Cerebral Metabolic Patterns In Neurodegeneration

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11. General Discussion and Future Perspectives

The aim of this thesis was to investigate patterns of altered cerebral glucose metabolism in neurodegenerative disorders using ^{18}F-FDG PET imaging and spatial covariance analysis. The results converge on the notion that the relevant pathophysiological information is indeed present in ^{18}F-FDG PET data, even in prodromal disease stages, and that this information can be extracted with spatial covariance analysis. Spatial covariance patterns provide insights into underlying pathophysiology, and subject scores on spatial covariance patterns are useful outcome measures to answer several clinical and research questions.

The PDRP Reflects Networks of Synaptic Dysfunction in Sporadic PD

The PD-related pattern (PDRP) has a central role in this thesis. The PDRP was first identified with SSM PCA by Eidelberg and colleagues in a North-American sample of 33 PD patients and 33 controls. We refer to this pattern as $PDRP_{USA}$. The $PDRP_{USA}$ was used for many 'benchmark' studies, as described in **Chapter 3**. In **Chapter 4**, we were able to identify a PDRP in Dutch, Italian and Spanish populations. The Dutch cohort consisted of PD patients who were scanned in the *off*-state with similar disease durations as the original $PDRP_{USA}$ identification sample. Indeed, the PDRP identified in the Dutch cohort ($PDRP_{NL}$) was similar to $PDRP_{USA}$ and subject scores on both patterns showed a very high correlation. It was important to establish the similarity between the 'reference' $PDRP_{USA}$ and our $PDRP_{NL}$, as an external validation of the method, and also to ensure that the $PDRP_{NL}$ could be used as a reliable outcome measure in subsequent studies.

It is striking that the topography of the PDRP is highly similar across populations, despite differences in demographics, clinical characteristics, medication use, scanning methods, and reconstruction algorithms. The PDRP is consistently characterized by relative hypermetabolism in the thalamus, putamen/pallidum, pons, cerebellum and motor cortex, and relatively decreased metabolism in the lateral premotor and parieto-occipital cortex. This topography reflects synaptic dysfunction in sporadic PD measured in vivo, and therefore provides important insights into the pathophysiology of PD.

The relatively hyperactive ('red') regions in the PDRP (i.e. thalamus, putamen/pallidum, pons, cerebellum and motor cortex) are central in brain network

dysfunction in PD. These structures comprise important nodes in widely distributed cortico-striatal-pallido-thalamo-cortical (CSPTC) circuits, which are modulated by dopaminergic input to the striatum (Rodriguez-Oroz et al., 2009, DeLong, Wichmann, 2007, Alexander, DeLong & Strick, 1986). The fact that these regions are hyper- rather than hypometabolic, may be explained by alterations in neuronal firing rates and firing patterns in networks of cortico-basal ganglia neurons, caused, at least in part, by focal loss dopaminergic drive to the striatum (Rivlin-Etzion et al., 2006).

Neuronal firing rates in the subthalamic nucleus (STN) and globus pallidus pars interna (GPi) are increased in PD. Positive correlations have been found between PDRP subject scores obtained from pre-operative ^{18}F-FDG PET data of PD patients undergoing deep-brain stimulation and intra-operatively recorded STN and GPi firing rates (Lin et al., 2008, Eidelberg et al., 1997). On a regional level, positive correlations were especially apparent between STN firing rates and putamen, globus pallidus, and primary motor cortex (Lin et al., 2008).

In addition to increased firing rates, the pattern of discharge is altered in the STN and GPi, with neighboring neurons shifting from spontaneous firing to synchronized oscillations (Oswal, Brown & Litvak, 2013, Wichmann, DeLong, 2003). Increased synchronicity of neural firing rates is associated with an increased functional coupling of neurons. In terms of network structure, networks that facilitate synchronization are associated with a small-world configuration. In small-world networks, there is a shortened communication distance between regions and increased connectivity between neighboring neurons. These properties normally serve to optimize the efficiency of information transfer in a network, at reduced energetic cost (Reijneveld et al., 2007). Excessive synchronization of local neural activity may be detrimental to circuit performance (Brittain, Brown, 2014).

