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CICLO XXX

COGNITIVE AND BRAIN IMAGING

CHANGES IN PARKINSONISM

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In examining disease, we gain wisdom about anatomy and physiology and biology. In examining the person with disease, we gain wisdom about life.

— Oliver Sacks

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ABBREVIATIONS LIST

ACE-R	Addenbrooke's Cognitive Examination Revised
AD	Alzheimer's Disease
ADL	Activities of Daily Living
ANOVA	One-way Analysis of Variance
ASST	Azienda Socio Sanitaria Territoriale
AUC	Area Under the Curve
Aβ	Amyloid-β
BA	Brodmann Area
CamPaIGN	Cambridgeshire Parkinson's Incidence from General Practitioner to Neurologist
CBD	Corticobasal Degeneration
CDT	Clock Drawing Test
Cx	Cortex
cp	Cerebral Peduncle
CSF	Cerebrospinal Fluid
CTO	Centro Traumatologico Ortopedico
DAED	Dopamine Agonist Equivalent Daily dose
DARTEL	Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra
DAT	Dopamine Transporter
DLB	Dementia with Lewy Bodies
DLPFC	Dorsolateral Prefrontal Cortex
DRS	Mattis Dementia Rating Scale
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSS	Digit Span Sequencing
dt_recon	Diffusion Tensor Reconnaissance (used in 'FreeSurfer dt_recon tool')
eTIV	estimated Total Intracranial Volume
FA	Fractional Anisotropy
FAB	Frontal Assessment Battery
FAST	FMRIB Automated Segmentation Tool software
Fx	Function
FLIRT	FMRIB Linear Image Registration Tool software
FMRIB	Functional Magnetic Resonance Imaging of the Brain
FNIRT	FMRIB Non-linear Image Registration Tool software
FSL	FMRIB Software Library developed by FMRIB Analysis Group, University of Oxford, United Kingdom
FSL-	FSL Smallest Univalue Segment Assimilating Nucleus
GCIs	Glia Cytoplasmic Inclusions
GLM	General Linear Model
GPi	Globus pallidus (internus)
HC	Healthy Controls

HD	Huntington's Disease
IADL	Instrumental Activities of Daily Living
[(123)I]FP-CIT	[(123)I]N-omega-fluoropropyl-2beta-carbomethoxy-3beta-{4-iodophenyl}nortropane
J	Youden's J statistic, Youden's index
IRCCS	Istituto di Ricovero e Cura a Carattere Scientifico
JLO	Judgment of Line Orientation Test
LB	Lewy Bodies
LEDD	Levodopa Equivalent Daily Dose
LN	Lewy Neuritis
LNS	Letter Number Sequencing
MCC	Middle Cingulate Cortex
MCI	Mild Cognitive Impairment
MDS	The International Parkinson and Movement Disorder Society
MDS-	MDS Unified Parkinson's Disease Rating Scales
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute and Hospital
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
mm	millimeter(s)
MSA	Multiple System Atrophy
MSA-C	MSA with predominant cerebellar symptoms
MSA-CI	MSA cognitively impaired
MSA-NC	MSA with normal cognition
MSA-P	MSA with predominant parkinsonism symptoms
N.acc	Nucleus Accumbens
NBM	Nucleus Basalis of Meynert
NC	Normal cognition
NINDS-	National Institute of Neurological Disorders and Stroke and Society for PSP
OPCA	Olivopontocerebellar atrophy
PAG	Periaqueductal gray
PAGF	PSP with progressive gait freezing
PD	Parkinson's disease
PD-CFRS	PD-Cognitive Function Rating Scale
PD-MCI	PD with mild cognitive impairment
PD-NC	PD with normal cognition
PDD	PD with dementia
PDQ-8	8-item Parkinson's Disease Questionnaire for quality of life
PET	Positron Emission Tomography
PMOD	Peripheral Module interface
PPC	Posterior parietal cortex
PPMI	Parkinson's Progression Markers Initiative

PSP	Progressive supranuclear palsy
PSP-P	PSP with parkinsonism phenotype
PSP-RS	PSP with Richardson's syndrome phenotype
R	Red Nucleus
RBD	Rapid eye movement Behavior Disorder
REM	Rapid Eye Movement
ROC	Receiver Operating Characteristic
ROCF	Rey-Osterrieth Complex Figure
SD	standard deviation
SDMT	Symbol Digit Modalities Test
SN	Substantia Nigra
SND	Striatonigral degeneration
SnPM	Statistical NonParametric Mapping
SPM	Statistical Parametric Mapping
SPECT	Single-Photon Emission Computed Tomography
STAI	State-Trait Anxiety Inventory form
STAI Y-1	STAI to assess state anxiety
STAI Y-2	STAI to assess trait anxiety
SUV	Standard Uptake Values
SUVRs	SUV Ratios
TIV	Total intracranial volume
TMT B-A	Trail Making Test part B-A
TMT-B	Trail Making Test part B
VBM	Voxel-Based Morphometry
VLa SN	Ventrolateral Substantia Nigra
VLPFC	Ventrolateral Prefrontal Cortex
VOIs	Volumes of Interest
VOSP	Visual Object and Space Perception Battery
VTA	Ventral Tegmental Area
WAIS-IV	Wechsler Adult Intelligence Scale–Fourth Edition

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SYNOPSIS

The present thesis comprises three main parts: one theoretical and two experimental.

The first part, composed of two chapters, will introduce the clinical and neuropathological features underlying parkinsonian disorders, namely in Parkinson's disease (PD) as well as in atypical parkinsonisms — multiple system atrophy (MSA) and progressive supranuclear palsy (PSP) (Chapter 1). In this regard, PD and MSA are defined as synucleinopathies due to the presence of synuclein aggregates; while PSP that is characterized by tau protein accumulations, is part of tauopathies. Further, Chapter 2 will provide an overview of the cognitive dysfunctions characterizing these disorders, as well as evidence on the biological mechanisms and structural changes underlying cognitive alterations.

The second and third parts are composed by studies I conducted during my doctoral research.

Namely, in Chapter 3, I report results of my studies on cognitive screening instruments most sensitive in detecting cognitive alterations in atypical parkinsonisms compared to PD.

In the following study, I characterized the progression of cognitive decline in these disorders (Chapter 4).

Finally, I investigated with magnetic resonance imaging the structural changes underlying cognitive alterations in PD (Chapter 5), and MSA (Chapter 6). I conclude this thesis by discussing the clinical consequences of these cognitive and imaging findings (Chapter 7).

PART I - Theoretical background

Chapter 1: Parkinsonian disorders

Parkinsonian disorders are characterized by different underlying pathologies. In PD and MSA there are synuclein aggregates respectively in dopamine neurons or in glial cells, while PSP patients present pathological aggregation of the tau-protein, resulting in neurofibrillary tangles formation (Daniel, de Bruin, & Lees, 1995; Dickson, 1999).

Clinical manifestations depend by the characteristics of protein aggregation and by the extent of disease spread to cortical and subcortical regions (Halliday, Holton, Revesz, & Dickson, 2011).

Thus, the present chapter will overview the underlying pathology of PD, MSA and PSP; and it will describe the different clinical features; and lastly review the most recent diagnostic criteria (e.g., Gelb, Oliver, & Gilman, 1999; Gilman et al., 2008; Höglinger et al., 2017).

Chapter 2: Cognitive features and their underlying mechanisms in parkinsonian disorders

Non-motor symptoms represent a crucial part of the parkinsonian disorders spectrum; and cognitive dysfunctions, including dementia, are probably the most relevant, since they affect functional independence of patients, increase caregiver burden as well as wield a considerable socioeconomic impact (Keranen et al., 2003; McCrone et al., 2011; Vossius, Larsen, Janvin, & Aarsland, 2011).

The first part of this chapter will provide an overview on cognitive dysfunctions in PD, MSA, and PSP. Moreover, the clinical criteria for the diagnosis of mild cognitive impairment and dementia in PD will be reported (Dubois et al., 2007; Emre et al., 2007; Litvan et al., 2012), while so far there are no available criteria to assess cognitive syndromes in PSP and MSA.

Lastly, the second and third parts of this chapter will review the evidence on biological mechanisms and structural changes underlying cognitive alterations in these disorders.

PART II - Studies on cognitive manifestations in parkinsonian disorders

Chapter 3: Montreal Cognitive Assessment and Mini-Mental State Examination performance in progressive supranuclear palsy, multiple system atrophy and Parkinson's disease

There is general agreement that cognitive dysfunctions are common in PD as well as in other parkinsonian disorders (Aarsland et al., 2017; Brown et al., 2010; Gerstenecker, 2017).

Brief screening cognitive scales can be adopted in routine care, to support the clinician in the diagnostic process (Marras, Troster, Kulisevsky, & Stebbins, 2014). The Mini-Mental State Examination (MMSE) is the most widely used (Folstein, Folstein, & McHugh, 1975) although MMSE is relatively insensitive in detecting cognitive deficits in parkinsonian disorders mainly because it does not investigate the fronto-executive domain (Hoops et al., 2009). Conversely, the Montreal Cognitive Assessment (MoCA), another brief cognitive screening tool widely used with PD patients (Nasreddine et al., 2005), showed high sensitivity and specificity in the assessment of cognitive dysfunctions in PD (Gill, Freshman, Blender, & Ravina, 2008; Hoops et al., 2009; Zadikoff et al., 2008), as well as also in several neurodegenerative conditions such as Alzheimer's disease, dementia with Lewy bodies (DLB) and Huntington's disease (Biundo et al., 2016b; Hoops et al., 2009; Nasreddine et al., 2005; Videnovic et al., 2010). However, MoCA has been poorly investigated in atypical parkinsonisms — especially in PSP and MSA (Kawahara et al., 2015).

Thus, this study's main aim was to determine if MoCA is more sensitive than the commonly used MMSE in detecting cognitive abnormalities in patients with probable PSP and MSA, compared to PD.

In this multicenter study across three European institutions, MMSE and MoCA were administered to 130 patients: 35 MSA, 30 PSP and 65 age, and education and sex matched-PD.

We assessed between-group differences for MMSE, MoCA, and their subitems and calculated receiver operating characteristic (ROC) curves.

Our results show that the mean MMSE is higher than the mean MoCA score in each patient group: MSA (27.7 ± 2.4 vs. 22.9 ± 3.0, $p<0.0001$), PSP (26.0 ± 2.9 vs. 18.2 ± 3.9, $p<0.0001$), and PD (27.3 ± 2.0 vs. 22.3 ± 3.5, $p<0.0001$). Furthermore, MoCA total score as well as its letter fluency subitem differentiates PSP from MSA and PD with high specificity and moderate sensitivity. Namely, a cut-off score of seven words or less per minute would support a diagnosis of PSP (PSP vs. PD: 86% specificity, 70% sensitivity; PSP vs. MSA: 71% specificity, 70% sensitivity). On the contrary, MMSE presented a ceiling

effect for most subitems, except for the 'bisecting pentagons', with PSP performing worse than MSA and PD patients.

These findings suggest that PSP and MSA, similar to PD patients, may present normal performance on MMSE, but reduced performance on MoCA. To conclude, MoCA is more sensitive than MMSE in detecting cognitive dysfunctions in atypical parkinsonisms, and together with its verbal fluency subitem can be a valuable test to support PSP diagnosis.

Chapter 4: Prospective assessment of cognitive dysfunctions in parkinsonian disorders

Clinical and research evidence suggests cognitive impairments in parkinsonian disorders are progressive. However, there are only a few longitudinal studies in the literature that investigated cognitive progression in PSP and MSA compared to PD (Dubois & Pillon, 2005; Rittman et al., 2013; Soliveri, 2000). In addition, previous studies are based on brief cognitive screening scales or on neuropsychological assessments that do not extensively investigate the full spectrum of cognitive abilities across the five cognitive domains (i.e., attention/working-memory, executive, memory, visuospatial and language).

Furthermore, even though clinical criteria for mild cognitive impairment (MCI) and dementia in PD have been formulated (Dubois et al., 2007; Litvan et al., 2012), it remains to be investigated whether similar criteria might be applied also for atypical parkinsonisms (Marras et al., 2014).

Based on these observations, the aims of the present study were to: i) assess the severity of cognitive dysfunctions in PSP and MSA patients using PD-criteria for cognitive statuses (i.e., MCI or dementia); ii) investigate the sensitivity of two widely used cognitive screening instruments, the MMSE and MoCA, in differentiating MSA, PSP and PD global cognitive profile; iii) characterize the progression of cognitive decline on the five cognitive domains and behavioral features; and to compare the 15-month follow-up profile across the parkinsonian diseases.

Our sample included 18 patients with PSP, 12 MSA; and 30 PD patients, matched for age, education and sex. They were evaluated at baseline and at a mean of 15-month follow-up.

Demographic and clinical variables were collected. From the cognitive standpoint, I selected a comprehensive neuropsychological battery specifically designed to target cognitive deficits in PD, according to Level II criteria (Dubois et al., 2007; Litvan et al., 2012; Marras et al., 2014). Thus, I applied these criteria also to MSA and PSP since there are no published criteria for atypical parkinsonisms. Statistical non-parametric analyses were used.

I found PSP patients had more severe cognitive decline compared to PD and MSA. Namely, after 15-month follow-up, we noted a marked decline in the executive and language domains in the PSP group. Baseline and follow-up evaluations agreed, showing that PSP had a worse performance than PD and MSA patients: especially, in the Stroop test, verbal fluencies (semantic and phonemic) and MoCA.

Assessing the severity of cognitive deficits, I found different percentages of cognitive status (i.e., normal cognition vs. MCI vs. dementia) among the three groups. In particular, the percentage of patients with dementia was higher in PSP compared to MSA (33% vs. no patients with dementia) even if disease duration was similar. Among MSA and PSP patients with multidomain MCI at baseline only PSP converted to dementia at follow-up. Then, although the disease duration was longer for PD patients compared with PSP, the proportion of patients who converted to dementia was lower in the PD group compared to PSP (7% vs. 16%), despite both groups having had similar baseline severity of MCI. Overall, these results suggest more rapid and severe cognitive decline in PSP while MSA patients generally have milder deficits.

MoCA showed higher sensitivity than MMSE in detecting cognitive changes, especially in PSP. But MoCA was less sensitive than MMSE in detecting cognitive decline at 15-month in PD, suggesting that MMSE is better if one wants to track cognitive changes in PD.

Neuropsychiatric features are more common in PSP than PD patients, especially apathy with accompanying low levels of anxiety and depression.

Lastly, analysis of subitems revealed that PSP patients had a 'clinically significant' worsening after 15-month in the attentive/executive subitems (Trial Making Test part B and Clock drawing). But it has been observed that some patients also improved in specific subtasks at the follow-up. This improvement could be related to their higher medication dose (although the dopaminergic treatment was not significantly different between the baseline and follow-up). However, noteworthy alterations in performance have been seen

for subitems sensitive to motor conditions (such as drawing figures and linking circles with a pen), which could affect cognitive outcome, leading to higher performance at follow-up. These limits of MoCA and MMSE scale have already been reported in PD patients (Biundo et al., 2016b; Hu et al., 2014), and maybe are more pronounced in atypical parkinsonisms.

Taken together, these findings show that PSP patients were markedly impaired in comparison to the other parkinsonian disorders (MSA and PD) and six years after first symptoms, 33 percent of patients have dementia. This severe progression is possibly associated with the distribution of tau pathology that involves also cortical structures. On the contrary, the pattern of cognitive impairment in MSA is less severe, possibly due to the predominance of subcortical pathology with cortical involvement occurring only secondary to these abnormalities.

Thus, these findings recommend using cognitive assessment to help differential diagnosis in atypical parkinsonisms, and to monitor disease progression.

PART III - Neuroimaging studies of synucleinopathies

Chapter 5: Amyloid depositions affect cognitive and motor manifestations in Parkinson's disease

Cognitive deficits, particularly executive problems, can be observed early in PD (Aarsland, Bronnick, Larsen, Tysnes, & Alves, 2009). Dysfunction of the frontostriatal dopaminergic system may influence the presence of executive and attention problems (Bruck, Aalto, Nurmi, Bergman, & Rinne, 2005), but so far, evidence from dopamine transporter (DAT) imaging are inconsistent (Delgado-Alvarado, Gago, Navalpotro-Gomez, Jimenez-Urbieta, & Rodriguez-Oroz, 2016).

In this regard, the neuropathology underlying cognitive impairment in PD is heterogeneous (Irwin, Lee, & Trojanowski, 2013; Kehagia, Barker, & Robbins, 2010) and amyloid deposit involvement with synuclein pathology remains poorly defined, particularly in the disease's early stages.

Thus, this study's aims were to investigate the interplay between amyloid depositions in frontostriatal pathways, striatal dopaminergic deficit and brain atrophy rates; and their contribution to cognitive defects (i.e., fronto-executive functions) in early-PD.

A multicenter cohort of 33 PD patients from the Parkinson's Progression Markers Initiative underwent [^{18}F]florbetaben positron emission tomography (PET) amyloid, [^{123}I]FP-CIT (see Abbreviations List) single-photon emission computed tomography (SPECT), structural magnetic resonance imaging (MRI), clinical and selective cognitive evaluations.

Our results showed that high amyloid levels were associated with reduced dopaminergic deficits in the dorsal striatum (as compared to low amyloid levels), increased brain atrophy in frontal and occipital regions and a tendency to show more frequent cognitive impairment in global cognition (as assessed by MoCA) and fronto-executive tests.

Of note, amyloid depositions in frontostriatal regions were inversely correlated with cognitive performance.

Overall, our findings suggest that early-PD patients with amyloid burden have higher brain atrophy rates and may experience more cognitive dysfunctions (i.e., executive) and motor impairment as compared to amyloid negative subjects.

In this regard, our results seem to be aligned with a recent neuropathological hypothesis that considers synaptic axonal damage and dysfunction as the PD key feature (Tagliaferro & Burke, 2016). Indeed, dopaminergic system neurons are particularly vulnerable to synuclein pathology due to their axonal characteristics — long, thin and unmyelinated. This is also confirmed by imaging studies with DAT-binding PET (Caminiti et al., 2017), suggesting that synuclein aggregations in PD can affect synaptic function, and thus signal transmission from the disease's very early stages.

Our findings suggested a possible interaction between synuclein and the coincident amyloid pathology, wherein amyloid burden may facilitate the spread of synuclein (i.e., Lewy bodies) (Toledo et al., 2016), and we speculate that this interaction can further contribute to axonal vulnerability.

Thus, consistently with this hypothesis, we conclude that possibly amyloid depositions act synergistically with synuclein pathology and affect PD clinical manifestations.

Chapter 6: Brain structural profile of multiple system atrophy patients with cognitive impairment

In contrast to other synucleinopathies (e.g., PD and DLB), presence of dementia is considered a non-supporting feature for MSA diagnosis (Gilman et al., 2008), however there is growing evidence that MSA patients can experience cognitive impairment ranging from executive dysfunctions to multiple-domain cognitive deficits, and in a few cases, also dementia (Gerstenecker, 2017).

MMSE is a commonly used global cognitive scale and recently a large multicenter study has suggested using a cutoff score below 27 to increase the MMSE sensitivity in identifying cognitive dysfunctions in MSA (Auzou et al., 2015).

Underlying mechanisms of cognitive impairment in MSA are still not understood, and in this regard evidence from MRI studies suggested a discrete cortical and subcortical contribution to explain cognitive deficits (Kim et al., 2015; Lee et al., 2016a), even though these findings were based on a relatively small number of patients at various disease stages as well as being single-center.

Thus, the aim of our multicenter study was to better characterize the anatomical changes associated with cognitive impairment in MSA and to further investigate the cortical and subcortical structural differences in comparison to a sample of healthy subjects.

We examined retrospectively 72 probable MSA patients and based on the MMSE threshold below 27, we defined 50 MSA as cognitively normal (MSA-NC) and 22 with cognitive impairment (MSA-CI). We directly compared the MSA subgroup, and further compared them to 36 healthy subjects using gray- and white-matter voxel-based morphometry and fully automated subcortical segmentation. Compared to healthy subjects, MSA patients showed widespread cortical (i.e., bilateral frontal, occipito-temporal, and parietal areas), subcortical, and white matter alterations. However, the direct comparison MSA-CI showed only focal volume reduction in the left dorsolateral prefrontal cortex compared with MSA-NC. These findings suggest only a marginal contribution of cortical pathology to cognitive deficits in MSA. Hence, we suggest that cognitive alterations are driven by focal frontostriatal degeneration that is in line with the concept of 'subcortical cognitive impairment'.

SINOSSI

La presente tesi è formata da tre parti principali: la prima teorica mentre le due seguenti sono sperimentali.

La prima parte, composta di due capitoli, introdurrà le caratteristiche cliniche e neuropatologiche sottostanti ai disturbi parkinsoniani, in particolare nella malattia di Parkinson (PD) e nei parkinsonismi atipici — atrofia multisistemica (MSA) e paralisi progressiva sopranucleare (PSP) (Capitolo 1). Nello specifico, PD ed MSA sono definite come sinucleinopatie per la presenza di aggregati di sinucleina, mentre la PSP che è caratterizzata dall'accumulo di proteina tau rientra a far parte delle tauopatie.

Invece, il Capitolo 2 fornirà una panoramica delle disfunzioni cognitive che caratterizzano questi disturbi e fornirà inoltre evidenze circa i meccanismi biologici e i cambiamenti strutturali che sono alla base delle alterazioni cognitive.

Nella seconda e la terza parte sono riportati alcuni studi che ho condotto durante il dottorato di ricerca. In particolare, nel Capitolo 3 riporto i risultati dei miei studi sugli strumenti di screening cognitivo più sensibili nel rilevare alterazioni cognitive nei parkinsonismi atipici rispetto ai pazienti con PD. Nel successivo studio invece ho investigato la progressione del declino cognitivo in questi disturbi (Capitolo 4).

Infine, ho investigato con studi di risonanza magnetica i cambiamenti strutturali che sottendono le alterazioni cognitive nel PD (Capitolo 5) e nella MSA (Capitolo 6).

Seguiranno le conclusioni generali, in cui discuto le conseguenze cliniche dei risultati ottenuti negli studi cognitivi e di imaging (Capitolo 7).

PARTE I – Background teorico

Capitolo 1: I disturbi parkinsoniani

I disturbi parkinsoniani sono caratterizzati da una diversa patologia sottostante. Nel PD ed MSA ci sono aggregati di sinucleina rispettivamente nei neuroni dopaminergici o nelle cellule gliali, mentre i pazienti con PSP presentano delle aggregazioni di proteina tau che determina la formazione di ammassi neurofibrillari (Daniel, de Bruin, & Lees, 1995; Dickson, 1999). Le manifestazioni cliniche dipendono dalle caratteristiche di aggregati

proteici e dall'entità di diffusione della malattia nelle regioni corticali e sottocorticali (Halliday, Holton, Revesz, & Dickson, 2011).

Quindi, il presente capitolo illustrerà la patologia sottostante nel PD, MSA e PSP, saranno poi descritte le diverse caratteristiche cliniche ed infine, saranno presentati i più recenti criteri diagnostici di questi disturbi (e.g., Gelb, Oliver, & Gilman, 1999; Gilman et al., 2008; Höglinger et al., 2017).

Capitolo 2: Caratteristiche cognitive e i sottostanti meccanismi nei disturbi parkinsoniani

I sintomi non-motori rappresentano una parte cruciale dello spettro dei disturbi parkinsoniani, in particolare le disfunzioni cognitive, inclusa la demenza, sono probabilmente tra i sintomi non-motori più rilevanti, in quanto influenzano l'autonomia funzionale dei pazienti, incrementano il carico di gestione del caregiver ed hanno un notevole impatto socioeconomico (Keranen et al., 2003; McCrone et al., 2011; Vossius, Larsen, Janvin, & Aarsland, 2011).

La prima parte di questo capitolo fornirà una panoramica sulle disfunzioni cognitive nel PD, MSA e PSP. Saranno inoltre riportati i criteri clinici per la diagnosi di declino cognitivo lieve e di demenza nel PD (Dubois et al., 2007; Emre et al., 2007; Litvan et al., 2012), al contrario invece non esistono al momento criteri disponibili per valutare le sindromi cognitive in PSP e MSA.

Infine, la seconda e la terza parte di questo capitolo forniranno evidenze sui meccanismi biologici e sui cambiamenti strutturali sottostanti alle alterazioni cognitive in questi disturbi.

PARTE II - Studi sulle manifestazioni cognitive nei disturbi parkinsoniani

Capitolo 3: Performance al Montreal Cognitive Assessment e Mini-Mental State Examination nella paralisi sopranucleare progresiva, atrofia multisistemica e malattia di Parkinson

Vi è un generale consenso nel riconoscere che le alterazioni cognitive siano frequenti nei PD e negli altri disturbi parkinsoniani (Aarsland et al., 2017; Brown et al., 2010; Gerstenecker, 2017).

Pertanto, nella pratica clinica possono essere adottate delle scale brevi di screening cognitivo, per supportare il clinico nel processo diagnostico (Marras, Troster, Kulisevsky, & Stebbins, 2014). Il Mini-Mental State Examination (MMSE) è la scala più utilizzata (Folstein, Folstein, & McHugh, 1975), anche se MMSE è relativamente insensibile nell'identificare rilevare disfunzioni cognitive nei disturbi parkinsoniani principalmente perché non indaga il dominio fronto-esecutivo (Hoops et al., 2009). Al contrario, il Montreal Cognitive Assessment (MoCA), un altro strumento di screening cognitivo ampiamente utilizzato nei pazienti con PD (Nasreddine et al., 2005), ha mostrato un'elevata sensibilità e specificità nell'identificazione di alterazioni cognitive nei PD (Gill, Freshman, Blender, & Ravina, 2008; Hoops et al., 2009; Zadikoff et al., 2008), come anche in altre malattie neurodegenerative come l'Alzheimer, la demenza da corpi di Lewy (DLB) e la malattia di Huntington (Biundo et al., 2016b; Hoops et al., 2009; Nasreddine et al., 2005; Videnovic et al., 2010). Tuttavia, vi sono poche evidenze sull'uso del MoCA nei parkinsonismi atipici, in particolare nella PSP ed MSA (Kawahara et al., 2015).

Pertanto, lo scopo del presente studio era di determinare se il MoCA fosse più sensibile del comunemente utilizzato MMSE nel rilevare alterazioni cognitive nei pazienti con probabile PSP e MSA, rispetto al PD.

In questo studio multicentrico, che ha coinvolto altri tre centri europei, sono state somministrate le scale MMSE e MoCA a 130 pazienti: 35 MSA, 30 PSP e 65 pazienti PD appaiati per età, scolarità e sesso.

Sono state valutate le differenze tra i gruppi per MMSE, MoCA, e i loro subitem; infine sono state calcolate le curve ROC (Receiver-Operating Characteristic).

Dai risultati emerge che la media del MMSE è superiore al punteggio medio del MoCA in ogni gruppo di pazienti: MSA (27.7 ± 2.4 vs. 22.9 ± 3.0, $p<0.0001$), PSP (26.0 ± 2.9 vs. 18.2 ± 3.9, $p<0.0001$), e PD (27.3 ± 2.0 vs. 22.3 ± 3.5, $p<0.0001$). Inoltre, il punteggio totale MoCA così come il suo subitem di fluenza fonemica è in grado di differenziare la PSP da MSA e PD con un'alta specificità e moderata sensibilità. Specificamente, un punteggio uguale o inferiore a sette parole al minuto sembra supportare una diagnosi di PSP (PSP vs PD: 86% specificità, sensibilità al 70%, PSP vs MSA: 71% specificità, sensibilità al 70%). Al contrario, nel MMSE è stato possibile osservare un 'effetto-soffitto'

per la maggior parte dei subitem, ad eccezione del subitem dei 'due pentagoni', in cui i pazienti con PSP hanno una prestazione peggiore rispetto a MSA e PD.

I nostri risultati suggeriscono che PSP ed MSA, similmente al PD, possono presentare una prestazione normale al MMSE ma deficitaria al MoCA.

In conclusione, il MoCA è più sensibile del MMSE nel rilevare disfunzioni cognitive nei parkinsonismi atipici ed insieme al suo subitem di fluenza verbale sembra essere un valido test per supportare una diagnosi di PSP.

Capitolo 4: Valutazione prospettica delle disfunzioni cognitive nei disturbi parkinsoniani

Evidenze in ambito clinico e di ricerca suggeriscono che le disfunzioni cognitive nei disturbi parkinsoniani siano progressive. Tuttavia, in letteratura vi sono pochi studi longitudinali che indagano la progressione cognitiva in pazienti con PSP ed MSA rispetto a pazienti PD (Dubois & Pillon, 2005; Rittman et al., 2013; Soliveri, 2000). In particolare, i precedenti studi si basano solo su scale globali di screening cognitivo, oppure su valutazioni neuropsicologiche parziali che non esaminano l'intero spettro delle abilità cognitive nei cinque domini (i.e., attenzione/memoria di lavoro, esecutivo, mnesico, visuospaziale e del linguaggio). Inoltre, sebbene siano stati formulati criteri clinici per la diagnosi di declino cognitivo lieve (MCI) e di demenza in pazienti PD (Dubois et al., 2007; Litvan et al., 2012), rimane ancora da investigare se tali criteri possano essere applicati anche nei parkinsonismi atipici (Marras et al., 2014).

Date tali premesse, gli obiettivi del presente studio sono stati: i) valutare la severità delle alterazioni cognitive in pazienti PSP ed MSA utilizzando i criteri validati nei pazienti PD, per identificare gli stati cognitivi (i.e., MCI o demenza); ii) esaminare la sensibilità di due strumenti di screening cognitivo ampiamente utilizzati, (i.e., MMSE e MoCA), nel differenziare il profilo cognitivo globale di pazienti MSA, PSP e PD; iii) caratterizzare la progressione del declino cognitivo nei cinque domini, il profilo comportamentale e infine confrontare il profilo cognitivo al follow-up tra i vari disturbi parkinsoniani. Il nostro campione includeva 18 pazienti con PSP, 12 MSA e 30 pazienti con PD appaiati per età, scolarità e sesso, che sono stati valutati alla baseline e al follow-up a 15 mesi. Sono stati raccolti dati demografici e clinici; inoltre dal punto di vista cognitivo è stata selezionata una

batteria di test neuropsicologici completa, specifica per l'identificazione di deficit cognitivi in pazienti PD, secondo i criteri pubblicati di 'Livello II' (Dubois et al., 2007; Litvan et al., 2012; Marras et al., 2014). Abbiamo quindi applicato tali criteri anche a pazienti MSA e PSP, dato che non esistono criteri pubblicati per i parkinsonismi atipici. Infine, sono state utilizzate analisi statistiche di tipo non-parametrico.

Dai nostri risultati emerge che i pazienti con PSP hanno un declino cognitivo più severo rispetto a pazienti PD ed MSA. Nello specifico, al follow-up è stato possibile osservare un marcato declino a carico del dominio esecutivo e del linguaggio nel gruppo con PSP. Le valutazioni cognitive alla baseline e al follow-up erano concordanti, ed entrambe confermano che i pazienti PSP hanno una prestazione peggiore rispetto ai pazienti PD ed MSA: in particolare, nello Stroop test, nelle fluenze verbali (semantica e fonematica) e nel MoCA.

Valutando la severità dei deficit cognitivi, abbiamo inoltre trovato diverse percentuali di diagnosi cognitive (i.e., profilo nella norma, MCI vs. demenza) tra i tre gruppi. In particolare, la percentuale più elevata di pazienti con demenza era nel gruppo con PSP rispetto ai pazienti MSA (i.e., 33% vs. nessun paziente con demenza), anche se la durata di malattia era simile. Inoltre, tra i pazienti MSA e PSP con un profilo MCI-multidominio alla baseline, solo pazienti con PSP passano ad una diagnosi di demenza al follow-up.

Infine nel gruppo di pazienti PD, nonostante avessero una durata di malattia più lunga, la percentuale di soggetti che passano ad una diagnosi di demenza era inferiore rispetto al gruppo con PSP (7% vs. 16%), nonostante entrambi i gruppi avessero una gravità di MCI simile alla baseline. Complessivamente questi risultati suggeriscono un più rapido e severo declino cognitivo in soggetti PSP, mentre i pazienti MSA mostrano generalmente deficit più limitati.

La scala globale MoCA sembra essere maggiormente sensibile, rispetto al MMSE, nel rilevare cambiamenti cognitivi, in particolare nella PSP. Tuttavia il MoCA mostra una sensibilità inferiore rispetto al MMSE nell'identificare un declino cognitivo al follow-up in pazienti PD; quindi il MMSE sembra essere uno strumento migliore per monitorare longitudinalmente cambiamenti cognitivi in pazienti PD.

Riguardo al profilo comportamentale, i pazienti PSP riportano più comunemente rispetto ai pazienti PD: apatia, ansia e depressione.

Infine, l'analisi dei subitem rivela che i pazienti PSP mostrano un peggioramento 'clinicamente significativo' dopo 15 mesi soprattutto nei subitem attentivo-esecutivi (Trial

Making Test parte B e il disegno di un orologio). Tuttavia è stato possibile osservare che alcuni pazienti hanno anche un miglioramento in specifici subitem al follow-up. Questo miglioramento potrebbe essere attribuibile ad una più elevata dose farmacologica (nonostante il trattamento dopaminergico alla baseline non fosse significativamente diverso al follow-up). Tuttavia, è importante notare che tali alterazioni erano presenti soprattutto in subitem sensibili alle problematiche motorie (i.e., disegno di figure e collegamento di cerchi con una penna) che quindi potrebbero aver alterato la performance. Questi limiti della scala MoCA e MMSE sono già stati osservati in precedenza nei pazienti con PD (Biundo et al., 2016b; Hu et al., 2014), e possibilmente sono ancora più pronunciati nei parkinsonismi atipici.

