New neuroimaging approaches in Parkinson's disease

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7 Summary, concluding remarks and future perspectives

In this thesis, the consequences of PD were studied using advanced approaches in neuroimaging, including structural as well as functional connectivity networks, examination of the role of aging in white matter degeneration in PD, and the use of machine learning classification models. Our main findings are summarized and discussed in the next paragraphs of this chapter, followed by possible directions for future research.

Summary

Chapter 1 is the introduction to this thesis. In **chapter 2**, we investigated the integrity of nine standardized structural covariance networks (SCNs) in relation to clinical severity of PD (Hafkemeijer et al., 2014). An SCN is based on interdependent regions of grey matter density. We found that two out of nine networks showed atrophy and a loss of integrity. We identified atrophy and loss of integrity in the anterior and posterior cingulate networks in PD patients. Abnormalities of both networks were associated with predominantly non-dopaminergic features, specifically cognition and excessive daytime sleepiness. Our findings suggest that (components of) the cingulate networks display a specific vulnerability to the pathobiology of PD and may operate as interfaces between networks involved in cognition and alertness.

DLB and PD are considered subtypes of the alpha-synucleinopathy spectrum that show similar and dissimilar clinical and morphological features. In **chapter 3**, we used the same technique as in chapter 2 (SCNs) to further our understanding of brain grey matter abnormalities that might differentiate PD and DLB more clearly, combined with voxel-based morphometry, volumetric measures and vertex-based shape analysis. This study showed atrophy of the hippocampus and parahippocampal gyrus in DLB compared with PD patients, with a differential involvement of the head and body of the hippocampus. Moreover, integrity of the posterior cingulate network, which also comprises (para)hippocampal regions, was significantly lower in DLB. The findings of this study show that regional hippocampal differences between DLB and PD may be important in the distinction between the two disorders.

As we identified a loss of integrity and atrophy beyond the strong effects of aging in two SNCs (see chapter 2 for explanation), we hypothesized that there may be an interaction effect of age and disease presence on white matter integrity in PD and this was addressed in **chapter 4**. White matter is especially prone to the effects of

aging, and it is increasingly recognized that white matter is already affected in early stages of PD. We found a similar age-related decline of white matter integrity for PD patients and healthy control subjects in this study, but PD patients had more white matter hyperintensities (WMHs) than expected for their age. The excess of WMHs was partly located in periventricular and deep frontal white matter of PD patients, and was, besides age, related to visuospatial functioning and postural instability and gait difficulty. Global microstructural integrity of the normal appearing white matter did not differ between patients and healthy control subjects, suggesting that PD-specific changes do not exceed normal age-associated change in white matter without lesions.

In **chapter 5**, we studied PD-related changes of the brain with network approaches again, but with a different method. We used a novel graph analytic approach in functional imaging (eigenvector centrality mapping; ECM), to examine changes in functional brain connectivity architecture on a whole brain network level in patients with PD. We found that frontoparietal regions display a stronger connectivity to the whole-brain network function in PD patients compared with healthy control subjects, while a decreased connectivity was found for frontal and occipital areas of the brain. Additionally, we used eight standardized resting-state subnetworks of the brain, which pointed at predominantly increased functional connectivity within the sensorimotor system and visual networks. Comparing both approaches highlighted altered functional connectivity in highly connected (hub) regions, particularly in the posterior cingulate cortex and precuneus, which may account for the distributed abnormalities across the whole brain network architecture in PD patients. The regional altered functional connectivity was not related to clinical measures in this study, indicating that changes on the level of functional connectivity architecture of the brain are not necessarily associated with clinical measures.

In **chapter 6**, we explored the use of machine learning techniques in combination with resting-state fMRI data to differentiate between PD patients and healthy control subjects at an individual level. We computed functional connectivity matrices and dynamics for full and partial correlations, resulting in four feature types, which have been used separately as well as combined in a cross-validated elastic net regularized logistic regression. We calculated the area under the curve (AUC) to determine classification performance. The AUC values ranged between 0.752 and 0.858. The highest values were found for partial correlation functional

connectivity dynamics. We also investigated whether particular clinical features contributed to the individual classification scores of PD patients and no associations were found. We showed that it is possible to achieve moderate to good PD classification using elastic net regularized logistic regression combined with resting-state fMRI data, in particular with the use of functional connectivity dynamics.