Spatial covariance patterns, such as the PDRP, are whole-brain maps in which each voxel is assigned a value according to its relative importance in the pattern (and by extension, its relative importance in determining the subject score). It discloses no information on connections between major regions. In other words, the PDRP represents changes in brain function but not network structure. To better understand the structural organization of hyper- and hypometabolic regions in the PD network, Ko et al. applied graph theoretical analysis to $PDRP_{USA}$ derivation ^{18}F-FDG PET scans (Ko, Spetsieris & Eidelberg, 2018). Graph theory independently identified the hypermetabolic ('red') PDRP regions as the core of an abnormal PD network with exaggerated small world properties. The authors concluded that there are pathological links between the 'red' regions, which incur a high energetic cost. In turn, the more weakly connected peripheral nodes in the graph corresponded to the hypometabolic regions of the PDRP.

The previous paragraphs explain why interventions that directly interrupt the aberrant pathways in the network core are effective in alleviating motor symptoms in PD. In **Chapter 3** it was discussed that $PDRP_{USA}$ expression decreased after subthalamotomy, DBS of the STN and levodopa treatment. In those studies, clinical improvement correlated with the degree of $PDRP_{USA}$ modulation. Of note, the $PDRP_{NL}$ described in **Chapter 4** was recently also used as an outcome measure in a study of magnetic resonance-guided focus ultrasound (MRgFUS) subthalamotomy (Rodriguez-Rojas et al., 2019). $PDRP_{NL}$ expression was significantly reduced following MRgFUS subthalamotomy ($P < 0.05$). There was a clear trend between clinical improvement and the degree of $PDRP_{NL}$ modulation 3 months after treatment (r=0.9, P=0.002). This study underscores that the $PDRP_{NL}$ may be used as a potential disease biomarker. Considering the large overlap between $PDRP_{USA}$ and $PDRP_{NL}$, it is likely that these patterns can be used interchangeably as outcome measures.

In the previous paragraphs, regions with positive voxel weights (i.e. the red regions), were directly interpreted as hypermetabolic. From a methodological perspective, this is not correct. Strictly speaking, positive and negative weights indicate the direction of the principal component vector with respect to the mean, and do not directly reflect hyper- or hypometabolism, respectively. In **Chapter 2** we illustrated that normalization to a mean is useful to eliminate subject-specific scaling factors in ^{18}F-FDG PET data, but can inherently induce artificial in- and decreases. This is a known issue in any imaging study where absolute values are not available, be it univariate or multivariate. It is therefore important that patients and controls have similar values of average ^{18}F-FDG brain uptake (i.e. global metabolic rate (GMR)). It has been posited that the PDRP reflects normalization artifacts due to GMR differences between controls and patients (Borghammer et al., 2008, Borghammer et al., 2009). Specifically, widespread cortical decreases, rather than subcortical increases, were suggested to be characteristic of the PD disease process (Borghammer et al., 2010). However, both theoretical and empirical evidence supports the contention that the PDRP topography holds true pathophysiologic meaning, and that the 'red PDRP nodes' are central to PD pathophysiology (Spetsieris, Eidelberg, 2011, Ma et al., 2009, Dhawan et al., 2012).

Cognitive Dysfunction in PD Is Reflected by a Metabolic Sub-Pattern

In **Chapter 4** we concluded that the PDRP is a common denominator for all PD patients. This appears to contrasts with many reports that there is remarkable heterogeneity between individuals with PD in terms of clinical manifestations and progression over time (Fereshtehnejad, Postuma, 2017). The PDRP was identified by applying PCA to combined data of controls and PD patients. By focusing on the major differences between PD patients and controls, differences within the PD

group may be obscured. That said, in **Chapter 4** we do describe some deviations from the typical PDRP topography in each population, which may be caused by differences in clinical characteristics.

