Chapter 9

Summary and general discussion

This thesis aimed at gaining more insight into the neural underpinnings of the cognitive heterogeneity among early-stage PD patients. For this purpose, we scanned 25 patients with PD who were not on dopamine replacement therapy, and 43 healthy controls while performing a set-shifting, working memory, and response inhibition task in an MRI scanner. We, furthermore, induced a "temporary lesion" in healthy participants to mimic the decreased activation of the left dorsal PFC in PD patients. Last, we compared the T1-weighted MRI scans from a large cohort of PD patients (N=93) with those of a group of ad hoc recruited healthy participants (N=46) to investigate between-group differences in brain structure, and assess the relation between structure related measures and performance on neuropsychological tasks within the group of PD patients.

We will first briefly discuss the main findings per research question based on the results reported in the corresponding chapter(s), and subsequently try to interpret our overall results in relation to the existing literature on PD and cognition. We will then try to deduce a working model about cognitive heterogeneity and compensation in PD, and discuss the consequences of our results for the classical model of the basal ganglia. Last, we will consider a number of important methodological issues that apply to this thesis, and end with recommendations for future investigations.

MAIN FINDINGS PER RESEARCH QUESTION

1. Do unmedicated PD patients show impaired set-shifting performance, associated with decreased neural activation and decreased task-related functional connectivity?

In **chapter 2** we compared our cohort of unmedicated PD patients with matched healthy controls while performing an in-house developed set-shifting task in an MRI scanner. Since task-performance on previous set-shifting paradigms also depended on other cognitive constructs, such as working memory, matching-to-sample, and visuo-spatial learning [99, 316, 317], we developed a new feedback-based set-shifting task. This task contained less of the potential confounding factors, hereby obtaining a less biased view on the behavioural and neural correlates of set-shifting in PD patients. PD patients made more errors during repeat, but contrary to our hypothesis and previous findings [93] displayed normal performance on the set-shift trials. Patients showed a decrease in task-related activity in the right inferior frontal gyrus (ventro-lateral prefrontal cortex (VLPFC)), and an increase in the bilateral parietal cortices and right superior frontal cortex during shift, when compared with repeat trials. We also found a negative correlation between DaT-SPECT uptake ratios and the degree of activation of the superior

frontal cortex in a sub-sample of patients. **Chapter 3** further investigated group differences in task-related functional connectivity during the set-shifting task discussed in chapter 2. We selected the bilateral DLPFC, bilateral superior frontal gyrus, and bilateral inferior parietal gyrus (IPG) as seed regions based on our own (described in chapter 2) and other findings [95, 96, 152]. For each region we assessed the brain areas with which it showed functional connectivity during the task using a general form of psycho-physiological interaction (gPPI) [133]. All six seed regions showed task-related functional connectivity with the posterior cingulate gyrus, medial prefrontal cortex, and angular gyrus. Although these areas were already known to be highly connected during rest, as reflected in the default mode network (DMN) [116, 117], their connectivity also appears to play an important role during task performance. We subsequently compared whether there were between-group differences in functional connectivity for each area and found that the seed regions of the PD patients were, overall, less functionally connected when compared with controls. Based on the results from chapter 2 and 3, we hypothesized that due to the striatal dopamine depletion in PD, task-related areas desynchronize [80], and are thereby less functionally connected. We further speculate that the hyper-activation during task performance compensates for the decreased task-related functional connectivity and hypo-activation of the ventral prefrontal cortex, in this way postponing behavioural impairments in set-shifting.

2. Is it possible to mimic set-shifting-related behavioural and neural impairments as seen in PD patients in healthy participants, using low-frequency (i.e. inhibiting) rTMS on the left dorsal PFC?

According to the classical model of the basal ganglia by Alexander and others [5, 6, 32, 33, 318], the striatal dopamine depletion in PD leads to a hypo-excitation of the DLPFC, which is thought to underlie executive dysfunction in general, and in set-shifting deficits in particular. We tested this hypothesis in **chapter 4** by applying low-frequency (i.e. inhibiting) rTMS to the left dorsal PFC in healthy controls, thereby creating a "temporary lesion" model of the set-shifting impairments in PD [146]. We measured the task-related brain activation of the left dorsal PFC in 33 healthy controls while performing our in-house developed set-shifting task in the MRI scanner twice: first during a baseline scan session, and subsequently after low-frequency rTMS at the area in the PFC that was most active during the baseline session in half of the group (verum), or at the vertex in the other half (sham). As hypothesized, behavioural performance, task-related activity, and functional connectivity selectively decreased over the sessions in the verum group, thereby further corroborating the role of the dorsal PFC in set-shifting. The area that displayed reduced activity was, however, not in the proximity of the stimulation site

(i.e. the left dorsal PFC), but in the left middle temporal gyrus. At the 2nd session, the stimulated area (left DLPFC) was less functionally connected during task performance with areas in the left post central gyrus, and left posterior insula. These results underline that functional connections within networks play an important role in brain function and disease [67], and that disturbances in one brain area can have consequences in remote, but connected, other regions.

3. Do unmedicated patients with PD show impaired working memory performance, associated with decreased neural activation and task-related functional connectivity?