In conclusione i nostri risultati rivelano che i pazienti PSP hanno una performance notevolmente alterata rispetto agli altri disturbi parkinsoniani (MSA e PD), e dopo circa 6 anni di durata di malattia, il 33% dei pazienti PSP ha una diagnosi di demenza. Questa severa progressione è probabilmente associata ad una diffusione di aggregati tau che coinvolge anche strutture corticali. Al contrario, il pattern di compromissione cognitiva in pazienti con MSA è meno severo, e probabilmente è associato ad una predominanza sottocorticale della patologia, con un coinvolgimento corticale solo secondario alle alterazioni sottocorticali. Pertanto, i nostri risultati suggeriscono che la valutazione neuropsicologica può essere utile nella differenziazione dei profili cognitivi nei parkinsonismi atipici e per monitorare la progressione della malattia.

PARTE III – Studi di neuroimmagine sulle sinucleinopatie

Capitolo 5: Effetti dei depositi di amiloide sulle manifestazioni cognitive e motorie nella malattia di Parkinson

Alterazioni cognitive, in particolare deficit esecutivi, possono essere osservati anche nelle prime fasi del PD (Aarsland, Bronnick, Larsen, Tysnes & Alves, 2009). La disfunzione frontostriatale del sistema dopaminergico può influenzare la presenza di problemi esecutivi ed attentivi (Bruck, Aalto, Nurmi, Bergman, & Rinne, 2005), tuttavia al momento le evidenze relative al trasportatore striatale di dopamina (DAT) sono inconsistenti (Delgado-Alvarado, Gago, Navalpotro-Gomez, Jimenez-Urbieta, & Rodriguez-Oroz, 2016).

I meccanismi neuropatologici che stanno alla base delle alterazioni cognitive nei PD sono eterogenei (Irwin, Lee, & Trojanowski, 2013; Kehagia, Barker & Robbins, 2010), ed il contributo del deposito di amiloide in aggiunta alla sinucleinopatia rimane ancora poco definito, soprattutto nelle prime fasi della malattia.

Pertanto, lo scopo del presente studio è stato quello di indagare l'interazione tra depositi di amiloide nel circuito frontostriatale, deficit dopaminergico striatale, grado di atrofia cerebrale ed il loro contributo nelle alterazioni cognitive (i.e., funzioni fronto-esecutive) nelle prime fasi del PD.

Una coorte multicentrica di 33 pazienti con PD ricavata dal 'Parkinson's Progression Markers Initiative' è stata sottoposta a una tomografia ad emissione di positroni (PET) con radiofarmaco [^{18}F]florbetaben, tomografia ad emissione di fotone singolo (SPECT) con radiofarmaco [^{123}I]FP-CIT, risonanza magnetica (MRI) strutturale, valutazione clinica e cognitiva.

Dai nostri risultati emerge che elevati livelli di depositi di amiloide erano associati ad una riduzione del deficit dopaminergico nello striato dorsale (rispetto ai bassi livelli di depositi di amiloide), ad un aumento dell'atrofia cerebrale in regioni frontali ed occipitali, e ad una tendenza a manifestare più frequentemente alterazioni cognitive globali (come valutato dal MoCA), ed in test fronto-esecutivi.

Inoltre, le deposizioni di amiloide nelle regioni frontostriatali erano inversamente correlate alla performance cognitiva.

Nel complesso i nostri risultati suggeriscono che pazienti con PD in fase iniziale di malattia e amiloidosi hanno un più elevato grado di atrofia cerebrale e possono esperire maggiori deficit cognitivi (i.e., disfunzioni esecutive) e alterazioni motorie rispetto a soggetti negativi all'amiloide.

I nostri risultati sembrano essere in linea con una recente ipotesi neuropatologica che considera il danno e disfunzione assonale a livello sinaptico come un elemento caratteristico del PD (Tagliaferro & Burke, 2016). Infatti, i neuroni del sistema dopaminergico sono particolarmente vulnerabili alla sinucleinopatia a causa delle loro caratteristiche assonali: gli assoni sono lunghi, sottili e non mielinizzati. Questa ipotesi è confermata anche da studi di neuroimmagine PET con traccianti che si legano al DAT (Caminiti et al., 2017), suggerendo che le aggregazioni di sinucleina nel PD possono influenzare la funzione sinaptica e la trasmissione di segnale sin dalle prime fasi della malattia.

I nostri risultati suggeriscono quindi una possibile interazione tra depositi di amiloide e sinucleinopatia, in cui la presenza di amiloide può facilitare la diffusione di sinucleina (i.e., corpi di Lewy) (Toledo et al., 2016), pertanto questa interazione può contribuire ulteriormente alla vulnerabilità assonale.

In linea con questa ipotesi, i nostri risultati sembrano confermare che le deposizioni di amiloide agiscono sinergicamente con la sinucleinopatia, influenzando le manifestazioni cliniche del PD.

Capitolo 6: Profilo neurostrutturale dell'atrofia multisistemica con alterazioni cognitive

A differenza di altre sinucleinopatie (e.g., PD e DLB), la presenza di demenza è considerata un criterio di esclusione nella diagnosi di MSA (Gilman et al., 2008), tuttavia vi è una crescente evidenza che pazienti affetti da MSA possano manifestare alterazioni cognitive, che includono disfunzioni esecutive ma anche deficit cognitivi multidominio, e in alcuni casi anche demenza (Gerstenecker, 2017).

Il MMSE è una scala cognitiva globale comunemente utilizzata nella pratica clinica, e recentemente uno studio multicentrico ha suggerito l'utilizzo di un cutoff <27 per aumentare la sensibilità di tale scala nell'identificare alterazioni cognitive in pazienti MSA (Auzou et al., 2015).

I meccanismi che sottendono le disfunzioni cognitive in soggetti MSA non sono ancora stati identificati ed evidenze da studi di MRI suggeriscono un discreto contributo corticale e sottocorticale per spiegare tali alterazioni cognitive (Kim et al., 2015; Lee et al., 2016a). Tuttavia questi risultati sono basati su un numero relativamente piccolo di pazienti e in vari stadi di malattia, inoltre sono studi basati su singoli centri.

Pertanto, lo scopo del nostro studio multicentrico è stato quello caratterizzare i cambiamenti anatomici associati ad alterazioni cognitive in pazienti MSA e di investigare le differenze strutturali corticali e sottocorticali rispetto ad un campione di soggetti sani.

Abbiamo quindi esaminato retrospettivamente 72 pazienti MSA, e definito 50 MSA come cognitivamente normali (MSA-NC) e 22 con alterazioni cognitive (MSA-CI) utilizzando il cutoff del MMSE <27. Abbiamo inoltre confrontato direttamente i due sottogruppi di MSA, e comparato l'intero gruppo di MSA ad un campione di 36 controlli

sani (HC) utilizzando un'analisi di 'morfometria basata sui voxel' che analizzava la sostanza grigia e bianca. Inoltre, abbiamo applicato anche una segmentazione automatizzata dei volumi sottocorticali.

Dai nostri risultati emerge che i pazienti MSA, rispetto a soggetti sani, hanno una diffusa atrofia corticale (i.e., che coinvolge bilateralmente aree frontali, occipito-temporali e parietali), sottocorticale ed alterazioni alla sostanza bianca. Tuttavia, nel confronto diretto, i soggetti MSA-CI mostrano solo una focale riduzione del volume a carico della corteccia prefrontale dorsolaterale sinistra rispetto a pazienti MSA-NC.

Tali risultati suggeriscono che la patologia corticale abbia un effetto marginale sulle alterazioni cognitive nei pazienti MSA. Suggeriamo quindi che le alterazioni cognitive siano piuttosto determinate da una degenerazione frontostriatale focale, che sembra essere in linea con il concetto di 'alterazioni cognitive sottocorticali'.

PART I

THEORETICAL BACKGROUND

CHAPTER 1

PARKINSONIAN DISORDERS

Two hundred years ago, in 1817, James Parkinson described for the first time some patients with movement disorders who showed the so-called "shaking palsy", altered posture and sense of weakness (Parkinson, 2002). Remarkably, in *An Essay on the Shaking Palsy*, motor aspects as well as non-motor features (i.e., behavioral, sleep, and autonomic dysfunctions) were reported, and a few decades later this disease was recognized by Jean-Martin Charcot as a complex disorder, defined as Parkinson's disease (PD) (Goetz, 2011).
PD is a common and disabling disorder that affects people in a wide age range, namely with a prevalence of about 1−2 percent in the population older than 60 to 65 years, or 0.3 percent in the general population. Indeed, PD is the second most common neurodegenerative disorder after Alzheimer's disease (AD) (De Lau & Breteler, 2006).

The etiology of PD remains unknown, since it is extremely difficult to disentangle the complex interactions between genetic causes and environmental agents. Several risk factors have been identified, including intoxications or familial history; although age remains the most significant factor so far (De Lau & Breteler, 2006); and since life expectancy is growing, the disease's prevalence is expected to dramatically increase, leading to health care issues (Dorsey et al., 2007).

From the motor standpoint, PD is characterized by a clinical manifestation known as parkinsonism, indicating a combination of four features: rigidity, tremor, slowing down of movements (bradykinesia), postural and gait dysfunctions. Initially, parkinsonism was considered specific to PD, however these symptoms were observed also in other neurodegenerative disorders, defined as atypical parkinsonisms. These include multiple system atrophy (MSA), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and dementia with Lewy bodies (DLB).

However, PD is by far the most common cause of parkinsonism accounting for up to 85 percent of the cases (Figure 1.1) (Colosimo, Riley, & Wenning, 2011).

As parkinsonism may occur in several clinical conditions, it is challenging to identify and distinguish these conditions from idiopathic PD. Although specific clinical diagnostic criteria are available to differentiate PD from other forms of atypical parkinsonism (Gilman et al., 2008; Höglinger et al., 2017), so far most of the available clinical features are

characterized by a poor sensitivity, leading to a high rate of misdiagnosis (Joutsa, Gardberg, Röyttä, & Kaasinen, 2014).

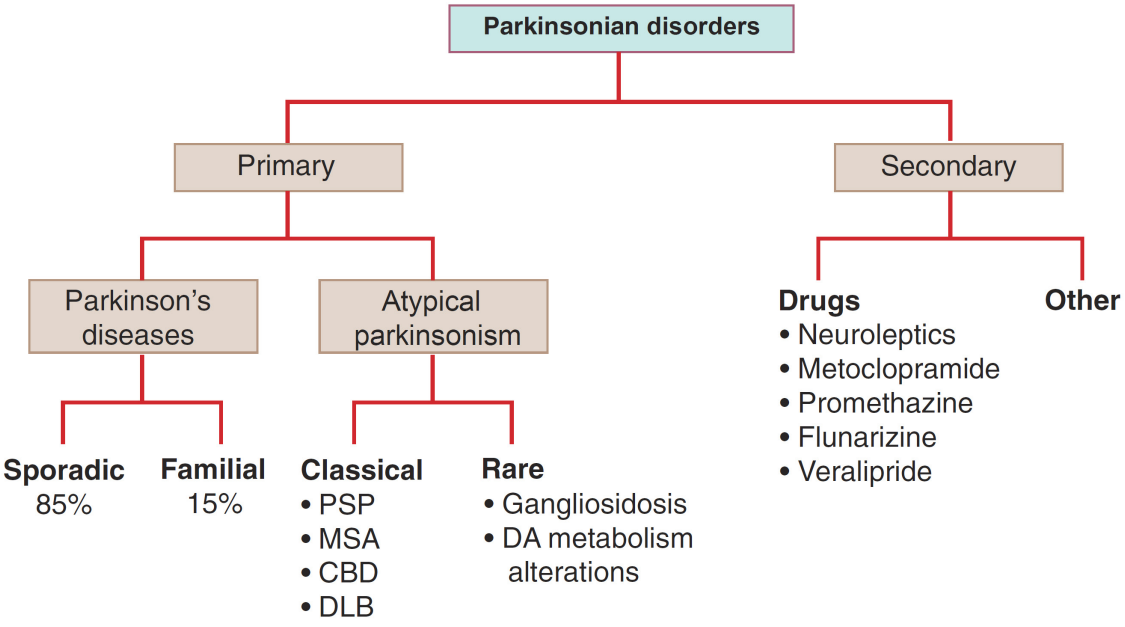


Figure 1.1 Summary of primary and secondary parkinsonian disorders. As primary and atypical parkinsonism are reported: progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal degeneration (CBD) and dementia with Lewy bodies (DLB). Modified from Schapira, Hartmann and Agid (2009).

Indeed, approximately 10 percent of patients, who were initially diagnosed as PD, ultimately are identified as atypical parkinsonisms, especially because of the significantly reduced (or absent) response to dopaminergic treatment, due to the degeneration of striatal neurons and thus the absence of postsynaptic dopaminergic receptors (Schapira, Hartmann & Agid, 2009). Making the distinction between PD and atypical parkinsonism is crucial for both clinical practice and research, as the prognosis and treatment of patients with atypical parkinsonism differ from PD, namely atypical parkinsonian disorders have a shorter survival time, more clinical complications since the early stage of the disease and in general a more severe prognosis (Litvan, 2005).

The present chapter will provide an overview of the current status of PD as well as of atypical parkinsonian disorders from a clinical and neuropathological viewpoint, in order to subsequently better understand the research work illustrated in this thesis.

1.1 CLINICAL AND NEUROPATHOLOGICAL FEATURES

An alternative classification of parkinsonian disorders is based on the underlying pathology; namely the aggregated proteins in the brain lesions, and thus it is possible to classify the disorders as synucleinopathies and tauopathies.

This classification comprises several neurodegenerative disorders, but the present thesis will focus only on PD and MSA as synucleinopathies, and on PSP as tauopathy.

1.1.1 Synucleinopathies: Parkinson's disease and multiple system atrophy

Synuclein is a 140-amino-acid protein that forms abnormal aggregates in PD, MSA and DLB.

The main pathological inclusions observed in the synucleinopathies are Lewy bodies (LB), Lewy neuritis (LN) and oligodendroglia cytoplasmic inclusions (Halliday et al., 2011; Spillantini & Goedert, 2000). Since these disorders have abnormal inclusions of synuclein, they are defined as synucleinopathies.

Of note, even though the common factor is synuclein, there are some features that can impact differently the clinical symptoms — the most important factor is the location of synuclein aggregates that will determine the clinical phenotype (Halliday et al., 2011).

Parkinson's disease

PD is characterized by the degeneration of nerve cells, in several brain regions particularly in the substantia nigra, due to filamentous inclusions in the form of LB and LN, whose major component is synuclein (Spillantini & Goedert, 2000).

Motor symptoms that characterize PD patients arise from the loss of nigrostriatal neurons that use dopamine as neurotransmitter to communicate within the striatal network.

According to the diagnostic criteria, PD diagnosis can be defined as: definite (i.e., assessed with postmortem autopsy), probable or possible. At present, only the criteria for the *probable* diagnosis will be briefly described since these are the diagnostic criteria subsequently used in the experimental studies (the detailed criteria are reported in the Appendices, page 199). For a *probable* diagnosis of PD, three of four cardinal symptoms should be observed (i.e., resting tremor, bradykinesia, rigidity and asymmetric onset) for at least three years and substantial response to levodopa therapy should be documented (Gelb et al., 1999). However, it has been demonstrated that motor symptoms are also

accompanied by non-motor symptoms, such as cognitive impairments, sleep disturbances and depression. Notably, it has been observed that non-motor problems can appear in the preclinical phase of the disease, before motor symptoms (Chaudhuri & Schapira, 2009).

The staging of LB distribution across the PD brain has been proposed by Braak and colleagues, receiving considerable attention (Braak et al., 2003; Braak, Ghebremedhin, Rub, Bratzke, & Del Tredici, 2004). This staging provides an explanation of the motor as well as non-motor symptoms based on the topographical distribution and extent of LB lesions. According to this staging LB and LN seem to start at specific sites, and subsequently follow a predictable topographical sequence (Figure 1.2).

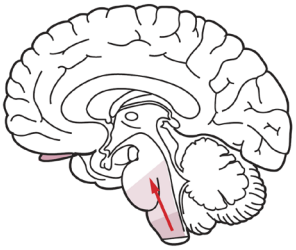
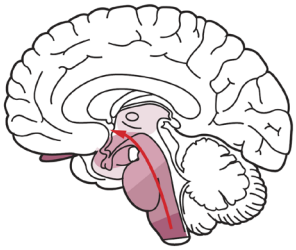
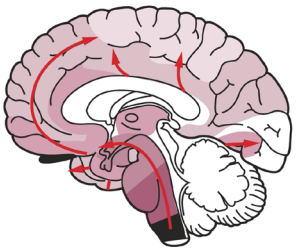
Braak stage		Lesion sites	Clinical features
1		• Mostly, medulla oblongata ◦ Dorsal IX/X motor nucleus ◦ and/or intermediate reticular zone • Frequently, anterior olfactory nucleus	• The areas involved in the preclinical stage 1 and 2 are thought to be key areas that mediate non-motor symptoms such as ◦ olfactory dysfunction (hyposmia) ◦ sleep disturbances (RBD*; restless legs syndrome) ◦ other autonomic abnormalities
2		• Caudal raphe nuclei • Gigantocellular reticular nucleus • Coeruleus-subcoeruleus complex	
3		• Midbrain ◦ Substantia nigra pars compacta ◦ Other deep nuclei of the midbrain	• Typical PD triad ◦ Tremor ◦ Rigidity ◦ Bradykinasia
4		• Forebrain ◦ Basal prosencephalon ◦ Mesocortex – Temporal mesocortex – Allocortex (neocortex is unaffected)	
5		• Neocortex ◦ High order sensory association areas of neocortex ◦ Prefrontal neocortex	• Neuropsychiatric symptoms • Cognitive impairment
6		• Neocortex ◦ First order sensory association areas ◦ Premotor areas ◦ Primary sensory areas (occasionally) ◦ Primary motor field (occasionally)	

Figure 1.2 Pathological staging scheme for Parkinson's disease according to the theory of Braak and colleagues. RBD, rapid eye movement behavior disorder. From Schapira, Hartmann and Agid (2009).

Braak stage I and II are defined as the presymptomatic, wherein the LB depositions are located mainly in the olfactory regions and anterior olfactory nucleus; and then in stage II the progression of pathology involves also the lower brainstem (dorsal motor nucleus of the vagus nerve and locus coeruleus). PD patients during these phases are characterized by mainly autonomic disturbances and non-motor symptoms (i.e., olfactory dysfunction, sleep disturbances, such as rapid eye movement (REM) sleep behavior disorder, restless legs syndrome). These symptoms usually precede the diagnosis of the disease, and can possibly occur also during the other phases of the disease.

Of note, typical PD motor symptoms (i.e., bradykinesia, rest tremor and rigidity) appear during Braak stages III and IV, implicated by the extension of synuclein pathology to the substantia nigra pars compacta, the basal forebrain and entorhinal cortex, leading to neurodegenerative processes associated with neural loss (and not only presence of LB depositions).

Finally, in Braak stages V and VI the presence of LB affects limbic regions and the neocortex (i.e., temporal, frontal and parietal). Thus in these phases PD patients can eventually experience cognitive impairments (including frank dementia), neuropsychiatric alterations (i.e., depression and apathy), and visual hallucinations, as consequence of the diffuse spread of synuclein pathology to the cerebral cortex.

This model of Braak and colleagues, is still object of debate and raised criticisms (Burke, Dauer, & Vonsattel, 2008; Jellinger, 2009), in light of the fact that neuropathological evidence did not confirm the proposed 'caudo-rostral progression pattern' of synuclein pathology in 47 percent of PD cases (Kalaitzakis, Graeber, Gentleman, & Pearce, 2008), and at autopsy patients had variable degrees of LB in several regions of the nervous system. Overall, the major criticisms are related to the fact that the Braak staging scheme is valid only to describe some of the PD clinical phenotypes.

In this regard, PD is a heterogeneous disease and can present with different clinical features, motor and non-motor manifestations, even though the clinical definition of PD is based on motor signs. The diverse clinical manifestations suggest the existence of different PD subtypes.

Indeed, a recent systematic review has identified four PD subtypes using a cluster analysis, considering motor as well as non-motor characteristics (i.e., age of onset, severity and type of motor impairments, rate of progression, and presence or absence of significant

cognitive impairment) (van Rooden et al., 2010). The four phenotypes were: early-onset, tremor dominant, postural instability and gait dominant, and old onset phenotype.

Interestingly, a model has been proposed that was able to combine the Braak PD staging with the four PD subtypes (Halliday et al., 2011), based on neuropathological evidence.

As shown in Figure 1.3, it has been demonstrated that the LB pathology distribution was similar for the 'early-onset' and 'tremor dominant' subtypes, suggesting that possibly the amount of LB depositions was different according to disease onset (i.e., early vs. late onset) (Halliday et al., 2011). Indeed, in patients with 'postural instability and gait' dominant as well as 'late onset' phenotype, there was a significantly broader distribution of cortical LB depositions with concomitant amyloid pathology compared to the other two phenotypes (e.g., tremor dominant and early onset) (Halliday et al., 2011; Selikhova et al., 2009).

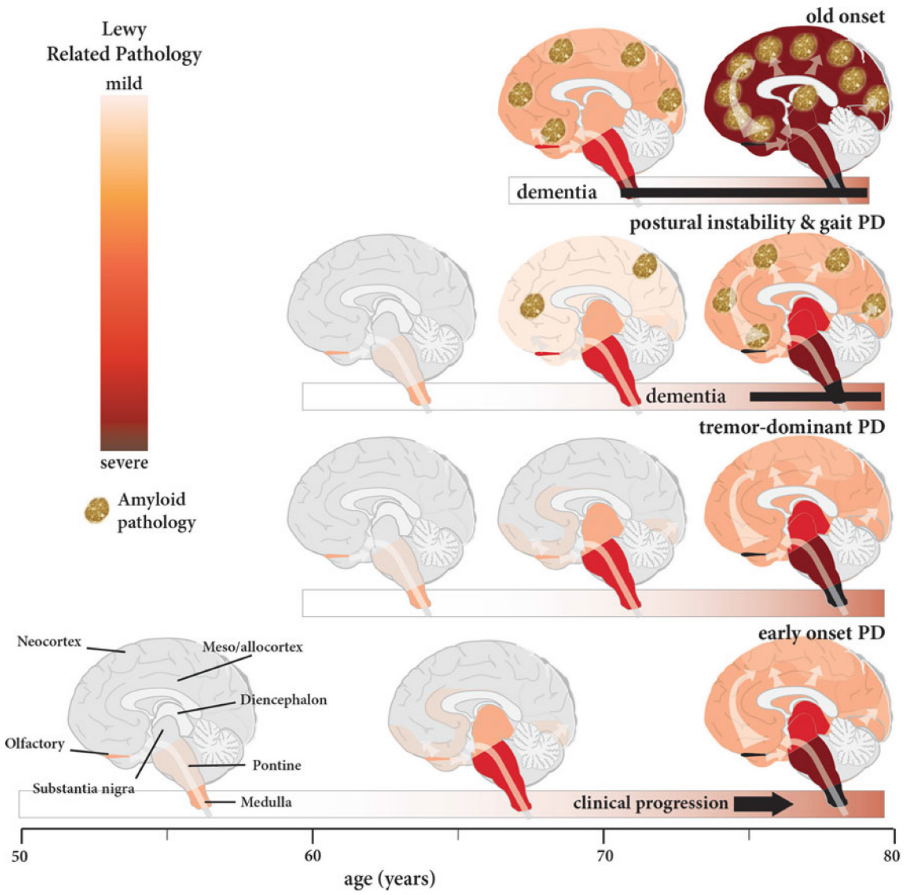


Figure 1.3 Schematic of the progression of pathology in the four main phenotypes of Parkinson's disease with Lewy body pathology (i.e., early onset PD, tremor dominant, postural instability and gait and old age onset). PD, Parkinson's disease. From Halliday et al. (2011).

The phenotype with the older onset had a higher rate of cortical LB depositions as well as amyloid pathology, suggesting that a faster rate of LB depositions is possibly associated with comorbid pathologies (i.e., amyloid-β).

This is aligned also with the recent evidence considering synaptic axonal damage and dysfunction as the key features of PD (Tagliaferro & Burke, 2016), supported by the fact that synuclein pathology affects dopaminergic neurons due to their vulnerability because of their axonal characteristics (i.e., long, thin and unmyelinated) (Braak et al., 2004). Axon damage can lead to protein accumulation, including amyloid precursor protein, which can be cleaved to form amyloid-β (Johnson, Stewart, & Smith, 2010; Stokin et al., 2005). In addition, interaction between synuclein and the coincident amyloid pathology has been suggested and this interaction can possibly facilitate the spreading of synuclein (Toledo et al., 2016).

Multiple system atrophy

MSA is a sporadic, adult onset, progressive neurodegenerative disease characterized by poorly levodopa-responsive parkinsonism, cerebellar ataxia, autonomic dysfunction and pyramidal signs (Gilman et al., 2008). Historically, three cardinal presentations were recognized — including patients with predominantly parkinsonian symptoms considered to be striatonigral degeneration (SND), patients with cerebellar symptoms considered to be sporadic olivopontocerebellar atrophy (OPCA), and patients with mainly autonomic dysfunctions considered to be Shy−Drager syndrome. However in 1969, the clinicopathological overlap of these disorders had been recognized and MSA had been proposed as an umbrella term (Graham & Oppenheimer, 1969). Subsequently, ubiquitin-positive glia cytoplasmic inclusions (GCIs) were discovered to be the common pathological hallmark of MSA, and thus MSA was defined as a member of the group of synucleinopathy (Papp, Kahn, & Lantos, 1989).

Clinical diagnostic criteria for the diagnosis of probable and possible MSA during life have been published, and include autonomic dysfunction, parkinsonism with poor response to levodopa therapy, pyramidal signs and cerebellar syndrome (Gilman et al., 2008). A definite diagnosis is provided post-mortem on a neuropathological basis, based on the presence of GCIs with concomitant neurodegenerative changes (striatonigral or olivopontocerebellar), which are the main constituents of MSA (Spillantini et al., 1998).

In the present chapter, only the criteria for the *probable* diagnosis will be briefly described since these are the diagnostic criteria subsequently used in the experimental studies (the detailed criteria are reported in the Appendices, page 199).

In the current criteria, *probable* MSA is defined as sporadic, progressive, adult-onset (> 30 years) characterized by autonomic failure of urogenital type (i.e., with erectile dysfunction in men), or of cardiovascular type (i.e., orthostatic reduction in blood pressure falls ≥30 mmHg systolic or ≥15 mmHg diastolic) in a context of a poor levodopa-responsive parkinsonism or cerebellar syndrome.

In addition, patients with predominant parkinsonism at first evaluation are defined as MSA with predominant parkinsonism symptoms (MSA-P) (with striatonigral involvement), while patients with a cerebellar syndrome are recognized as MSA-C (with olivopontocerebellar involvement). Even though it is acknowledged that with disease progression, these distinguishing features can both be present (Gilman et al., 2008). Interestingly, the distribution of the phenotypes shows some ethnic variations: namely, in Europe, 58 percent usually are MSA-P and the remaining are classified as MSA-C — on the contrary, in Japan the most frequent phenotype is represented by MSA-C, accounting for 84 percent (Geser et al., 2006; Yabe et al., 2006).

MSA, has an equal distribution in both genders, and usually its disease duration varies from seven to nine years. The mean age at onset is approximately 60 years; instead, the prevalence rate is about 4.4 per 100,000 (Schrag, Ben-Shlomo, & Quinn, 1999; Schrag, Wenning, Quinn, & Ben-Shlomo, 2008).

Due to the high variability in disease severity and in the regional distribution of the MSA pathology (i.e., regarding both neural loss and GCIs), Wenning, Seppi, Tison, and Jellinger (2002) proposed a disease staging system.

This model (specific for SND) suggested that at grade I, neural loss was restricted to the substantia nigra, then extending to the putamen (grade II), and finally affecting the caudate nucleus and globus pallidus at grade III. The present classification was able to interpret the spread of MSA pathology, but it did not take into account the OPCA (Wenning & Fanciulli, 2013).

Another classification was proposed by Halliday et al. (2011), wherein both the MSA variants (MSA-P an MSA-C) have been illustrated (Figure 1.4). It is possible to note that although patients may present with a predominant SND or OPCA degenerative pattern, then at autopsy, synuclein pathology and neuronal loss are usually widespread and are not

confined to striatonigral nor olivopontocerebellar regions respectively. Hence, usually there is an overlap of these two conditions.

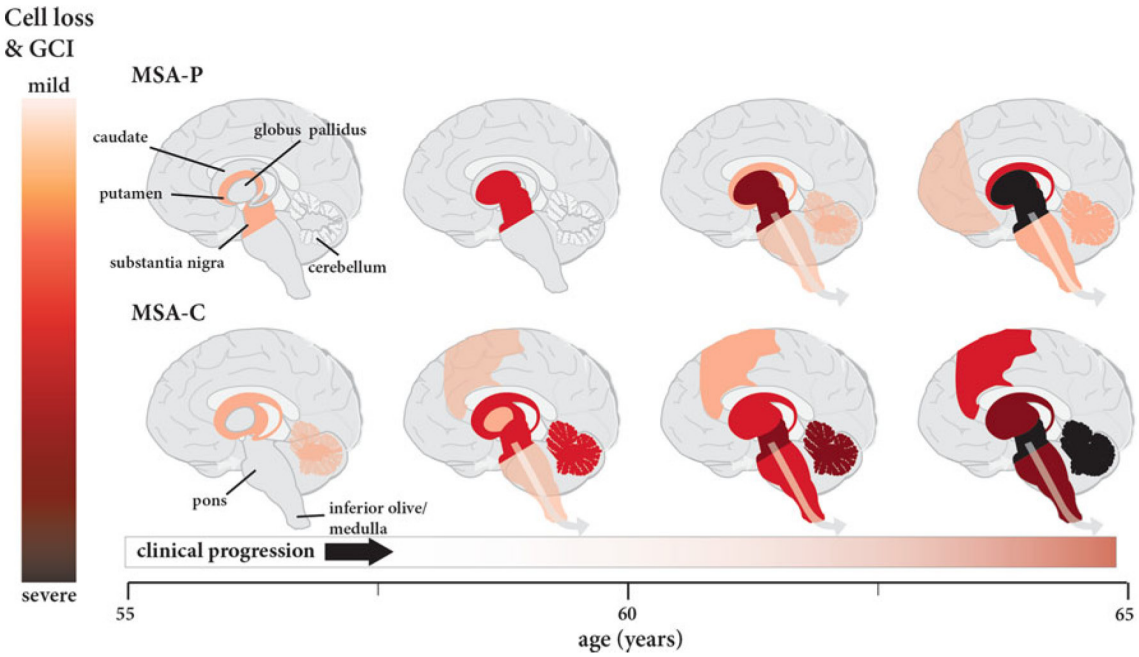


Figure 1.4 Schematic of the amount and progression of pathology in the two major clinical phenotypes of MSA with oligodendroglial cytoplasmic inclusions (GCIs). MSA-P, multiple system atrophy parkinsonian variant; MSA-C, multiple system atrophy cerebellar variant. From Halliday et al. (2011).

In agreement with this observation, a study that included 100 MSA confirmed cases from the Parkinson's UK Brain Bank showed that in the grading of neuronal loss, 34 percent was affected mostly on striatonigral regions, while 17 percent on olivopontocerebellar regions and the remaining half of the cases were equally affected in both regions (Ozawa et al., 2004).

In another neuropathological study that included 203 cases, the severity of gliosis and neurodegeneration in the substantia nigra, globus pallidus and putamen was associated with akinesia, while rigidity correlated only with neuronal loss in the putamen. On the contrary, limb and gait ataxia were more associated with Purkinje cell depletion, inferior olives and pontine nuclei (Wenning, Tison, Ben Shlomo, Daniel, & Quinn, 1997). Interestingly, patients with less changes in the putamen were more responsive to the levodopa treatment. Thus, it seems conceivable that in general a poor response to treatment in MSA is due to an extensive putaminal degeneration (Fearnley & Lees, 1990).

It is also of note that the density of GCIs and the degree of neural loss are positively associated (Ozawa et al., 2004). This suggests that GCIs have an important role in the neurodegenerative process in MSA, although this relationship was previously object of debate (Papp & Lantos, 1994).

Finally, autonomic dysfunctions — a prominent clinical feature that can also precede motor symptoms — seem to be associated with pathological changes in components of the autonomic system (e.g., parasympathetic preganglionic nuclei in Onuf's nucleus and in the inferior intermediolateral nucleus of sacral spinal cord) (Ozawa, 2007).

However, in order to develop a MSA staging scheme similar to the Braak staging proposed for PD, further studies are needed with larger cohorts to establish the earliest site of involvement to develop MSA (Wenning & Fanciulli, 2013).

1.1.2 Tauopathy: Progressive supranuclear palsy

Different neurodegenerative diseases (e.g., PSP, AD, CBD, Pick's disease) are denoted by the presence of tau protein accumulation. Tau is a phosphoprotein, which is involved in the regulation of tubulin assembly and is an intracellular accumulation. So far, six tau isoforms have been identified that can include three or four of the repetitive patterns (i.e., 3R or 4R tau) (Goedert, Wischik, Crowther, Walker, & Klug, 1988). PSP is characterized by 4R tau isoform; and this thesis will focus only on PSP among the tauopathies.

Progressive supranuclear palsy

The first detailed description of PSP came from J.C. Steel, J.C. Richardson and J. Olszewski in the 1960s, to denote 'a heterogeneous degeneration involving brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia' (Steele, 1964). In this article, they described nine cases that now are defined as PSP Richardson's syndrome (PSP-RS) phenotype. So far, many phenotypes have been described, and after PSP-RS, the second most common is PSP-parkinsonism (PSP-P), in which the disease duration is longer (Respondek et al., 2014; Williams et al., 2005).