One aspect of clinical heterogeneity within PD populations pertains to cognitive decline (Hely et al., 2008, Buter et al., 2008). Identifying patients at risk for dementia is important for patient management. Eidelberg and colleagues had previously identified a metabolic pattern related to cognitive dysfunction within a cohort of PD patients (n=15). SSM PCA was applied to PD patients and principal components were selected that correlated to scores on several neuropsychological tests. The resulting cognition-related pattern ($PDCP_{USA}$) correlated with performance on multiple tests in the executive and memory domains (Huang et al., 2007, Huang et al., 2008). In **Chapter 5**, we were able to replicate the PDCP in an independent Dutch cohort ($PDCP_{NL}$; n=19). $PDCP_{NL}$ and $PDCP_{USA}$ were both expressed in patients with multiple domain MCI, but not in patients with normal cognition.

Both PDCPs were characterized by metabolic reductions in medial frontal and paietal association areas, and relative metabolic increases in the cerebellum. There is probably an association between the PDCP and degeneration of dopaminergic networks that project to the frontal cortex (the fronto-striatal and mesocortical networks) (Gratwicke, Jahanshahi & Foltynie, 2015). Modest associations have been found between $PDCP_{USA}$ expression and uptake of dopaminergic tracers in the anterior striatum (Holtbernd et al., 2015, Niethammer et al., 2013). The association between the PDCP and the dopaminergic system is further supported by a previous study by Mattis et al. This study showed that only PD patients with high $PDCP_{USA}$ expression scores (z-score>1) improved cognitively after levodopa treatment. The change in performance on a neuropsychological test after dopaminergic treatment was correlated to the degree of $PDCP_{USA}$ modulation. Patients with low PDCP scores did not improve cognitively after levodopa treatment. Interestingly, treatment with a placebo also gave cognitive benefit but did not modulate PDCP scores (Mattis et al., 2011). The PDCP may therefore be used as a biomarker evaluating treatment-mediated changes in cognition in PD.

We suggested that the PDCP may be used for predicting dementia onset in PD patients. However, the presence of frontostriatal executive dysfunction is probably not sufficient to explain full development of dementia in PD. Evidence is accumulating that development of dementia is associated with more widespread and posterior cortical changes secondary to dysfunction of the cholinergic system (Strafella et al., 2017). Cross-sectional, univariate studies comparing ^{18}F-FDG uptake in PD patients with normal cognition (PDNC), PD patients with MCI, and patients with PDD in a voxel-by-voxel manner have shown a trend of progressive posterior cortical hypometabolism (Garcia-Garcia et al., 2012, Pappata et al., 2011, Tang et al., 2016, Hosokai et al., 2009, Lyoo et al., 2010). Two small longitudinal

^{18}F-FDG PET studies support these findings (Bohnen et al., 2011, Tard et al., 2015). These studies thus seem to challenge the contention that PDCP expression may predict dementia in incident PD cases. However, the association between cholinergic dysfunction and PDCP expression remains to be investigated, and the true predictive value of PDCP expression can only be determined in a longitudinal study.

If the PDCP is indeed to be considered as a potential biomarker for cognitive decline, it should also first be replicated in multiple populations, as was done for the PDRP. We also calculated $PDCP_{NL}$ expression in the PD patients from the Italian and Spanish cohort from chapter 4, and found that indeed the $PDCP_{NL}$ was expressed in PDMCI, but not in PDNC (unpublished data). Analyses to identify a PDCP in the Spanish and Italian datasets from **Chapter 4** are underway, but prove to be more difficult due to differences in neuropsychological testing scores between populations.

The PDRP Is Expressed in Prodromal Patients

More than 80% of patients with idiopathic RBD will develop a parkinsonian (α-synucleinopathy) neurodegenerative disorder, usually PD or dementia with Lewy bodies (DLB), within a 12-14 year range after diagnosis. Idiopathic (or rather, isolated) RBD should thus be considered a form of prodromal parkinsonism and presents the unique opportunity to study patients with PD (or DLB) at an early disease stage. Moreover, early identification of α-synucleinopathic conditions via iRBD might be the first step towards successful prevention trials of late-life neurodegenerative diseases. In order to identify which iRBD patients will convert to a specific condition, and at what time interval, neuroimaging biomarkers may be essential.