This question was investigated in **chapter 5**. Our cohort of unmedicated patients with PD performed a visuo-spatial *n*-back paradigm while in an MRI scanner. Since the *n*-back task has been employed in numerous other investigations, we had strong hypotheses about which areas would be involved in this task [168, 319]. We used the bilateral DLPFC, bilateral caudate nucleus and bilateral inferior parietal cortex (IPC) as regions-of-interest (ROIs) to assess between-group differences in task-related activation, and investigated i) their individual activity, ii) the functional connectivity of the bilateral DLPFC, using gPPI, and iii) the effective connectivity within the fronto-striatal (i.e. bilateral DLPFC, bilateral caudate nucleus) and fronto-parietal (i.e. bilateral DLPFC, bilateral IPC) networks, using dynamic causal modelling (DCM). Contrary to our hypotheses, we found no behavioural deficit, and increased (instead of decreased) activity in the left DLPFC in the PD group. Both connectivity analyses showed decreased connectivity in the fronto-parietal network, but no differences in connectivity within the fronto-striatal network in PD patients. Exploratory regression analyses in a sub-sample of PD patients revealed a positive relation between DaT-SPECT uptake ratios, behavioural performance, and task-related functional connectivity between the left DLPFC and parietal areas. In line with the findings in chapter 3, we hypothesized that task-related functional connectivity between (remote) brain regions decreases due to the striatal dopamine depletion, but that this is compensated for by a hyper-activation of task-related areas, thereby forestalling behavioural deficits.

4. Do unmedicated patients with PD show impaired response inhibition, associated with decreased task-related brain activation?

In chapter 6 we described the background and results of the study on response inhibition in our unmedicated cohort of PD patients and healthy controls. Since we had strong *a priori* hypotheses about which brain areas were involved in task performance, we employed the bilateral inferior frontal gyri, inferior parietal lobes, caudate nuclei, and right pre-supplementary motor area as ROIs. In accordance

with our hypothesis, we found that the patients were slower at response initiation, but not at inhibiting their responses, as measured with the stop-signal reaction time (SSRT). This behavioural deficit was accompanied by a hypo-activation of the bilateral inferior frontal gyri and the left inferior parietal lobe. Disease related measures (i.e. UPRDS-III scores) correlated negatively with the task-related activity of the left inferior frontal gyrus. We hypothesize that due to the PD related pathology, task-related areas were hypo-active and task performance decreased accordingly. We further speculate that, because this task exerted too much pressure on an already affected cognitive system [251], patients were not able to compensate, in contrast to our observations in chapters 2 and 5.

5. What is the contribution of variation in regional brain structure across PD patients to differences in task-performance on neuropsychological tasks; and what is the best analysis technique to study this?

In **Chapter 7** we investigated this research question with the often employed volume-based method voxel-based morphometry (VBM). Although several other studies have also investigated this question with the same technique [45-47], a strong point of our study was the large and well-characterized cohort of PD patients (N=93), and the availability of neuropsychological test data for the PD patients. When correlating neuropsychological test scores with GM volume, our most important findings were positive correlations between the (i) parahippocampalgyrus and verbal memory, (ii) medial temporal lobe and putamen and visuospatial memory, and (iii) middle temporal gyrus and frontal lobe and verbal fluency. Additionally, we found in PD patients relatively small clusters of reduced GM volume in frontal, parietal and temporal cortices and in the cerebellum. Because GM volume is an unspecific measure, consisting of the product of surface area and cortical thickness, we also studied structural differences using the surface-based method FreeSurfer, while using the same cohort of patients and controls as in chapter 7. This analysis is described in **Chapter 8**. Only a handful of other studies have of yet investigated the relation between cortical thickness, surface area and (sub)cortical volume in relation to cognition in PD [52, 56, 58, 320-322] and, to ourknowledge, only one [323] has compared these results with the findings obtained by a voxel-based method. We found cortical thinning in occipital, parietal, and frontal areas in the PD group, and negative correlations between (i) cortical thickness in occipital areas and performance on a verbal memory task, (ii) surface area of the prefrontal cortex and interference susceptibility, (iii) surface area and cortical volume of the operculum and visuo-spatial memory performance, and, (iv) volume of the right thalamus and scores on two verbal fluency tasks. We concluded that cortical thickness, but not surface area, is affected in PD, and that cognitive

heterogeneity between patients is related to differences in cortical thickness, cortical surface area and (sub)cortical volume. We also found surprisingly little overlap between the results of chapter 7 and chapter 8, despite using (nearly) the same cohort. We attribute this discrepancy to i) between-technique differences in statistics / methodology, and ii) the relatively subtle structural alterations at this early disease stage. In sum, we concluded that variation in local brain structure contributes to the between-patient variation in cognitive performance but does not distinguish between patients and controls. Based on these results, we advise to use both a volume- and surface-based technique when investigating structural differences in PD, since both measures are sensitive to specific aspects of brain structure which might be overlooked when applying only a single method.