Concluding remarks

Structural and functional connectivity

Chapter 2, 3 and 5 in this thesis showed that network approaches provide valuable insights in the structural and functional brain architecture of patients with PD. It is hypothesized that neurodegenerative diseases show a selective, network-driven neuronal vulnerability (Seeley et al., 2009). The findings in chapter 2 and 3 showed specific structural network degeneration in PD and DLB, namely degeneration of the anterior and posterior cingulate network. The anterior cingulate network shows spatial overlap with a network described in a previous study that identified a structural network that was also degenerated in PD (Zeighami et al., 2015). The cortical regions in this network included the anterior cingulate cortex as well (Zeighami et al., 2015). Another study used this network in their analysis of subtypes with "mild motor-predominant", "diffuse malignant" and "intermediate" PD. This study showed more pronounced atrophy in the anterior cingulate cortex in "diffuse malignant" PD patients compared with the other two subtypes (Fereshtehnejad et al., 2017). These findings indicate that the cingulate cortex is particularly vulnerable in PD, and that the changes in the networks in which the cingulate cortex is involved, relate to disease progression in PD.

Further we found regionally altered functional connectivity in the precuneus and posterior cingulate cortex, which are important regions of the posterior cingulate structural connectivity network (chapter 5). The precuneus and posterior cingulate cortex are key nodes in the default mode network, which is a functional connectivity network that is frequently described to be disrupted in PD, and is possibly associated to cognitive deficits in PD (Baggio et al., 2014). These structures are considered as central and highly connected brain regions (Hagmann et al., 2008; van den Heuvel and Hulshoff Pol, 2010), and are suggested to be part of a set of posterior medial and parietal cortical regions that form a structural core of the brain (Hagmann et al., 2008). The structural core may have a central role in integrating information across structurally segregated and functionally specialized brain

regions (Hagmann et al., 2008). Changes in the structural core may therefore have widespread changes across the brain network architecture. Combined, these findings point out an important role of these highly connected (hub) regions in structural and functional covariance in PD, and connectional variability of the precuneus and posterior cingulate cortex may relate to specific subsets or characteristics of PD patients, such as our finding in chapter 2 that loss of integrity of the posterior cingulate network related to predominantly non-dopaminergic features.

Age

Several studies suggested that age is an important modulating factor in the disease progression and phenotype expression of PD. For example, advancing age is associated with a more rapid decline in motor function and more severe cognitive impairment in patients with PD (Alves et al., 2005; Levy, 2007). The exact role of aging in PD, however, and changes that underlie the relationship between age and PD, are unclear. Numerous neuroimaging studies have shown an age-related decline in MRI parameters of brain structure and function (Ferreira and Busatto, 2013; Good et al., 2001; Hafkemeijer et al., 2012; Jernigan et al., 2001; Raz et al., 2005; Resnick et al., 2003). Our results showed grey matter atrophy in DLB and PD patients compared with age-matched healthy control subjects in regions that explicitly show age-dependent decreased grey matter volume, such as the hippocampus and cingulate cortex (Good et al., 2001; Jernigan et al., 2001; Raz et al., 2005; Resnick et al., 2003). Further, regional altered functional connectivity was mainly expressed in the precuneus and posterior cingulate cortex, which are also regions that are associated with functional disruption at high age (Ferreira and Busatto, 2013; Hafkemeijer et al., 2012). These findings suggests that the pathobiology of PD has a preferential susceptibility for regions that are already affected by the aging process, or that the age-effect is possibly influenced by disease in a region dependent manner (Claassen et al., 2016). Our results of chapter 4, combined with the results of previous studies, suggest a similar age-related decline of global white matter integrity in PD and healthy control subjects. The results of chapter 4 further showed that WMHs in PD may already be present in an early phase of the disease. This is in line with other studies suggesting that white matter may be affected in the very early stages of PD (Dadar et al., 2018; Duncan et al., 2016; Veselý and Rektor, 2016). A possible explanation could be that PD patients show a non-linear trajectory of an early accelerated age-related decline, which stabilizes with advancing age (Claassen et al., 2016).This explanation

is supported by the absence of any relation between disease duration and WMHs in chapter 4.