In **Chapter 6**, we reviewed neuro-imaging studies in iRBD. We concluded that presynaptic dopaminergic imaging studies have provided clear and consistent results in iRBD. Decreased striatal uptake of a presynaptic dopaminergic tracer in an individual with iRBD indicates imminent phenoconversion. Although iRBD patients rarely develop MSA, early differentiation between PD and MSA is relevant for prognosis, management and inclusion in trials. In early-stage patients with clinical parkinsonism, differentiation between PD and MSA could be achieved with ^{18}F-FDG PET spatial covariance analysis (Tang et al., 2010b, Tripathi et al., 2015), but not with DAT-SPECT (Holtbernd et al., 2014, Brooks, 2016). In addition, a particularly interesting observation is that some patients with DLB have normal DAT scans at first presentation which become abnormal with disease progression (van der Zande et al., 2016). This could imply that iRBD patients with a normal DAT scan could still be at risk for developing DLB. In this respect, ^{18}F-FDG PET studies may complement DAT-SPECT scans.

In **Chapter 7**, we calculated $PDRP_{NL}$ subject scores in 21 patients with idiopathic RBD. Two previous studies had already shown that PDRP expression was

significantly higher in iRBD patients compared with controls, and lower compared with PD patients (Wu et al., 2014, Holtbernd et al., 2014). In a longitudinal clinical study of 17 iRBD patients, high baseline PDRP subject scores were associated with greater likelihood of developing PD/DLB within five years (Holtbernd et al., 2014). Our results were fully in line with these two previous studies. $PDRP_{NL}$ was significantly expressed in iRBD patients compared with controls. Although on average, PDRP *z*-scores were lower in iRBD patients compared with PD/DLB, 57% of iRBD patients already had a PDRP *z*-score in the range of PD/DLB patients. Patients could be identified (5/12) who had a normal DAT scan, but already had clearly elevated PDRP *z*-scores. This could indicate that PDRP expression is an earlier marker for phenoconversion than DAT-SPECT.

It is unclear if a differentiation between PD and DLB can be achieved with ^{18}F-FDG PET pattern analysis, let alone in the prodromal stages. DLB patients in our study and a previous one (Ko, Lee & Eidelberg, 2016) expressed the PDRP. Moreover, the DLB related pattern (DLBRP) is highly similar to the PDRP (unpublished data). In addition, a longitudinal study reported no differences in cerebral blood flow between iRBD patients converting to PD (n=5) and those converting to DLB (n=5) (Dang-Vu et al., 2012).

Holtbernd et al. described three iRBD patients with low baseline PDRP *z*-scores who converted to MSA within four years; MSARP expression was not reported (Holtbernd et al., 2014). We show that combined PDRP and MSARP *z*-scores can potentially differentiate between PD/DLB and MSA in iRBD patients (**Chapter 6**, Figure 3). However, longitudinal data are needed to corroborate this claim.

In addition to PDRP expression, we also investigated DAT binding and olfaction in our cohort, and found considerable heterogeneity in these markers. We did not find significant correlations between PDRP subject scores and DAT binding in iRBD, probably due to the small sample size (only 9 out of 21 iRBD subjects had an abnormal DAT scan). This claim is supported by a recent larger dual-tracer study investigating the relationship between PDRP expression and DAT binding in iRBD (*n*=37) (Huang et al., 2019). Within the iRBD group with an abnormal DAT scan (*n*=18), there was a significant correlation between DAT uptake in the posterior putamen (most affected hemisphere) and PDRP expression (*r*=-0.6, *P*=0.01). This trend was absent in iRBD patients with a normal DAT scan (*n*=19). In PD studies, correlations between DAT binding and PDRP expression have always been modest (R^2 of approximately 0.3) (Niethammer, Eidelberg, 2012). It is likely that the PDRP reflects more extensive underlying pathophysiological factors, which are not captured by presynaptic dopaminergic imaging alone.

Some of the subjects investigated in **Chapter 7** and **Chapter 8** have recently been included in a follow-up study. To date, twenty iRBD subjects of

the total cohort (*n*=30) underwent repeat ^{18}F-FDG PET scanning, approximately four years after the initial ^{18}F-FDG PET scan. Preliminary results show that 90% (18/20) show an increased PDRP expression compared to baseline. Additionally, several of these subjects now show early motor signs of PD.