OVERALL RESULTS IN RELATION TO OTHER LITERATURE ON PD AND COGNITION

Task-related activity and cognition

Research questions 1, 2 and 4 relate to the behavioural and neural consequences of PD on set-shifting, working memory, and response inhibition, respectively, three important aspects of executive functions [29]. Although PD patients typically show impairments on these tests [12, 13], it is also well recognized that there is a large between-patient variability in cognitive performance, already early in the disease [14]. One of the unique features of this thesis is that we can compare both behavioural and neural responses of the same cohort of patients and controls over different tasks. A first remarkable similarity was that both in the set-shifting and the response inhibition task we found a decrease in activation in the right inferior frontal gyrus, more specifically, in the VLPFC (BA 47).This area is strongly associated with motor response inhibition [324], as measured with the Stop-Signal task. While we strived to design our set-shifting task in a way that it measured the cognitive construct set-shifting as accurately as possible, a certain extent of response inhibition is inherent to set-shifting [29, 325]. We therefore speculate that both tasks put response inhibition-related demand on this area, which could be compensated for by the fronto-parietal hyper-activation in the set-shifting task, but not during the Stop-Signal task. This suggests that the ventral system in general, and the VLPFC in particular, is a vulnerable region in PD. Although this fits the classical model in which the PFC is vulnerable to PD pathology [5, 32], traditionally the dorsal, and not ventral, areas are affected first. Future research should further investigate why this particular area seems to be vulnerable for task-related pressure in PD.

Another noteworthy pattern that emerged over the three tasks was that patients displayed hyper-activity and intact behavioural performance on the set-shifting and

working memory task, and hypo-activation and decreased behavioural performance in the response inhibition task. This pattern fits within the Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) [196, 326], which is often employed in the aging literature. This theory states that older and younger healthy participants can behaviourally perform equally well, but that the elderly need to hyper-activate their task-related network in order to do so. Conversely, when this compensatory mechanism fails, hyper-activation turns into hypo-activation, and behavioural performance declines accordingly. In line with this theory, we argue that the hyper-activation, as seen in our patients during the working memory and set-shifting task, is compensatory, thereby forestalling behavioural deficits. In contrast, we saw only hypo-activation of task-related areas in the response inhibition task, and decreased task performance. The task demands of the Stop-Signal task apparently put too much cognitive pressure on the task-related neural network in the PD patients to be able to compensate for by hyper-activation [251]. This is also in line with other studies on response inhibition in early PD that employed a cognitively less demanding Go/No-Go task, and again found no behavioural deficits and hyper-activation in the PD patients [327, 328]. The CRUNCH model also concurs with our findings from chapter 4, in which we used the non-invasive technique rTMS to decrease the activity of the left dorsal PFC in healthy participants, prior to performing a set-shifting task in an MRI scanner. We found, as hypothesized, a decrease in activation which was accompanied by a decrease in behavioural performance.

It remains an open question why the same patients were able to compensate for difficult tasks such as the *n*-back and switch, but not for the Stop-Signal task. Whereas working memory and set-shifting are most strongly associated with the dorsal (prefrontal) circuit [168, 329], response inhibition is associated with the ventral (prefrontal) network [324]. According to the classical model of Alexander [5] and that of later work, the dorsal part of the striatum and PFC are most severely affected by the PD-related pathology, whereas the ventral system is relatively preserved [38]. As previously discussed, however, PD is not a 'dopamine-only' disease, and also the serotonergic and noradrenergic systems are affected. Two recent studies found that selective serotonin [226] and noradrenalin [330] re-uptake inhibitors improved response inhibition performance in PD, whereas a different study found that dopaminergic medication did not [331]. These findings are in line with the general view of serotonin in inhibitory response control [40]. One could thus speculate that in early PD the ventral circuit is relatively spared with respect to behavioural performance that depends on ventral dopaminergic projections, such as reward based reversal learning [37, 332], but that behavioural and neuronal impairments can be detected on tasks that also depend on ventral serotonergic / noradrenergic projections. In general, these results suggest that the dorsal circuit

was either i) less affected by the PD-related pathology, or ii) had more compensatory abilities than the ventral cognitive network. These findings again underline the complexity of the PD-related pathology in relation to cognition. Another more practical explanation relates to the consequences of the standardized order in which participants had to perform the three neuropsychological tasks in the MRI scanner. Since scan sessions were often in the evenings, and the Stop-Signal task was always the last task to be completed, patients might have been more tired than controls, leading to the decreased neural activity and behavioural performance. The high prevalence of sleeping problems among PD patients further strengthens this possibility [11, 333]. Future research should randomise the task order to exclude these potentially biasing order effects.