The prevalence of PSP is approximately five per 100,000 and accounts for five percent of all parkinsonian disorders (Nath et al., 2001); PSP affects both genders with a slight predominance in males. Clinical symptoms commonly begin in the seventh decade and the median age is about 63 years. The most frequent symptom reported at the onset is postural

instability, characterized by frequent falls, and followed by cognitive impairment, bulbar and visual deficits (Nath et al., 2001). The disease can be easily recognized when all the clinical features are present — vertical supranuclear gaze palsy, history of falls, postural instability, symmetrical bradykinesia, axial rigidity, dysarthria, dysphagia and dysexecutive syndrome. Although, patients usually present with atypical symptoms, leading to difficulties in the diagnostic process.

In this regard, the International Parkinson and Movement Disorder Society (MDS) PSP study group has recently revised the diagnostic criteria for PSP (Höglinger et al., 2017), wherein the main aim was to optimize the sensitivity and specificity of PSP diagnosis. Since the previous criteria, proposed by the National Institute of Neurological Disorders and Stroke and Society for Progressive Supranuclear Palsy (NINDS-SPSP) (Litvan et al., 1996a), did not take into account all the variants of PSP syndrome and were focused only on the PSP-RS variant.

According to the diagnostic criteria, PSP diagnosis can be defined as: definite, probable or possible. Also the new diagnostic criteria require neuropathological confirmation to establish a PSP definite diagnosis, where the appropriateness of the definition is based on unique neuropathological features: namely, presence of intracellular aggregates of microtubule-associated protein tau (4R-tau) in neurofibrillary tangles, oligodendrocytic coils and astrocytic tufts (Dickson, 1999; Höglinger et al., 2017; Kovacs, 2015).

The mandatory features are: a sporadic occurrence, minimum age at onset of the first symptoms at 40 years and gradual progression of PSP-related symptoms.

In the present chapter, only the criteria for *probable* diagnosis will be briefly described since these are the diagnostic criteria subsequently used in the experimental studies (the detailed criteria are reported in the Appendices, page 199).

The core clinical features for a *probable* diagnosis require the presence of a combination of these symptoms: ocular motor dysfunction (i.e., clear limitation of voluntary gaze range especially in the vertical plane), postural instability (i.e., spontaneous loss of balance while standing and history of more than one unprovoked falls, within 3 years), akinesia (i.e., sudden and transient motor block, within 3 years) and cognitive dysfunctions (i.e., defined as speech/language disorders or frontal cognitive deficits).

Interestingly, PSP with the previous diagnostic NINDS-SPSP criteria was underdiagnosed (Respondek et al., 2017), as the criteria did not identify variants other than PSP-RS. Thus, the actual criteria included also other variants of PSP manifestation.

For instance, the 'probable' diagnostic criteria distinguish between PSP-RS, PSP-P, PSP with progressive gait freezing (PAGF) and PSP with predominant frontal presentation (Höglinger et al., 2017).

A recent study by Williams et al. (2005) has tried to correlate the heterogeneity of clinical features (in PSP-RS, PSP-P and PAGF) with the pathological variations, based on regional differences in tau load or in the type of tau lesions. Thus, the neurofibrillary tangles, coiled, tufted astrocytes bodies and thread pathology were quantified, and a grading system was established accordingly for each region (Dickson, 1999) (Figure 1.5).

The substantia nigra, subthalamic nucleus and globus pallidus were the regions most affected by tau pathology. Of note, tau severity was higher in PSP-RS compared to PSP-P and PAGF in all brain regions.

The PSP-tau score (as the sum of the coiled bodies and the thread pathology in the substantia nigra, caudate and dentate nucleus) was a valuable marker for pathological disease progression (Williams et al., 2007).

The examined cases were then grouped according to the PSP-tau score. Namely, the involvement was: with a score of 0–1, limited to the striatum and premotor cortex (Figure 1.5B); scores of 2–3, moderate in the basal ganglia, pontine nuclei and dentate nucleus and absent in parietal lobe (Figure 1.5C); scores 4–5: severe in the basal ganglia and dentate nucleus and moderate in the frontal and parietal lobes (Figure 1.5D); scores 6–7: moderately severe in the basal ganglia, pontine nuclei, parietal and frontal lobes (Figure 1.5E); score >7: severe in the subthalamic nucleus, substantia nigra, internal globus pallidus as well as neocortical areas, pontine nuclei and cerebellar structures (Figure 1.5F) (Williams et al., 2007).

Overall, PSP-RS had a significantly higher PSP-tau score compared to PSP-P cases, and no PSP-P had a score higher than 5, suggesting that PSP-P had also a topographically restricted distribution of tau-pathology.

To summarize, tau pathology was more widespread in PSP-RS variant compared to the other variant (PSP-P), and this is aligned also with the clinical symptoms and cognitive dysfunctions (Bigio, Brown, & White, 1999; Josephs et al., 2006) (see also Section 2.1.3).

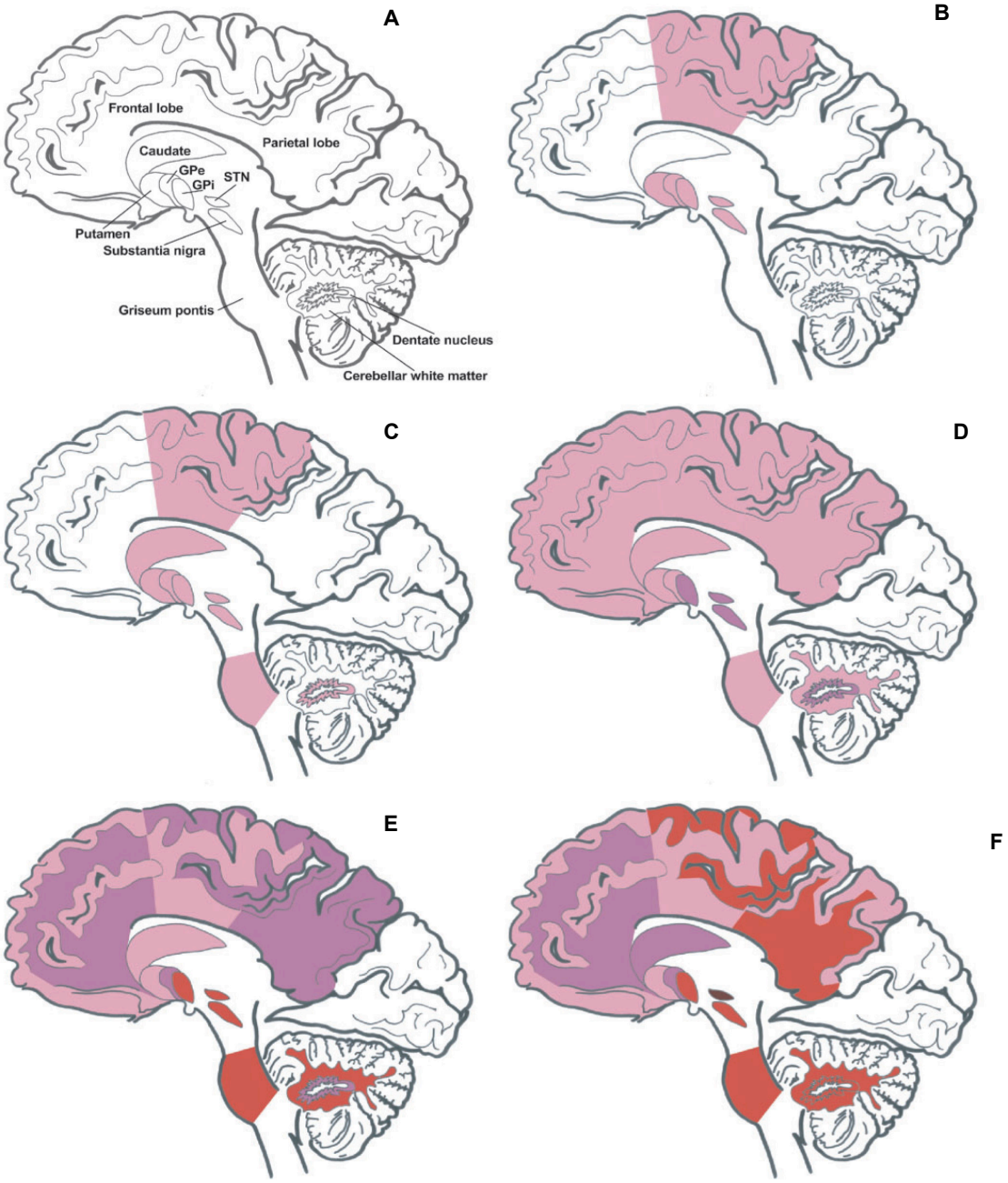


Figure 1.5 Distribution of median coiled body plus thread tau pathology, according to PSP-tau score. Color/median grade per PSP-tau score: pink/grade 1; purple/grade 2; red/grade 3; brown/grade 4. A=legend; B = PSP-tau scores 0-1; C=PSP-tau scores 2-3; D=PSP-tau scores 4-5; E=PSP-tau scores 6-7; F=PSP-tau scores >7. Modified from Williams et al. (2007).

CHAPTER 2

COGNITIVE FEATURES AND THEIR UNDERLYING MECHANISMS IN PARKINSONIAN DISORDERS

As introduced in Chapter 1, atypical parkinsonisms (MSA and PSP) are characterized by rapid disease progression, poor response to dopaminergic treatment, and the presence of features that are atypical for PD (e.g., severe autonomic dysfunctions, cerebellar or pyramidal signs, early postural instability, or dementia in the early phase of the disease). Overall, the survival time is shorter and motor complications occur in the earlier stages of the disease and with higher degree of severity than in PD patients (Colosimo et al., 2011; Litvan, 2007; Muller et al., 2000).

Recently, it has been recognized that also non-motor symptoms in movement disorders represent a crucial part of the parkinsonian disorders spectrum. Of note, non-motor symptoms include psychiatric symptoms (i.e., depression, anxiety, apathy); cognitive impairment; sleep disorders (i.e., restless leg syndrome, REM sleep behavior disorder, daytime somnolence); sensory and other symptoms (i.e., pain, fatigue) (Chaudhuri, Healy, & Schapira, 2006; Schapira et al., 2009). These symptoms, which can also appear early or even precede characteristic parkinsonian motor symptoms, are the ones that frequently trouble patients and caregivers the most, contributing significantly to quality of life (Colosimo et al., 2010; Duncan et al., 2014; Martinez-Martin, Rodriguez-Blazquez, Kurtis, Chaudhuri, & Group, 2011; Schapira et al., 2009).

Thus, the present chapter will provide an overview on the state of the art regarding cognitive dysfunctions in parkinsonian disorders (namely, in PD, MSA, and PSP). Further, evidence on the biological mechanisms and structural changes underlying cognitive alterations in these disorders will be provided.

2.1 COGNITIVE PROFILING

Among the non-motor symptoms in parkinsonian disorders, perhaps cognitive deficits are probably the most relevant, since they can potentially predict dementia, which affects significantly patients' autonomy, caregivers' burden, as well as wields a considerable socioeconomic impact (Keranen et al., 2003; McCrone et al., 2011; Vossius et al., 2011).

In addition, assessing cognitive dysfunctions could help the diagnostic process, since distinct and specific neuropsychological profiles can be useful in defining the clinical picture in the diagnostic work up (Pillon, Dubois, & Agid, 1996). In this regard, in the early stage of the disease when motor symptoms are mild and not sufficiently evident, it could be challenging to differentiate parkinsonian disorders and establish the correct diagnosis. Indeed, misdiagnoses are not infrequent (Joutsa et al., 2014).

As shown in Table 2.1, the cognitive profiles seem to be characteristic enough to differentiate cognitive impairment observed in PD from that found in PSP, or to distinguish MSA from PSP or PD with dementia (PDD). However, this is not the case when comparing MSA and PD, since their cognitive pattern is almost identical, as well as PSP versus PDD (Schapira et al., 2009).

Hence, the following paragraphs will focus on cognitive features characterizing these three parkinsonian disorders: PD, MSA and PSP, respectively.

Table 2.1 Neuropsychological profile in patients with movement disorders

	PD	**PDD**	**MSA**	**PSP**
Dementia				
Global impairment	−	+	−	+
Fluctuations	−	−	−	−
Memory				
Storage disorders	−	−	−	−
Recall disorders	+	**++**	+	**++**
Instrumental disorders				
Linguistic	−	±	−	±
Praxic	−	±	−	±
Executive disorders				
Planning	+	**++**	+	**++**
Behavior	±	+	±	**++**

Note. Abbreviations: PD, Parkinson's disease; PDD, Parkinson's disease with dementia; MSA, multiple system atrophy; PSP, progressive supranuclear palsy; − = absent; ± = mild or discussed; + = moderate or present in a proportion of patients; ++ = severe and present in a majority of patients. The double crosses underline the neuropsychological characteristics of each disease. Adapted from Pillon et al. (1996).

2.1.1 Parkinson's disease

Despite an initial controversy in 1817, about the existence of cognitive dysfunctions in PD (Parkinson, 2002), Dr. Jean-Martin Charcot, who named this disorder, conversely, underlined that in PD 'the mind becomes clouded and the memory is lost' (Charcot, 1889).

Today, cognitive impairment and dementia are well recognized as the most prevalent and invalidating non-motor symptoms in PD. Robust evidence showed that compared to age-matched healthy subjects, PD patients experience more severe cognitive changes in several domains — namely, executive, attentive, visuospatial and also memory domains (Aarsland et al., 2017).

Early PD patients are twofold more likely to develop mild cognitive impairment (MCI) than are healthy elderly (Aarsland et al., 2009; Foltynie, Brayne, Robbins, & Barker, 2004), and between the 20−57 percent of patients will experience MCI within the first five years of the disease (Janvin, Larsen, Aarsland, & Hugdahl, 2006; Williams-Gray, Foltynie, Brayne, Robbins, & Barker, 2007).

Indeed, cognitive deficits in PD are defined as a heterogeneous entity, as they vary in severity from 'subjective cognitive decline', to MCI (i.e., insufficient to affect daily functioning), and to frank dementia (Marras et al., 2014). While severe cognitive deficits are observed in the advanced stage of the disease, mild cognitive changes are more common in the early phase of PD, affecting mostly the fronto-executive functions (Schapira et al., 2009).

Regarding fronto-executive dysfunctions, usually experienced by early-PD, untreated patients or even by the prodromal phase PD (Goldman, Williams-Gray, Barker, Duda, & Galvin, 2014; Santangelo et al., 2015), these dysfunctions mirror the deficits usually observed in patients with frontal lesions — as assessed by the Tower of London test, Wisconsin Card Sorting Test, and Odd-Man-Out Reaction Time test that evaluate planning, working memory, switching and concept formation (Kehagia et al., 2010; Morris et al., 1988; Owen et al., 1992; Taylor & Saint-Cyr, 1995). In line with these findings, PD has been considered as a frontostriatal syndrome (Kehagia et al., 2010); and in agreement with this observation, the first studies on dopaminergic treatment reported beneficial drug-effects on cognition (i.e., on fronto-executive tasks) (Taylor & Saint-Cyr, 1995). Although dopaminergic treatment showed no effect on other cognitive functions (e.g., attentive set-shifting, verbal memory, associative learning and spatial recognition memory), and further

showed a detrimental effect on another group of cognitive abilities (e.g., reversal learning, decision making and gambling) (Cools, Barker, Sahakian, & Robbins, 2003; Kehagia et al., 2010). These findings explained the non-linear effect of dopaminergic treatment on cognition and are in line with the hypothesis of 'dopamine overdose' (Biundo, Weis, & Antonini, 2016a). Dopaminergic drugs, while improving motor symptoms (through dorsal striatum), can eventually overdose the ventral striatum and consequently the orbitofrontal cortex, leading to side effects on cognitive functions associated with this network (Swainson et al., 2000).

Since dopaminergic treatment restores only a proportion of PD cognitive deficits, it is evident not all-cognitive dysfunctions are dopamine-related. Indeed, neurotransmitter systems such as acetylcholine, noradrenaline and serotonin are involved in cognition, leading to a heterogeneous picture (Biundo et al., 2016a; Kehagia et al., 2010) (see Section 2.2.1 for a description).

Mild cognitive impairment and dementia diagnostic criteria

In this regard, cognitive deficits deserve attention from early disease stages, as they can possibly precede cognitive decline.

Recently, considerable interest has been shown for 'subjective cognitive complaints' — initial cognitive deficits perceived by the patient or caregiver in a context of a normal performance at the cognitive evaluation. Of note, subjective cognitive complaints have been reported in patients with PD, and evidence showed these can possibly herald further cognitive alterations (Erro et al., 2014).

The full 'spectrum of cognitive statuses' can be observed in PD, from normal cognition to PD with MCI (PD-MCI) and PDD. In recent decades, attention has focused particularly on PD-MCI, denoting a status of impaired cognition (as compared to a 'normal' age-matched sample) suggesting a transitional status within a continuum from normal cognitive functions to dementia (Figure 2.1).

Among patients with PD, approximately 25 to 30 percent are PD-MCI, and MCI syndrome is present at diagnosis in 10 to 20 percent (Svenningsson, Westman, Ballard, & Aarsland, 2012). Thus, PD-MCI seems to be associated with a shorter time (and so a higher risk) to develop dementia, even though considerable variability could be observed, and some patients can possibly revert to normal cognition (Pedersen, Larsen, Tysnes, & Alves, 2013); or remaining stable as PD-MCI or PD cognitively normal (Figure 2.1).

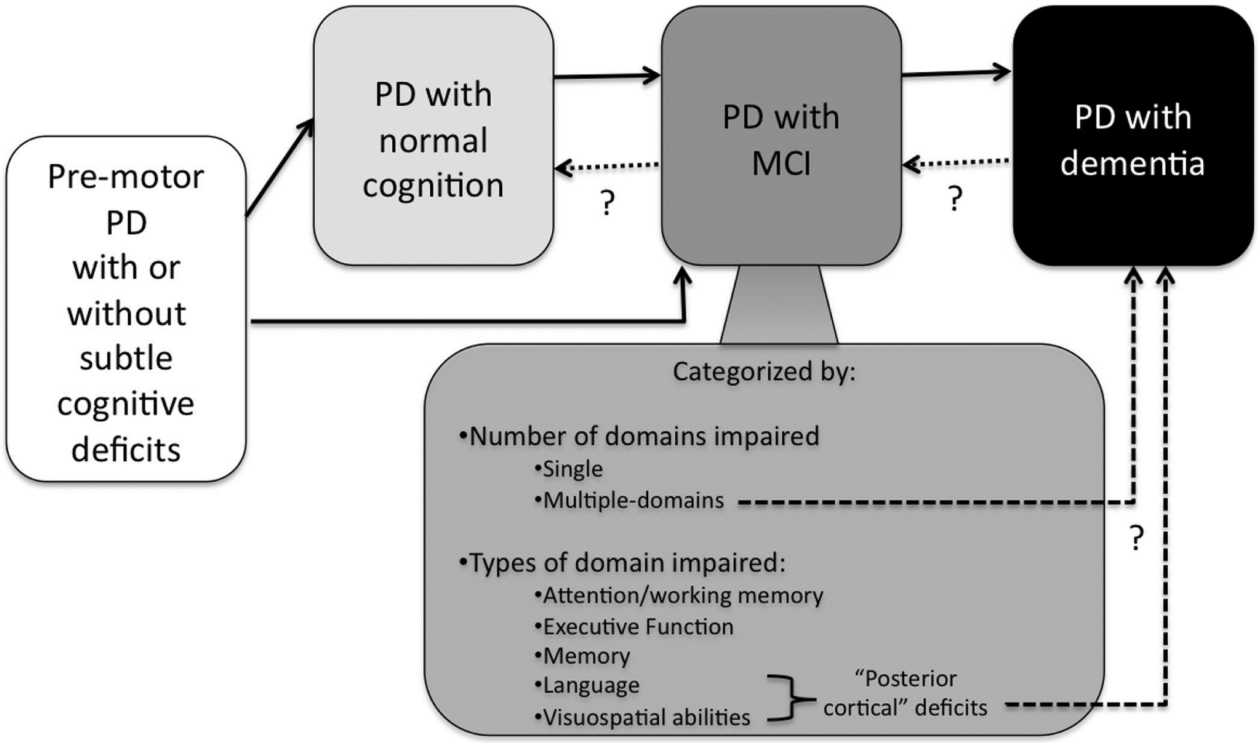


Figure 2.1 Parkinson's disease cognitive impairment spectrum. Although potential modifiers can contribute to progression, stability or reversion across the PD cognitive categories: demographic data, biological data (i.e., gene susceptibility, environmental factors, neuropathology), and clinical data (i.e., neuropsychological patterns or PD-MCI subtypes, cognitive tests performance, other neuropsychiatric features such as depression, apathy, sleepiness etc.), medical treatments (e.g., for motor, cognitive or other symptoms). MCI, mild cognitive impairment; PD, Parkinson's disease. Adapted from Goldman et al. (2014).

The concept of PD-MCI has been developed and defined in the Diagnostic and Assessment Guidelines of MDS, in order to: i) clinically characterize the earliest stage of PD cognitive impairment; ii) identify the predictors that better explain the conversion from PD-MCI to PDD; iii) determine to which extent PD-MCI influences quality of life and functional autonomy; iv) identify the more suitable patients for interventions that can possibly reduce cognitive decline; and iv) help clinicians in clinical practice and research (Litvan et al., 2012).

According to the MDS criteria, PD-MCI syndrome can be evaluated with an abbreviated assessment (Level I) or with a comprehensive neuropsychological assessment (Level II). Namely, according to:

- Level I: PD patients should be impaired on global cognitive abilities or impaired on at least two tests of a limited neuropsychological battery (i.e., if the battery included less than two tests per cognitive domain, or assessed less than five cognitive domains); and
- Level II: PD patients should be impaired on two tests within each of the five cognitive domains (i.e., attention and working memory, executive, language, memory and visuospatial) or impaired on two tests within one cognitive domain.

According to these criteria, the cutoff values for impairment are set between 1.0 and 2.0 standard deviation (SD) below the appropriate norms. Furthermore, PD-MCI can be classified in two subtypes (only by means of Level II assessment): namely, as MCI-single domain, when the deficits are within a single domain; or MCI-multiple domain, when abnormalities are based on at least one test in two or more domains (Litvan et al., 2012) (the detailed criteria are reported in the Appendices, page 199).

Several studies applied these standardized criteria, but the PD-MCI prevalence estimates remain highly variable (ranging from 20−62%) (Goldman et al., 2014). This high variability is possibly due to the different applied methodologies: indeed, there is still no consensus on which and how many cognitive tests are needed in the neuropsychological battery as well as which is the best cutoff value to define the impairment (1.0 or 1.5 or 2.0 SD) (Biundo et al., 2016a).

However, although the current criteria seem to have some weaknesses, a recent validation study supported their predictive validity and demonstrated that PD-MCI syndrome (as assessed by Level II) is highly related to the risk of turning into dementia (Hoogland et al., 2017). More specifically, the risk to develop dementia was 3.5-fold higher as compared to healthy subjects, and this effect was comparable to an age increase of 14 years and increased PD severity of 37 points at the motor part of the MDS Unified Parkinson's Disease Rating Scales (MDS-UPDRS III). This is aligned also with previous findings reporting that PD-MCI is at risk of developing dementia (Janvin et al., 2006; Pedersen et al., 2013).

Overall according to this evidence, PD-MCI syndrome seems to be not a 'static entity', but a 'transitional status', with a high risk for developing PD dementia.

Diagnostic criteria for the diagnosis of PDD have been also recommended by the MDS Task Force (Dubois et al., 2007; Emre et al., 2007), and the diagnostic procedures for the PDD diagnosis have been established, by means of two series of tests. Namely, Level I, a practical test that requires no specific expertise in neuropsychology and can be used also at bedside; and Level II that is more comprehensive and reliable (the detailed criteria are reported in the Appendices, page 199). Specifically, according to:

- Level I: patients should develop Parkinson before the onset of dementia; the Mini-Mental State Examination (MMSE) should be below 26 (Folstein et al., 1975); cognitive severity should be severe enough to impair functional autonomy (as assessed by the caregiver interview or the Pill Questionnaire); impairment should be present in at least two of the following tests:
    - Months reversed, or Seven backward (MMSE)
    - Lexical fluency, or Clock drawing
    - MMSE Pentagons
    - 3-Word recall (MMSE)

  As supporting features, at least also one behavioral symptom should be present (i.e., among hallucinations, anxious or depressed mood, excessive daytime sleepiness, apathy, and delusions).
- Level II: this assessment should follow the Level I, in order to better characterize the impaired components of PDD. Cognitive deficits include impairment in at least two of the four core cognitive domains and the presence of at least one behavioral symptom. Four areas will be investigated: global cognitive efficacy, subcortico-frontal functions (i.e., executive functions, long-term memory process and retrieval ability), instrumental cortically mediated functions (i.e., language and complex visual-functions), and neuropsychiatric features.

Objectively demonstrated cognitive deficits are the core features of these criteria, although another primary aspect to establish presence of dementia is that cognitive deficits must be severe enough to interfere and impair functional independence (i.e., personal care, social and occupational functioning), independently of the impairment related to motor or

autonomic symptoms (Emre et al., 2007). In this regard, measuring the specific impact of cognitive decline in patients with PD, minimizing the impact of the disease motor symptoms, is challenging and requires elements of clinical judgment (Aarsland et al., 2017; Marras et al., 2014).

Furthermore, the lack of valid instruments capable of measuring the specific cognitive impact on functional independence, made this evaluation even more complex (Marras et al., 2014).

Recent MDS PDD criteria suggest using the Pill Questionnaire to assess functional autonomy — however previous evidence reported its low sensitivity and specificity (Reginold et al., 2012). But now, there are two newer PD-specific instruments: the Brief Penn Daily Activities Questionnaire (Weintraub et al., 2013) and the PD-Cognitive Function Rating Scale (PD-CFRS) (Kulisevsky et al., 2013).

These tools need to be validated across countries, but can possibly be useful in identifying alterations in the instrumental activities of daily living (IADL) due to cognitive deficits.

Mild cognitive impairment and the risk of turning into PD dementia

In the recent literature, there is great interest in the identification of biomarkers for the conversion from PD-MCI to dementia. However, the association between the first cognitive symptoms and the subsequent development of dementia has not been clearly determined.

Of note, recently detailed characterization of patients with PD-MCI has led to the recognition that PD-MCI is more heterogeneous than what was previously expected, and this provides another point of confusion (Goldman et al., 2015). Namely within the 'PD-MCI classification' there are several types of MCI patients (i.e., closer to the normal cognition vs. closer to dementia syndrome), and so far the current criteria are unable to assess and operationalize this heterogeneity within the PD-MCI definition (Biundo et al., 2016a).

This clinical heterogeneity can possibly reflect also a range of different pathologies, and combining all PD-MCI together into a single entity can create confusion in the research as well as the clinical and pathophysiological field (Goldman et al., 2015) (for further detail see Section 1.1.1).

The point-prevalence of dementia in PD is approximately 30 percent (Aarsland, Zaccai, & Brayne, 2005; Emre et al., 2007), and approximately 80 percent of PD patients progressed to dementia after 15 to 20 years from the beginning of the disease (Figure 2.2) (Aarsland & Kurz, 2010; Halliday, Hely, Reid, & Morris, 2008; Hely, Reid, Adena, Halliday, & Morris, 2008).

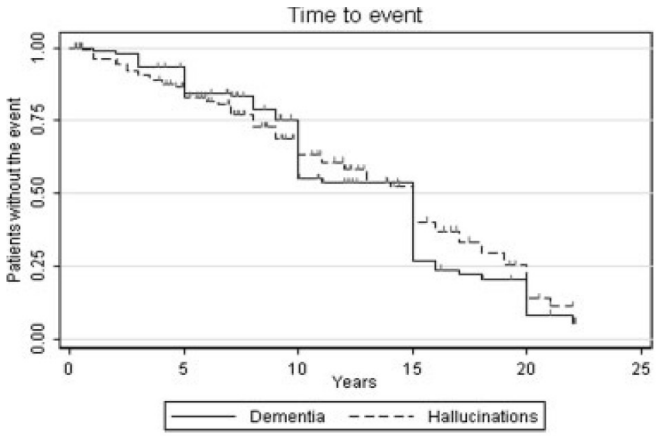


Figure 2.2 Kaplan–Meier plot of time (years of disease duration) to presence of dementia and hallucinations. From Hely et al. (2008).

The average rate of cognitive decline in PDD is about 2.3 points per year on the MMSE, similar to the decline observed in patients with AD, while in non-demented PD is about one point per year (Aarsland et al., 2004; Burn et al., 2006). Typically, patients with PD are characterized by a time period with a minimal decline, followed by an 'inflection point' after which a more severe decline is observed and interestingly the time of this inflection can vary a lot between patients (Aarsland, Muniz, & Matthews, 2011) (Figure 2.3).

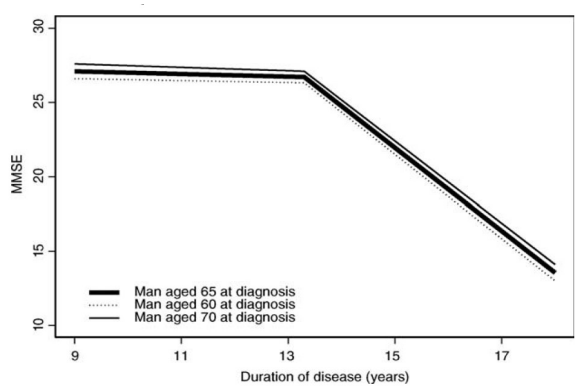


Figure 2.3 Estimated mean Mini-Mental State examination trajectory. From Aarsland et al. (2011).

Thus, defining whether a specific cognitive or motor pattern can predict the progression to dementia has been widely investigated, but this is still unclear (Troster, 2008, 2011).

In this regard, some studies demonstrate that there are some distinct types of PD-MCI with different prognostic implications.

A longitudinal study on PD motor-subtypes demonstrated that patients characterized by older-age, severe parkinsonism, as well as 'postural instability and gait difficulty' were associated with a higher risk to develop dementia, compared to the tremor-dominant type (Alves, Larsen, Emre, Wentzel-Larsen, & Aarsland, 2006). This is also aligned with the neuropathological evidence reported in the previous chapter (for further details see Section 1.1.1).

Interestingly, another study by Aarsland, Andersen, Larsen, Lolk, and Kragh-Sorensen (2003a) found an association between the presence of hallucinations and an akinetic-dominant phenotype and the further turning into dementia (at 8 years of follow-up). In this regard, another study confirmed these results, demonstrating that hallucination increased the hazard of developing dementia at 4 to 5 years follow-up (Anang et al., 2014).

With regard to the cognitive profile, clinical evidence from the CamPaIGN cohort showed two phenotypes of PD-MCI patients — the former, an executive dysfunction/frontostriatal type; while the latter, a posterior-cortical type with visuospatial (intersecting pentagon copying) and semantic naming deficits (Williams-Gray et al., 2009; Williams-Gray et al., 2013); and they found that the posterior-cortical phenotype was probably more associated with dementia. In agreement with these findings, a recent study showed that only PDD patients were impaired on executive and visuospatial tasks (Biundo et al., 2014).

Another longitudinal study (5-year follow-up) observed that older PD-MCI with deficits in episodic memory, category fluency and mental flexibility tasks were more likely to develop dementia (Domellof, Ekman, Forsgren, & Elgh, 2015).

These findings seem to be aligned with the so called 'dual syndrome hypothesis' (Kehagia, Barker, & Robbins, 2013), which identified two cognitive syndromes in PD — the 'executive syndrome' related to frontostriatal dysfunctions secondary to dopaminergic deficits, and the 'posterior syndrome' associated with cortical alterations and thus with mainly visuospatial and semantic deficits. According to this model the 'posterior syndrome' and thus cortical abnormalities can possibly herald a further progression to dementia.

Coversely, another recent study demonstrated that frontal dysfunctions were associated with the conversion from PD-MCI syndrome to PDD (Lee et al., 2014)

A further study of Besser et al. (2016), reported distinct cognitive and clinical characteristics in PD–MCI compared to MCI patients with AD, wherein PD-MCI are characterized by slower decline and different cognitive profiles; suggesting the need to use different instruments to monitor disease progression as well as for diagnostic purposes.

Taken together, these findings seem to suggest that both the 'dysexecutive' and 'posterior' syndromes can be a harbinger of PDD; but while the 'dysexecutive' syndrome is possibly a long-range predictor, the posterior cortical syndrome indicates a more imminent turning to dementia (Biundo, Weis, Fiorenzato, & Antonini, 2017).

2.1.2 Multiple system atrophy

According to a current consensus statement on the diagnosis of MSA (Gilman et al., 2008), dementia is listed among the nonsupporting features.

In the original paper of Quinn (1989), dementia has been reported as an exclusion criterion for 'practical purposes' as MSA patients usually showed a 'preservation of intellect' compared to the motor and autonomic dysfunctions. It was recognized that AD pathology could occur also in MSA, but amyloid depositions rates were not higher than in healthy subjects.

In addition, few cases showing parkinsonism and dementia, presented cortical and diffuse LB at autopsy, suggesting not a diagnosis of MSA. Thus, dementia was listed as an exclusion criterion to not avoid misdiagnoses (Quinn, 1989).

While there has been some reluctance to recognize cognitive deficits in MSA patients in previous clinical criteria, there is now abundant evidence that cognitive disorder can be early in the course of MSA (Kitayama, Wada-Isoe, Irizawa, & Nakashima, 2009; Konagaya, Sakai, Matsuoka, Konagaya, & Hashizume, 1999; Wakabayashi, Ikeuchi, Ishikawa, & Takahashi, 1998), and also cognitive disorders can precede motor symptoms (Kitayama et al., 2009).

In the literature, the debate about the existence and severity of this cognitive decline is ongoing (Gerstenecker, 2017), however listing dementia as an exclusion criterion has been demonstrated to lead to misdiagnoses — MSA patients with cognitive impairment tended to be misdiagnosed as other neurodegenerative disorders (i.e., PSP, PD and DLB) (Koga et

al., 2015; Koga et al., 2016). As reported in a study by Kitayama et al. (2009), a subgroup of MSA patients with cognitive disorders preceding motor symptoms were misdiagnosed as AD during life.