Early Synaptic Dysfunction in PD is Reflected by the iRBD-RP

Idiopathic REM sleep behavior disorder presents a unique opportunity to test the Braak staging hypothesis (Braak et al., 2003). Braak and colleagues hypothesized that α-synuclein pathology in PD starts in the peripheral autonomic nervous system and spreads via autonomic nerves in a predictable caudorostral manner to the medulla (stage I), and then to the pons (stage II). When the pathology arrives at the midbrain (stage III), the substantia nigra is damaged, which results in parkinsonism. In later stages, the amygdala (stage IV) and cortex (stages V and VI) are affected. According to the Braak hypothesis, subjects with iRBD should have more extensive damage to the autonomic nervous system and the noradrenergic system in the pons compared to the nigrostriatal dopaminergic system.

In **Chapter 8**, we aimed to identify the metabolic pattern of iRBD, to study its topography and determine its position between healthy controls and PD. Although the study is cross-sectional, this could provide a further understanding of disease progression in PD from its prodromal stages. The iRBD related pattern (iRBDRP) indeed showed such remarkable overlap with the PDRP, that we concluded that the iRBDRP reflects the early, pre-motor stages of PD.

In previous sections, the relevance of the hypermetabolic (i.e. 'red') regions in the PDRP was discussed, in the context of the CSPTC circuits, and the nigro-striatal dopaminergic system. Unlike in the PDRP, the putamen did not reliably contribute to the iRBDRP. This can be explained from the fact that (by definition) none of the iRBD subjects had motor symptoms, and only 9/21 had an abnormal DAT scan. In other words, the motor networks are not (yet) significantly involved in iRBD, and this is reflected in the pattern.

An interesting observation is that hypermetabolism was already rather extensive in the cerebellum, pons and hippocampus in iRBD, perhaps reflecting alterations in ponto-cerebello-limbic pathways. This would fit Braak stage II. The graph theoretical analysis study by Ko *et al* identified these regions, along with the amygdala, frontal cortex and cingulum, as part of a cluster separate from the CSPTC circuit in a PD network study (Ko, Spetsieris & Eidelberg, 2018). This cluster also had a clear hypermetabolic core (cerebellum, pons, hippocampus, amygdala) with small-world properties, and a periphery of hypometabolic regions (frontal cortex, cingulum).

It could be hypothesized that the metabolic alterations in iRBD reflect monoamergic deficiencies other than dopamine. A key candidate may be

noradrenaline (Vermeiren, De Deyn, 2017). The noradrenergic locus coeruleus (LC), which has a long trajectory in the pons, is affected early and extensively in the disease process, and is associated with RBD and a variety of other non-motor symptoms. The LC provides the sole source of norepinephrine to the neocortex, hippocampus, cerebellum, and thalamus, and also exerts control over other nuclei, such as the substantia nigra and the raphe nuclei.

Following from the above, it appears that the iRBDRP could fit within the Braak hypothesis. In the absence of a (readily available) α-synuclein tracer (Jovalekic et al., 2017), multi-modality imaging is currently the only way to further investigate this topic in vivo. Recently, Knudsen et al. combined several imaging techniques in healthy controls, iRBD and PD to assess Braak stages I-III (Knudsen et al., 2018). Braak stage I was assessed with ^{11}C-donepezil PET–CT (cholinergic innervation of the gut) and ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy (noradrenergic innervation of the heart). These two imaging studies can be used to determine the integrity of the dorsal motor nucleus of the vagus nerve in the medulla. Integrity of the pontine locus coeruleus (stage II) was studied with neuro-melanin sensitive MRI and ^{11}C-methylreboxetine (MeNER) PET, a noradrenergic tracer. Presynaptic dopaminergic imaging (^{18}F-FDOPA PET) was done to characterize stage III. The authors showed that iRBD patients have abnormal studies in Braak stages I and II, similar to PD patients. Only approximately 30% of iRBD patients had abnormal ^{18}F-Dopa PET scans, in contrast to 100% of the PD patients. There was a striking separation of controls versus iRBD/PD on ^{123}I-MIBG. This suggests that any patient with iRBD who has abnormal findings on ^{123}I-MIBG has an α-synucleinopathy. It would be interesting to investigate the position of cerebral glucose metabolism, and more specifically PDRP expression, in such a multi-modality context. ^{123}I-MIBG shows a clear floor effect and may be useful for screening, whereas presynaptic dopaminergic imaging and PDRP expression may be valuable progression markers (Boeve, Kantarci, 2018).