Dopaminergic medication

Except for a group of drug-naïve patients, the patients in the studies described in this thesis were scanned while taking their usual medications. MRI scanning of PD patients without dopaminergic treatment is problematic, as tremor may result in motion-related artifacts (Tahmasian et al., 2015) and participation may be jeopardized when patients have to travel from home during the off-state. Resting-state fMRI is particularly prone to noise due to motion, and the effects of head motion should be considered when it differs between groups (van Dijk et al., 2012). The reduction of motor symptoms through dopaminergic treatment may reduce the effects of excessive involuntary head motion, in addition to the other approaches used in this thesis for motion artifact removal (Jenkinson et al., 2002b; Pruim et al., 2015a, 2015b; Tahmasian et al., 2015). However, scanning PD patients in the "on-medication state" may also hamper the interpretation of data since functional connectivity changes secondary to dopaminergic therapy may occur in PD patients (Tahmasian et al., 2017). fMRI studies have reported a normalizing effect of dopaminergic medication on abnormal functional connectivity associated with PD, which might suggest that our findings would have been more pronounced if patients were scanned in the "off-medication-state" (Tahmasian et al., 2015). The results of other studies suggest that dopaminergic medication may also alter the functional connectivity between brain regions that are not necessarily involved in the improvement of symptoms of PD (Ng et al., 2017). However, there is evidence that these effects of dopaminergic medication are small, in particular in the well-established resting-state networks that we used in chapter 5 (Flodin et al., 2012). Moreover, scanning PD patients in the "off-medication-state" may result in group functional connectivity differences secondary to dopaminergic treatment as well, due to changed brain organization caused by the long-term use of dopaminergic medication (Kaasinen et al., 2003; Kurani et al., 2015). De novo patients are therefore the only population to study pathophysiology independent of dopaminergic therapy. In PD populations using dopaminergic treatment the dopaminergic state of PD patients should be considered when interpreting or comparing results of MRI studies.

Study population

The research presented in this thesis was performed in a cross-sectional setting in PD and DLB patients. Some studies described in this thesis required the use of a control group. These control subjects were selected from the Leiden Longevity Study (Altmann-Schneider et al., 2013), which comprises a large pool of healthy subjects from which matched controls can be selected. It should be considered that there were some slight differences in some of the variables collected and the methods of data collection between patients and control subjects. The MRI acquisition in our control group and PD group was performed with a different type of head coil (8-channel versus 32-channel, respectively), which may have influenced our findings, although we used MRI approaches relatively insensitive to a potential coil effect (Geng et al., 2012; Panman et al., 2019; Paolini et al., 2015). However, the use of different head coils may hamper the analysis of MRI data and standardization of head coils across groups of interest is recommended in future study designs (Panman et al., 2019). Patients and control subjects were matched for age and gender, two known confounders of particular importance in MRI studies (Barnes et al., 2009). A critical consideration related to the control group selected for the study presented in chapter 4 is that control subjects were not matched for cardiovascular risk profile, in addition to age and gender, although it should be noted that patients and control subjects did not differ in those cardiovascular risk factors that were assessed in both the PROPARK and Leiden Longevity study.

Future perspectives

Multimodal neuroimaging studies

We studied brain MRI in PD and DLB patients with five different MRI sequences in this thesis, which, amongst other things, highlighted a spatial overlap of altered structural and functional connectivity, in particular of the cingulate cortex. Structural and functional MRI are increasingly combined to provide more informative insights into both structural as well as functional connectivity in neurodegenerative diseases (Arbabshirani et al., 2017; Seeley et al., 2009). Combining data of different imaging modalities, such as MRI, Positron-Emission Tomography (PET), magnetoencephalography (MEG) or electroencephalography (EEG), may yield even more insights into the relationships between function and structure (Arbabshirani et al., 2017; Calhoun and Sui, 2016). In schizophrenia, several studies combined MRI data with EEG or MEG data (Calhoun and Sui, 2016). One study showed considerable spatial consistency between function (MEG) and