Increasing evidence suggests that the presence of cognitive dysfunctions is frequent, and this varies from 22 to 37 percent in MSA (with autoptic confirmation) (Homma, Mochizuki, Komori, & Isozaki, 2016; Wenning et al., 1997). Although so far the cognitive impairment in MSA remains poorly characterized (Stankovic et al., 2014).

Furthermore, previous studies reported that motor severity is a predictor of cognitive decline (Brown et al., 2010; Kawamura et al., 2010), while controversial findings are reported for disease duration (Balas, Balash, Giladi, & Gurevich, 2010; Chang et al., 2009; Kawamura et al., 2010).

Among patients who survived at least 8-year, the point prevalence of cognitive deficits was approximately 50 percent (Brown et al., 2010), as assessed with a score below the fifth percentile on the Mattis Dementia Rating Scale (DRS) (Mattis, 1988). While the interval between disease onset and the presence of significant cognitive deficits was about 6.5 years (O'Sullivan et al., 2008), the deficits were defined as interfering with the ability to perform tasks of daily living as described in Diagnostic and Statistical Manual of Mental Disorders (DSM) Fourth Edition criteria (APA, 1994). MSA patients with longer disease duration reported dementia after 13.5 to 17 years (Petrovic et al., 2012).

Overall, these findings seem to suggest that dementia could be observed among the MSA clinical symptoms. This is in agreement also with PD cognitive decline, wherein dementia appears at an advanced disease stage (see also Figure 2.2, in Section 2.1.1) (Hely et al., 2008).

Thus, possibly the majority of MSA patients did not experience dementia due to the short time of survival (indeed the disease duration is of approximately 7-9 years; for further details see Section 1.1.1) (Stankovic et al., 2014).

Notably, previous studies assessed the presence of dementia in MSA using PD dementia criteria (Auzou et al., 2015; Kim et al., 2013), DSM-IV criteria (Kim et al., 2013; Kitayama et al., 2009; O'Sullivan et al., 2008), cut-off values of the Clinical Dementia Rating Scale (Chang et al., 2009; Kitayama et al., 2009), DRS (Brown et al., 2010), using a threshold of MMSE $\leq$ 24 (Kitayama et al., 2009), or of MMSE below 26 (Kim et al., 2013).

On the basis of this heterogeneity on the assessment of dementia in MSA, comparing the findings of previous studies seems difficult. Dementia was defined with different

clinical criteria, and frequently also with a 'combination of criteria'. However, varying the thresholds, and the applied methods can possibly affect the frequencies as well as the clinical profiles of dementia.

This heterogeneity on the methods used is due to the fact that criteria for the diagnosis of MCI and dementia in atypical parkinsonisms have not been formulated; hence, few studies tried to apply the MDS PD criteria for the diagnosis of MCI or dementia (Emre et al., 2007; Litvan et al., 2012) (for further details see also Section 2.1.1). However, it remains unclear whether these criteria can be applied also in atypical parkinsonisms.

Interestingly, some studies also used the DSM-IV criteria for the diagnosis of dementia. These criteria specify that memory deficits must be present, but it is well recognized that MSA patients exhibited more fronto-executive deficits (Brown et al., 2010; Gerstenecker, 2017). Thus, the DSM-IV criteria for the diagnosis of dementia are probably not the most feasible to use in this population. Conversely, the latest criteria for dementia of DSM-5 (i.e., defined as major-neurocognitive disorder) did not require memory deficits (APA, 2012), but still it remains unclear whether it is better to apply the MDS PD criteria or the DSM-5 in atypical parkinsonisms.

Another important issue is that to assess the presence of dementia, the cognitive deficits should 'significantly' affect functional performance. However, the assessment of dementia in MSA is challenging as activities of daily living (ADL) and IADL are usually impaired due to motor dysfunctions from the disease's first stages, and isolating the cognitive component of already impaired functional tasks requires elements of clinical judgment.

Hence, if clinicians do not consider properly the motor impact of the disease on functional independence, there could be an overestimation of the extent to which cognitive dysfunctions are contributing to functional tasks (Feldman et al., 2001; Marras et al., 2014).

Cognitive impairment in MSA covers a wide spectrum, and even though the executive dysfunctions are prominent, also alterations in the other cognitive domains have been observed (Gerstenecker, 2017; Stankovic et al., 2014)

Regarding fronto-executive dysfunctions, up to 69 percent of MSA showed executive deficits (Auzou et al., 2015; Brown et al., 2010; Koga et al., 2016; Lyoo et al., 2008; Siri et al., 2013). Namely, over 40 percent had an impaired performance on verbal fluency tasks (phonemic and category fluencies) (Dujardin, Defebvre, Krystkowiak, Degreef, & Destee, 2003; Kawai et al., 2008; Soliveri, 2000). Interestingly, category fluency was more impaired

than the phonemic fluency in some cases (Bak, Crawford, Hearn, Mathuranath, & Hodges, 2005a; Kao et al., 2009).

Further, also impairment in response inhibition tasks (as assessed by Stroop tests) (Meco, Gasparini, & Doricchi, 1996), mental flexibility, planning and problem solving were observed (Dujardin et al., 2003; Kao et al., 2009; Robbins et al., 1994). When comparing the performance of MSA-P versus MSA-C, the latter (MSA-C) were slower than the former (Chang et al., 2009).

Also, deficits on the attentive/working-memory domain were observed, as assessed by Trail Making Test part B (TMT-B) (Chang et al., 2009; Kao et al., 2009). Instead, regarding working-memory tasks, MSA patients showed a similar performance to the other parkinsonisms (Kao et al., 2009; Stankovic et al., 2014), but another study demonstrated that MSA patients were able to apply some effective strategies in working-memory tasks (Gerstenecker, 2017; Robbins et al., 1994).

In addition, also memory disorders were reported, namely up to 16 percent of MSA patients showed an altered performance on learning and long-term verbal memory task (Siri et al., 2013), also delayed and immediate recall (Brown et al., 2010; Kim et al., 2013; Lyoo et al., 2008). In the direct comparison of the two subtypes, MSA-C showed a worse performance than MSA-P on immediate as well as delayed recall (Balas et al., 2010; Chang et al., 2009).

Regarding visuospatial abilities, there is inconsistent evidence in the literature, since some studies reported that visuospatial abilities were preserved in MSA (Bak, Caine, Hearn, & Hodges, 2006; Brown et al., 2010; Burk, Daum, & Rub, 2006); while a worse performance compared to healthy controls in visuospatial and visuo-constructive was also observed (Kawai et al., 2008; Kim et al., 2013; Lyoo et al., 2008).

Language skills seem to be not consistently impaired in MSA (Gerstenecker, 2017; Kao et al., 2009), with the exclusion of verbal fluencies that are listed among the executive tasks in the majority of the studies. However, Kim et al. (2013) reported an altered performance in naming tasks in MSA with dementia compared with MSA without cognitive defects.

Furthermore, studies investigating differences of cognitive performance in the MSA subtypes found controversial results. Evidence reported that MSA-P and MSA-C were compromised to a similar extent in executive and memory tasks (Burk et al., 2006; Siri et al., 2013); while Chang et al. (2009), examining the same cognitive domains, reported a worse performance in MSA-C patients. Another study reported that MSA-P patients were

cognitively more impaired than MSA-C, specifically the former reported multidomain deficits and the latter only visuospatial dysfunctions (Kawai et al., 2008).

Recently, Koga and others (2016) observed that cognitive impairment was more evident on the comprehensive neuropsychological assessment rather than on the brief screening cognitive tests; thus the authors suggest to assess cognition extensively if MSA patients reported subjective complaints on cognition (Koga et al., 2016).

Hence, if comprehensive assessments will be used in future studies, probably these will be useful to better define the MSA cognitive profile, as in the literature there are still inconsistent findings.

Overall, executive dysfunction, followed by memory, attention and visuospatial deficits are observed in the cognitive profile of MSA with cognitive alterations (Wenning & Fanciulli, 2013); although according to the controversial results, no specific deficits were observed in the two MSA variants (i.e., MSA-P and MSA-C).

Notably, the cognitive pattern of MSA patients seems to overlap the PD profile, characterized by mild impairment especially in executive functions as expression of the subcortical degeneration (Pillon et al., 1996; Pillon et al., 1995), resulting in a more severe profile only with the progression of the disease. In support of this observation, so far there is no neuropsychological testing able to differentiate the two synucleinopathies, while considerable differences have been observed compared to PSP (Pillon et al., 1995; Stankovic et al., 2014).

2.1.3 Progressive supranuclear palsy

Severe cognitive dysfunctions were reported in the first cases of PSP (Steele, 1964); ten years later, Albert, Feldman, and Willis (1974) described the cognitive and behavioral changes of seven patients with PSP and defined this specific pattern as 'subcortical dementia'. Of note, this subcortical pattern was different from the one observed in patients with AD, while the observed deficits were more similar to those of patients with frontal lobe damage (Albert, 2005; Bak et al., 2005a).

'Subcortical dementia' is a syndrome characterized primarily by slowness of thought (bradyphrenia), executive dysfunctions, impaired memory retrieval and behavioral changes (i.e., apathy, irritability); in the absence of aphasia, agnosia, and apraxia.

Thus, clinically different from 'cortical dementia' syndrome characterized by memory, language, perception and praxis disorders (Albert, 2005; Cummings, 1986).

Cognitive and behavioral changes occur in the early stages of PSP, and bradyphrenia has been observed in 52 percent of patients in the first year of the disease (Dubois & Pillon, 2005). Furthermore, approximately 70 percent will progress to dementia during the disease's course (Daniel, de Bruin, & Lees, 1995; Pillon, Dubois, Ploska, & Agid, 1991). Evidence showed that cognitive dysfunctions rapidly progressed, namely in a sample of 24 PSP, where 38 percent reported cognitive deficit at their first evaluation, while after 15-month, this percentage significantly increased to 70 percent (Dubois & Pillon, 2005).

It is worth noting, as previously mentioned for the MSA population that criteria for the diagnosis of MCI and dementia in atypical parkinsonisms have not been formulated (for further details see also Section 2.1.2).

Previous studies on PSP defined dementia profiles with different clinical criteria: namely, using the DSM-IV (APA, 1994), as well as trying to apply the MDS PD criteria for the diagnosis of dementia (Emre et al., 2007) (for further details see also Section 2.1.1). Varying the methods can possibly affect the frequencies as well as the clinical profiles of dementia in PSP. Hence, there also is a strong need to define specific criteria for the diagnosis of dementia and MCI for PSP patients.

Executive dysfunctions are the most common cognitive defect in PSP (Gerstenecker et al., 2013; Lee, Williams, & Anderson, 2016b; O'Keeffe et al., 2007) and are more severe than those observed in the other parkinsonian disorders (Dubois & Pillon, 2005).

Bradyphrenia appears evident in PSP, who slowly answer questions as well as need more time to solve simple problems; this is also confirmed from the neuropsychological assessment, wherein PSP had an impaired performance on the Tower of London test (planning and problem-solving task) (Robbins et al., 1994). PSP patients also performed poorly in other executive tasks (i.e., Wisconsin Card Sorting Test that requires shifting and concept formation abilities), where a tendency to perseverate was observed (Pillon et al., 1991).

Cognitive slowing is not related to motor dysfunctions, but is a 'genuine' slowing of cognitive processes also observed in reaction time tasks (Dubois & Pillon, 2005; Dubois, Pillon, Legault, Agid, & Lhermitte, 1988); indeed, bradyphrenia can possibly also contribute to poor performance on verbal fluency tasks, which are more severely impaired in PSP compared to other parkinsonian disorders (PD and MSA).

This deficit has been reported in phonemic and category fluencies (Grafman, Litvan, & Stark, 1995; Soliveri, 2000).

However, it is worth noting that especially phonemic fluency is severely impaired in PSP (Bensimon et al., 2009; Brown et al., 2010; Burrell, Hodges, & Rowe, 2014; O'Keeffe et al., 2007; Pillon et al., 1991).

Interestingly, Donker Kaat et al. (2007) reported that 50 percent of PSP named less than three words per minute beginning with a given letter, and 80 percent less than five, and 85 percent less than nine words.

In addition, a recent work demonstrated that phonemic fluency was able to differentiate PSP from PD patients with high specificity and sensitivity (0.85 and 0.83, respectively), at early PSP stage (3-year of disease duration). Seven or less words per minute suggests a diagnosis of PSP instead of PD and MSA; while the differentiation was slightly poorer when combining category and phonemic fluency (i.e., combined fluency) (Figure 2.4) (Rittman et al., 2013).

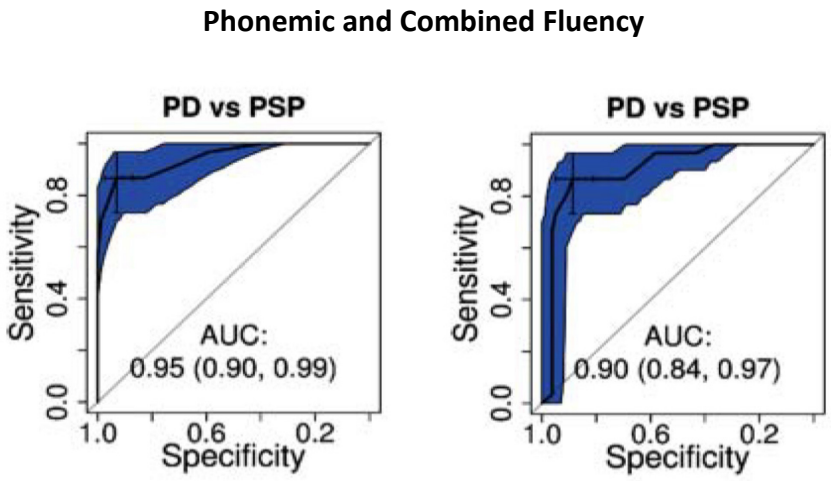


Figure 2.4 Verbal fluency is very sensitive in differentiating PSP from PD. On the left side, phonemic fluency and on the right side, combined fluency (phonemic and category fluency). Here, receiver operating characteristics curves are reported distinguishing between PSP versus PD in verbal fluencies (both subscores of ACE-R). Thresholds were chosen by means of a 'top left corner' algorithm. Confidence intervals are shown for sensitivity and specificity in parenthesis. ACE-R, Addenbrooke's Cognitive Examination Revised; AUC, area under the curve; PD, Parkinson's disease; PSP, progressive supranuclear palsy. Adapted from Rittman et al. (2013).

Fluency deficits are not entirely associated with motor deficits, since motor involvement is quite minimal (Rittman et al., 2013). Possibly this is more associated with bradyphrenia that characterized PSP patients; namely the impaired performance is related to difficulties

in words recalled rather than motor deficits. In support of this observation, Rittman et al. (2013) noted PSP patients tend to generate a small number of 'low frequency words' (e.g., 'perambulator'), instead of 'high frequency words' (i.e., 'people', 'phone', etc.).

These findings suggest that easy and brief tests such as phonemic and category fluency can also be used in clinical practice with a diagnostic purpose.

Of note, several brief cognitive scales include verbal fluency: such as the Montreal Cognitive Assessment (MoCA)(Nasreddine et al., 2005), the Frontal Assessment Battery (FAB) (Dubois, Slachevsky, Litvan, & Pillon, 2000), the Addenbrooke's Cognitive Examination-Revised (ACE-R) (Mioshi, Dawson, Mitchell, Arnold, & Hodges, 2006), suggesting that these scales are valuable tools to briefly assess cognitive functioning of PSP patients (Burrell et al., 2014). Conversely, MMSE seem to be very insensitive to PSP's most impaired domains (i.e., executive and attentive) (Bak et al., 2005b; Bensimon et al., 2009; Lagarde et al., 2013).

Regarding memory domain, one third of patients reported memory deficits — in long and short-term memory (Pillon et al., 1994; Robbins et al., 1994). Notably, it was observed that performance on memory retrieval tasks improved significantly when facilitations were presented (i.e., cueing or recognition) (Dubois & Pillon, 2005), suggesting memory deficits in PSP are not a 'poor amnesia', but the retrieval processes are possibly the most impaired (Pillon et al., 1994). Further, this would be aligned with the 'subcortical' memory profile, wherein retrieval deficits are frequently observed (Cummings, 1986).

However, findings on memory domain are still controversial, since Aarsland et al. (2003b) found that PSP patients had no deficits in the memory subtest of the DRS (Mattis, 1988). But these results are based on a subtest of a brief cognitive scale, thus possibly they are less reliable than the more complex memory measures used in previous studies (Pillon et al., 1994; Robbins et al., 1994).

With regard to attention and working-memory domain, patients with PSP performed poorly on TMT-B as well as on other measures of attentional set-shifting (Grafman et al., 1995; Paviour et al., 2005; Robbins et al., 1994).

In addition, at 21-month follow-up, PSP patients showed a significant decline in attentive tasks (Soliveri, 2000).

Evidence on visuospatial domain is also still uncertain, as PSP patients performed worse than MSA and PSP at Benton's Judgment of Line Orientation Test (JLO) (Soliveri, 2000),

while showing no impairment in other visuospatial tasks of the Visual Object and Space Perception Battery (VOSP) (Bak et al., 2006).

Also language deficits were observed in PSP patients (in naming tasks), however further studies need to verify this finding (Cotelli et al., 2006).

Other features worth mentioning are the behavioral aspects that can be strictly connected with neuropsychological assessment as well as interfere with it (Bak, Crawford, Berrios, & Hodges, 2010; Brown et al., 2010).

In this regard, PSP patients usually exhibit profound apathy that is among the supporting features of the PSP diagnosis. Apathy is observed in PSP almost as a rule — in up to 91 percent of cases (Litvan, Mega, Cummings, & Fairbanks, 1996b) and often it is one of the earliest symptoms (Burrell et al., 2014). Along with apathy severity, PSP patients usually develop disinhibited behaviors, impulsivity and show poor judgment (Litvan et al., 1996b). This can be easily evaluated by means of the 'clapping sign': namely, when asked to clap their hands three times consecutively, usually PSP patients tend to clap more (i.e., four or five times) (Dubois & Pillon, 2005).

Overall, this neuropsychological evidence was observed in the PSP-RS variant, suggesting that cognitive changes are more frequent in PSP-RS rather than PSP-P phenotype, in which fewer cognitive defects have been observed (Burrell et al., 2014).

Tröster and Browner (2013) have proposed some guidelines and modifications that should be applied to the standard administration of the neuropsychological evaluation. Indeed, PSP patients are characterized by more severe motor dysfunctions than PD patients (i.e., vertical gaze palsy) that can hamper the cognitive evaluation. Thus, to not overestimate the cognitive deficits due to motor symptoms, some modifications should be applied to the standard administrations. For instance, it has been recommended to present the tests at about 45 cm from a patient's face, due to downward gaze palsy impairments (Marras et al., 2014).

To summarize, PSP striatocortical dysfunctions are so severe, they frequently lead to considerable executive dysfunctions (i.e., planning, monitoring) and recall deficits, followed by attention and visuospatial disorders and finally evolving toward dementia (Pillon et al., 1996).

Diffuse cortical changes probably secondary to subcortical alterations can produce composite pictures. Further, other factors (i.e., age at onset and disease duration) can possibly alter the neuropsychological profile at different time-point.

However, specific neuropsychological batteries, tailored for PSP patients and atypical parkinsonisms, can be a useful method not only for diagnosis work up, but also to better understand the underlying pathology and neuronal pathways affected by the disease (Dubois & Pillon, 2005).

2.2 BIOLOGICAL MECHANISMS UNDERLYING COGNITIVE DEFICITS

A large body of evidence recognized the heterogeneity of cognitive impairment in PD as well as in atypical parkinsonisms (for reviews see Gerstenecker, 2017 and Kehagia et al., 2010; Pillon et al., 1996); and recently several studies aimed to identify the widespread pathological changes in the brain underpinning cognitive dysfunctions (Irwin et al., 2013; Kehagia et al., 2010).

In this regard, understanding the nature of cognitive alterations and the interplay between cognitive, neurochemical and pathological entities, associated with cognitive deficits in parkinsonian disorders, is one of the major challenges. This will be crucial also for practical implications, such as advancing new treatments, as well as for prognostic purposes. Advances have been made in disentangling the neurochemical and neuropathological substrate of cognitive deficits in PD, and to a lesser extent, also in atypical parkinsonisms (MSA and PSP).

Thus, the following paragraphs will provide a brief overview about the diverse biological mechanisms (i.e., neuropathological and neurochemical) underlying cognitive impairments in parkinsonian disorders.

2.2.1 Parkinson's disease

As reported in the previous Section (2.1.1), cognitive alterations, particularly in the form of executive dysfunctions, are common in early-stage PD — although there is not a universal or uniform profile, and variable risks as well as progression rates to dementia are observed (Kehagia et al., 2010). The cognitive profile of patients who eventually develop dementia can differ from the typical dysexecutive profile seen in early-PD. Specifically, the roles of visuospatial and language deficits have been emphasized in those patients at a higher risk to develop dementia (Williams-Gray et al., 2007).

This heterogeneous constellation of cognitive deficits (caused by PD) can be explained by a combination of biological mechanisms (Kehagia et al., 2013), such as uneven striatal dopaminergic loss (Lewis & Barker, 2009; Sawamoto et al., 2008), or by the deficits of other neurotransmitter systems (i.e., acetylcholine and norepinephrine) (Halliday, Leverenz, Schneider, & Adler, 2014; Kehagia et al., 2010) as well as neurodegenerative pathologies (i.e., cortical LB and other non-parkinsonian features as a consequence of aging) (Kempster, O'Sullivan, Holton, Revesz, & Lees, 2010). Together, these heterogeneous processes that include neural and multiple neurochemical alterations can possibly interact, leading to the complex cognitive picture that characterizes PD patients, from the disease's early stage.

Hence, this Section will briefly introduce the neurobiological basis underlying cognitive impairment in PD patients: first, the neurochemical changes will be presented, and subsequently the neuropathological degenerative processes.

Neurotransmitter systems underlying cognitive impairment

PD is characterized by various neurotransmitter dysfunctions that can possibly contribute to explain its cognitive impairment. Among the neurotransmitter systems that have been emphasized are the importance of the dopaminergic dysfunctions (that underlines the diagnosis of this disorder), and the noradrenergic and the cholinergic dysfunctions (Halliday et al., 2014). Also serotonergic dysfunctions can possibly be involved in cognitive processes, but these will not be described in the present Section, since evidence is still limited (Kerenyi et al., 2003; Švob Štrac, Pivac, & Mück-Šeler, 2016).

Interestingly, Kehagia and others (2010) proposed a 'model' able to describe the PD neuropsychological profile and its underlying neurochemical mechanisms. According to this model, executive dysfunctions characterizing the neuropsychological profile of PD-MCI in the early stage of the disease are associated mainly to frontostriatal *dopaminergic* dysfunctions (depicted as the blue pathway in Figure 2.5).

Further, although it remains to be verified, *noradrenergic* dysfunctions possibly can be related with attentional set-shifting impairment that forms part of the PD dysexecutive syndrome (depicted as the green pathway in Figure 2.5). Finally, also some frontal *cholinergic* dysfunctions can be observed in the early stage of the disease that possibly compromise PD cognition (depicted as the red pathway in Figure 2.5). However, cholinergic abnormalities seem to have a crucial role in the progression to PDD. Indeed, even though

diffuse cortical degeneration has been observed in PDD patients, the presence of specific deficits in the visuospatial and memory domains suggests a cholinergic involvement. In support of this observation, it has been demonstrated that cholinesterase inhibitors improve cognitive performance in PDD (Rolinski, Fox, Maidment, & McShane, 2012) — by contrast anticholinergic treatments accelerate the onset of cognitive alterations (Ehrt, Broich, Larsen, Ballard, & Aarsland, 2010). Of note, as shown in Figure 2.5, neuropsychological deficits in PDD include dysexecutive alterations (associated with dopaminergic and noradrenergic changes) as well as marked acetylcholine-based visuospatial and memory deficits.

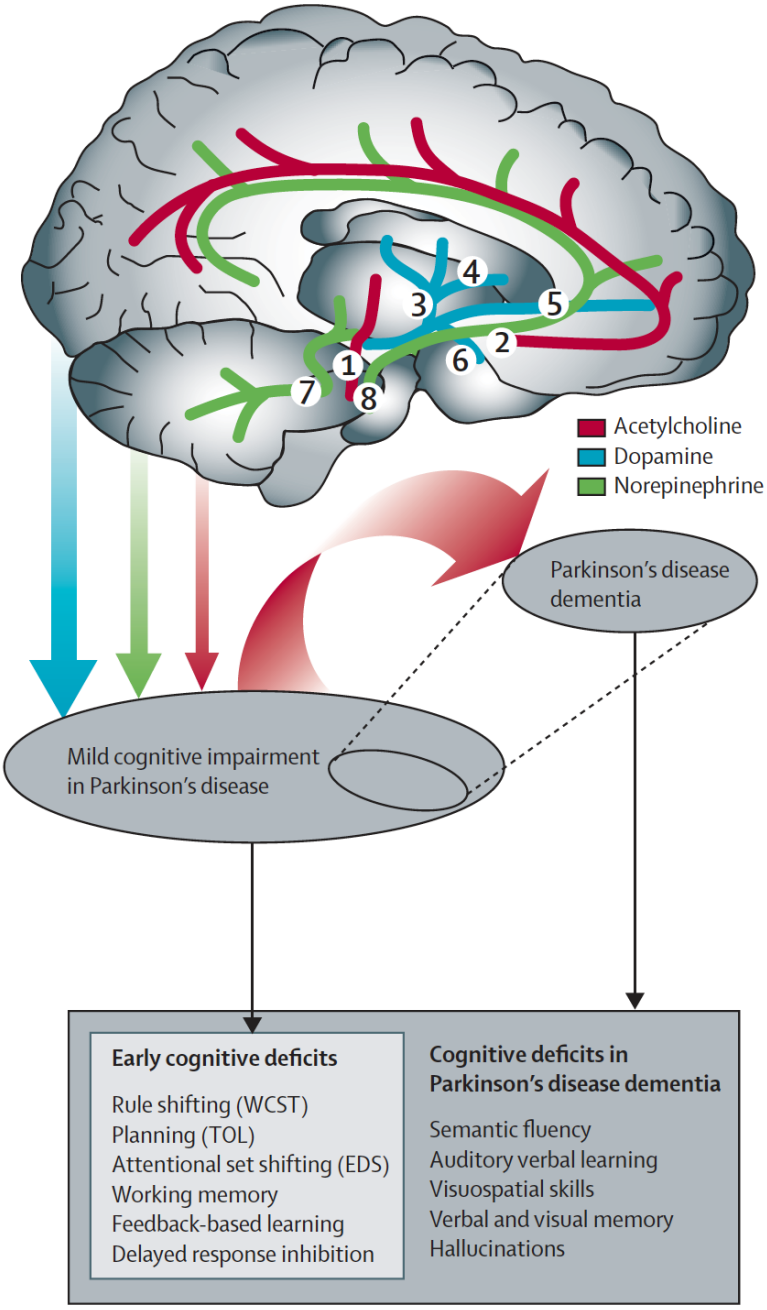


Figure 2.5 Neurochemical pathways possibly implicated in PD cognitive deficits. Cholinergic pathways extend from: 1) the pedunculopontine nucleus to the thalamus and 2) the basal nucleus of Meynert to the neocortex. Dopaminergic pathways are the 3) nigrostriatal, from the substantia nigra (pars compacta) to the striatum; 4) mesolimbic, from the ventral tegmental area to the nucleus accumbens; 5) mesocortical, from the ventral tegmental area to the frontal cortex and 6) tuberoinfundibular, from the hypothalamus to the pituitary gland. The noradrenergic pathways are from 7) the lateral tegmental nucleus to the amygdala and hippocampus and 8) the locus coeruleus to the hypothalamus, thalamus, amygdala, cortex, and cerebellum. WCST, Wisconsin Card Sorting Test; TOL, Tower of London test; EDS, extra-dimensional shifting. From Kehagia et al. (2010).

Among the pathways compromised by PD and implicated in its cognitive dysfunctions, there is the dopaminergic pathway (Figure 2.6) including the nigrostriatal system that projects from the substantia nigra (pars compacta) to the striatum; the mesolimbic system that projects from the ventral tegmental area to the nucleus accumbens (limbic regions), and the mesocortical system that projects from the ventral tegmental area to the frontal cortex (Halliday et al., 2014). The *nigrostriatal pathway,* which provides feedback to the striatum necessary to control actions and cognition; especially the projections to the putamen, degenerate early in PD, even before LB formation (Milber et al., 2012). Of note, nigrostriatal dopaminergic degeneration is almost identical in PD and PDD (Colloby et al., 2005), and this is aligned also with evidence from neuroimaging studies in PD (Klein et al., 2010; Pavese, Rivero-Bosch, Lewis, Whone, & Brooks, 2011). With regard to the *mesolimbic pathway,* greater degeneration is reported in PDD patients (Zweig, Cardillo, Cohen, Giere, & Hedreen, 1993); namely cognitive changes are possibly associated to cell loss in the caudate nucleus and ventral striatum (Mattila et al., 2001). In addition, this pathway plays a key role in behavioral features (e.g., impulsivity) (Reyes, Cottam, Kirik, Double, & Halliday, 2013).

Instead, the findings from functional imaging about the *mesocortical pathway* suggest a reduction in cortical dopamine in PDD patients (Ito et al., 2002), but the effects of dopaminergic treatment on mesocortical system are still not completely understood (Delgado-Alvarado et al., 2016).

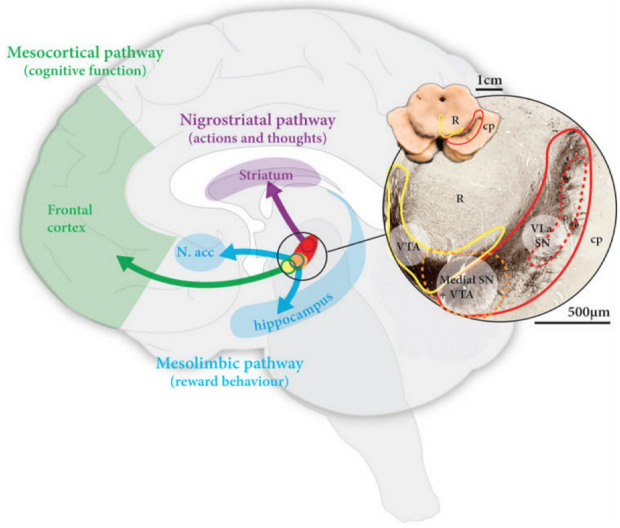


Figure 2.6 Dopaminergic pathways affected in PD and PDD: in red the substantia nigra (SN); in dotted red line the ventrolateral substantia nigra (VLa SN); in yellow the ventral tegmental area (VTA); in dotted orange the medial SN and VTA which give rise to mesolimbic projections affected in PDD. PDD, Parkinson's disease with dementia; cp, cerebral peduncle; N.acc, nucleus accumbens; R, red nucleus. From Halliday et al. (2014).

The noradrenergic pathways extend from the lateral tegmental nucleus to the amygdala and hippocampus, and from the locus coeruleus to the hypothalamus, thalamus, amygdala, cortex, and cerebellum (Figure 2.7) (Halliday et al., 2014). Although there is less evidence about the noradrenergic effects on cognition in PD, it has been suggested that alterations of this pathway can contribute to another subset of higher-order cognitive flexibility deficits (i.e., attentional set-shifting) (Kehagia et al., 2010). In agreement with this observation, a pilot study demonstrated that a noradrenaline reuptake inhibitor improved executive functions, attention, and verbal memory in PD patients (Marsh, Biglan, Gerstenhaber, & Williams, 2009). Marked alterations of noradrenergic pathways are reported in PDD, but there is little evidence on its contribution to the progression of dementia (Del Tredici & Braak, 2013; Zweig et al., 1993). Furthermore, degeneration of this pathway is associated with alterations of cholinergic neurons in the basal nucleus of Meynert (Halliday et al., 2014; Zweig et al., 1993).

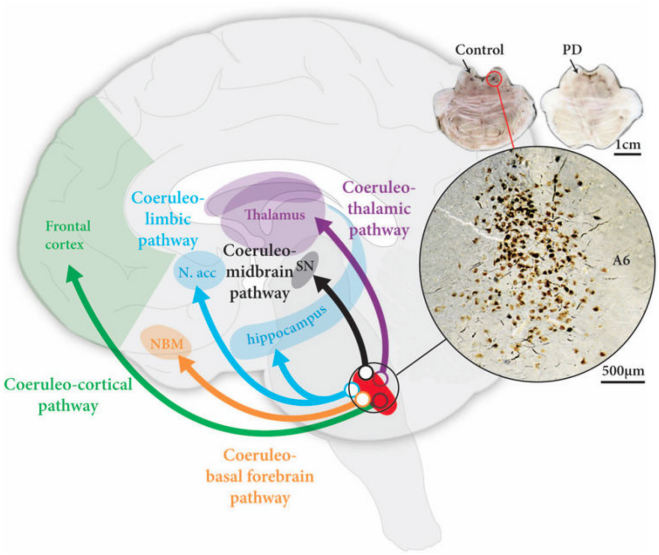


Figure 2.7 Noradrenaline pathways affected in PD and PDD: noradrenergic neurons innervate most of the brain including the substantia nigra (SN) and thalamus, pathways affected in patients with PD, as well as the nucleus basalis of Meynert (NBM), cortical and limbic regions, pathways affected in PDD. N. acc=nucleus accumbens. From Halliday et al. (2014).

