhibitors for cognitive impairment, have benefi-
cial effects in both diseases. With regard to neuropathological changes, both PDD and DLB are characterized by widespread α-synuclein pathology, as demonstrated in Chapter 6 of this thesis and in numerous previous studies.

Arguments against the idea that PDD and DLB represent phenotypes of a single disease are based on the observation of differences in the specific nature, prevalence and temporal sequence of clinical symptoms. Dementia, for example, occurs more than 8 years after the onset of motor symptoms in the vast majority of PDD patients, while it is often the presenting symptom in DLB patients. Parkinsonism is usually bilateral in DLB, as opposed to the unilateral and asymmetric presentation in PDD, and tremor is less frequently seen in DLB patients. Also, although dopaminergic medication may improve motor function in DLB patients, it is not as effective as in PDD. Complex VH occur early in DLB, which is in contrast to the typical gradual development of hallucinations in PDD, and delusions are far more common in DLB than in PDD. Furthermore, most DLB patients are highly sensitive to treatment with neuroleptics. As mentioned above, the nature of the cognitive impairments is somewhat different between PDD and DLB, with memory impairment arising earlier and being more severe in DLB. Lastly, overall disease progression arising earlier and being more severe in DLB.

In addition to these clinical differences between PDD and DLB, there is evidence for neuropathological differences as described in Chapters 5 and 6 of this thesis and reported previously. These include differences in the degree of cell loss in the PPN, and the distribution and severity of concomitant Aβ pathology.

Considering the arguments mentioned in the preceding paragraph, distinguishing between PDD and DLB seems justified for routine patient care, choice of symptomatic treatment and clinical research. Patients with a primary movement disorder and dementia later in the disease course – i.e. patients diagnosed with PDD – and patients with the primary symptom complex of dementia – i.e. patients diagnosed with DLB – require different symptomatic treatments, particularly in the early phase of disease. At the same time, a distinction between PDD and DLB may be less relevant in studies focusing on a better understanding of the relationship between α-synuclein accumulation and neurodegeneration, as well as in future studies assessing the effects of disease-modifying treatments targeting α-synuclein.

In spite of the widespread nature of α-synuclein pathology in both PDD and DLB, there is differential involvement of brainstem nuclei, notably the PPN
and the substantia nigra\textsuperscript{58}, which suggests heterogeneity in selective vulnerability of specific brain areas and/or a different pattern of progression of α-synuclein pathology.\textsuperscript{58,78} Moreover, there are differences in the prevalence and severity of Aβ pathology between PDD and DLB, as described in Chapter 6 of this thesis, once more emphasizing the presence of neuropathological differences. To better answer the question whether PDD and DLB represent the same or different disease entities, the temporal development of the different pathologies in these diseases needs to be studied in more detail in future neuropathological studies that include prospectively diagnosed PDD and DLB patients, who have died at different stages of disease, ideally including the prodromal stages.

**PD(D) AND DLB: TOWARDS TAILORED THERAPY**

Taking together the results described in the different Chapters of this thesis, it appears that clinical phenotypic differences (in particular the occurrence of VH and the presence and temporal sequence of dementia), both among PD patients and between PD, PDD and DLB patients, are to a large extent determined by neurobiological and neuropathological differences. The presence of VH in PD is associated with a specific cholinergic mediated cognitive profile (Chapter 2), a widespread loss of functional interactions between brain regions (Chapter 3) and disrupted cholinergic tracts between the NBM and the posterior cortices (Chapter 4). Thus, PD patients with VH are likely to benefit from specific therapeutic interventions targeting the cholinergic system, including both pharmacological treatment with acetylcholine enhancing medication and directed NBM-DBS, and from therapies (such as rTMS) that target specific disconnected brain regions. These treatment options may not be beneficial for PD patients without VH.

The presence of early-stage dementia in DLB appears to be the result of extensive cortical and striatal Aβ pathology, which is less abundant in PDD patients and virtually absent in PD patients (Chapter 6). Hypothetically, cortical and striatal Aβ pathology, together with cortical α-synuclein pathology, occur early in DLB and drive the early-stage and severe dementia in this disease. At a later disease stage, α-synuclein pathology appears in the brainstem and may cause parkinsonism in a selection of DLB patients. By contrast, in PD(D), α-synuclein pathology ascending from the brainstem to the cerebral cortex comes first, initially giving rise to parkinsonism. Later in the course of the disease, cortical α-synuclein pathology and – in a selection of patients – co-occurring Aβ pathology contribute to the development of dementia. This hypothetical course of neuropathological events should be tested in rigorous future histopathological studies, which are highly relevant
to provide the optimal targets for the development of disease-modifying therapies.

To conclude, the acknowledgement of the above-mentioned neuropsychological, neuroimaging and neuropathological determinants of VH and dementia in PD and DLB may prove relevant for the future development of both targeted disease-modifying therapies as well as tailored symptomatic treatments. Awaiting the much-wanted development of disease-modifying therapies, the development of symptomatic treatments tailored to the individual patients should not be neglected as these could considerably improve the quality of life of our patients. Tailored symptomatic treatment requires a better understanding of the underlying substrates of clinical heterogeneity in PD(D) and DLB. Hence, future studies using a dual approach of histopathological analysis and high-field brain imaging, both post-mortem and in vivo, in prospectively followed, well-phenotyped cohorts should be a high-priority.
Conclusions of this thesis

VH in PD are associated with a specific cognitive profile

- The presence of visual hallucinations (VH) in non-demented Parkinson’s disease (PD) patients is associated with a cognitive profile characterized by impairments in verbal learning and attention.
- This specific cognitive profile suggests the involvement of other neurotransmitter systems than dopamine in the etiology of VH in PD, especially a cholinergic deficit.
- Other non-motor symptoms, namely anxiety, depression and sleep disturbances, are associated with VH in PD.
- The presence of VH in PD may be part of a ‘malignant’ clinical PD subtype, which is characterized by a rapid disease progression.

VH in PD are associated with a loss of functional interactions between brain regions and cholinergic denervation

- PD patients with VH show a widespread loss of resting-state functional connectivity suggesting a global loss of network efficiency in these patients.
- This global loss of network efficiency could drive disturbed attentional and visuospatial processing, leading to VH in PD.
- The disruption of white matter tracts between the nucleus basalis of Meynert (NBM) and posterior cortical brain regions in PD patients with VH may partly explain the global loss of network efficiency in these patients.
- The selective involvement of tracts between the NBM and posterior, i.e. parietal and occipital, brain regions in PD patients with VH suggests a more severe involvement of the intermediate part of the NBM.

A neuropathological substrate for clinical differences between PD(D) and DLB

- Degeneration of the pedunculopontine nucleus (PPN) is severe and consistent in PD(D) patients, but variable in dementia with Lewy bodies (DLB) patients, which suggests a different pattern of degeneration of cholinergic output structures in both diseases.
- Amyloid-β pathology is different in PD(D) and DLB with most frequent, widespread and severe Aβ pathology in DLB patients, less in PDD patients and least in PD patients.
- The load, extent and localization of Aβ pathology may contribute to the development of dementia in PD (PDD) with disease progression and the early-stage, severe dementia of DLB patients.
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