structure (white matter integrity), with regional reduced MEG amplitude and FA in a posterior visual network, which was related to cognitive performance (Stephen et al., 2013). There is increasing knowledge on the integrated analysis of multimodal data. The combination of deep learning and multimodal data or multimodal classification may reveal unique information that cannot be obtained when using one modality (Arbabshirani et al., 2017; Calhoun and Sui, 2016). Several combinations of PET and MRI results have been applied in PD and DLB, indicating striatal dopamine modulation of functional connectivity networks (Baik et al., 2014; Lebedev et al., 2014; Weingarten et al., 2015), and showing that combined data distinguished DLB from Alzheimer's disease better than measurements from one modality (Kantarci et al., 2012; Weingarten et al., 2015).

Artificial intelligence and big data

We used machine learning in chapter 6, which is a current application of artificial intelligence based on algorithms that can learn from data and subsequently construct a model in order to make predictions or decisions without being explicitly programmed. Machine learning approaches are increasingly applied to MRI data to study neurodegenerative diseases. A promising unsupervised machine learning method that allows for the exploration of previously unidentified disease subtypes based on neuroimaging, is cluster-wise ICA (C-ICA) (Durieux, 2015). C-ICA aims to cluster subjects in a data-driven fashion into homogeneous groups based on similarities and differences in the functional connectivity patterns that characterize them. The functional connectivity patterns that are associated with these subgroups could further provide information about neurodegenerative diseases such as PD. Components of a thus identified cluster may serve as a marker for the differentiation of subtypes with differing patterns of abnormal functional connectivity and potentially distinct clinical symptom profiles. Possible extensions of the C-ICA model include applications to other types of data as EEG data (Durieux, 2015).

Despite promising results of some studies, the use of machine learning in PD is still in its early stage. This is likely caused by the limited sample sizes (Arbabshirani et al., 2017). However, a unique international, multicenter clinical, imaging and biologic specimens dataset of PD patients has been collected, managed and made available by the Parkinson's Progression Markers Initiative (PPMI) (Marek et al., 2011). A recent study using PPMI data investigated strategies to handle challenges of large datasets, such as heterogeneity or missing data, and subsequently

produced models for PD classification and prediction (Dinov et al., 2016). Future studies can possibly provide additional insights on how to address availability, integration and analytics of big data to advance the usability of MRI of PD in clinical practice.

Individual classification

Advanced neuroimaging approaches are of special interest for their potential to characterize unique morphological characteristics and connectivity patterns of neurodegenerative diseases (Agosta et al., 2017). For example, due to the high availability of 3 Tesla MRI scanners, the analysis of hippocampal subfields has gained increasing attention (Agosta et al., 2017). We showed that regional hippocampal differences between DLB and PD may be important in the distinction between the two disorders in chapter 3. However, except for the model that was built to identify PD patients at an individual level in chapter 6, the results in this thesis cannot be applied to individual patients. Neuroimaging studies in PD have largely provided average estimates at a group level. Although these studies are valuable for the discovery of relevant disease markers, the discriminative ability at the individual level of these neuroimaging measures is typically not evaluated. Neuroimaging-based single-subject prediction of neurodegenerative diseases has gained attention in recent years. In PD, a study using machine learning for the analysis of resting-state fMRI data showed that it was possible to predict motor scores (Movement Disorder Society unified Parkinson's disease rating scale; MDS-UPDRS - part III) on an individual patient level (correlation = 0.35, $p = 0.001$; mean sum of squares = 222.17, $p = 0.002$) (Hou et al., 2016). Another study showed that machine learning in combination with multimodal MRI data for the differentiation of PD patients with a non-postural instability and gait difficulty subtype from patients with a postural instability and gait difficulty subtype resulted an accuracy of 92.3% (specificity = 97.0%, sensitivity = 84.2% and AUC = 0.96) (Gu et al., 2016). These findings provide preliminary evidence that neuroimaging-based inference in the individual heterogeneity in clinical features, disease course, and treatment response of PD might be possible.