Task-related functional connectivity and cognition

It is important to realize that behavioural manifestations resulting from neurological damage are not purely the consequence of one abnormally functioning area, but represent changes in the whole brain network [67]. In our set-shifting and working memory study we therefore investigated, besides neural activity, also task-related functional connectivity. Although the exact seed-regions differed between studies, we found in both cases that the task-related functional connectivity of our ROIs was decreased in the PD patients. Also in our rTMS study we found that the perturbation of a specific area (i.e. the left dorsal PFC) led to a reduction of task-related functional connectivity with a remote location, again stressing the need for understanding the brain in a less modular and more network-type fashion. Neuronal oscillations are a likely mechanism through which individual, and populations of, neurons synchronize and thereby functionally connect, either in rest or during task performance [68]. To maintain the high-precision timing in firing frequency between the phase-locked oscillating neuronal assemblies, neurons are dynamically tuned by biasing the membrane conductance through neurotransmitters such as glutamate and GABA, or modulatory neurotransmitters, such as acetylcholine, serotonin, or dopamine [334, 335]. We hypothesize that primarily because of the striatal dopamine depletion, but probably in addition to changes in the serotonergic, noradrenanergic, and cholinergic system [9, 10, 39, 41, 43], the membrane conductance of neurons is no longer properly fine-tuned, leading to a desynchronized firing pattern within and between neuronal assemblies. We furthermore hypothesize that this desynchronization underlies the decreased task-related functional connectivity in our functional MRI studies. This interpretation is in line with findings which show that optimal dopamine levels lead to "quelling" within neuronal populations, and thus to an increased signal-to-noise ratio [70], and that the relation between dopamine and cognitive performance is often described as an

inverted U-shape [329]. Numerous neurophysiological resting-state studies in PD have confirmed changes in local power and global synchronization [72, 74, 76], and argued that these changes can be used as a marker of disease progression [77] and declining cognition [73, 78, 79]. Also electrophysiological data from deep brain stimulation (DBS) electrodes indicates that the striatal dopamine depletion in PD leads to a pathological increase in beta band oscillations within the cortico-basal ganglia circuit that correlate with motor dysfunction [80, 336, 337]. Last, a few studies investigated the effect of dopamine replacement therapy on functional connectivity during resting-state fMRI [130] and task performance [120, 131] and found a normalization of functional connectivity following medication in the PD patients. Although it is difficult to compare functional connectivity between task-related and resting-state studies, these studies suggest an association between pathological neural oscillations / functional connectivity and impaired cognition.

Structure and cognition

Numerous imaging studies have consistently shown a negative correlation between structural brain measures and cognition in PD [50-52, 54, 320]. For example, PD patients with MCI have a faster rate of cortical thinning over time than patients without MCI [58] and non-demented PD patients who developed PDD within two years had a faster rate of cortical thinning than those who did not develop PDD [57]. These studies further corroborate the association between structure and cognition in a longitudinal design. Other studies have found correlations between task performance on neuropsychological tests and GM structure within groups of PD patients, thereby further strengthening the relation between brain structure and cognition [262]. We also found positive correlations between cortical thickness / cortical surface area / GM volume and task performance on several neuropsychological tests in our studies. Furthermore, we found relatively small areas of reduced GM volume / cortical thickness in our cohort of patients, which corresponds with findings from other investigations in groups of cognitively preserved PD patients [46-48], although it is important to emphasize that our cohort was not selected to represent a unitary cognitive status (e.g. not cognitively impaired / cognitively impaired).

Even though there is still no definite consensus, studies suggest that differences in cortical thickness and GM volume primarily represent differences in neuronal structural complexity (i.e. synapses and dendritic arborisation) and not neurons *per se*, although the influence of neuronal size, (mircro)glia, and blood vessels cannot be fully excluded [338]. This fits with observations in which cognitive training increased GM volume [339, 340] or cortical thickness [341] in task-related areas. Relating this to our previous findings suggests that an optimal structural complexi-

ty (e.g. synapses) in some areas results in better task-performance on some neuropsychological tasks, and that structure and function can influence each other [342].

Table 9.1 Demographic, clinical, and behavioural characteristics of the whole cohort.

	HC (N = 43)	PD (N = 25)	p-value
Demographics			
Age (years)	57 ± 9 (38 – 75)	60 ± 11 (38 – 78)	.24[a]
Gender (% men)	23 (54 %)	19 (76 %)	.08[c]
Handedness (right)	37 (86 %)	21 (84 %)	.92[b]
DART score	104 ± 14 (73 – 132)	102 ± 18 (72 – 42)	.69[a]
Education[#]	6 (3 – 7)	6 (1 – 7)	.58[b]
Clinical measures			
BDI	2 (0 – 13)	5 (0 – 22)	< .001[d]
BAI	1 (0 – 11)	5 (0 – 19)	.02[d]
UPDRS	NA	20 (2 – 35)	-
H&Y stadium	NA	2 (1 – 3)	-
Behavioural test scores			
MMSE	29 (24 – 30)	29 (24 – 30)	.18[d]
RAVLT (total)	47 ± 8 (31 – 63)	43 ± 9 (26 – 61)	.05[a]
RAVLT (recall)	10 ± 3 (2 – 14)	8 ± 3 (2 – 14)	.07[a]
Category Fluency	27 ± 6 (17 – 40)	24 ± 6 (12 – 40)	.06[a]
Letter Fluency	43 ± 10 (26 – 73)	38 ± 10 (22 – 54)	.07[a]
Stroop Interference Score	38 (13 – 118)	41 (24 – 95)	.27[d]
ROCFT (copy)	36 (13 – 36)	34 (27 – 36)	.02[d]
ROCFT (3-min recall)	20 ± 8 (4 – 33)	19 ± 8 (5 – 31)	.69[d]
ROCFT (30-min recall)	20 ± 7 (4 – 31)	20 ± 7 (6 – 30)	.98[d]
TMTB-A	26 (3 – 78)	36 (-4 – 114)	.08[d]
Digit span (total items)	15 (9 – 25)	16 (10 – 20)	.82[d]
ToL (overall percentage correct)	89 (62 – 97)	85 (45 – 91)	.001[d]
ToL (overall RT in sec)	11 ± 2 (7 – 17)	14 ± 4 (8 – 24)	< .001[a]