It must be emphasized that this discussion only applies to the pathological process in iRBD patients who later develop PD. Approximately 24% of patients with idiopathic PD has concomitant RBD (Zhang, Xu & Liu, 2017). PD patients with RBD tend to have a disease subtype with accelerated cognitive decline (Duarte Folle et al., 2019). As discussed above, current studies seem to point to a distinct caudorostral gradient in individuals with iRBD, paralleling the Braak hypothesis, but it is unclear if this also holds for RBD-negative PD patients.

^{18}F-FDG PET May Predict Phenoconversion From MCI to Alzheimer's Disease

A prodromal disease stage can also be recognized in Alzheimer's disease dementia (AD). The prodromal stage of AD is characterized by the onset of first symptoms that do not meet the criteria for dementia. Identifying individuals with mild cognitive

impairment (MCI) who are at risk of phenoconversion to dementia will provide an opportunity to study the early disease stages of AD and to possibly alter its course. In **Chapter 9**, we compared AD-related pattern (ADRP) expression between MCI individuals who converted and those who did not (mean clinical follow-up of 7 years). ADRP *z*-scores in converters were indeed in the range of patients with established AD, whereas ADRP *z*-scores in non-converters were similar to controls. These findings parallel those of PDRP expression in iRBD. However, there was overlap between groups and several of the non-converters had high ADRP z-scores, which may limit its applicability as a predictor for conversion. It would be of interest to study the value of ADRP *z*-scores in tracking disease progression in MCI cohorts enriched with other biomarkers such as amyloid imaging.

Parkinsonism in Spinocerebellar Ataxia Type 3

In **Chapter 10**, we investigated the metabolic pattern in patients with spinal cerebellar ataxia type 3 (SCA3). Several brain networks are affected by the neurodegenerative process in SCA3, including the basal-ganglia-thalamocortical loop and midbrain cholinergic and pontine noradrenergic systems. Due to overlap in affected networks, interesting parallels can be drawn between SCA3, PD and MSA. For instance, parkinsonism and RBD are relatively common in SCA3, and RBD may precede gait ataxia in SCA3 by several years (Pedroso et al., 2013).

Studies have shown neuronal loss in the substantia nigra pars compacta in SCA3, which was associated with decreased dopamine transporter binding in the putamen and caudate in SCA3 patients (Rub, Brunt & Deller, 2008, Braga-Neto et al., 2012b, Yen et al., 2000) and pre-symptomatic gene carriers (Yen et al., 2002). Even in early disease stages, dopamine transporter binding was in the range of patients with PD. Although this early, severe presynaptic dopaminergic deficit is observed in most SCA3 patients, not all patients develop parkinsonism. It has been suggested that co-occurrence of neuronal loss in the motor territory of the subthalamic nucleus (STN) prevents parkinsonism in SCA3 patients (Schols et al., 2015). An alternative explanation is that cerebellar degeneration itself counteracts the motor effects of striatal denervation (Haugarvoll, Bindoff & Tzoulis, 2016).

Interestingly, the metabolic profile of SCA3 resembles the MSARP rather than the PDRP (Teune et al., 2013, Poston et al., 2012). In the PDRP, cerebellum, thalamus and putamen/pallidum are hyperactive, whereas in MSA and SCA3, these structures show decreased metabolism. These findings illustrate that the relationship between dopaminergic deficiencies, local pathology, and resulting alterations in basal ganglia firing rates and patterns are complex, and that parkinsonism, which is a feature of all three disorders, can be caused by different underlying mechanisms.

Methodological Issues

As exemplified in this thesis, spatial covariance analysis of ^{18}F-FDG PET provides a useful tool to determine disease patterns and to quantify disease activity. However, several methodological challenges exist which deserve attention.