Instead, the cholinergic pathways that are more involved in cognition extend from the pedunculopontine nucleus to the thalamus, and from the nucleus basalis of Meynert to the neocortex (Figure 2.8) (Halliday et al., 2014). Cholinergic deficits originated from changes in the basal forebrain (e.g., associated with degeneration as well as LB pathology) and

ascending cholinergic pathways affecting amygdala, hippocampus and cortical regions (i.e., frontal, parietal, and superior temporal cortex) that are more pronounced in PDD compared to non-demented PD and AD patients (Bohnen & Albin, 2011; Kuhl et al., 1996).

Neuropathological evidence showed that reduced choline acetyltransferase in the temporal cortex was associated with cognitive impairment (Mattila et al., 2001; Perry et al., 1985), and progression to dementia correlated with the cholinergic degeneration of the basal forebrain (Ruberg, Rieger, Villageois, Bonnet, & Agid, 1986). Further, this agreed also with the findings from in-vivo neuroimaging studies, wherein PDD were characterized by a broad loss of cortical acetylcholine (Bohnen & Albin, 2011; Yarnall, Rochester, & Burn, 2011).

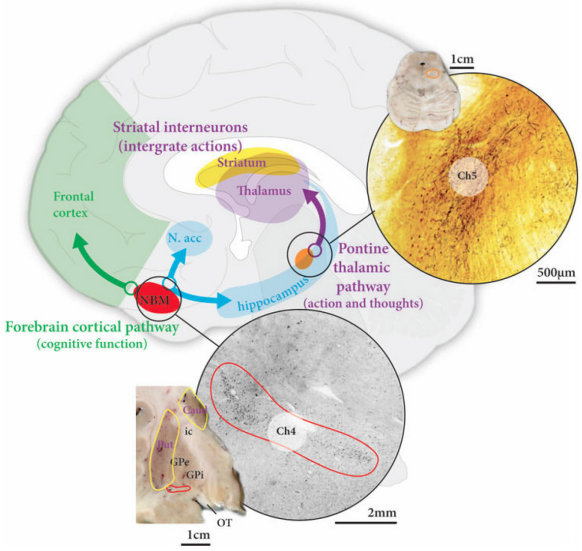


Figure 2.8 Acetylcholine pathways affected in PD and PDD: The two major acetylcholine nuclei with projections to the forebrain are the nucleus basalis of Meynert (NBM), and the pedunculopontine nucleus. The pedunculopontine nucleus projects to the thalamus (this is affected in PD), while the NBM projects to limbic regions and cortex (this is affected in PDD). Caud=caudate nucleus, GPe=external globus pallidus, GPi=internal globus pallidus, ic=internal capsule, OT=optic tract, Put=putamen, N. acc=nucleus accumbens. From Halliday et al. (2014).

To summarize, heterogeneity of cognitive impairment in PD reflects the complexity of the neurodegenerative disease process that includes the interaction of the dopaminergic, noradrenergic and cholinergic systems. A recent model tried to explain this heterogeneity with the 'dual syndrome hypothesis', which distinguishes between a profile characterized by

frontostriatal deficits in PD/PD-MCI, and a profile characterized by a greater involvement of the cholinergic system in PDD — with distinctive cholinergic memory, visuospatial psychiatric deficits (Kehagia et al., 2013) (for further details see Section 2.1.1).

Nevertheless, this hypothesis recognizes some degree of overlap and interaction between the two 'syndromes' and their underpinning systems; indeed, PDD could not exist without a significant disruption of the dopaminergic system, given nigrostriatal degeneration is the core feature of PD (Kehagia et al., 2013).

Furthermore, mesolimbic and mesocortical dopamine dysfunctions contribute to executive deficits, but it is worth noting that these systems include also the parietal and temporal cortexes (De Keyser, Ebinger, & Vauquelin, 1989); thus, possibly also their involvement contributes to the 'posterior' deficits. By contrast, cholinergic deficits will probably also contribute to executive dysfunctions since their system also innervates some frontal regions (Kehagia et al., 2013). Although this model distinguished two specific 'syndromes', it does consider the presence of underlying 'interacting' patterns of neurodegeneration — recognizing the complexity of the disease processes underpinning cognitive impairment heterogeneity.

This agrees also with a recent model proposed by Gratwicke, Jahanshahi and Foltynie (2015), which provides a neural network approach to the mechanisms underlying cognitive features of PDD (see Figure 2.9 for further details). The authors hypothesized the involvement of specific brain networks (i.e., frontostriatal, mesocortical, cholinergic, fronto-parietal, medial temporal and noradrenergic networks), influenced by dopaminergic, noradrenergic and cholinergic deficits. Namely, executive dysfunction in PDD is mostly associated with disruption of the frontostriatal and mesocortical dopaminergic pathways, followed by dysfunction of the noradrenergic and cholinergic networks (Gratwicke et al., 2015). Attention deficits, in particular 'top-down' executive control deficits are mediated by dysfunction of the fronto-parietal network, while 'bottom-up' orienting of attention deficits are associated with cholinergic and noradrenergic networks dysfunctions. Further, according to this model, memory deficits, in terms of memory storage and retrieval, are mediated by medial temporal lobe structures; whereas memory encoding as well as visuoperceptual deficits are associated with nucleus basalis of Meynert cholinergic network.

Overall both Gratwicke's neural network model (Gratwicke et al., 2015) and the 'dual syndrome hypothesis' (Kehagia et al., 2013) supported the predominance of cholinergic dysfunction as predictor of dementia in PD. Namely, the former model identified the

nucleus basalis of Meynert cholinergic network as involved in all cognitive domains dysfunction.

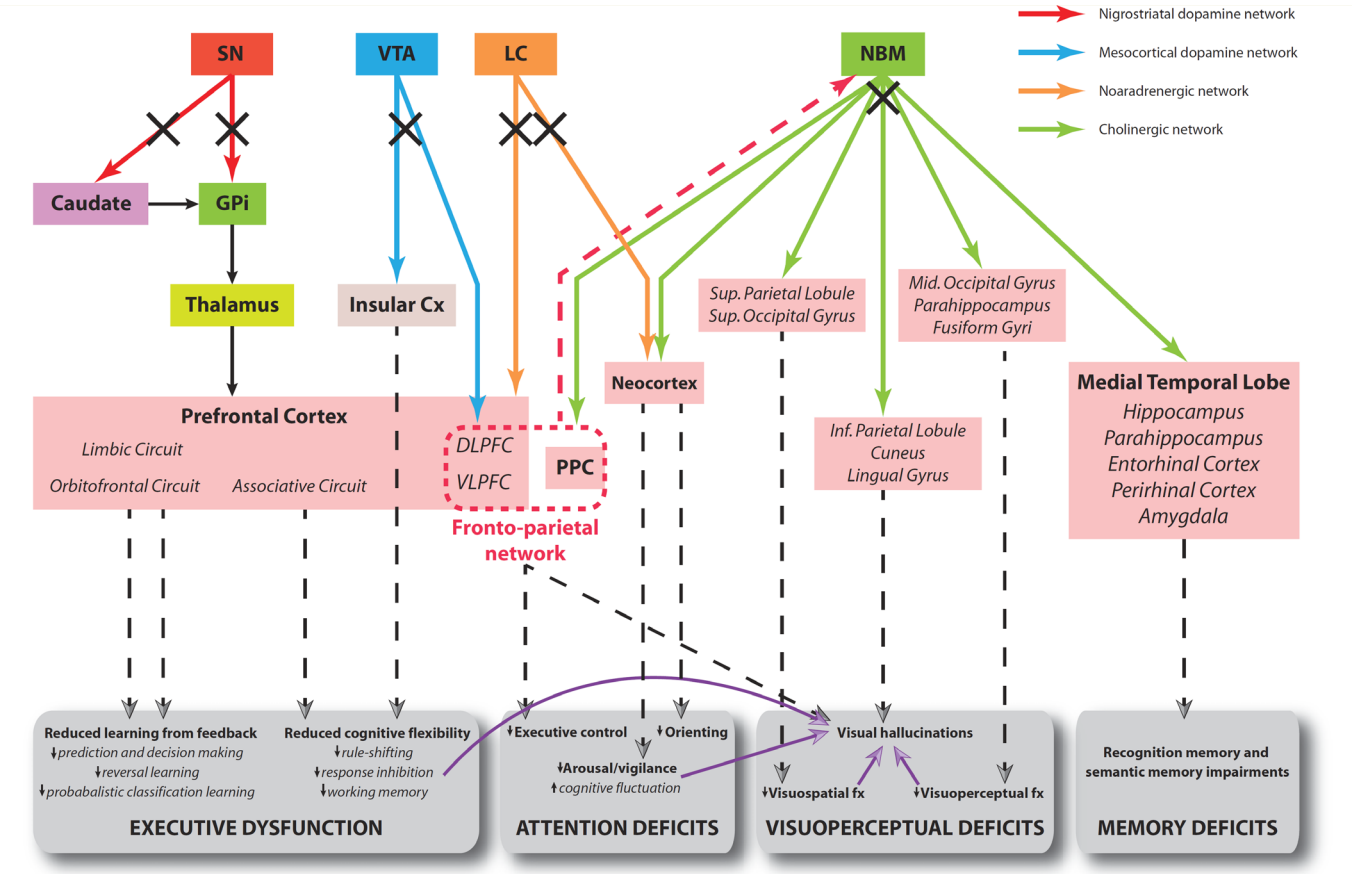


Figure 2.9 Model of neural networks and neurotransmitter affected in PDD, and associated cognitive deficits. As shown in the legend, colored arrows correspond to the neural networks and neurotransmitter involved. Black crosses indicate dysfunction in the network, black dashed arrows associate the dysfunctional network with the cognitive deficits. Purple arrows show that cognitive deficit in a specific domain can contribute to deficits among other cognitive domains. The red dashed arrow indicates the connection between prefrontal areas to the nucleus basalis of Meynert. The frontostriatal, orbitofrontal, limbic and associative circuits correspond to the parallel organization linking basal ganglia and cortex by Alexander et al. (1986). Cx = cortex; DLPFC = dorsolateral prefrontal cortex; fx = function; GPi = globus pallidus (internus); NBM = nucleus basalis of Meynert; PPC = posterior parietal cortex; SN = substantia nigra; VLPFC = ventrolateral prefrontal cortex; VTA = ventral tegmental area. Modified from Gratwicke et al., 2015.

Neuropathology underlying cognitive impairment

Also, the neuropathological substrate of cognitive alterations in PD appears heterogeneous in nature and includes mainly LB, AD pathology and cerebrovascular lesions (Halliday et al., 2014; Irwin et al., 2013).

The majority of studies observed that PDD has higher levels of cortical synuclein pathology than PD (Apaydin, Ahlskog, Parisi, Boeve, & Dickson, 2002; Compta et al., 2011; Irwin et al., 2012; Jellinger & Attems, 2008; Kempster et al., 2010).

In this regard, the progression of synuclein pathology was inversely correlated with cognitive performance (on several cognitive measures) (Aarsland et al., 2003a; Kovari et al., 2003; Mattila, Rinne, Helenius, Dickson, & Roytta, 2000). Notably, a large autopsy cohort of 48 non-demented PD and 92 PDD cases found that cortical and limbic LB and LN were the strongest predicators of dementia in PD (Irwin et al., 2012).

However, overall, evidence from the literature suggests that cortical and limbic synuclein are not necessarily required for the development of PDD (Irwin et al., 2013) — non-demented PD with marked burden of cortical and limbic synuclein pathology has been observed (Hurtig et al., 2000), as well as PDD with minimal cortical synuclein pathology at autopsy (Galvin, Pollack, & Morris, 2006; Irwin et al., 2012; Pletnikova, 2005).

Thus, Irwin et al. (2013) suggested that PDD with reduced cortical and limbic synuclein pathology can possibly be associated with the presence of comorbid pathologies (i.e., amyloid-β pathology).

Interestingly, there is evidence that disease progression in PDD is also faster in presence of comorbid amyloid pathology — in particular, without amyloid-β pathology the mean survival was about 10.1 years while it was about 4.5 years in presence of comorbid amyloid pathology (Jellinger, 2003; Jellinger, Seppi, Wenning, & Poewe, 2002). PDD with amyloid comorbidity was also associated with older age-at-onset of motor symptoms (Irwin et al., 2012; Jellinger et al., 2002; Sabbagh, 2009).

In this regard, although the key role of synuclein pathology in PDD has been frequently demonstrated, some studies observed that also amyloid pathology burden as well as tau-neurofibrillary tangles were inversely associated with cognitive defect in PDD (Compta et al., 2011; Jellinger, 2007; Jellinger & Attems, 2008; Kovari et al., 2003). A recent study, wherein cortical synuclein and amyloid pathologies were quantitatively assessed, found that when combining both the two pathologies the correlation with PDD was higher (Compta et al., 2011).

PDD patients with amyloid- related pathology have higher levels of cortical and limbic synuclein compared to PDD patients without the comorbidity (Compta et al., 2011; Pletnikova, 2005). In addition, increased severity of amyloid pathology and tau-neurofibrillary tangles correlated with a higher burden of cortical synuclein (Compta et al., 2011; Harding & Halliday, 2001; Jellinger, 2007; Lashley, 2008), suggesting a possible interaction (Toledo et al., 2016). In agreement with this observation, recent evidence from neuropathological studies suggests coincident amyloid pathology may alter the spread of LB in PD (Toledo et al., 2016)

To conclude, it seems very likely that there is a synergistic effect between age, synuclein and amyloid pathologies, which has been considered as the main trigger of cognitive decline in PD — as the presence of amyloid in PD patients can possibly lead to a PD-subtype with an older-age of onset, which is characterized by a more 'malignant prognosis' (Compta et al., 2011; Halliday & McCann, 2010; Irwin et al., 2013).

Lastly, recent evidence from neuroimaging studies confirmed these patterns of underlying pathologies associated with PD cognitive decline (Duncan, Firbank, O'Brien, & Burn, 2013),

In this regard, a further Section will briefly overview the neuroimaging evidence assessing such structural changes underpinning cognitive deficits in PD (for further details see Section 2.3.1).

2.2.2 Multiple system atrophy

In MSA, pathological changes affect primarily subcortical structures, while cortical pathology is not a predominant feature of this disorder (Papp & Lantos, 1994) (for further details see Section 1.1.1). Namely, MSA is characterized by putaminal and nigral degeneration (Wenning et al., 1997), and by a disruption of striato–pallido–thalamocortical circuit possibly secondary to the subcortical degeneration (Stankovic et al., 2014).

Furthermore, evidence from neuropsychological studies showed that executive dysfunctions are the prominent cognitive symptoms in MSA (Gerstenecker, 2017) (for further details see Section 2.1.2), thus it has been suggested that the concept of 'subcortical dementia' may partially explain cognitive features of MSA (Bak et al., 2005a; Cummings, 1986; Stankovic et al., 2014).

Recently, a few studies investigated the underlying neuropathology of cognitive alterations in MSA, but the results are controversial, and the underlying mechanisms are still not completely understood.

Notably, a neuropathological study reported no significant difference between cognitively impaired MSA and MSA without cognitive impairment — GCIs and neural cytoplasm inclusions in limbic or cortical regions as well as secondary pathological conditions (AD-related pathology) were not more severe in MSA with cognitive alterations (Asi et al., 2014). The authors noted that cognitive deficits in MSA are independent of AD-pathology or other secondary pathologies, suggesting that cognitive impairment can be possibly intrinsic of the MSA disease process, but without being associated with GCIs or neural cytoplasm inclusions.

By contrast, other studies demonstrated that neural cytoplasm inclusions in limbic or cortical regions (rather than GCIs) were related to cognitive dysfunctions in MSA (Cykowski et al., 2015; Homma et al., 2016; Koga et al., 2016).

Recently, Koga et al. (2016) reported a greater burden of neural cytoplasm inclusions in the limbic regions (i.e., hippocampal dentate gyrus) that were associated with cognitive impairment in MSA. Although in this study, they did not identify any correlations between cognitive measures and the respective neuroanatomical regions: suggesting that MSA were characterized by a predominant fronto-subcortical pattern of cognitive dysfunction.

Then, in another study, it was demonstrated that presence of LB-like inclusions in the neocortex was strongly associated with cognitive alterations, suggesting that neural pathology plays an important role both in cognition and disease progression (Cykowski et al., 2015).

Finally, Homma and others (2016) reported that MSA with dementia was characterized by frequent globular neural cytoplasm inclusions in the medial temporal region (i.e., subiculum). Furthermore, they did not find any association between dementia and AD-pathology (i.e., neurofibrillary tangles and senile plaques). Thus, the author suggested that neural cytoplasm inclusions in medial temporal regions are one of the most important features in MSA with dementia, also in a context of absent cortical atrophy.

More recently, in a single-case study, an MSA patient with dementia showed numerous neural cytoplasm inclusions in the perirhinal cortex's anterior portion, but without hippocampal involvement (Saito et al., 2017).

However, further neuropathological studies are still required to elucidate the underlying mechanism of cognitive impairment in MSA, in this regard also correlations of cognitive measures and the responsible neuroanatomical regions will be necessary (Koga & Dickson, 2017).

2.2.3 Progressive supranuclear palsy

Neuropathology of PSP is widespread and comprises several structures; namely the substantia nigra, subthalamic and red nucleus, pontine tegmentum, striatum, oculomotor nucleus, medulla, and dentate nucleus (Litvan et al., 1996a) (for further details see Section 1.1.2).

Cognitive dysfunctions are a prominent feature of PSP patients, especially of PSP-RS phenotype (Gerstenecker, 2017; Gerstenecker et al., 2013) (for further details see Section 2.1.3), and due to the prevalence of executive dysfunctions as well as bradyphrenia, their cognitive impairments profile has been described with the concept of 'subcortical dementia' (Albert, 2005; Albert et al., 1974; Bak et al., 2005a). In this regard, striatofrontal dysfunction is frequently severe and leads to dramatic monitoring, planning, and recall deficits, progressing toward dementia (Dubois & Pillon, 2005).

Despite the lack of detailed neuropsychological studies in patients with pathological confirmed PSP, there is little neuropathological evidence that reported correlations between global cognitive performance and underlying pathology.

In this regard, Cordato, Halliday, Harding, Hely, and Morris (2000) found that PSP patients had a greater frontal lobe atrophy (compared with PD and DLB patients) that correlated with clinical dementia. Furthermore, the authors reported significant frontal atrophy without clinical dementia, although overall, the most severe frontal atrophy correlated with dementia. Of note, hippocampal volumes were relatively well preserved in both demented as well as in non-demented PSP patients.

Interestingly, a factorial analysis in a study by Verny, Duyckaerts, Agid, and Hauw (1996) was able to isolate the cortical from the subcortical factor. The results showed the extent of cortical pathology was not entirely determined by the severity of the subcortical changes, suggesting it possibly was due to an independent disease mechanism.

As mentioned above, dementia with predominant frontal features is common in PSP (Brown et al., 2010; Burrell et al., 2014). Since at first, the cortex was thought to be spared, Albert and others (1974) hypothesized a subcortical origin of cognitive symptoms — leading to the concept of 'subcortical dementia' (Albert, 2005; Albert et al., 1974; Verny et al., 1996), possibly due to the deafferentation from thalamus or reticular structure.

However, correlations between dementia and lesions in the entorhinal region have been found (Braak, Jellinger, Braak, & Bohl, 1992); and further, the presence of neurofibrillary tangles in frontal regions leaves the possibility that some cognitive deficits are associated with these lesions (Verny et al., 1996).

Clinicopathological studies that aim to explain the underlying pathology of cognitive impairment in PSP are still limited and further studies with autopsy-based correlates are necessary to elucidate the pathological mechanisms of dementia in PSP.

2.3 STRUCTURAL NEUROIMAGING UNDERLYING COGNITIVE DEFICITS

In recent decades, several neuroimaging techniques have considerably been used to advance our understanding of the complex mechanisms underpinning development of cognitive impairment as well as the potential progression to dementia in parkinsonian disorders (Aarsland et al., 2017; Biundo et al., 2016a; Delgado-Alvarado et al., 2016).

Structural magnetic resonance imaging (MRI) can identify and localize differences in regional tissue volume (i.e., cortical and subcortical) between groups of individuals.

The following Sections will overview the most recent literature on structural changes underlying cognitive dysfunctions in PD, MSA and PSP.

2.3.1 Parkinson's disease

The major aim of neuroimaging studies in PD has been to identify potential biomarkers of the progression to dementia and MCI — despite the elevated number of studies, so far no explicit biomarker has been identified. A possible explanation for this is that the majority of these studies are based on the PD-MCI available criteria, leading to a very heterogeneous PD-MCI sample (for further details see Section 2.1.1) (Biundo et al., 2016a;

Litvan et al., 2012). Of note, also the inclusion of small samples as well as heterogeneous neuroimaging methodological approaches could also have increased variability (Biundo et al., 2016a).

As expected, several cross-sectional studies have reported broader brain atrophy in PDD as compared to PD-MCI patients and PD with normal cognition (PD-NC) (Apostolova et al., 2012; Biundo et al., 2013; Compta et al., 2012; Delgado-Alvarado et al., 2016; Melzer et al., 2012; Summerfield et al., 2005); namely in the parietal, occipital, temporal, and frontal areas, as well as in other specific regions (i.e., hippocampus, amygdala, caudate, putamen, thalamus, and substantia innominate) (Figure 2.10) (Delgado-Alvarado et al., 2016).

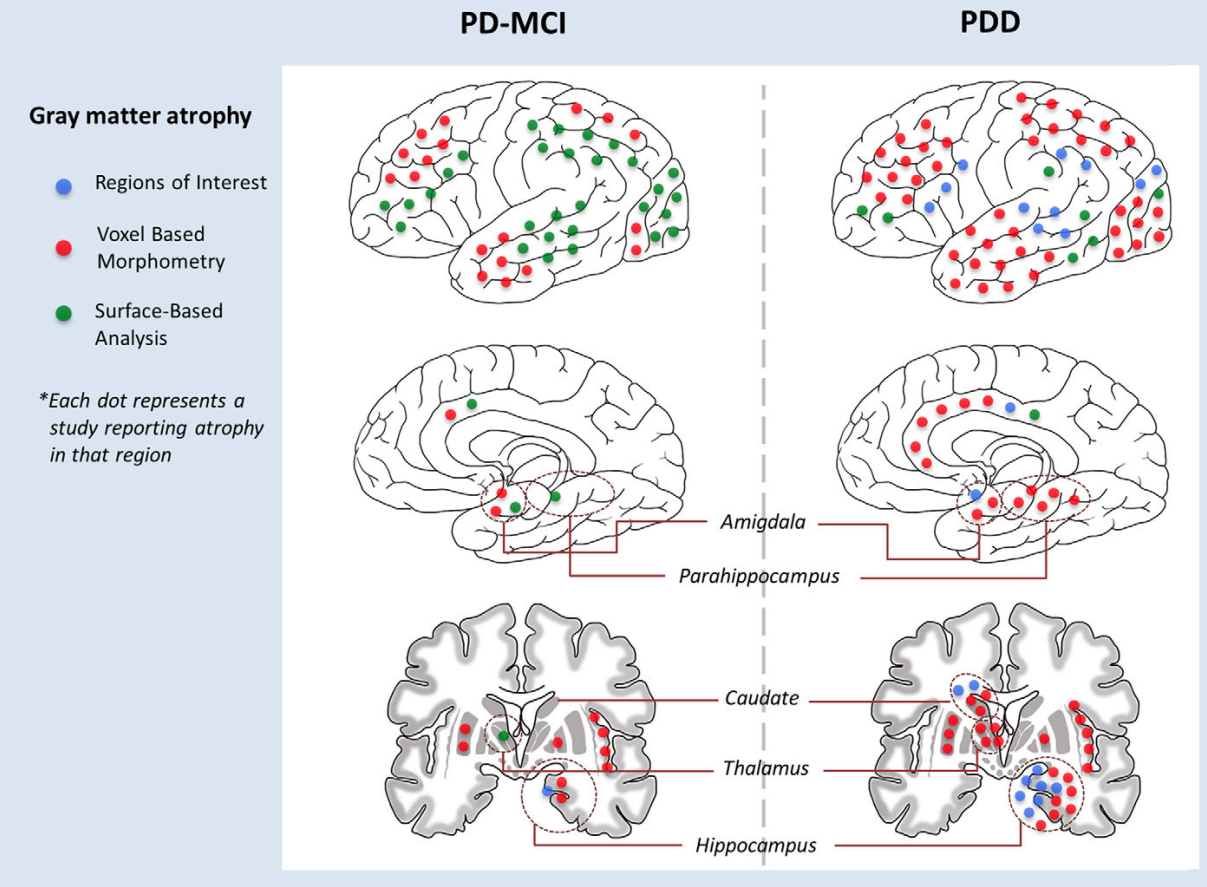


Figure 2.10 Summary of the potential biomarkers of PD with dementia (PDD) and PD with mild cognitive impairment (PD-MCI) found in studies of magnetic resonance imaging. Adapted from Delgado-Alvarado et al. (2016).

In this regard, PDD patients showed more cortical thickness than PD-MCI in the anterior cingulate and entorhinal and orbitofrontal cortices as well as in the parahippocampus, temporal pole, precuneus, and fusiform and lingual areas (Pagonabarraga et al., 2013). Furthermore, PDD also showed cortical volume reductions in several temporal and prefrontal areas (Song et al., 2011), including the amygdala compared to PD-MCI (Choi et al., 2012).

Interestingly, evidence on PD-MCI reported cortical volume reduction in the frontal, parietal and posterior areas, as well as atrophy in the hippocampus, which correlated with memory deficits (Mak et al., 2015; Pereira et al., 2015). Further, longitudinal studies demonstrated that patients with PD-MCI showed a cortical thinning in frontal, temporal, parietal and occipital cortices, and loss of hippocampal volume that was related to cognitive decline (Hanganu et al., 2014; Mak et al., 2015). Hippocampal volume has also been identified as a predictor of the further conversion to PD-MCI (Kandiah et al., 2014), and other evidence using Bayesian network classifiers was aligned with this finding (Morales et al., 2013). Indeed, Morales et al. (2013) demonstrated that it was possible to classify PDD, PD-MCI and PD-NC with high sensitivity and specificity — PDD patients were identified by left hippocampal and right entorhinal cortex atrophy and by lateral ventricles enlargement, while PD-MCI patients were characterized by brain stem and left hippocampus atrophy.

Another 2-year longitudinal study showed that atrophies in the caudate nuclei, prefrontal and insular cortex were associated with a further cognitive decline and thus turning into dementia (Lee et al., 2014). Lastly, another study identified volume reduction in the occipital cortex as able to differentiate PDD from PD without cognitive defects (Burton, McKeith, Burn, Williams, & O'Brien, 2004).

To summarize, several studies reported a typical cortical thinning pattern that identified PDD. Specifically, this pattern is associated with gray matter loss in the frontal, temporal, parietal, occipital cortices; in conjunction with volume loss in the insula, hippocampus, parahippocampus, and cingulate gyrus, which is associated with cognitive decline (Hwang et al., 2013; Pagonabarraga et al., 2013; Rektorova et al., 2014; Zarei et al., 2013).

Taken together these findings seem i) to suggest that reduced volume or thickness in several cortical areas as well as in the hippocampus appear to be associated with the further progression to PD-MCI or PDD; ii) to be aligned with the 'dual syndrome hypothesis'.

Indeed, progressive thinning of 'posterior' regions together with 'frontal' atrophy can possibly herald a progression towards dementia.

2.3.2 Multiple system atrophy

In the literature, there is little evidence of structural MRI studies investigating the pattern underlying cognitive impairment in MSA. This is probably associated to the fact that cognitive deficits are still listed as an exclusion criterion among the MSA diagnostic criteria (Gilman et al., 2008), as well as due to the rare nature of this disease.

Several studies tried to identify a specific pattern underpinning MSA pathology — and indeed, a characteristic pattern was found — with cortical atrophies in the frontal, temporal, and parietal areas in MSA-P (Brenneis et al., 2007; Brenneis et al., 2003; Minnerop et al., 2007) and in MSA-C (Brenneis et al., 2006; Hauser et al., 2006; Specht et al., 2003; Specht, Minnerop, Müller-Hübenthal, & Klockgether, 2005).

Interestingly, about the pattern underpinning cognitive alterations in MSA, Kim et al. (2013) found MSA with dementia was characterized by focal cortical atrophies on parahippocampal and lingual cortices compared to MSA without dementia, which was not, suggesting a pattern resembling those found in AD or PDD (even though more widespread in these two disorders).

Instead, a previous study by Paviour, Price, Jahanshahi, Lees, and Fox (2006) found a correlation in MSA patients between pontine, midbrain, and cerebellar atrophy and impairment in attentive/executive domains as well as global cognition (i.e., FAB), advancing the hypothesis of cortical deafferentation secondary to subcortical regions alterations. In this regard, this hypothesis also was in agreement with another study demonstrating that short disease duration was associated with atrophy in the striatum, while longer disease duration was associated with increasing atrophy in the cortical and cerebellar areas (Brenneis et al., 2007).

Interestingly, a study by Chang et al. (2009), wherein more cognitive domains were assessed (i.e., memory, attention and executive), noted that prefrontal atrophy correlated with the memory performance of MSA patients. Further, evidence from a functional study reported a dorsolateral prefrontal hypoperfusion associated with executive dysfunction in MSA-P (Kawai et al., 2008). Overall, these findings seem to suggest an important role of

frontal regions in understanding the underpinning mechanisms of cognitive deficits in MSA.

Recently, another study on MSA-P demonstrated that cortical, cerebellar atrophy and striatal degeneration were associated with the presence of cognitive impairment (i.e., immediate and recall memory, and executive functions) in these patients (Kim et al., 2015).

By contrast, Lee et al. (2016a) found that cortical thinning in the fronto-temporo-parietal regions and volume reduction in subcortical structures were significantly correlated with attentional, executive and visuospatial dysfunctions in MSA-C patients: suggesting cognitive dysfunctions in MSA-C can possibly result from functional disruption of the corticostriatal and pontocerebellar circuit, mediated by primary cortical, cerebellar or thalamic pathology.

To summarize, overall evidence from structural neuroimaging studies in MSA seem to suggest that both deafferentation from subcortical structures and intrinsic cortical pathology have a crucial role in cognitive decline, wherein cortical involvement is possibly secondary to a subcortical alteration (Stankovic et al., 2014). However, these hypotheses need to be verified by further studies applied in larger cohorts and with reliable cognitive measures, possibly not biased by motor dysfunctions.

2.3.3 Progressive supranuclear palsy

Although cognitive dysfunctions are widely recognized as a prominent feature of PSP (Brown et al., 2010), there is still little neuroimaging evidence in literature about the underlying structural deficits associated with cognitive impairment.

Regarding the distribution of atrophy associated with the disorder, it has been noted that the pattern is less broad from neuroimaging evidence (i.e., voxel-based morphometry)(Cordato, Duggins, Halliday, Morris, & Pantelis, 2005; Ghosh et al., 2012; Josephs et al., 2008; Padovani et al., 2006), than in neuropathological studies (Schofield, Hodges, Bak, Xuereb, & Halliday, 2012): suggesting that maybe tau inclusions have only a weak correlation with the extensive cell loss that underpins atrophy detected by MRI studies (Burrell et al., 2014).

Neuroanatomical changes underlying cognitive deficits were reported by Cordato et al. (2005), namely PSP presented symmetrical atrophies in the frontal cortex (i.e., orbitofrontal

and medial frontal cortex), subcortical nuclei (i.e., midbrain, caudate and thalamic) and periventricular white matter that correlated with cognitive measures (MMSE).

Furthermore, Paviour et al. (2006) found that alterations in the brainstem and subcortical structures contribute to cognitive deficits in PSP, namely an association between increased midbrain atrophy and a worse performance on the FAB was observed. Interestingly, the association with the midbrain was even stronger than the frontal atrophy. In addition, they found a correlation between increasing lateral ventricles atrophy (possibly explained by changes at the lateral ventricle/caudate nucleus boundary) and decline at the FAB. Hence, the authors suggested that the atrophy in the caudate can possibly contribute to worsening of the subcortical dementia.

Overall, the evidence about neuroanatomical changes associated with cognitive deficits in PSP is very limited; but these findings seem to be promising and in line with the concept of 'subcortical dementia' (Albert et al., 1974).

PART II

STUDIES ON COGNITIVE MANIFESTATIONS IN PARKINSONIAN DISORDERS

CHAPTER 3

MONTREAL COGNITIVE ASSESSMENT AND MINI-MENTAL STATE EXAMINATION PERFORMANCE IN PROGRESSIVE SUPRANUCLEAR PALSY, MULTIPLE SYSTEM ATROPHY and PARKINSON'S DISEASE[1]

3.1 Introduction

Cognitive dysfunctions are frequently reported in PD as well as in atypical parkinsonisms — at early disease phase, absence of marked cognitive changes makes a diagnosis of PD and MSA more probable, while presence of dysexecutive symptoms are more suggestive of a diagnosis of PSP that is considered the typical form of 'subcortical dementia' (Bak et al., 2005a; Dubois & Pillon, 2005; Lee et al., 2012).

However, the identification and magnitude of cognitive abnormalities are frequently not routinely assessed in most clinical settings, and performing a comprehensive neuropsychological assessment is impractical in most clinical settings (Marras et al., 2014). Thus, to make cognitive assessment more practical during routine care, brief screening cognitive scales can be adopted, to support the clinician in the diagnostic process.

Indeed, brief cognitive scales have some advantages over extensive neuropsychological evaluation: brevity, low cost and higher patient acceptability (Bak et al., 2005a; Burrell et al., 2014). Cognitive screening tools should be sensitive in detecting parkinsonism-related cognitive dysfunctions, possibly brief and easy to be administered.

Among the generic screening tests adopted in PD cognitive assessment, MMSE is the most widely used (Folstein et al., 1975). Although MMSE was designed for assessing AD, it has been applied to several disorders also in PD populations, but MMSE was relatively insensitive in detecting MCI or dementia, mainly as it does not investigate the fronto-executive domain (Hoops et al., 2009).