Values are presented as mean ± standard deviation or median (range) unless indicated otherwise.
Abbreviations: *HC* healthy controls, *PD* patients with Parkinson's disease, *DART* Dutch adult reading test, *NA* not applicable, *BDI* Beck depression inventory, *BAI* Beck anxiety inventory, *UPDRS* Unified Parkinson's disease rating scale, *H&Y* Hoehn and Yahr, *MMSE* mini-mental state examination, *RALVT* Rey's auditory verbal learning test, *ROCFT* Rey-Osterrieth complex figure test, *TMT* Trail making test, *ToL* Tower of London test.
[a] = Independent samples t-test [b] = Pearson's χ^2 test [c] = Fisher's exact test [d] = Independent samples Mann-Whitney *U*-test
[#] = Education level was measured in 7 levels ranging from 1 (no finished education) to 7 (university training)

Cognitive reserves and compensation

We found behavioural deficits on the response inhibition task, but intact performance on the set-shifting and working memory task in our cohort of PD patients. When investigating our complete cohort (see Table 1), we did find behavioural performance differences on other conventional neuropsychological tests that we collected outside the scanner and measured cognitive domains (e.g. verbal memory, executive functions, visuo-spatial abilities). By excluding patients who were not able to perform the MRI tasks, we selected a group of high-performing patients, despite PD-related pathology. An often employed cognitive framework to explain why some people can tolerate more age- or pathology-related neuronal damage than others while maintaining normal cognitive functioning, are cognitive reserves [343-345]. Epidemiological studies have consistently shown that, amongst others, increased educational exposure postpones the age-of-onset of dementia, a phenomenon that is also described in PD [25]. Since the average educational level of our cohort was relatively high, we theorize that this might partly explain why their cognitive abilities were still preserved. We speculate that when the PD-related pathology further progresses, cognitive reserves will become exhausted, consequently leading to neural hypo-activation and cognitive deficits. This scenario is illustrated in a positron emission tomography (PET) study in which PD patients were scanned during a sequence learning task. At baseline, normal task-performance was accompanied with neural hyperactivity in frontal and parietal areas in patients [346]. After two years task-related activity levels in these regions had decreased, together with behavioural performance [347]. Similar observations have been made in other neurodegenerative diseases, such as multiple sclerosis [348, 349] and Alzheimer's disease [344].

Although task-related hyper-activation is often interpreted as compensatory and vital for maintaining normal cognitive function, it is important to emphasize that this is still an unresolved issue. Some authors have argued that increased neural activation reflects pathological disinhibition [350] or simply increased mental effort [196, 351]. There are also indications that increased neuronal activity might be toxic, or can cause damage to the function of synapses, as is, for example, seen in hippocampal atrophy in medial temporal lobe epilepsy [352, 353]. One pioneering study on this topic showed a striking overlap between areas with a high metabolism rate, such as the posterior cingulate cortex, in young and healthy participants and amyloid deposition in patients with Alzheimer's disease. The results suggested that metabolically highly active areas might be extra vulnerable to amyloid pathology [354], a finding that was further substantiated by subsequent investigations [355-357]. Indeed, there have been findings of cortical amyloid-beta in PD [57, 59] that

were associated with faster progression to PDD [63, 358]. A recent glucose PET study investigated the relation between cognitive status, metabolism at rest, and cortical atrophy in PD patients and found a large overlap between areas in which there was hypo-metabolism and atrophy in PDD patients, and areas that were hypo-metabolic, but not atrophic in MCI patients [359]. The authors suggested that brain areas first suffer from synaptic dysfunction, reflected in hypo-metabolism as seen in the MCI patients, which is followed by a loss of dendritic arborisation and synapses, thus accounting for the large reduction in GM volume in the PDD patients. Taken together, one could speculate that increased neural metabolism might lead to progressive synaptic dysfunction, and eventually to synaptic damage, indicating the transition to hypo-metabolism and MCI. Although the topological pattern of the progression of amyloid pathology has not yet systematically been investigated in PD, post mortem studies in Alzheimer's disease patients and healthy controls show that the initial amyloid depositions are found in the neocortex, and subsequently progress in a descending fashion [360]. The hypothesis that cortical hyper-activity might be neurotoxic or induces amyloid pathology could provide an explanation as to why amyloid beta is found in cortical areas, and why some cortical brain areas show volume reductions, such as decreases in prefrontal GM volume, in a pre-PDD stage [57].

WORKING MODEL OF COGNITIVE HETEROGENEITY AND COMPENSATION IN PD

Combining previous findings in the literature with our own, I have tried to deduce a simplified working model to explain cognitive heterogeneity in (early) PD patients (see Figure 1).