In a recent study, we show that PDRP (raw) scores are systematically influenced by a variety of PET/CT system and reconstruction-related factors (Kogan et al., 2019). Some of these effects are non-linear, suggesting that simple linear score corrections may not provide an adequate solution. This is a substantial problem, because this implies that raw PDRP scores acquired from different centers cannot be directly compared or pooled. This impedes efforts to properly implement the PDRP and other validated disease-related patterns as robust imaging biomarkers for disease progression in multicenter and international studies. Accurate and comparable differential diagnosis among multiple subject groups is possible provided that either the PDRP used is derived using the same methods as the subject groups tested, or that appropriate calibration to a local healthy control cohort is applied (i.e. a *z*-transformation) (Tomse et al., 2017b, Tomse et al., 2018). This solution was also applied in this thesis. However, obtaining a healthy control cohort is not always feasible. Additionally, *z*-scoring to controls in different centers has the potential drawback of introducing additional factors of human variation which could influence pattern expression score comparability between centers.

Another issue pertains to the selection of principal components that together form the disease pattern. Throughout this thesis, we retained components that explained the top 50% of variance accounted for in the data. This decision is arbitrary and assumes that this amounts to reduction of the data to the components that contain the most relevant effects. A logistic regression model was subsequently used to identify the linear combination of components that best discriminated patients from controls. In this model, each component is efficiently evaluated to determine whether it discriminates between patients and controls, and whether it gives a relevant contribution to discriminatory power in combination with other candidate components. These decisions are based on pre-fixed values and hypothesis testing. Ideally, such parameters should be optimized first in a separate dataset. A further limitation is that this approach can only deal with one imaging modality, and two classes at a time.

These issues may be solved by applying more sophisticated machine-learning algorithms after principal component decomposition. Our group has indeed explored the combination of SSM PCA features with several algorithms, including decision trees, Generalized Matrix Learning Vector Quantization (GMLVQ), and Support Vector Machines (SVMs). These methods indeed seem feasible, but generally require larger datasets for optimal training and are therefore hampered by center-specific properties (Mudali et al., 2015, van Veen et al., 2018, Mudali et al., 2016). Recently,

we explored the application of deep-learning algorithms (convolutional neural networks), which may be more resilient to center-specific effects (Manzanera et al., 2019).

Conclusions and Future Perspectives

^{18}F-FDG PET imaging is relevant in the study of neurodegenerative disorders, because it provides a direct, physiologically specific signal that can be quantified. ^{18}F-FDG PET provides a proxy for synaptic dysfunction, which is a core aspect of neurodegeneration. The studies in this thesis exemplify that consistent disease-related information can be extracted from ^{18}F-FDG PET data with PCA, without making any prior assumptions. This was possible even in the early disease stages.

Clinically, there is a pressing need for ascertaining a precise diagnosis in each subject with a possible neurodegenerative disease for both prognostic and treatment purposes. ^{18}F-FDG PET is widely available and affordable in most hospitals, and in combination with SSM PCA, may provide a realistic answer to this problem. Before this method can be implemented in clinical practice, further validation and expansion of disease-related (sub)patterns is needed, as well as further validation of classification algorithms for differential diagnosis (Tang et al., 2010b, Tripathi et al., 2015). Obviously, larger longitudinal studies in iRBD and MCI are needed to corroborate claims that these patterns can predict phenoconversion. Pattern identification may be optimized by application of more advanced machine-learning algorithms. Furthermore, it is essential that we overcome center-specific properties that can affect data acquisition.

Throughout this thesis, we have shown that individual clinical syndromes consistently match their metabolic patterns and vice versa. However, these syndromes are not necessarily synonymous with specific underlying proteinopathies, as we did not study autopsy-confirmed patients for obvious reasons. The fact that neurodegenerative diseases have overlapping clinical manifestations (the outcome of neuronal dysfunction) may imply that they also have overlapping changes in metabolic connectivity, and that accurate classification with ^{18}F-FDG PET will remain difficult in some cases. It is possible that ^{18}F-FDG PET will have greater value in quantifying disease progression and treatment effects, than in actual diagnosis of the underlying proteinopathy. It would be of interest to study the cerebral metabolic consequences of specific proteinopathies, perhaps even regardless of clinical phenotype. This may become possible in the near future with the advent of tracers that target specific proteins such as α-synuclein and tau.