[1] *Published: Fiorenzato, E., Weis, L., Falup-Pecurariu, C., Diaconu, S., Siri, C., Reali, E., ... & Biundo, R. (2016). Montreal Cognitive Assessment (MoCA) and Mini-Mental State Examination (MMSE) performance in progressive supranuclear palsy and multiple system atrophy. Journal of Neural Transmission, 123(12), 1435-1442.*

On the contrary, the MoCA, another brief cognitive screening tool widely used in PD patients (Nasreddine et al., 2005), showed high sensitivity and specificity in the assessment of cognitive dysfunctions in PD (Gill et al., 2008; Hoops et al., 2009; Zadikoff et al., 2008).

Indeed, previous evidence reported higher sensitivity and adequate psychometric properties with MoCA, when compared to MMSE in detecting cognitive changes, particularly in the general population (Luis, Keegan, & Mullan, 2009), and in several neurodegenerative conditions such as AD, DLB, and Huntington's disease (HD) (Biundo et al., 2016b; Hoops et al., 2009; Nasreddine et al., 2005; Videnovic et al., 2010).

However, MoCA has been poorly investigated in atypical parkinsonisms — especially in PSP and MSA (Kawahara et al., 2015).

Thus, we hypothesize that as observed in PD, MoCA should have a higher sensitivity than MMSE also in atypical parkinsonisms due to the lack of ceiling effect and the more challenging attention-executive subitems (Hoops et al., 2009; Zadikoff et al., 2008).

This present study's objective was to investigate the sensitivity of MoCA and MMSE scales in detecting cognitive dysfunctions in atypical parkinsonisms, probable MSA and PSP, as compared to PD patients.

3.2 Methods

3.2.1 Study population and data collection

One hundred thirty patients with parkinsonian disorders were evaluated. Namely, 35 patients with MSA, 30 with PSP and 65 age, sex and education matched PD patients. Data were collected across three European centers specialized in movement disorders: San Camillo Hospital Foundation, Venice, Italy; Parkinson Institute, ASST G. Pini-CTO, Milan, Italy and at the Faculty of Medicine, Transilvania University, Brasov, Romania. All the patients fulfilled the clinical 'probable' level of the diagnostic criteria and the diagnoses were based on the more recent published criteria (Gelb et al., 1999; Gilman et al., 2008; Höglinger et al., 2017). Patients with PSP were all of Richardson's syndrome phenotype.

Ethical approval was obtained from the Venice Research Ethics Committee, Venice, Italy and before study enrolment, patients gave their written, informed consent. This study was conducted in accordance with the Declaration of Helsinki.

The two brief cognitive scales, MMSE and MoCA, were performed at each center on two separate occasions within five to seven days, in a random order and administered in the morning ON medication.

Demographic variables (i.e., age, education, sex) and clinical characteristics (i.e., disease duration, age of onset) were collected; in addition, motor symptoms were assessed by means of the motor session of the MDS-UPDRS III administered by neurologists with experience in movement disorders.

Exclusion criteria were presence of psychiatric and other neurological comorbidity, and surgical treatment with deep brain stimulation.

Levodopa equivalent daily dose (LEDD) and dopamine agonist equivalent dose (DAED) were calculated accordingly to Tomlinson and others (2010).

3.2.2 Statistical analyses

All statistics were performed using SPSS version 20 (IBM SPSS, Chicago, IL). Statistical significance threshold was set at $p < 0.05$ and Bonferroni correction was used to control for multiple comparisons.

One-way Analysis of Variance (ANOVA) was run to analyze the clinical and demographic data of the whole sample, while the Pearson's chi-squared test was adopted to analyze categorical variables. To compare the mean difference in MMSE, MoCA and MoCA's letter fluency task across the MSA, PSP and PD group, ANOVA was run. Further, if clinical and demographic variables differed among the groups, analysis of covariance (ANCOVA) was run to compare MMSE, MoCA and MoCA's letter fluency scores controlling for the previously mentioned variables. Lastly, to evaluate the presence of ceiling or floor effects in MMSE and MoCA scales, we calculated the 25 to 75 percent percentiles for each group. To compare the MSA subgroups (MSA-P and MSA-C), we ran a non-parametric analysis due to the non-normal distribution of several outcome variables; the Kruskal-Wallis test was applied for the between-groups comparison.

Backward linear regression analysis was also run to investigate if clinical or demographic characteristics explained the variance between the two scales. Thus, we set MMSE and MoCA scores as dependent variable; and age, education, disease duration, MDS-UPDRS III score, LEDD and DAED as independent variables. Pearson's correlation coefficients were calculated between the scales in each group.

Regarding the analysis of the subitems, first we normalized the subitems scoring differences in each scale, dividing the subitem's obtained scores by the subitem's maximum score, and then the 'weighted subitems means' were converted to percentage. Lastly, we ran Pearson's chi-squared analyses to compare the performance on MMSE and MoCA subitems. Receiver Operating Characteristic (ROC) curves with Area Under the Curve (AUC) (95% CI) were also obtained. Then, for each scale's total scores (MMSE and MoCA) and for the subitems reaching the significance, we calculated the AUCs, optimal cut-off, sensitivity, specificity, positive predictive value and negative predictive value. The optimal cut-off point was defined as the value optimizing the combination of sensitivity and specificity scores of each test by means of Youden's J statistic (J) (Youden, 1950), and the difference in discriminative power (i.e., AUC) of each cut-off score for between-group comparisons was tested with DeLong's method (DeLong, DeLong, & Clarke-Pearson, 1988).

3.3 Results

3.3.1 Demographic and clinical features

Table 3.1 shows the demographic and clinical data and the corresponding group comparisons. Data of MSA subtypes, MSA-P and MSA-C are presented in Table 3.2.

Table 3.1 Demographic and clinical characteristics of MSA, PSP and PD groups

	MSA (n = 35)				**PSP (n = 30)**				**PD (n = 65)**				**MSA vs. PSP**	**PSP vs. PD**	**MSA vs. PD**
	Mean (SD)	Mdn	min	max	Mean (SD)	Mdn	min	max	Mean (SD)	Mdn	min	max	*p* value		
Age, y	64.5 (6.1)	64.5	49	77	69.8 (7.4)	70.0	57	88	67.5 (7.5)	68.0	49	85	*		
Sex (m/ f)	18/17				12/18				40/25						
Education, y	10.1 (4.1)	9.0	5	18	9.0 (3.2)	8.0	4	17	9.6 (4.2)	8.0	4	18			
Disease duration, y	4.5 (1.9)	4.0	1	10	5.4 (3.1)	4.5	2	14	7.7 (4.5)	7.0	0	22		*	***
Age of onset, y	60.1 (5.9)	61.5	44	69	64.4 (7.5)	64.0	48	80	59.7 (8.7)	59.0	43	74		*	
MDS-UPDRS-III	38.6 (19.4)	35.5	11	93	41.2 (16.6)	37.5	16	73	24.0 (11.9)	22.0	4	68		**	**
LEDD	602.1 (340.0)	621	0	1340	476.1 (303.0)	500	0	1208	897.3 (552.3)	800	0	2511		**	*
DAED	85.9 (96.9)	9	0	240	53.0 (84.7)	0	0	249	143.3 (108.3)	160	0	400		**	*

Note. The p values were obtained by one-way analysis of variance. Chi-squared analyses were used for categorical variables. Significant difference Bonferroni-corrected for multiple comparison at: * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$. MSA, multiple system atrophy; PSP, progressive supranuclear palsy; PD, Parkinson's disease; Mdn, median; min, minimum; max, maximum; MDS-UPDRS III, Movement Disorder Society Unified Parkinson's Disease Rating Scale III; LEDD, levodopa equivalent daily dose; DAED, dopamine agonist equivalent dose.

Table 3.2 Demographic data of the MSA sample (MSA-P and MSA-C subtypes)

	MSA-P (n = 26)				MSA-C (n = 9)				MSA-P vs. PSP	MSA-P vs. PD
	Mean (SD)	Mdn	min	max	Mean (SD)	Mdn	min	max	*p* value	
Age, y	64.8 (5.3)	64.0	51	70	63.8 (8.2)	64.5	49	72		
Sex (m/ f)	13/13				5/4					
Education, y	9.9 (4.0)	8.0	5	18	10.6 (4.6)	10.0	5	18		
Disease duration, y	4.4 (1.7)	4.0	1	8	4.7 (2.6)	4.0	2	10		*
Age of onset, y	60.4 (5.3)	61.0	47	68	59.2 (7.6)	62.0	44	67		
MDS-UPDRS III	39.8 (19.4)	40.0	15	93	35.00 (20.2)	33.0	11	78		*
LEDD	620.0 (350.1)	660.0	0	1340	550.2 (319.2)	400.0	200	1338		
DAED	97.7 (95.4)	100.0	0	240	52.0 (98.5)	0.0	0	240		
MMSE	27.7 (2.3)	28.0	21	30	27.4 (2.7)	28.0	23	30		
MoCA	23.1 (2.6)	23.0	18	28	22.3 (4.2)	22.0	15	28	**	
MoCA letter fluency	10.1 (4.9)	9.5	3	22	11.8 (4.7)	12.0	5	20		

Note. The p values were obtained by non-parametric Kruskal-Wallis test and Bonferroni post hoc test to compare groups. Chi squared analyses were used for nominal variables. Comparison was considered significant at: * = $p < 0.05$, ** = $p < 0.001$. No differences between MSA-C vs. MSA-P, MSA-C vs. PD, MSA-C vs. PSP. MSA, Multiple System Atrophy; MSA-P, MSA Parkinsonian type; MSA-C, Cerebellar type; PSP, Progressive Supranuclear Palsy; PD, Parkinson's Disease; Mdn, median; Min, minimum score; Max, maximum score; MDS-UPDRS III, Movement Disorder Society Unified Parkinson's Disease Rating Scale III; LEDD, Levodopa Equivalent Daily Dose; DAED, Dopamine Agonist Equivalent Dose; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

Patients with MSA were younger than PSP ($p = 0.01$), while the PD group agreed in age with PSP and MSA patients. No differences were found between-groups in terms of education and sex. The PD group had longer disease duration than PSP ($p = 0.01$) and MSA groups ($p < 0.0001$); and PSP patients were older at disease onset than PD ($p = 0.02$) but not older than MSA patients.

We also noted that PD patients had lower motor severity (MDS-UPDRS III) than PSP ($p = 0.00003$) and MSA ($p = 0.00004$) as well as PD patients had higher LEDD and DAED than PSP ($p = 0.0002$; $p = 0.0004$ respectively) and MSA patients ($p = 0.007$; $p = 0.02$ respectively).

3.3.2 Cognitive results

MMSE mean total score was higher than the mean of MoCA in the whole group (27.05 ± 2.43 vs. 21.54 ± 3.93), as well as in PSP (26.0 ± 2.9 vs. 18.2 ± 3.9, $p < 0.0001$), MSA (27.7 ± 2.4 vs. 22.9 ± 3.0, $p < 0.0001$), and PD patients (27.3 ± 2.0 vs. 22.3 ± 3.5, $p < 0.0001$). Correlation analysis showed that MMSE and MoCA total scores were highly correlated (PSP: $r = 0.75$, $p < 0.0001$, MSA: $r = 0.63$, $p < 0.0001$, and PD: $r = 0.68$, $p < 0.0001$). Interestingly, MSA subtypes (MSA-P and MSA-C) had a similar performance on MoCA and MMSE scales and we found no significant differences when comparing their performances on both scales (Table 3.2). Thus in the present thesis, we decided to analyze all MSA subjects as a whole group without differentiating them according to their subtypes.

To investigate a possible floor and ceiling effect of the scales, we ran some descriptive analyses and we noted that MMSE scores of 29 or 30 were found in 13 percent of PSP, 48 percent of MSA and 28 percent of PD patients. Indeed, these higher scores corresponded with the 75th percentile (Figure 3.1, Table 3.3), while in MoCA the maximum scores did not correspond with the 75th percentile and overall MoCA scale showed a broader distribution.

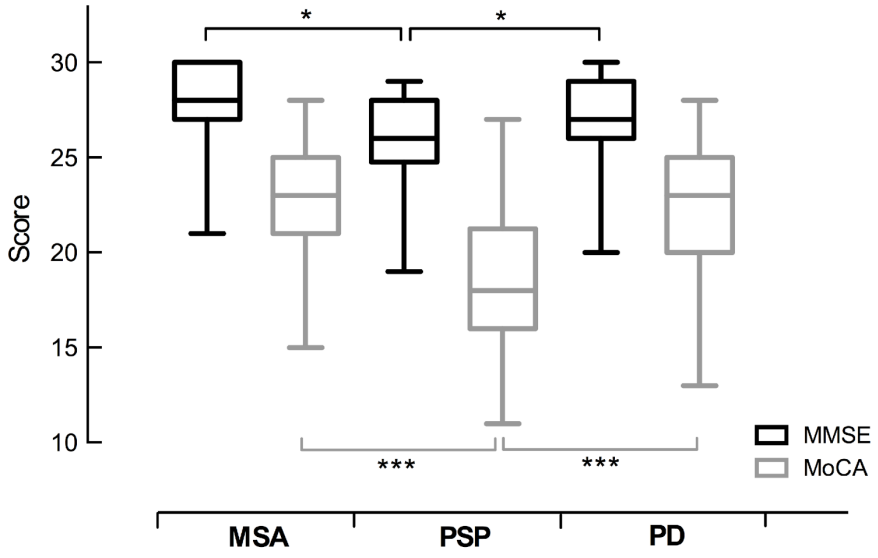


Figure 3.1. Between-group differences for MMSE and MoCA total scores. Box plots show the medians with upper (75th percentile) and lower quartiles (25th percentile). The T-bars (whiskers) that extend from the boxes indicate maximum and minimum values. The *p* values were obtained by one-way analysis of variance. Significant differences Bonferroni-corrected for multiple comparison at: * = $p < 0.05$, *** = $p < 0.0001$. MSA, multiple system atrophy; PSP, progressive supranuclear palsy; PD, Parkinson's disease; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

Table 3.3 Between-group differences for MMSE and MoCA scores

	MSA (n = 35)					PSP (n = 30)					PD (n = 65)					MSA vs. PSP	PSP vs. PD
	Mean (SD)	Mdn	min	max	25 – 75P	Mean (SD)	Mdn	min	max	25 – 75P	Mean (SD)	Mdn	min	max	25 – 75P	*P* value	
MMSE	27.7 (2.4)	28.0	21	30	27 – 30	26.0 (2.9)	26.0	19	29	24.8 – 28	27.3 (2.0)	27.0	20	30	26 – 29	*	*
MoCA	22.9 (3.0)	23.0	15	28	21 – 25	18.2 (3.9)	18.0	11	27	16 – 21.25	22.3 (3.5)	23.0	13	28	20 – 25	***	***
MoCA letter fluency	10.5 (4.8)	10	3	22	7 – 14	6.6 (3.6)	7	2	17	2 – 17	10.1 (4.5)	12	3	21	9 – 14	**	***

Note. The p values were obtained by one-way analysis of variance. MSA, Multiple System Atrophy; PSP, Progressive Supranuclear Palsy; PD, Parkinson's Disease; Mdn, median; Min, minimum score; Max, maximum score; 25-75P, 25-75th percentile; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.
Significant difference Bonferroni-corrected for multiple comparison at: * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$.

Results from backward linear regression analysis showed that in the PSP group 52 percent of MMSE variance was explained by age, LEDD and education, and 34 percent of MoCA variance was explained by age and LEDD (Table 3.4).

In MSA group, 15 percent of MMSE variance was explained by education, while 41 percent of MoCA variance was explained by education associated with age. Instead, in the PD group the only factor that explained the variance in MMSE and MoCA scales was education, accounting for the 12 percent and 16 percent of variance, respectively.

Overall, we noted that there was a tendency for a positive correlation between LEDD and MMSE ($r = 0.373$, $p = 0.051$), and LEDD and MoCA ($r = 0.373$, $p = 0.05$).

Between-group comparisons showed that PSP patients had a mean MoCA score (18.2 ± 3.9) lower than MSA and PD patients (22.9 ± 3.0, $p < 0.00001$; 22.3 ± 3.5, $p < 0.00001$, respectively). While this difference was less significant when comparing PSP mean MMSE score (27.7 ± 2.4) with MSA and PD performances (26.0 ± 2.0, $p = 0.004$; 27.3 ± 2.0, $p = 0.01$, respectively). Further, since we found between-group differences in age (only between MSA and PSP group), disease duration, motor severity and LEDD, we used those variables as covariates. Results from ANCOVA confirmed that PSP had a significantly lower MoCA compared to MSA ($p < 0.001$) and PD ($p < 0.011$), while the difference for MMSE was no more significant when comparing PSP with MSA ($p < 0.064$) and PD ($p < 0.079$).

Figure 3.2 showed the results of the MMSE and MoCA subitems analyses. On the MMSE scale, PSP patients had a lower performance in the 'copy of the pentagons' compared to MSA and PD patients (p = 0.0223; p = 0.0121, respectively).

While on the MoCA scale, the PSP group performed worse than MSA and PD patients in several subitems, respectively: in the 'digit forward task' (50% vs. 80%, p = 0.0223 and 50% vs. 78%, p = 0.0121), 'abstraction task' (37% vs. 71%, p = 0.0125 and 37% vs. 62%, p = 0.0402) and 'letter-fluency' (13% vs. 43%, p=0.0173 and 13% vs. 62%, p = 0.00001). We also found additional differences between the three groups, which are reported in Table 3.5. However, applying Bonferroni correction for multiple comparisons, the significant results were noted only on the following MoCA subitems: namely, in the 'copy of the cube' where PSP performed worse than MSA (corrected p = 0.047) and in the 'letter fluency' where PSP had a highly significant lower performance than PD (corrected p = 0.0012).

Table 3.4 Impact of clinical and demographical variables in explaining MMSE and MoCA score variance

		R^2	R^2-adjusted	Independent variables	Coefficient	Std. Error	*t*	*p* value
MSA				(Constant)	25.204	1.021	24.682	< 0.0001
	MMSE	0.172	0.147	Education	0.241	0.093	2.582	0.015
				(Constant)	33.163	4.659	7.118	< 0.0001
	MoCA	0.449	0.414	Age	-0.219	0.071	-3.098	0.004
				Education	0.381	0.097	3.931	< 0.0001
PSP				(Constant)	36.758	5.542	6.632	< 0.0001
	MMSE	0.587	0.518	Age	-0.233	0.072	-3.214	0.005
				Education	0.377	0.174	2.167	0.044
				LEDD	0.004	0.001	2.836	0.011
				(Constant)	37.316	7.618	4.898	< 0.0001
	MoCA	0.404	0.342	Age	-0.298	0.108	-2.756	0.013
				LEDD	0.005	0.002	2.246	0.037
PD				(Constant)	25.605	0.591	43.328	< 0.0001
	MMSE	0.138	0.124	Education	0.179	0.057	3.174	0.002
				(Constant)	19.038	1.007	18.912	< 0.0001
	MoCA	0.168	0.155	Education	0.343	0.096	3.564	0.001

Note. Backward regression analyses were run including Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) as dependent variable and as independent variables: age, education, disease duration, Movement Disorder Society Unified Parkinson's Disease Rating Scale III (MDS-UPDRS III), Levodopa Equivalent Daily Dose (LEDD) and Dopamine Agonist Equivalent Daily Dose (DAED).

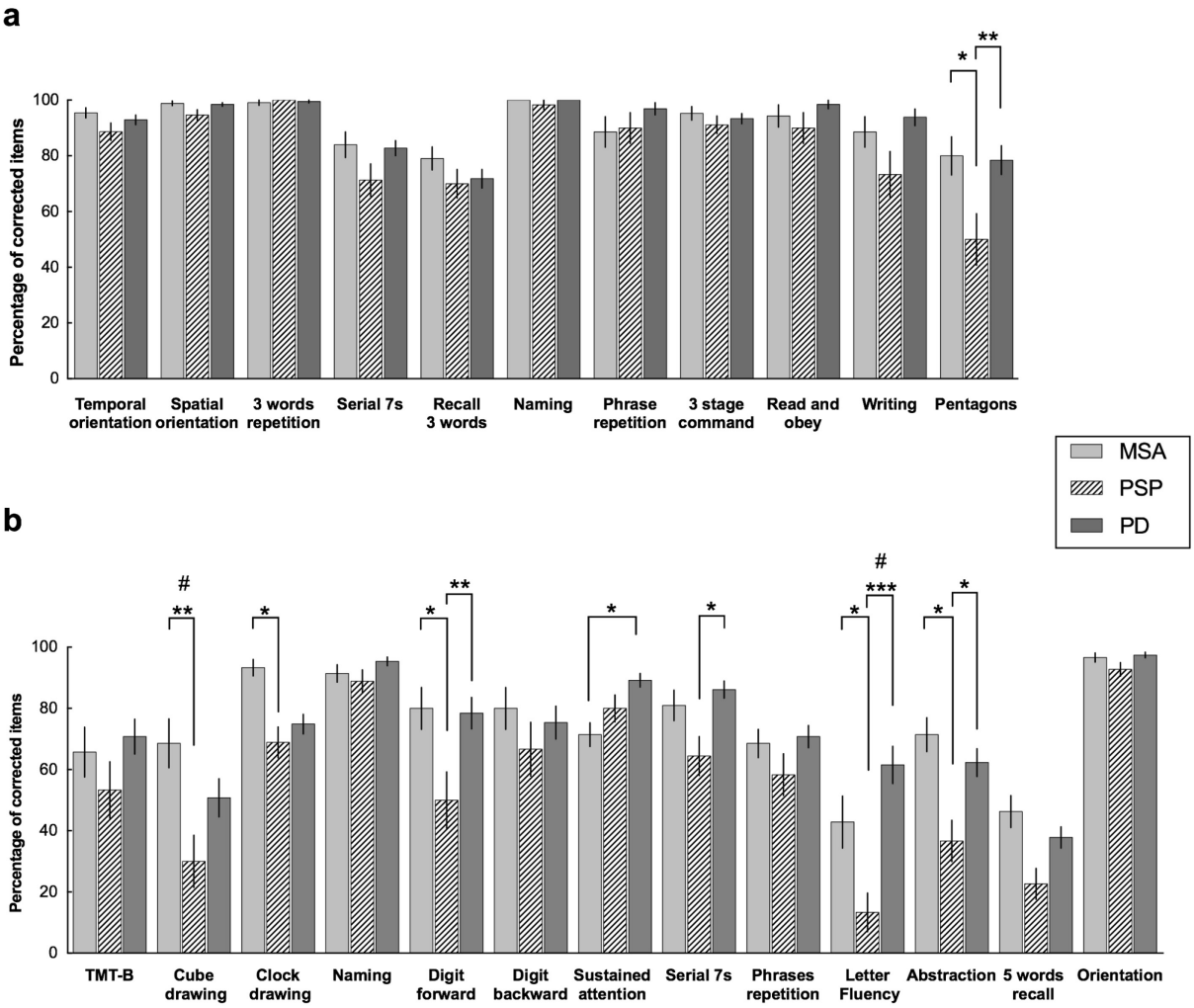


Figure 3.2 Between-group differences (obtained by Pearson's Chi-squared analyses) in MMSE and MoCA subitems. In figure a) performance on MMSE and figure b) performance on MoCA. MSA, multiple system atrophy; PSP, progressive supranuclear palsy; PD, Parkinson's disease; TMT-B, Trail Making Test (part B). * = $p \leq 0.05$ ** = $p \leq 0.01$, *** = $p \leq 0.001$, # Significant after Bonferroni correction $p < 0.05$.

Results of the ROC curve analyses for MMSE and MoCA total scores as well as the MoCA letter fluency subitems are reported in Table 3.6.

MoCA total score and its fluency subitem were able to discriminate PSP from PD patients with AUC exceeding 0.80, as shown in Figure 3.3.

In addition, a MoCA total score equal to 19 was able to differentiate PSP patients from MSA (AUC 0.83, specificity 0.89, sensitivity 0.70) as well as PSP from PD (AUC 0.78, specificity 0.80, sensitivity 0.70). While a cut-off score at the letter fluency subitem equal to 7-words per minute was able to distinguish PSP from MSA patients (AUC 0.75, specificity 0.71, sensitivity 0.70) as well as from PD (AUC 0.82, specificity 0.86, sensitivity 0.70).

Table 3.5. MMSE and MoCA subitems comparison between MSA, PSP and PD groups

MMSE subitems				**MoCA subitems**			
	MSA vs. PSP	**PSP vs. PD**	**PD vs. MSA**		**MSA vs. PSP**	**PSP vs. PD**	**PD vs. MSA**
Temporal orientation	0.6627	0.7969	0.9690	TMT-B	0.4164	0.1385	0.7721
Spatial orientation	0.8269	0.8799	0.6538	Cube	**0.0039**#	0.0905	0.1276
3 words repetition	0.8269	0.4300	0.2921	Clock	**0.0290**	0.7157	0.0532
Serial 7s	0.8269	0.2856	0.8781	Naming	0.8829	0.5239	0.7254
Recall 3 words	0.5844	0.9652	0.5995	Digit forward	**0.0223**	**0.0121**	0.9816
Naming	0.6453	0.8031	0.2921	Digit backward	0.3641	0.5725	0.7517
Phrase repetition	0.7839	0.3521	0.2364	Sustained attention	0.5829	0.3892	**0.0463**
3 stage command	0.8855	0.9407	0.9690	Serial 7s	0.2073	0.0294	0.7137
Read & obey	0.8924	0.2253	0.6353	Phrases repetition	0.5082	0.3091	0.9835
Writing	0.1806	0.1376	0.6172	Letter Fluency	**0.0173**	**< 0.0001**#	0.1069
Pentagons	**0.0223**	**0.0121**	0.9816	Abstraction	**0.0125**	0.0402	0.4959
				5 words recall	0.0943	0.2270	0.5734
				Orientation	0.8655	0.7258	0.5389

Note. P values are reported. Significant differences ($p < 0.05$) are reported in bold type. #Significant values after Bonferroni correction for multiple comparisons ($p < 0.05$). MMSE, Mini-Mental State Examination subitems; MSA, multiple system atrophy; PSP, progressive supranuclear palsy; PD, Parkinson's disease; MoCA, Montreal Cognitive Assessment; TMT-B, Trail Making Test (part B).

Table 3.6 Receiver operating characteristic for between-group comparisons

	Cut-off	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**PPV**	**NPV**
PSP vs. MSA					
MMSE	26*	0.77 (0.60 to 0.90)	0.57 (0.37 to 0.75)	0.68	0.68
MoCA	19*	0.89 (0.73 to 0.97)	0.70 (0.51 to 0.85)	0.84	0.78
MoCA Fluency	7*	0.71 (0.54 to 0.85)	0.70 (0.51 to 0.85)	0.68	0.74
PSP vs. PD					
MMSE	26*	0.74 (0.62 to 0.84)	0.57 (0.37 to 0.75)	0.50	0.79
MoCA	19*	0.80 (0.68 to 0.89)	0.70 (0.51 to 0.85)	0.62	0.85
MoCA Fluency	7*	0.86 (0.75 to 0.94)	0.70 (0.51 to 0.85)	0.70	0.86
PD vs. MSA					
MMSE	28	0.46 (0.29 to 0.63)	0.72 (0.60 to 0.83)	0.71	0.47
MoCA	20	0.77 (0.60 to 0.90)	0.32 (0.21 to 0.45)	0.72	0.38
MoCA Fluency	10	0.54 (0.37 to 0.69)	0.61 (0.47 to 0.74)	0.66	0.49

Note. MSA, multiple system atrophy; PSP, progressive supranuclear palsy; PD, Parkinson's disease; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PPV, positive predictive value; NPV, negative predictive value. * Tests that survived post-hoc DeLong test threshold $p < 0.005$ (DeLong et al. 1988), after Bonferroni correction.

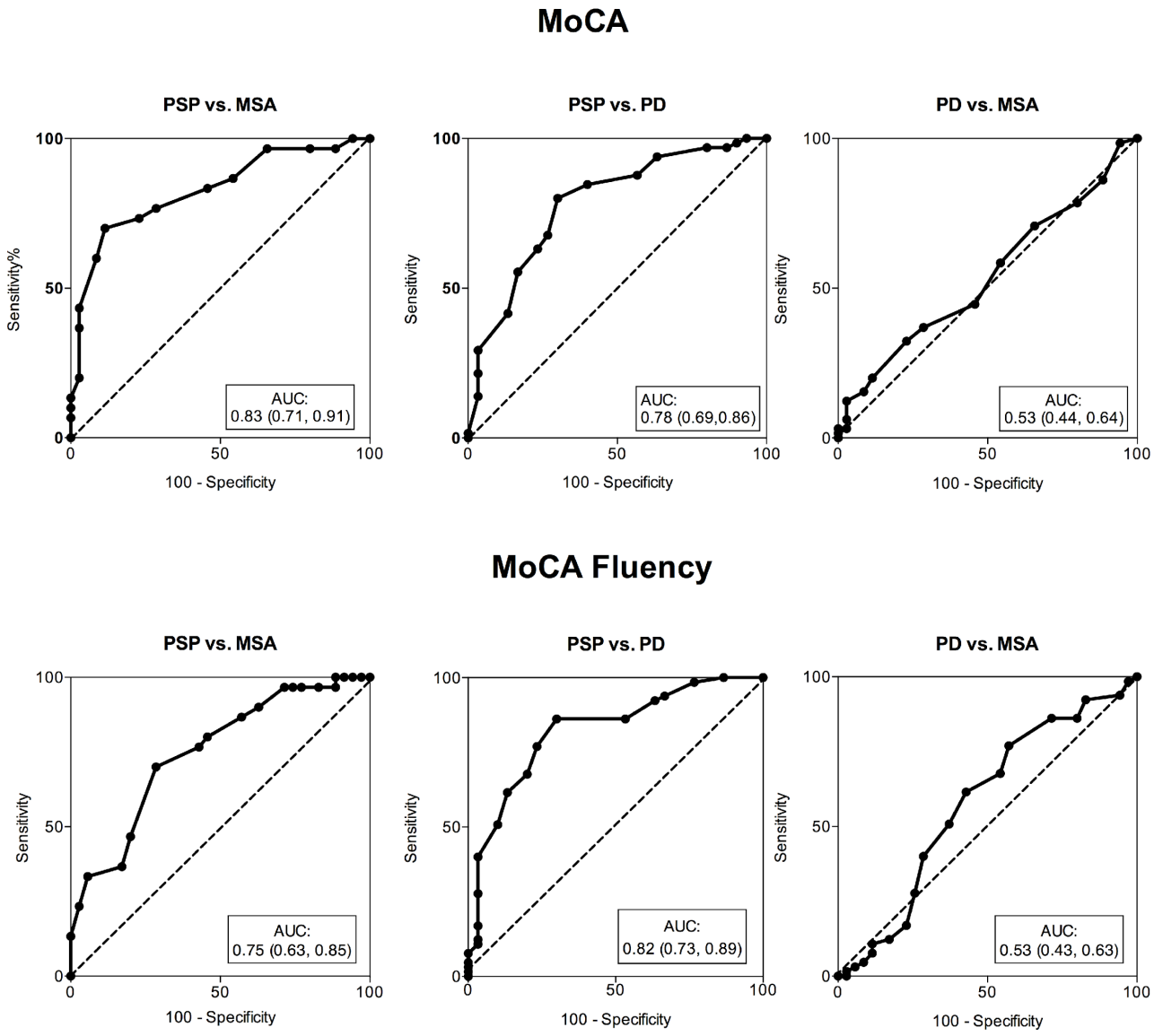


Figure 3.3 Receiver operating characteristic (ROC) curves distinguishing between diagnostic groups using MoCA and its letter fluency subitem. Confidence intervals are shown for sensitivity and specificity. MSA, multiple system atrophy; PSP, progressive supranuclear palsy; PD, Parkinson's disease; AUC, Area Under the Curve; MoCA, Montreal Cognitive Assessment.

3.4 Discussion

Our multicenter study showed that MoCA is more sensitive than the commonly used MMSE in detecting cognitive alterations in atypical parkinsonisms, especially in the PSP group. This is aligned with previous evidence showing that MMSE is relatively insensitive to the attentive and executive deficits, which are the most affected cognitive domains in MSA and PSP patients (Rittman et al., 2013; Siri et al., 2013). Indeed, previous studies

showed the superiority of other brief cognitive scales compared to MMSE, in detecting cognitive changes in MSA and PSP — namely, the ACE-R, the FAB and the DRS (Aarsland, 2003; Bak et al., 2005a; Paviour et al., 2005; Rittman et al., 2013; Siri et al., 2013). Our findings showed the presence of a ceiling effect in the performance of MMSE and this effect was noted in all the three groups, the 25th to 75th percentile in MSA was 27 to 30, in PSP 19 to 29, and 20 to 30 in the PD group. While MoCA did not show a ceiling effect, this was probably due to the fact that MoCA includes also the assessment of attentive and executive domains. Noteworthy, MoCA scores were distributed in a wider range than the MMSE scores, and did not show floor effect. This suggests that MoCA is more challenging than MMSE and overall clinically more meaningful and reliable in the assessment of general cognition in PSP as well as MSA.

Dysfunctions on the executive and attention/working-memory domain are frequently reported in parkinsonian disorders, and characterize the neuropsychological profile of MSA and PSP (Auzou et al., 2015; Dujardin et al., 2003; Gerstenecker, 2017; Lange et al., 2003). Based on MoCA subitems analyses, overall, we found a tendency of PSP patients to perform worse than MSA and PD in tasks of the executive (i.e., 'clock drawing, 'letter fluency' and 'abstraction' subitem), and attentive/working-memory domains (i.e., 'sustained attention', 'digit forward' and 'calculation' subitem).