Phase 1: Neurotransmitter producing nuclei in the brain stem and midbrain areas degenerate due to the Lewy-body pathology, leading to a desynchronisation of the oscillations that functionally connect local and distant neuronal assemblies. This sub-optimal synchronization can be measured as altered functional connectivity during resting state or task performance.

Phase 2: Either to compensate for the sub-optimal functional connectivity, or as a consequence of it, (primarily dorsal cortical) brain areas display increased activity levels during task performance, measured as hyper-activation. While sustaining this hyper-activation, cognitive deficits are postponed. The increased metabolism, however, might lead to synaptic dysfunction, possibly resulting from the accumulation of amyloid pathology near the synapses following the hyper-activation. As

PD-related pathology progresses, load on the cognitive networks increases, which needs to account for an increasing degree of functional and structural damage.

Phase 3: Synaptic dysfunction becomes so pronounced that local processes can no longer be sustained, and the task-related activation in specific brain areas converts from hyper-activation to hypo-activation, resulting in cognitive deficits or MCI. We hypothesize that an optimal amount of GM volume or cortical thickness (i.e. synapses and dendritic arborisation) might serve (at least in some areas) as a protective mechanism to be able to withstand more synaptic dysfunction before converting into hypo-activation. When synapses degenerate after having suffered too much damage, cortical atrophy becomes apparent.

Phase 4: Lewy-body pathology reaches the neo-cortex in Braak stages V and VI, and patients convert from MCI to PDD.
Especially the transition from phase 2 to phase 3 is individually determined and I believe that this conversion can be postponed by more optimal structural brain measures in critical brain regions. It is also likely that not all brain circuits simultaneously convert from phase 2 to phase 3, but that some are able to stay longer in phase 2 than others. This also applies to our own data, in which we found behavioural deficits and hypo-activation on the response inhibition task, but not on the set-shifting and working memory task.

Although this is a highly simplified model of the complex interplay between brain structure and function, it combines a number of elements which are found persistently throughout the literature on (pathological) aging and cognition. More importantly, it presents a working model that can be further expanded or modified by future research.

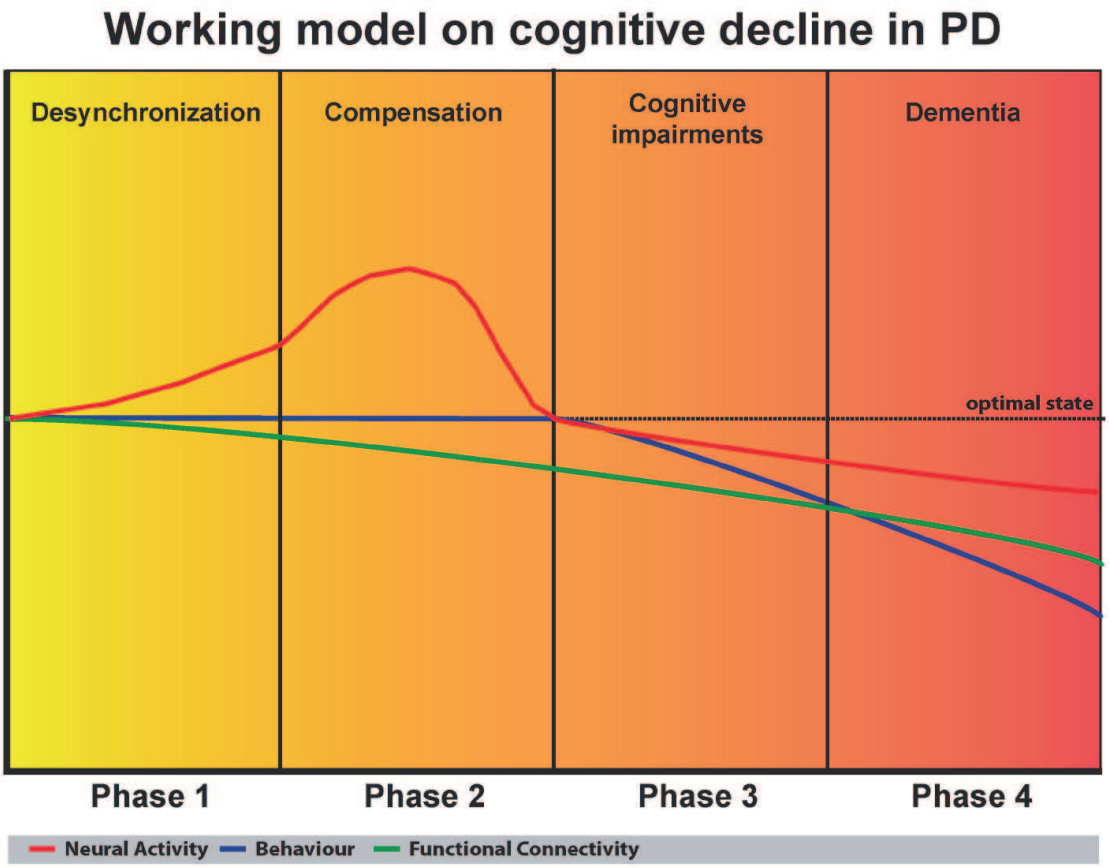

Figure 9.1 Working model on cognitive decline in PD
Phase 1: Desynchronization / decrease in functional connectivity between local and distant neuronal assemblies due to the degeneration of the neurotransmitter producing nuclei in the brainstem and midbrain.
Phase 2: Individual brain areas increase activity levels during task performance, which might lead to synaptic dysfunction. While sustaining this hyper-activation, cognitive deficits are postponed.
Phase 3: Synaptic dysfunction becomes so pronounced that local processes no longer properly function, and the task-related areas convert to hypo-activation, resulting in cognitive deficits or MCI.
Phase 4: Lewy-body pathology reaches the neo-cortex in Braak stages V and VI, and patients convert from MCI to PDD.