According to previous evidence, visuospatial dysfunctions have been reported in PSP patients (Borroni et al., 2008; Cordato, Halliday, Caine, & Morris, 2006; Nichelli & Magherini, 2005). This is in line with our findings, since we found a trend for PSP patients to have a worse performance than MSA and PD, in the MoCA 'cube drawing' task and in the MMSE subitem of the 'bisecting pentagons'. Interestingly, Cordato and others (2006) noted that PSP patients were frequently impaired in the 'copy of the bisecting pentagons,' where the most common error was the incorrect positioning of the right pentagon. However, these findings should be considered cautiously, since in these visuospatial tasks, motor involvement as well as oculomotor scanning are required and thus, it is possible that specific clinical features of PSP (e.g., vertical gaze palsy) hamper the execution of these visuospatial tasks (Bak et al., 2006).

Another important observation is that our study underlined the presence of prominent cognitive alterations, particularly in PSP patients. Interestingly, PSP were severely impaired on the letter fluency and we found that this task was able to differentiate PSP from PD patients with a high specificity and moderate sensitivity (Lange et al., 2003; Rittman et al.,

2013; Soliveri, 2000). Letter fluency is impaired also in MSA (Pillon et al., 1995), and PD (Henry & Crawford, 2004; Lange et al., 2003), but their performance is not impaired as in PSP patients. The letter fluency task requires producing words beginning with a given letter in one minute; thus, it is a demanding task and its execution requires the involvement of a wide network, including the anterior–frontostriatal pathway to retrieve the words from the semantic network in the posterior regions (medial temporal and parietal cortex) (Koziol & Budding, 2009). The frontal cortex has a crucial role in the execution of letter fluency task. Indeed, previous evidence showed that patients with a brain lesion in the left dorsolateral and/or striatal region had an impaired fluency (Stuss et al., 1998). Hence, we hypothesize that the poor performance of PSP is possibly associated with a broader distribution of pathology, with a consistent involvement of cortical regions, compared to MSA and PD (Bak et al., 2005a).

Our results provide additional support for superiority of MoCA over MMSE, and we recommend the use of MoCA, particularly of its letter fluency subitem, as seven or fewer F-words per minute would support a diagnosis of PSP, at least the Richardson's syndrome subtype (Burrell et al., 2014; Rittman et al., 2013). Noteworthy, the same letter fluency cut-off score is valid not only in the differential diagnosis with PD, but also between MSA and PSP. Thus, we recommend the use of MoCA in the clinical routine assessment of atypical parkinsonisms, as it could be a useful tool in the diagnostic process, especially in conjunction with other clinical features (Lee et al., 2012).

Several studies highlighted the presence of cognitive deficits in MSA, which have a reduced magnitude compared to those in PD and PSP patients (Gerstenecker, 2017; Lee et al., 2012; Meco et al., 1996; Soliveri, 2000). However cognitive impairments are still considered as exclusion criteria on the current consensus criteria for the MSA diagnosis (Gilman et al., 2008). Further our results add to the view that MSA is associated with cognitive alterations and that current criteria need to be revised, considering the presence of cognitive alterations in MSA.

Taken together our findings show MoCA is brief, easy to administer (requires about 10 minutes) and provides important clinical information, particularly in the context of the differential diagnosis. General cognitive testing is crucial in the diagnostic work up of atypical parkinsonisms, even though in this study, our patients had no pathological confirmation and patients with probable diagnosis were included.

In addition, we found that age and education contribute to explain MMSE and MoCA performance, but this is not surprising as we used uncorrected data for both scales since age and education specific normative data were unavailable for the Romanian subsample.

There are other limitations in our multicenter study: first, the sample size was relatively small, due to the rare nature of the investigated disorders. Then, the disease duration was not matched across the three diseases, as we preferred to match patients for age, sex and education. In addition, we included PSP patients in a moderate to advanced stage of the disease and patients of the more frequent phenotype (i.e., PSP-RS), which makes our results not generalizable to other PSP variants, like the parkinsonian variant. However, the features and the extent of the degenerative process are similar in all PSP manifestations, and possibly the underlying mechanisms of cognitive impairments of our population can be applied also to the other phenotypes. Lastly, we did not include other screening cognitive scales (i.e., ACE-R, DRS, and FAB) and further studies can be necessary to assess whether these tools would be better suited than MoCA in the assessment of cognitive changes in these disorders. Although our findings suggest that a simple, brief and easy to administer test – such as the letter fluency task − can be sufficient to differentiate PSP from MSA and PD.

To conclude, in the present multicenter study we found that MoCA is a brief cognitive scale able to test cognitive abilities in atypical parkinsonisms. Further, we strongly suggest the use of phonemic fluency task since seven or fewer words per minute would support the PSP diagnosis. Additional studies are needed to assess the sensitivity of MoCA and of letter fluency in the early phases of the disease and to validate them in longitudinal studies.

CHAPTER 4

PROSPECTIVE ASSESSMENT OF COGNITIVE DISFUNCTIONS IN PARKINSONIAN DISORDERS[2]

4.1 Introduction

Similar to PD, atypical parkinsonisms were primarily classified as motor disturbances; indeed, the hallmarks of MSA and PSP are characterized by a combination of motor, cognitive and behavioral symptoms (Gerstenecker, 2017).

Cognitive deficits were initially categorized as 'subcortical dementia' (Albert et al., 1974; Brown & Marsden, 1988), as patients present mainly fronto-executive abnormalities (Brown et al., 2010) different from the Alzheimer's-like pattern consisting of 'genuine amnesia' or abnormal instrumental functions (e.g., agnosia and apraxia) (Pillon et al., 1996). Although cognitive alterations are now recognized features in parkinsonian disorders, the specific neuropsychological profile for each disorder is poorly characterized as well as their neuropathological underpinnings. The different distribution of pathology could determine distinct neuropsychological profiles as well as specific characteristics of motor and clinical symptoms (Bak et al., 2005a; Pillon et al., 1996).

Clinical neuropsychology can therefore contribute to better diagnostic accuracy among parkinsonian diseases (Hughes, Daniel, Ben-Shlomo, & Lees, 2002; Joutsa et al., 2014).

In PD, cognitive impairment is characterized by heterogeneous manifestation during the disease's course, ranging from normal cognition, through MCI to frank dementia (Biundo et al., 2016a). Impairment in fronto-executive functions has been described since the early stage of the disease, while deficits in visuospatial and semantic memory abilities seem to be highly sensitive in detecting transition to dementia (Kehagia et al., 2010; Williams-Gray et al., 2009). Prevalence of dementia in PD has been reported in approximately 80 percent after 15 to 20 years of disease (Halliday et al., 2008).

[2] *Part of this study has been published: Fiorenzato, E., Antonini, A., Wenning, G.,* & *Biundo, R. (2017). Cognitive impairment in multiple system atrophy. Movement Disorders 32(9), 1338-1339.*

Regarding atypical parkinsonisms, cognitive impairments are prominent and primary features of PSP patients. Overall cognitive dysfunctions and behavioral abnormalities are more frequent and severe in PSP than in MSA (Gerstenecker, 2017), and evidence suggests that this reflects a different distribution underlying pathology (Bak et al., 2005a). Interestingly, a previous study conducted with PSP, reported that the frequency of dementia, described as subcortical-frontal, increased greatly from 38 to 70 percent after a 15-month follow-up (Dubois & Pillon, 2005). For MSA patients there are contradictory results: the current consensus criteria for MSA diagnosis consider dementia as non-supporting feature, while a few studies reported dementia in some cases (Stankovic et al., 2014).

Although clinical and research experience suggests that cognitive impairments in parkinsonian disorders are progressive, there are only a few longitudinal studies in the literature, which investigated the progression in atypical parkinsonisms compared to PD (Dubois & Pillon, 2005; Rittman et al., 2013; Soliveri, 2000). Furthermore, these previous studies are based on screening scales or on brief neuropsychological assessments that did not extensively investigate the full spectrum of cognitive abilities (attention/working-memory, executive, memory, visuospatial and language domains).

Although clinical criteria for MCI and dementia in PD have been formulated (Dubois et al., 2007; Litvan et al., 2012), it remains unclear whether similar criteria might be applied also for atypical parkinsonisms (Marras et al., 2014).

Based on these considerations, the aims of the present study are:

1) To assess the severity of cognitive dysfunctions in PSP and MSA patients using PD criteria for cognitive statuses;
2) To investigate the sensitivity of two widely used cognitive screening instruments, the MMSE and prospectively MoCA, in differentiating MSA, PSP and PD global cognitive profile;
3) To characterize the progression of cognitive decline on the five cognitive domains, behavioral features and to compare the 15-month follow-up profile across the parkinsonian diseases.

4.2 Materials and methods

4.2.1 Study population

Eighteen PSP and 12 MSA patients referred consecutively, and 30 PD patients matched for age, education and sex were evaluated at baseline and at a mean of 15-month follow-up (range 12−18 months). Patients were recruited at the Parkinson's Disease and Movement Disorders Unit, San Camillo Hospital IRCCS, Venice, Italy, from June 2012 to August 2017. All PD patients fulfilled the Queen Square Brain Bank criteria for a diagnosis of probable PD (Gelb et al., 1999), and standard diagnostic criteria were applied for the diagnosis of probable MSA and probable PSP patients (Gilman et al., 2008; Höglinger et al., 2017). PSP patients were all of Richardson's syndrome phenotype. The exclusion criteria of this study were the presence of: 1) only one cognitive and motor evaluation at baseline, without a follow-up assessment, 2) deep brain stimulation, and 3) psychiatric or neurological comorbidity. Patients gave written informed consent (according to the Declaration of Helsinki) before study enrollment, and ethical approval was obtained from the Venice Research Ethics Committee, Venice, Italy.

4.2.2 Clinical and neuropsychological assessment

Demographic variables (i.e., age, education, and sex), and clinical characteristics (i.e., disease duration, age of onset, and motor symptoms) were collected by neurologists with experience in movement disorders.

Motor function was assessed using the MDS-UPDRS: namely, motor aspects interfering with daily living were assessed by MDS-UPDRS II and motor symptoms by means of the motor section (MDS-UPDRS III). LEDD and DAED were calculated (Tomlinson et al., 2010).

With regard to cognitive evaluation, we selected a comprehensive neuropsychological battery specifically designed to target cognitive deficits in PD, according to Level II criteria (Dubois et al., 2007; Litvan et al., 2012; Marras et al., 2014) (see also Chapter 2 for further details). We also applied these criteria to MSA and PSP since there are no published criteria for atypical parkinsonisms.

The neuropsychological tests were performed on two separate occasions within 5 to 7 days and administered in the morning ON medication.

The cognitive battery included MMSE and MoCA to assess general cognitive functions, Attention and working memory domain was tested with the Trail Making Test part B-A (TMT B-A) (Giovagnoli et al., 1996) and Digit Span Sequencing (DSS) of Wechsler Adult Intelligence Scale–Fourth Edition (WAIS–IV) (Wechsler, 2008). Executive functions were evaluated with the Stroop Color and Word test, phonemic fluency, WAIS-IV similarities, and the Clock Drawing Test (CDT) (Caffarra et al., 2011; Caffarra, Vezzadini, Dieci, Zonato, & Venneri, 2002; Novelli, Papagno, Capitani, & Laiacona, 1986b; Wechsler, 2008). Memory was assessed with the delayed recall of Rey-Osterrieth complex figure test (ROCF)(Caffarra et al., 2002), word paired associated task, and prose memory tests (Novelli, Papagno, Capitani, & Laiacona, 1986a). Language was tested with the semantic fluency task, and Novelli's naming test (Novelli et al., 1986b). Visuospatial and visuoperceptive functions were assessed by Benton's JLO (Gullett et al., 2013), VOSP incomplete letters recognition subtask (Warrington & James, 1991), and a copy of ROCF (Caffarra et al., 2002).

We assessed the presence of depression, anxiety, apathy and quality of life using the Beck Depression Inventory (BDI-*II*), State-Trait Anxiety Inventory forms (STAI Y-1 to assess state anxiety, STAI Y-2 to assess trait anxiety), Starkstein Apathy Scale and an 8-item version of the Parkinson's Disease Questionnaire for quality of life (PDQ-8), respectively (Yamanishi et al., 2013). All the test scores were adjusted for age and education, according to the specific norm available.

Subjective cognitive complaints and their impact on daily functioning were assessed during the clinical interview using the PD-CFRS (Kulisevsky et al., 2013), as well functional autonomy was evaluated with ADL and IADL scales (Katz, 1983).

First, z-scores were calculated for each test and subject, based on standardized, published Italian normative data. We classified patients as having MCI if the z-score for a given test was at least -1.5 SD below appropriate norms on two tests within a single cognitive domain or at least one test in two or more cognitive domains (Litvan et al., 2012). Presence of dementia was assessed based on the MDS Task Force recommendations (Dubois et al., 2007), which included cognitive examination, functional autonomy and neuropsychiatric assessment. Patients without cognitive alterations were defined as having a 'normal cognition.'

Then, we defined the percentage of impaired subjects for each cognitive domain, when they had a performance below -1.5 SD.

Since our neuropsychological battery had more than two tests per cognitive domain, for diagnostic classification purpose (of cognitive statuses), we excluded the exceeding tests, and the tests whose performance could be hampered by motor deficits (i.e., ROCF and CDT) since atypical parkinsonisms usually showed severe motor impairment. Table 4.1 shows the neuropsychological tests used and their associated norms for each cognitive domain.

The outcomes of the clinical assessment and the extensive neuropsychological battery were compared at baseline as well as at follow-up.

Table 4.1 Neuropsychological tests and their associated norms for each cognitive domain

Cognitive Domains	Neuropsychological tests and their associated norms
Executive	Stroop Color/ Word test (Caffarra et al., 2002)
	Similarities (WAIS-IV) (Wechsler, 2008)
Attentive/ working memory	Trail Making Test part B-A (TMT B-A) (Giovagnoli et al., 1996)
	Digit Span Sequencing (DSS) (WAIS–IV) (Wechsler, 2008)
Memory	Word paired associated task (WPAT) (Novelli et al., 1986a)
	Prose memory test (Delayed recall) (Novelli et al., 1986a)
Visuospatial	Incomplete letters subtask (VOSP) (Warrington & James, 1991)
	Benton's Judgment of Line Orientation Test (Benton- JLO) (Gullett et al., 2013)
Language	Semantic fluency task (Novelli et al., 1986b)
	Novelli's Naming test (Novelli et al., 1986b)

Note. WAIS IV, Wechsler Adult Intelligence Scale—Fourth Edition; VOSP, Visual Object and Space Perception.

4.2.3 Statistical analyses

All statistics were performed using SPSS version 20 (IBM SPSS, Chicago, IL) and statistical significance threshold was set at $p < 0.05$.

Between-group comparisons were run with the non-parametric Kruskal-Wallis analysis of variance, ANOVA; and post hoc comparisons, followed by Bonferroni correction for multiple comparisons, were applied when appropriate. Within-group comparisons were

performed using the non-parametric Mann–Whitney *U* test and categorical variables were analyzed using Pearson's Chi-squared test.

To investigate changes over time on MMSE and MoCA performance, we calculated the percentage change of total score as well as of the subitems. Subitems scores were normalized, dividing the scores of each subitem by its maximum score.

4.3 Results

4.3.1 Demographic and clinical characteristics at baseline

As reported in Table 4.2, the PD cohort was matched for age, sex and education with MSA and PSP groups. PD patients had longer disease duration than PSP and MSA, and a tendency to show less severe motor impairments (as assessed by MDS-UPDRS III), even though this difference was not significant after correction for multiple comparisons (PD vs. PSP, p = 0.064; PD vs. MSA p = 0.052). There were no differences in levodopa and dopamine-agonist dose.

Table 4.2. Baseline demographic and clinical characteristics of PD, MSA and PSP groups

	PD (n=30)	**MSA** (n=12)	**PSP** (n=18)	*p value*
Age, y	68.40 (7.02)	65.08 (4.85)	70.00 (6.82)	0.076
Education, y	9.10 (4)	8.92 (4.58)	10.39 (4.79)	0.496
Sex (m/f)	23/7	5/7	11/7	0.091
C/P subtypes	na	4/8	na	na
Disease Duration, y	8.63 (4.78)#§	3.92 (2.02)#	5.11 (3.18)§	**0.001**
MDS-UPDRS II	13.43 (7.76)	19.00 (10)	19.33 (8.52)	0.101
MDS-UPDRS III	25.30 (12.9)	40.64 (20.34)	38.53 (16.24)	**0.018**
MDS-UPDRS Tot	57.93 (28.78)	61.36 (30.64)	57.93 (23.26)	0.937
LEDD	798.41 (586.81)	625.85 (274.14)	518.58 (405.58)	0.248
DAED	111.62 (115.04)	78.17 (100.73)	45.28 (71.05)	0.248

Note. Values are means and SD. Post hoc comparison adjusted by the Bonferroni correction for multiple tests: p<0.05; #PD vs. MSA, §PD vs. PSP. PD, Parkinson's disease; MSA, multiple system atrophy; PSP progressive supranuclear palsy; C, cerebellar subtypes; P, parkinsonian subtypes; na, not applicable; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; LEDD, levodopa equivalent daily dose; DAED, dopamine agonist equivalent dose.

4.3.2 Neuropsychological and behavioral assessment at baseline

PSP group performed significantly worse than PD patients at the MoCA (p = 0.024), Stroop test (time), phonemic and category fluencies. We found no differences in the other cognitive tests (Table 4.3). The most impaired domain in PSP patients was the executive domain — 44 percent of patients had a performance below normality. Regarding the behavioral outcomes, the PSP group was characterized by reduced functional autonomy (ADL), more apathy and depression when compared to PD.

Table 4.3 Between-group comparisons at baseline evaluation of behavioral and neuropsychological measures across PD, MSA and PSP groups

	PD	**MSA**	**PSP**	*p value*
Behavioral measures				
ADL	5.67 (0.61)	5.00 (1.41)	4.47 (1.74)	**0.017**§
IADL	5.00 (1.74)	5.75 (1.96)	4.76 (2.11)	0.306
PD-CFRS	2.96 (3.55)	2.38 (1.77)	4.54 (4.91)	0.333
PDQ-8	9.07 (5.95)	13.00 (6.81)	11.07 (4.98)	0.220
Apathy scale	14.91 (6.10)	16.14 (5.76)	21.82 (7.86)	**0.048**§
STAI Y-1	38.57 (11.60)	40.90 (8.17)	39.07 (7.34)	0.544
STAI Y-2	41.04 (12.46)	44.91 (8.91)	45.73 (10.58)	0.514
BDI-*II*	10.17 (7.21)	14.25 (7.30)	18.06 (10.35)	**0.024**§
Neuropsychological assessment				
MMSE	26.41 (2.20)	27.25 (1.89)	24.12 (4.18)	0.061
MoCA	23.33 (3.73)	23.33 (2.64)	21.00 (3.14)	**0.025**§
ROCF- copy	29.27 (6.38)	31.14 (5.14)	24.69 (8.54)	0.052
ROCF - delayed	12.60 (5.96)	15.77 (5.70)	11.09 (3.61)	0.100
WPAT	8.88 (2.86)	10.54 (3.00)	10.00 (3.41)	0.265
TMT B-A	206.80 (175.15)	183.17 (157.62)	235.69 (186.47)	0.759
STROOP (Time)	31.21 (17.30)	27.94 (11.40)	53.08 (25.10)	**0.013**§
STROOP (Errors)	2.83 (4.87)	2.50 (3.66)	6.50 (5.33)	0.062
Phonemic Fluency	29.41 (8.71)	28.83 (10.49)	19.18 (8.72)	**0.003**§
Semantic Fluency	33.17 (6.45)	37.92 (8.23)	26.94 (10.51)	**0.021**§
Similarities (WAIS-IV)	17.48 (4.37)	18.67 (3.55)	16.38 (3.69)	0.335
DSS- SPAN (WAIS-IV)	4.72 (1.56)	5.58 (1.24)	5.07 (1.16)	0.250
Prose Memory Test - immediate	9.77 (4.48)	10.83 (4.24)	9.88 (5.01)	0.730
Prose Memory Test - delayed	12.55 (5.22)	12.75 (4.61)	11.50 (5.05)	0.724
Clock- CDT	12.70(2.56)	13.58 (1.56)	11.31 (3.14)	0.095
VOSP	17.13 (3.33)	18.75 (0.97)	17.00 (2.90)	0.168
Benton - JLO	22.08 (5.36)	21.00 (7.01)	19.69 (6.87)	0.605
Naming Task Novelli	30.13 (2.40)	30.89 (1.54)	30.08 (1.61)	0.406
Subjects with an impaired performance per domain				
Attention/ working memory	13%	8%	25%	
Executive	33%	17%	44%	
Memory	17%	0%	19%	
Visuospatial	33%	8%	31%	
Language	10%	8%	12%	

Note. Significant values are in bold type. Post hoc comparison adjusted by the Bonferroni correction for multiple tests: p<0.05; §PD vs. PSP. PD, Parkinson's disease; PSP, progressive supranuclear palsy; MSA, multiple system atrophy; BDI-II, Beck Depression Inventory-II; State-Trait Anxiety Inventory (STAI Y-1, Y-2); MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ROCF, Rey–Osterrieth complex figure; WPAT, Word Paired associated task; TMT, Trail Making Test; DSS, Digit Span Sequencing; WAIS IV, Wechsler Adult Intelligence Scale—Fourth Edition; CDT, Clock Drawing task; VOSP, Visual Object and Space Perception; JLO, Judgment of Line Orientation Test.

4.3.3 Clinical, behavioral and neuropsychological assessment at 15-month follow-up

At the second assessment, the three groups differed significantly on disease severity (as assessed by MDS-UPDRS III), and the post hoc comparison analysis revealed that MSA and PSP were more impaired than PD ($p = 0.012$ and 0.007, respectively). Motor aspects also affect functional autonomy; indeed, MDS-UPDRS II was significantly different between MSA and PSP compared to the PD group ($p = 0.002$ and 0.017, respectively). This is in line also with the ADL and IADL score, where PSP patients showed a lower score in comparison to PD.

Interestingly, functional autonomy in PSP was not associated only with motor impairments but also with cognition deficits, since PSP patients showed a higher score at PD-CFRS compared to MSA patients ($p = 0.032$) and there was a trend also in comparison to PD ($p = 0.092$), suggesting that cognitive alterations in PSP patients could interfere with their functional autonomy.

These results agreed also with the PDQ-8 score, in which MSA and PSP ($p = 0.029$ and $p = 0.051$, respectively) reported a poor quality of life in comparison to PD. In addition, PSP patients reported more apathy and anxiety in comparison to PD.

From a cognitive perspective, the between-group comparison showed again that PSP patients had a worse performance compared to PD on Stroop tests (error), phonemic and category fluencies (Table 4.4). As well, PSP had a lower score on MoCA scale when compared to PD and MSA (even though this difference did not reach significance after multiple comparisons, $p = 0.079$ and $p = 0.060$, respectively). The most impaired domain was the executive domain, with 65 percent of PSP patients below normality.

Table 4.4 Between-group comparisons at 15-month follow-up of behavioral and neuropsychological measures across PD, MSA and PSP groups.

	PD	**MSA**	**PSP**	*p value*
Clinical measures				
MDS-UPDRS-II	12.52 (8.78)	26.77 (13.34)	24.82 (12.06)	**0.002**§,#
MDS-UPDRS-III	25.90 (10.81)	48.89 (23.11)#	46.18 (16.91)§	**0.001**§,#
LEDD	818.74 (493.49)	698.52 (460.05)	542.84 (363.03)	0.143
DAED	112.22 (96.18)	59.00 (79.83)	41.56 (60.32)	**0.026**§
Behavioral measures				
ADL	5.33 (0.88)	4.00 (2.14)	3.00 (2.00)	**<0.001**§
IADL	4.53 (1.43)	3.91 (2.39)	3.17 (2.07)	**0.041**§
PD-CFRS	4.48 (4.31)	2.80 (2.90)	9.41 (7.95)	**0.022***
PDQ-8	8.62 (5.59)	14.00 (5.73)	13.44 (5.23)	**0.009**#
Apathy scale	15.62 (5.04)	17.70 (6.60)	23.33 (6.52)	**0.003**§
STAI Y-1	35.24 (9.68)	37.36 (5.26)	39.25 (10.19)	0.289
STAI Y-2	37.75 (10.41)	45.00 (11.36)	46.67 (7.51)	**0.041**§
BDI-*II*	9.90 (6.13)	15.82 (11.05)	13.53 (8.37)	0.167
Neuropsychological assessment				
MMSE	25.46 (1.99)	26.10 (2.08)	23.71 (5.15)	0.445
MoCA	22.57 (3.81)	23.55 (2.98)	18.78 (5.57)	**0.031**
ROCF- copy	27.03 (5.68)	24.95 (4.70)	20.88 (11.38)	0.174
ROCF - delayed	11.88 (6.58)	14.50 (4.47)	8.75 (6.61)	0.076
WPAT	9.98 (4.74)	10.68 (3.93)	10.00 (3.91)	0.921
TMT B-A	271.83 (195.46)	235.10 (197.18)	318.47 (211.87)	0.561
STROOP (Time)	32.99 (16.31)	32.30 (15.46)	48.63 (28.45)	0.155
STROOP (Errors)	3.48 (6.33)	2.80 (4.57)	10.97 (11.11)	**0.039**§
Phonemic Fluency	28.64 (9.16)	27.36 (10.13)	18.94 (10.38)	**0.006**§
Semantic Fluency	33.30 (9.45)	35.09 (10.52)	25.76 (11.33)	**0.030**§
Similarities (WAIS-IV)	16.38 (5.45)	19.00 (3.22)	17.12 (5.38)	0.281
DSS- SPAN (WAIS-IV)	4.55 (1.27)	5.17 (1.19)	4.06 (1.39)	0.075
Prose Memory Test - immediate	8.92 (4.52)	10.36 (3.20)	8.94 (5.78)	0.647
Prose Memory Test - delayed	10.73 (5.77)	12.09 (3.48)	10.59 (6.22)	0.622
Clock- FD	12.17 (2.21)	12.90 (1.60)	10.29 (3.31)	0.069
VOSP	17.21 (3.23)	17.83 (1.90)	16.56 (3.85)	0.811
Benton - JLO	20.00 (7.06)	19.60 (4.40)	16.38 (6.68)	0.304
Naming Task Novelli	29.79 (2.57)	30.73 (0.79)	29.18 (3.00)	0.612
Subjects with an impaired performance per domain				
Attention/ working memory	20%	25%	31%	
Executive	33%	18%	65%	
Memory	27%	9%	29%	
Visuospatial	37%	17%	38%	
Language	13%	0%	35%	

Note. Significant values are in bold type. Post hoc comparison and adjusted by the Bonferroni correction for multiple tests: p<0.05; §PD vs. PSP, #MSA vs. PSP, * PSP vs. MSA. PD, Parkinson's disease; PSP, progressive supranuclear palsy; MSA, multiple system atrophy; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; LEDD, levodopa equivalent daily dose; DAED, dopamine agonist equivalent dose; BDI-II, Beck Depression Inventory-II; State-Trait Anxiety Inventory (STAI Y-1, Y-2); MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ROCF, Rey–Osterrieth complex figure; WPAT, Word Paired associated task; TMT, Trail Making Test; DSS, Digit Span Sequencing; WAIS IV, Wechsler Adult Intelligence Scale—Fourth Edition; CDT, Clock Drawing task; VOSP, Visual Object and Space Perception; JLO, Judgment of Line Orientation Test.

4.3.4 Sensitivity to motor and cognitive change after 15-month follow-up

We further investigated which clinical and neuropsychological tests were the most sensitive in detecting a change at 15-month follow-up (Table 4.5). The within-group comparison analysis (follow-up vs. baseline) showed that the MDS-UPDRS-II was able to detect a change both in PSP and MSA group. MDS-UPDRS-III did not change significantly after 15 months, although there was a strong trend within the PSP group to show a higher motor severity ($p = 0.050$). ADL and IADL scores decreased significantly for each group: in addition, PD patients showed also a significant change in the PD-CFRS (2.96 ± 3.55 vs. 4.48 ± 4.31), only seen as a strong trend in the PSP group probably due to the small sample size (4.54 ± 4.91 vs. 9.41 ± 7.95; $p = 0.052$).

With regard to the cognitive assessment, within the PD-group the most sensitive tests to detect cognitive change over time were the MMSE scale, the ROCF-copy, TMT B-A and the CDT. While in the MSA, the only test that was able to detect a change was the ROCF-copy. Finally, in the PSP-group, MoCA was the most sensitive scale, together with the ROCF-delayed recall, TMT B-A, category fluency, DSS-span and Benton's JLO.

Table 4.5 Neuropsychological, clinical and behavioral measures most sensitive to cognitive decline at 15-month follow-up for each group

	Mean score at baseline and follow-up								
	PD			**MSA**			**PSP**		
	Baseline	Follow-up	*p* value	Baseline	Follow-up	*p* value	Baseline	Follow-up	*p* value
Clinical measures									
MDS-UPDRS-II	13.43 (7.76)	12.52 (8.78)	ns	19.00 (10.00)	26.77 (13.34)	**0.012**	19.33 (8.52)	24.82 (12.06)	**0.039**
MDS-UPDRS-III	25.30 (12.90)	25.90 (10.81)	ns	40.64 (20.34)	48.89 (23.11)	ns	38.53 (16.24)	46.18 (16.91)	0.050
LEDD	798.41 (586.81)	818.74 (493.49)	ns	625.85 (274.14)	698.52 (460.05)	ns	518.58 (405.58)	542.84 (363.03)	ns
DAED	111.62 (115.04)	112.22 (96.18)	ns	78.17 (100.73)	59.00 (79.83)	ns	45.28 (71.05)	41.56 (60.32)	ns
Behavioral measures									
ADL	5.67 (0.61)	5.33 (0.88)	**0.004**	5.00 (1.41)	4.00 (2.14)	**0.043**	4.47 (1.74)	3.00 (2.00)	**0.002**
IADL	5.00 (1.74)	4.53 (1.43)	**0.038**	5.75 (1.96)	3.91 (2.39)	**0.011**	4.76 (2.11)	3.17 (2.07)	**0.002**
PD-CFRS	2.96 (3.55)	4.48 (4.31)	**0.022**	2.38 (1.77)	2.80 (2.90)	ns	4.54 (4.91)	9.41 (7.95)	ns
Neuropsychological assessment									
MMSE	26.41 (2.20)	25.46 (1.99)	**0.042**	27.25 (1.89)	26.10 (2.08)	ns	24.12 (4.18)	23.71 (5.15)	ns
MoCA	23.33 (3.73)	22.57 (3.81)	ns	23.33 (2.64)	23.55 (2.98)	ns	21.00 (3.14)§	18.78 (5.57)	**0.029**
ROCF- copy	29.27 (6.38)	27.03 (5.68)	**0.042**	31.14 (5.14)	24.95 (4.70)	**0.008**	24.69 (8.54)	20.88 (11.38)	ns
ROCF - recall	12.60 (5.96)	11.88 (6.58)	ns	15.77 (5.70)	14.50 (4.47)	ns	11.09 (3.61)	8.75 (6.61)	**0.038**
TMT B-A	206.80 (175.15)	271.83 (195.46)	**0.030**	183.17 (157.62)	235.10 (197.18)	ns	235.69 (186.47)	318.47 (211.87)	**0.036**
Semantic Fluency	33.17 (6.45)	33.30 (9.45)	ns	37.92 (8.23)	35.09 (10.52)	ns	26.94 (10.51)	25.76 (11.33)	**0.017**
DSS- SPAN (WAIS-IV)	4.72 (1.56)	4.55 (1.27)	ns	5.58 (1.24)	5.17 (1.19)	ns	5.07 (1.16)	4.06 (1.39)	**0.005**
Clock- CDT	12.70 (2.56)	12.17 (2.21)	**0.049**	13.58 (1.56)	12.90 (1.60)	ns	11.31 (3.14)	10.29 (3.31)	ns
Benton - JLO	22.08 (5.36)	20.00 (7.06)	ns	21.00 (7.01)	19.60 (4.40)	ns	19.69 (6.87)	16.38 (6.68)	**0.035**

Note. Significant values are in bold type. MSA, multiple system atrophy; ns, not significant; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ROCF, Rey–Osterrieth complex figure; TMT, Trail Making Test; DSS, Digit Span Sequencing; WAIS IV, Wechsler Adult Intelligence Scale—Fourth Edition; CDT, Clock Drawing task; JLO, Judgment of Line Orientation Test. §PD vs. PSP.

4.3.5 Changes in cognitive profile at 15-month follow-up

To investigate which cognitive domains were the most sensitive to detect cognitive decline over time (15-month), we calculated the difference of impaired subjects (as assessed by a cognitive performance below the normality, SD ≤ -1.5) at follow-up versus baseline (Figure 4.1).

We observed significant changes in executive and language domain. Namely, compared to PD and MSA groups, which showed none or very little (0% and 2% respectively) variation in number of subjects with executive alterations. Up to 21 percent of PSP subjects further fell in these domains and the increment was significant compared to the PD group ($p < 0.0098$).

With regard to the language domain, up to 24 percent of PSP patients further fell in this ability compared to the baseline evaluation; this percentage was significantly different in comparison to the PD (3%, $p = 0.262$) and MSA groups (-8%, $p = 0.0071$).