THE CLASSICAL ALEXANDER-MODEL OF THE BASAL GANGLIA:

In the traditional model of the basal ganglia [5, 6, 32, 33, 318], the striatal dopamine depletion leads to an irreversible hypo-activation of the areas that are anatomically connected to the striatal regions, in turn leading to the observed cognitive deficits. We, however, found hyper-activation in frontal regions and intact behavioural performance within our cohort of PD patients while performing a working memory and set-shifting task. Although this model focuses exclusively on the dopamine system, ignores cortico-cortical connections, and does not consider

the effects of the evolving Lewy body pathology, it is still widely used to explain cognitive deficits in PD. It seems that the model describes what happens at a neuronal level when cognitive deficits are present (also see e.g. [34]), but does not correctly predict the consequences of the dopaminergic depletion when cognition is still preserved. In order to predict what the cortical net-result of the striatal dopaminergic depletion will be (i.e. hypo-activation vs hyper-activation), an intermediate stage should be incorporated into the model, which takes the cognitive status of the patient into account. To what degree the modification of the model is applicable to the motor- or limbic circuit remains to be elucidated by future research.

METHODOLOGICAL CONSIDERATIONS:

PD patients typically display cognitive deficits on neuropsychological tasks, which was also true for our entire cohort of patients (N=25). We, however, excluded patients from our MRI analyses who were not able to perform the tasks, thereby selecting a group of high performing patients. This procedure has introduced a selection bias and compromises the generalisation of our results to all PD patients. We believe, however, that our cohort provides a unique insight into the underlying neural mechanisms which are involved in the maintenance of behavioural performance in PD. A second issue is that our patients were not using anti-parkinsonian medication, while many other studies have recorded patients in an ON or OFF stage. Although our cohort contains less potential biasing factors, it is important to realise that this issue also makes it more difficult to compare studies.

Another important aspect to consider, also for future research, is the relative straightforwardness of the set-shifting task we developed to measure the cognitive construct set-shifting more accurately and was used in chapters 2, 3, and 4. Although we argue that we have succeeded in developing such a task, as can be seen in the robust main effect activation maps, we believe, in retrospect, that the task might not have been cognitively challenging enough to properly distinguish patients from controls based on task performance or neural activity. This consequently might have led to an underestimation of the measured behavioural and neural deficit, both in the PD patients as in the group which received real rTMS.

For the response inhibition task we used a more conservative measure to calculate the stop-signal reaction time (SSRT) than previous studies used, since the traditional measure consistently overestimates the SSRT. We did find a behavioural deficit when employing the conventional method, but not when employing the more conservative calculation. Since this was the first study on *de novo* PD patients

in relation to response inhibition, we warrant caution with respect to the absence of the behavioural deficit, and recommend further investigation in future studies.

Also, by employing a ROI approach for our task-related functional connectivity analyses, we might have overlooked potentially relevant effects outside the seed-region. Although we based our regions on previous literature this issue needs to be considered when interpreting our results.

An important methodological issue is the large discrepancy in findings between the VBM and FreeSurfer method. Although we followed standard procedures, and used a well-powered cohort (N=149), it is remarkable that these two methods specialized in structural measurements yield such different results. We think that these differences are attributable to i) differences in employed statistics (uncorrected vs corrected results), ii) between-method differences in technique (voxel-based vs atlas-based), and iii) the relatively subtle structural alterations at this early disease stage. We argue that if the structural differences had been more pronounced, both techniques would have detected them.

A last statistical consideration is that we not always applied a correction for multiple comparisons when assessing between-group interactions. Because our patients were still in an early disease stage, we wanted to be maximally sensitive to subtle, but potentially meaningful between-group differences, and thus to avoid false-negative findings. This issue further stresses the importance that future research replicates our findings before making any definite statements.

FUTURE PERSPECTIVES

In this thesis, we have found a decrease in task-related functional connectivity in PD patients. It would be interesting to investigate whether this decrease is also present during rest, thereby relating task-related and resting-state functional connectivity data to each other. It would furthermore be interesting to investigate the role of structural connectivity (i.e. white matter fibre tracts) using diffusion tensor imaging (DTI) and try to relate this to patterns we found in both task- and resting-state functional connectivity results. Such an analysis might provide further important insights into how changes in structural and functional connectivity relate to neural activity, and thereby compensation. Both DTI and resting state fMRI data were obtained during data acquisition, but are, as of yet, not analyzed. However, plans are underway to investigate both data in a combined analysis in the near future in collaboration with Dr. Clare Mackay of the Oxford Parkinson's Disease Centre (OPDC) at the University of Oxford (UK).

Several large cohort studies have followed PD patients longitudinally with respect to behavioural performance on neuropsychological tasks, neural structural measures, and connectivity measures during resting-state, gaining valuable insight into the progression of the disease and its relation to cognition. Longitudinal studies on task-related neural activity or task-related functional connectivity, however, are still scarce. A study design that would follow a cohort of cognitively intact patients through time, while obtaining task-related and resting-state fMRI, sMRI, together with an extensive neurological and neuropsychological assessment, would be greatly beneficial for our understanding of the neural mechanisms allowing some patients to maintain cognitive performance while others convert to MCI or PDD. Since different tasks tap into different cognitive networks, it would also be insightful to investigate disease progression-related changes in activity for different cognitive networks. For this reason, we now re-scanned a large part of the unmedicated patients and controls cohort one to three years after the initial investigation, again obtaining task-related fMRI, resting state fMRI, and DTI. The data analysis is now in progress, and the results will provide more insight into the neural changes in PD during disease progression in relation to cognitive performance. They will, furthermore, supply further evidence for our 4 stage working model of cognitive heterogeneity in PD.

We also encourage the use of a multi-modal imaging approach, such as EEG / MEG, MRI and SPECT/PET to obtain complementary measures on the same patients, and thereby increasing the understanding of the underlying pathologic mechanisms. In our working model there is an important role for the desynchronization of the oscillations, and their relation with neural activity. Examining patients using both M/EEG, with its high temporal resolution, and functional MRI, with its high spatial resolution, while performing the same task, could yield unique information on the relation between task-related functional connectivity, activity, and cognition. Our unmedicated cohort of patients and controls was scanned while performing the same *n*-back working memory task in both an MRI and MEG scanner. Some first steps have already been taken in preprocessing this unique data set, and will hopefully be fully analyzed in the near future. Also imaging different neurotransmitter systems (i.e. dopamine, serotonin, noradrenalin, acetylcholine) with PET/SPECT and relating these to, for example, BOLD or MEG derived measures could yield important information about how the functional changes relate to neural activity or functional connectivity.

According to our working model on compensation in PD the striatal dopaminergic depletion primarily underlies the desynchronzation between frontal-parietal task-related brain areas during task-performance, which is compensated for by hyper-

activity. This hypothesis could be tested in a task-related ON-OFF medication design. Especially the link between dopamine and task-related functional connectivity in PD in relation to behavioural performance is still a relatively unexplored area, but a promising field of research. These studies would ideally also employ a multi-modal approach to study the effects of dopaminergic medication on both activity and oscillations and would provide further evidence about our proposed working model.

PD-related pathology acts at the microscopic scale of synapses, dendrites, and neurons. Only after a certain amount of changes or damage is it possible to detect these disease-related alterations on the macroscopic scale using modern neuroimaging techniques. What is thus seen at the macroscopic scale is the consequence of countless alterations at the microscopic scale. In order to better understand how these scales relate to each other, one could think of correlating neuropsychological task performance of patients during life to post mortem microscopic alterations (e.g. amyloid deposition, Lewy body inclusions, damage to synapses / neurons). Another possibility relates to using advanced neuroimaging techniques such as high field-strength structural MRI (e.g. 7 Tesla or higher) to image sub-millimetre structural details or damage *in vivo* and relate these to neuropsychological task performance. In order to fully understand changes in behaviour and cognition, one needs to understand what is happening at the level of the synapse, and all the levels in between.

There is, unfortunately, still no remedy against cognitive dysfunction in PD. A more detailed understanding of the pathological mechanisms that underlie the cognitive deficits in PD might provide a better starting point for neurorehabilitation or cognitive training [361], or a combination of both. One might hypothesize that if behavioural task-related deficits are primarily induced by a desynchronization (i.e. decrease in functional connectivity) between frontal and parietal areas, rTMS or transcranial direct current stimulation (tDCS) could be used to artificially restore (theta) frequencies between the two areas, thereby diminishing cognitive complaints (see e.g. [362]). Indeed, a recent review suggested that (non)-invasive brain stimulation modulates functional brain networks and that the stimulation site needs to be functionally connected with the targeted network in order to be effective [363]. This further emphasizes why a more elaborate understanding of (functional) connectivity might be valuable in the future.

Taken together, we found in a cohort of relatively early unmedicated patients with PD decreased task-related functional connectivity, neural hyper-activation, and intact behavioural performance and hypo-activation and behavioural deficits. We

furthermore found in a more naturalistic cohort of PD patients that structural differences contribute to the cognitive heterogeneity between PD patients. Based on these findings, we have proposed a working model to understand how PD-related pathology might affect functional connectivity, neural activity, and brain structure and how we could use these between-patient differences to better understand cognitive heterogeneity in PD. Future research should systematically test and validate the hypotheses proposed by the model, make adjustments where necessary, and expand it with novel insights. A better understanding of the cognitive heterogeneity among PD patients is an important starting point for the development of innovative treatment alternatives targeting the specific networks. This could lead to new cognitive rehabilitation strategies to improve or maintain cognitive status in spite of the progressive neurodegeneration.