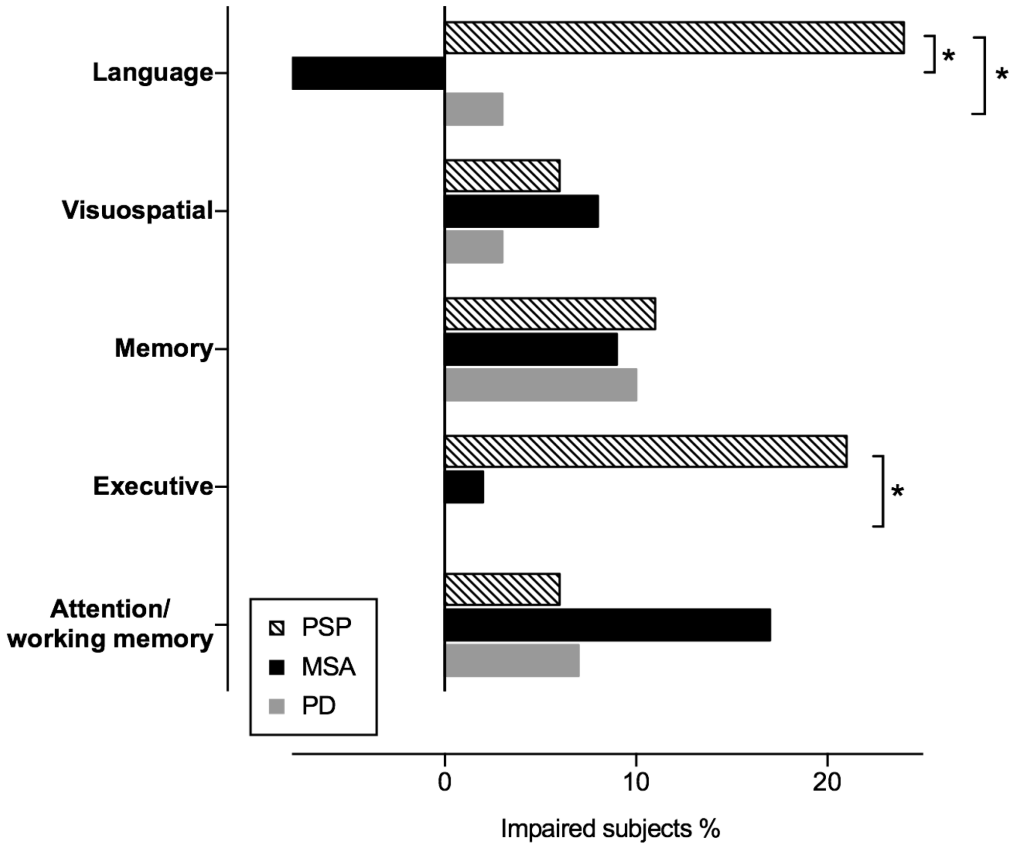


Figure 4.1 Percentage of impaired subjects between follow-up and baseline (T_1-T_0) across the cognitive domain. *Note.* T_1, follow-up; T_0, baseline.

4.3.6 Cognitive statuses

In the context of similar disease duration, MSA and PSP groups showed a different percentage of cognitive statuses at baseline (Figure 4.2). Namely, in the MSA group, 75 percent showed normal cognition, 25 percent showed MCI, and no patient had diagnosis of dementia. While in the PSP group at baseline, 22 percent was classified as cognitively normal, 61 percent showed MCI and 17 percent had a diagnosis of dementia.

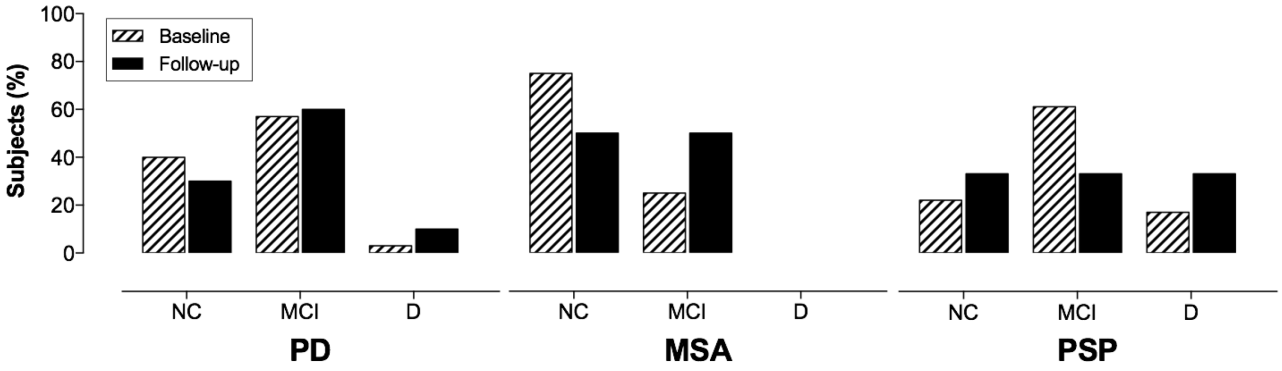


Figure 4.2 Percentage of subjects (PD, MSA and PSP) across cognitive statuses. NC, normal cognition; MCI, mild cognitive impairment; D, dementia.

At 15-month follow-up, thus approximately at six years of disease duration in both the diseases, 25 percent of MSA converted to MCI and no patients converted to dementia; while in the PSP group, 11 percent of patients with MCI improved and was classified as cognitively normal, and another subgroup (16%) converted to dementia.

Of note, PSP patients who converted from MCI to normal cognition presented improved performance on one test of the attentive or executive domain (i.e., TMT B-A or Stroop test).

Interestingly, in MSA and PSP, MCI patients at baseline evaluation all presented with multidomain deficits, but only some PSP patients converted to dementia.

In PD, although the disease duration was longer compared with PSP and MSA patients, at baseline 40 percent was classified as PD-NC, 57 percent as PD-MCI (88% of whom showed multidomain deficits) and three percent as having dementia. Fifteen months later, 10 percent of PD-NC converted to MCI and seven percent of PD-MCI to dementia.

4.3.7 MMSE versus MoCA change over time

As reported in Table 4.3, both MMSE and MoCA scores declined after 15-month. At follow-up, the mean MMSE score was higher than the mean MoCA total score in all parkinsonian disorders. Namely, in PD (25.46 ± 1.99 vs. 22.57 ± 3.81, $p < 0.0001$), MSA (26.10 ± 2.08 vs. 23.55 ± 2.98, $p < 0.013$), and PSP group (23.71 ± 5.15 vs. 18.78 ± 5.57, $p < 0.0001$). Although a more comprehensive evaluation, by means of the full neuropsychological battery, showed a global decline over time at follow-up. We found MMSE scores of either 29 or 30 in 30 percent of the PD, 33 percent of MSA and 25 percent of PSP patients. In contrast, a MoCA score over 28 was only present in one PD patient. This result suggests possibly the presence of a MMSE ceiling effect in the three groups.

Exploratory analyses of the percentage changes on MMSE and MoCA between the follow-up and baseline revealed a greater decline of the PSP group on MoCA score (-11%) in comparison to the MSA and PD groups – namely, the score declined by 2.2 points in 15 months (Figure 4.3). While on MMSE scale, MSA patients showed the greatest deterioration of performance, even though the change was relatively small (-4%), corresponding to a decline of 1.15 points.

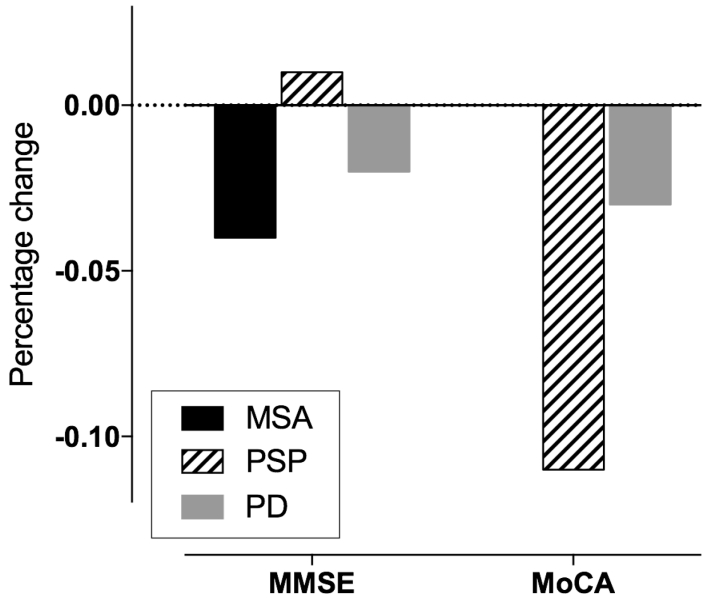


Figure 4.3 Percentage change (between follow-up and baseline) on MMSE and MoCA for patients with PD, PSP and MSA.

To investigate which subitems on MMSE and MoCA were more vulnerable to change over time, we calculated the 'percentage change' of the subitems and we considered a change as 'clinically significant' if greater than 30 percent, as reported previously (Soliveri, 2000).

Interestingly, in MMSE we found that PSP patients had a better performance in comparison to baseline in the 'copy of the pentagons', while MSA and PD groups showed a tendency to perform worse (Figure 4.4a).

Regarding the analysis of MoCA subitems, PSP patients showed a significant greater decline in TMT-B and the 'clock drawing,' but they improved in 'cube drawing.' Instead, MSA and PD patients' performance improved in the TMT-B task as well as in 'clock drawing' (Figure 4.4b).

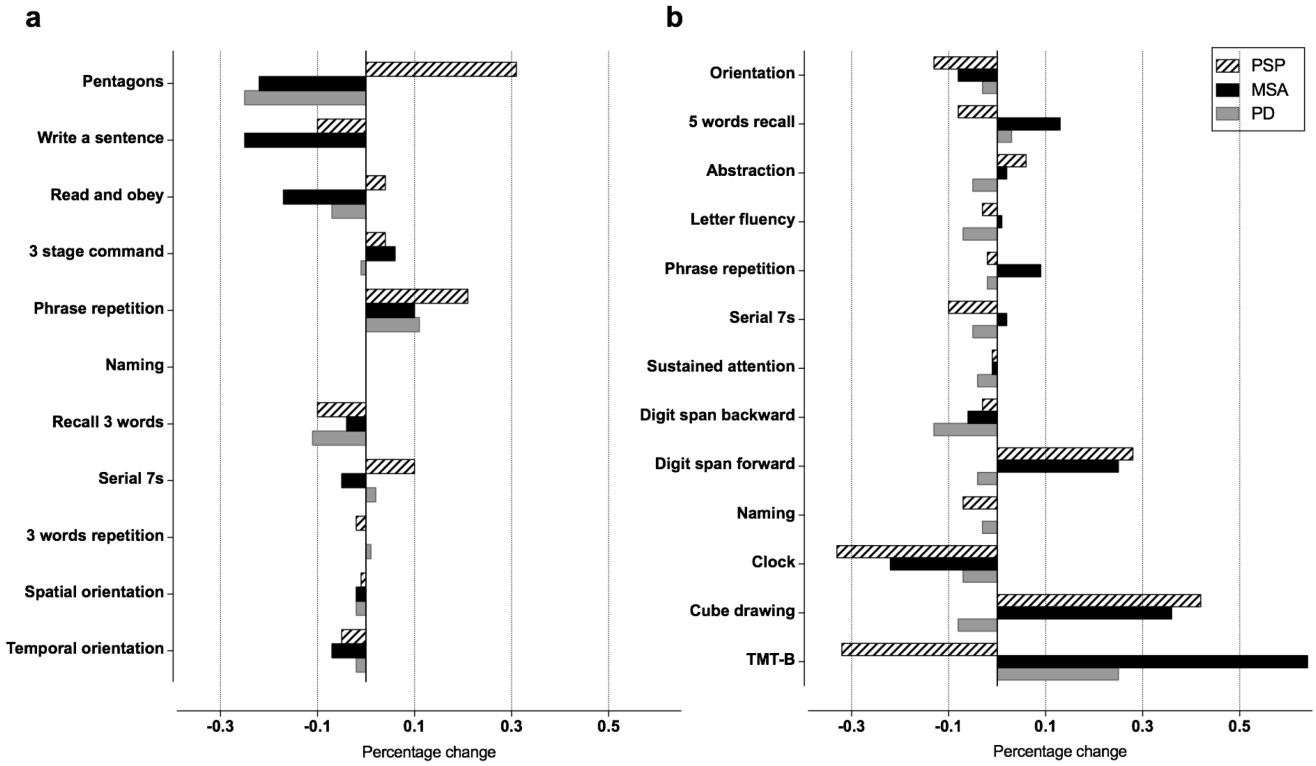


Figure 4.4 a) MMSE and b) MoCA subitems percentage change (between follow-up and baseline) for patients with PD, PSP and MSA. A percentage change greater than 30 percent was considered 'clinically significant', as reported previously (Soliveri, 2000).

4.4 Discussion

The main finding of our prospective study was that PSP patients have more severe cognitive decline than PD and MSA, matched for age and education. After 15-month follow-up, we found greater decline in the executive and language domains in PSP group compared to the others. This was the first study in which an extensive neuropsychological battery investigating the five cognitive domains was administered to atypical parkinsonisms as well as PD patients. Baseline and follow-up evaluations agreed, and showed that PSP patients had a worse performance than PD and MSA patients: especially, in the Stroop test, verbal fluencies (semantic and phonemic), and MoCA scale. These results are in line with previous findings that reported fronto-executive deficits as the predominant features of PSP neuropsychological profile (Brown et al., 2010; Gerstenecker et al., 2013)

Verbal fluency tasks, and more specifically phonemic fluency, were particularly impaired in PSP and as previously reported they differentiated PSP from PD patients (Bak et al., 2005a; Fiorenzato et al., 2016; Rittman et al., 2013). The consistent decline in verbal fluency is not exclusively related to motor problems (i.e., dysarthria), since this task requires minimal motor function (Rittman et al., 2013). Overall, PSP patients generated few words in the semantic fluency task, but even less in the phonemic task (at follow-up: 26 words vs. 19 words in 3 minutes, respectively). This is aligned with previous evidence, showing that letter fluency was more impaired than category fluency across all parkinsonian syndromes, compared to healthy control or to AD performance (Bak et al., 2005a). Thus, it has been demonstrated that especially phonemic fluency is a 'distinctive deficit' of PSP, and a cut-off of 7-words per minute is able to differentiate PSP from PD patients, with a high specificity and sensitivity (0.92 and 0.87, respectively) (Rittman et al., 2013).

Verbal fluency tasks probably reflect subcortical alterations. Indeed, patients with brain lesions in the frontal lobe were found to have altered verbal fluency, only if they had a concomitant or isolated striatal lesion (Stuss et al., 1998). And in turn, evidence from functional imaging studies, showed that frontal regions play a key role during the execution of verbal fluency tasks, namely Brodmann area (BA) 44 (pars opercularis of inferior frontal gyrus) seems to be involved in phonological processes while BA 45 (pars triangularis of inferior frontal gyrus) in semantic processes (Heim, Eickhoff, & Amunts, 2009). Hence, the more severe performance of PSP patients, compared to PD and MSA, could reflect a more

extensive frontal alteration, associated with cortical neuroglial tau pathology (Bigio et al., 1999; Williams et al., 2007).

It is worth noting that PSP patients showed a decline also in their language domain. In our neuropsychological battery, language abilities were assessed by naming and semantic fluency tasks: namely, it has been shown that the latter has a stronger relationship with language functions rather than with the executive domain (Whiteside et al., 2016). Exploratory analyses of our sample indicate that a performance below normality in the language domain was driven by both the cognitive tests (naming and semantic fluency), and not by one in particular. Our finding is consistent with previous evidence, showing that the language domain was the second most impaired domain (after the executive) in PSP patients (Bak et al., 2005a).

Assessing the severity of cognitive deficits, we found different percentages of cognitive statutes (i.e., normal cognition vs. MCI vs. dementia) among the three groups. Namely, in the MSA and PSP groups, in the context of similar disease duration (approximately six years at the follow-up) the percentage of patients with dementia was higher in the PSP group compared to MSA (33 percent vs. no patients with dementia). Noteworthy, MSA and PSP patients with MCI at baseline, all had multidomain deficits, but only patients with MCI in the PSP group converted to dementia.

Another important observation is that although the disease duration was longer for PD patients compared with PSP, the percentage of patients who converted to dementia was still higher in the PSP group compared to PD (16 vs. 7 percent), despite both groups seeming to have similar severity of MCI status at baseline. Overall this suggests a more rapid and severe cognitive decline in PSP. Conversely, cognitive impairment in MSA is milder compared to the other two disease groups, suggesting a pattern of cognitive dysfunctions very similar but less pronounced, and this is in line with previous findings that tried to characterize the cognitive decline in MSA (Bak et al., 2005a; Soliveri, 2000). Our findings should be confirmed by longitudinal studies in larger cohorts and with longer follow-up.

MSA and PSP have a different distribution of their pathology. In particular, MSA is characterized by alterations primarily in subcortical structures while cortical pathology is not considered as a predominant feature (Papp & Lantos, 1994). This notion is supported by a recent neuropathological study that failed to identify neuroanatomical regions associated with cognitive impairment in MSA (Koga et al., 2016), and is in line with also

our findings in a volumetric study, where we found only a focal atrophy in the frontal region associated with cognitive alterations, suggesting a marginal contribution of cortical pathology to cognitive defects in MSA (Fiorenzato et al., 2017) (see also Chapter 6 for further details).

By contrast, in PSP, the pathological tau burden extends from the frontal cortex to the dentate nucleus of the cerebellum. Although the most severely affected regions are the subthalamic nucleus, substantia nigra, and globus pallidum (Hauw et al., 1994; Williams et al., 2007); several studies reported the association between cortical tau pathology and the severity of cognitive and behavioral dysfunctions (Bergeron, Pollanen, Weyer, & Lang, 1997; Cordato et al., 2002).

PD patients had longer disease duration in comparison to MSA and PSP. At follow-up 60 percent was classified as MCI and 10 percent with dementia. However, the disease duration of patients with dementia was ranging between 11 to 21 years. Our findings suggest that PD patients deteriorate more slowly than MSA and PSP patients, and possibly longer follow-up is necessary to observe a sufficient decline in this disease (Soliveri, 2000).

Interestingly, in this study we did not find MSA patients with dementia, and in the literature there are controversial results (Stankovic et al., 2014). A previous study, based on the Level II criteria showed that dementia was ranging between eight to 11 percent in a MSA sample (Auzou et al., 2015). Based on the MDS criteria as well as DSM-5 criteria for the diagnosis of dementia (APA, 2012; Dubois et al., 2007), cognitive impairment should be severe enough to impact daily functioning. The assessment of dementia in atypical parkinsonisms is challenging, as ADL and IADL are usually impaired due to motor dysfunctions from the first stages of the disease, and isolating the cognitive component of already impaired functional tasks is difficult (Marras et al., 2014). In order to address this clinical issue, we used for the first time the PD-CFRS scale with atypical parkinsonisms to measure the functional impact of cognitive impairments (Kulisevsky et al., 2013). We administered this scale to patient as well as caregiver to help the clinician in the diagnostic process.

Results from the behavioral measures confirmed that neuropsychiatric features are more common in PSP patients than PD (Gerstenecker, 2017), especially apathy with accompanying low level of anxiety and depression. Also MSA patients usually reported numerous behavioral features, however in this study we found only a group difference between MSA and PD in the level of quality of life that was lower in the MSA group. Even

though we did not find other between group differences, level of apathy, anxiety and depression were always 'higher' compared to PD but 'lower' in respect to PSP.

Another interesting finding from the analyses of the brief cognitive scales (MMSE and MoCA) was the higher sensitivity of MoCA in detecting cognitive changes, especially in PSP. This is possibly associated to the characteristics of MoCA's subitems that evaluate executive and attentive abilities (Hoops et al., 2009). This finding is aligned with a previous study, where we demonstrated the superiority of MoCA (compared to MMSE) in detecting cognitive impairments in atypical parkinsonisms (Fiorenzato et al., 2016) (for further details please see Chapter 3). Interestingly, MoCA was less sensitive than MMSE in detecting cognitive decline at 15-month follow-up in PD patients, and this is consistent with previous evidence suggesting that MMSE was better for tracking cognitive changes in PD (Lessig, Nie, Xu, & Corey-Bloom, 2012). Analysis of subitems revealed that PSP patients had a 'clinically significant' worsening after 15-month in the attentive/executive subitems (TMT-B and the Clock drawing). However, it has been observed that some patients also improved in specific subtasks at the follow-up. This improvement could be related to a higher medications dose (although the difference was not significant when comparing the LEDD at baseline vs. follow-up).

It is worth noting that alterations (>30% change) in performance have been seen for subitems sensitive to motor conditions (such as drawing figures and linking circles with a pen), which could have affected cognitive outcome leading to higher performance. These limits associated with MoCA and MMSE scale, already showed for PD patients (Biundo et al., 2016b; Hu et al., 2014), maybe exacerbate in atypical parkinsonisms.

Significant differences between groups were also found in the disease severity assessment, where MSA and PSP showed more severe motor impairments compared to PD. For comparability purposes, we assessed the motor severity with the MDS-UPDRS, although we are conscious that this scale is tailored for PD patients rather than atypical parkinsonisms and possibly was unable to evaluate all the motor impairments of PSP and MSA.

Our study has also other important limitations. First, the lack of pathological confirmation of the clinical diagnosis, although we used the most recent clinical consensus criteria published (Gilman et al., 2008; Höglinger et al., 2017), we are aware that there is a high risk of misdiagnosis across the atypical parkinsonisms (Hughes et al., 2002; Joutsa et al., 2014; Koga et al., 2015). However, the clinical diagnoses were made by movement

disorders specialists and the patients were monitored for at least three years, due to the longitudinal nature of this study. Second, another limitation is the small number of our sample size, which is due not only to the rare nature of these disorders but also to the high drop-out rate because patients died or were too disabled. So, ideally future longitudinal multicenter studies should be conducted in order to ameliorate this issue. Third, we focus only on PSP-RS phenotype and this makes our findings unapplicable to the other PSP subtypes (i.e., the PSP parkinsonian variant). Lastly, the disease duration of our PD sample was not matched with PSP and MSA, since we focus on matching the patients for age and education.

In conclusion, the contribution of cognitive assessment can be useful in conjunction with other clinical information (e.g., disease progression, response to medication, motor and clinical features) to differentiate atypical parkinsonism and thus make an accurate clinical diagnosis (Lee et al., 2012).

Taken together, our findings showed that PSP patients were markedly impaired in comparison to the other parkinsonian disorders (MSA and PD) and within six years from the first symptoms, 33 percent of patients develop dementia. This severe progression is possibly associated with the distribution of tau pathology that involves also cortical structures. By contrast, the pattern of cognitive impairment in MSA was less severe than in the PSP patients, suggesting a distribution of underlying pathology in MSA, mostly in subcortical structures with a cortical involvement only secondary to these abnormalities. However, our results demonstrated that cognitive dysfunctions are frequent in MSA and strongly suggest the need to revise the current consensus criteria for the diagnosis of MSA by including the presence of MCI in the diagnostic criteria. This would be useful also to minimize the risk of misdiagnosis, since patients with parkinsonisms plus cognitive impairment tend to be misdiagnosed with another neurodegenerative disease (Koga et al., 2015). Thus, our findings recommend the use of cognitive assessment, as it could be useful to differentiate diagnosis across atypical parkinsonisms, and particularly to better understand the underlying pathology and its progression.

PART III

NEUROIMAGING STUDIES ON SYNUCLEINOPATHIES

CHAPTER 5

AMYLOID DEPOSITIONS AFFECT COGNITIVE AND MOTOR MANIFESTATIONS IN PARKINSON'S DISEASE[3]

5.1 Introduction

Cognitive dysfunction, particularly executive-attentive problems, can be present early in PD. Although very common, they are not universal and different cognitive profiles are associated with variable risk of progression rate of dementia (Aarsland et al., 2003a; Aarsland et al., 2009; Biundo et al., 2014; Kehagia et al., 2010). Prevalence of cognitive impairment in PD is about 30 percent and following the disease's course, approximately 80 percent of patients develop dementia (Emre et al., 2007; Halliday et al., 2008), with negative consequences on quality of life and survival (Levy et al., 2002). Several neuropathological, biochemical and anatomical changes may partially explain the heterogeneous profile of PD cognitive impairment and dementia (Irwin et al., 2013). In particular, presence of cortical and limbic LB (associated with synuclein), uneven dopamine loss across the basal ganglia circuitry (Lewis, Dove, Robbins, Barker, & Owen, 2003; Sawamoto et al., 2008), degeneration of basal forebrain cholinergic neurons, and AD like pathology (with amyloid-β [$A\beta_{42}$] plaques and tau neurofibrillary tangles) have been identified (Irwin et al., 2013; Mattila et al., 2000). This is also associated with presence of specific metabolic and structural brain deficits (Biundo et al., 2013; Peppard et al., 1992; Song et al., 2011). Indeed, it has been suggested that amyloid pathology and synuclein may act in synergy, leading to a worse prognosis (Compta et al., 2011; Irwin et al., 2013).

Dysfunction of the frontostriatal dopaminergic system may influence the presence of executive and attention problems in PD (Bruck et al., 2005), even though the evidence from dopamine transporter (DAT) imaging so far is inconsistent (Delgado-Alvarado et al., 2016). Deficits in memory and visuospatial functioning, defined as posterior cortically

[3] *Published as: Fiorenzato, E., Biundo, R., Cecchin, D., Frigo, A.C., Kim, J., Strafella, A.P., Antonini, A. Amyloid deposition affects cognitive and motor manifestations in Parkinson disease: the PPMI dataset (under review).*

based dysfunctions, have been related to non-dopaminergic alterations (i.e., cholinergic deficits) and the cortical LB (Williams-Gray et al., 2009). Furthermore, visuospatial deficits were also associated with amyloid depositions in the posterior and parietal cingulate cortices (Gomperts et al., 2008). Recent evidence from positron emission tomography (PET) imaging has suggested that in PD amyloid burden may contribute over time to cognitive, but not motor manifestations (Gomperts et al., 2013). Although in vivo evidence is still limited, particularly in early patients.

The relationship between amyloid-β and synuclein pathology has been intensely debated and investigated. It has been suggested that synuclein facilitates deposition of other protein aggregates (Irvine, El-Agnaf, Shankar, & Walsh, 2008) — although this seems unlikely given the presence of findings from post-mortem studies showing presence of concomitant pathologies in only a small proportion of PD (Irwin et al., 2012; Mattila et al., 2000); besides, recently, it has been posited that the presence of amyloid depositions can possibly facilitate the spread of synuclein strains (Toledo et al., 2016). This hypothesis seems likely and is in line with recent evidence that in PD, synuclein pathology is mainly located in axons, resulting in synaptic axonal damage, and consequently dysfunction (Tagliaferro & Burke, 2016). Hence, in agreement with this model, amyloid deposition facilitates the spread of synuclein, increasing axonal vulnerability, and also leading to neuro-inflammation processes (Edison et al., 2013).

To our knowledge, no study has previously combined PET-amyloid, DAT imaging and MRI-based measures of atrophy in order to investigate the relationship between amyloid depositions and synuclein pathology in the early phase of PD.

In the present study, we investigated the extent to which amyloid depositions in frontostriatal circuit, striatal dopaminergic dysfunction and brain atrophy rates can interact and contribute to frontostriatal based cognitive impairment. Specifically, we hypothesized that amyloid depositions can possibly act synergistically with synuclein pathology, interfering with axonal transmission; and thus we expect that PD patients with amyloid burden will be more vulnerable to cognitive and motor alterations.

5.2 Methods

5.2.1 Study design and participants

We obtained approval to access the Parkinson's Progression Markers Initiative (PPMI) database and to analyze the neuroimaging, cognitive and clinical data in early-PD (Marek et al., 2011). Briefly, the PPMI started in 2010 and is an ongoing international, multicenter, observational study of patients with PD and healthy controls in 33 sites in the USA, Europe, Israel and Australia; whose aim is to identify biomarkers of PD progression. Study aims, methodology, and details of the assessments have been published and are available on the PPMI website[4]. The Institutional Review Board of each participating institution approved the PPMI study. All participants gave their written informed consent to participate in the program.

Participants selected from the PPMI dataset for the current study were all those with [^{18}F]florbetaben PET images available and a diagnosis of PD. As of May 2017, these were 33 patients who had been recruited at five institutions worldwide. For this study, assessment included single-photon emission computed tomography (SPECT) with [^{123}I]FP-CIT (DaTSCAN by GE Healthcare) imaging, MRI, clinical evaluation of motor and non-motor features and cerebrospinal fluid (CSF) examination, as described previously (Kang et al., 2016). The [^{18}F]florbetaben PET and the clinical evaluations were performed between visit 4 and 8, [^{123}I]FP-CIT-SPECT between visit 6 and 10 (approximately within a year from the PET scanning). MRI scanning and CSF examination were collected at baseline (approximately within 2 or 3 years from the PET scanning). The participants of our sample have been evaluated between November 1, 2010 and August 1, 2016. Regarding the clinical evaluations, these subjects were monitored at least over three years.

5.2.2 Motor and non-motor outcomes

Demographic and clinical variables comprised age, sex, education, disease duration, LEDD, depression evaluated with the shorter 15 item Geriatric Depression Scale, disease severity assessed by means of the motor part of the Movement Disorder Society Unified Parkinson's Disease Rating Scale (in ON state), first motor symptoms at the onset and

[4] http://www.ppmi-info.org/study-design

functional autonomy using the ADL. We also assessed CSF for $A\beta_{42}$, synuclein, total tau and phosphorylated tau.

Global cognition was evaluated by means of MoCA test scores assessed at the time of neuroimaging examination. Further, to evaluate fronto-executive abilities, we analyzed performance on the Letter Number Sequencing test (LNS) to evaluate working-memory and executive function and the Symbol Digit Modalities Test (SDTM), which measures attention and executive functions. The LNS requires one to recall strings of digits and letters and to re-organize them, while the SDTM requires one to pair a specific number with geometric figures based on a references key.

5.2.3 Data acquisition and image processing: PET and SPECT

Images were acquired at PPMI institutions as specified in the imaging protocol for the PPMI scans[5]. Assessment of amyloid depositions was made using available, already processed data on the PPMI database. [^{18}F]florbetaben PET images were imported to PMOD Biomedical Image Quantification Software (PMOD Technologies, Zurich, Switzerland) for processing and analysis, following scientific and technical quality control performed at an imaging core lab (Institute for Neurodegenerative Disorders, New Haven, Connecticut). Dynamic PET frames were assessed for motion and if necessary, motion correction was performed. These files were then averaged (time weighted mean) to create a single PET volume. The PET volume was normalized to standard Montreal Neurological Institute and Hospital (MNI) space, to have all scans in the same anatomical alignment. The normalized PET volume was then converted to standard uptake values (SUV). Volumes of Interest (VOIs) from the MNI modified Automated Anatomical Labeling template, including cortical and subcortical regions, were applied to the SUV PET volume and adjusted as needed for subject atrophy (Tzourio-Mazoyer et al., 2002). The VOI placement was saved specifically for each subject. Semi-quantitative measurements (average SUV per voxel) were extracted from the regions to calculate the regional SUV Ratios (SUVRs) using the cerebellar gray matter as reference.

A composite SUVR for each subject was computed by calculating the mean SUVRs from six regions of interest, typically associated with increased tracer uptake (Rowe et al., 2008): namely, frontal cortex, lateral temporal cortex, anterior cingulate cortex, posterior

[5] http://www.ppmi-info.org/wp-content/uploads/2015/12/PPMI-AM10_protocol.pdf

cingulate cortex, parietal cortex, and occipital cortex. Composite SUVR values ≥ 1.45 were considered positive, indicating presence of amyloid-β depositions in the range expected for AD (Jennings et al., 2015; Ong et al., 2013). Thus, we defined PD Aβ+ and Aβ- according to this SUVR cut-off.

SPECT with the DAT tracer [^{123}I]FP-CIT was obtained in all 33 participants, and the striatal binding ratio was calculated for the right and left caudate and putamen separately, using as a reference region the occipital lobe. Detailed explanations of data acquisition and processing have been described previously (Siepel et al., 2014), and this information is also available in the PPMI imaging protocol reported above.

5.2.4 Structural MRI acquisition

Non-contrast enhanced 3D volumetric T1-weighted brain MRI scans were acquired using 1.5 or 3 Tesla scanners and were available for the majority of the sample (30 out of 33 subjects), after excluding low quality scans on visual inspection. The majority of subjects were scanned using a 3 Tesla MR scanner (n = 24) and the remaining 6 with 1.5 Tesla MR scanner. To minimize bias in data collection between different institutions, the PPMI optimized acquisition protocols across centers to maximize the data comparability. Hence, typical MRI parameters were as follows: repetition time 5 to 11 ms; echo time 2 to 6 ms; slice thickness 1 to 1.5 mm; inter slice gap 0 mm; voxel size 1*1*1.2 mm; matrix 256* minimum 160.[6]

5.2.5 Pre-processing and statistical analysis for voxel-based morphometry

Voxel-based morphometry analysis was performed using the DARTEL toolbox (Ashburner, 2007), as implemented in SPM12 as follows (Statistical Parametric Mapping; Wellcome Trust Centre for Neuroimaging, London, UK).[7] i) The origin (coordinate x, y, z = 0, 0, 0) of all T1-weighted structural images were manually centered to the anterior commissure for normalization improvement. ii) An individual image was segmented into gray matter, white matter, and CSF tissue in native space using the New Segment procedure. To improve automatic segmentation for subcortical structures, the new tissue

[6] Further details at http://www.ppmi-info.org/wp-content/uploads/2010/07/Imaging-Manual.pdf
[7] http://www.fil.ion.ucl.ac.uk/spm/software/spm12/

probability maps were used (Lorio et al., 2016). iii) The resulting segmented images were first spatially normalized to MNI space and used to acquire the study-specific template in DARTEL. The structural images were convolved using Jacobian determinant estimated during the previous step, so that the intensity of voxel in the image could reflect the volumes of the brain tissue class in the given voxel. iv) The images were eventually normalized to MNI space using affine spatial normalization. v) Gray matter images were smoothed by convoluting the Gaussian kernel with an 8 mm full width at half maximum. A final smoothed and spatially normalized GM image represents the regional volume of gray matter tissue and was used for statistical analysis. Total intracranial volume (TIV), the global volumes of individuals, were calculated by summing the value from the segmented gray matter, white matter, and CSF images using a 'tissue volume' utility implemented in SPM 12 (Malone et al., 2015), and used as covariate to control the head size difference across individuals.

For statistical analysis to determine group differences of gray matter volumes, the normalized and smoothed gray matter images were analyzed with a two-sample *t*-test in SPM12. Age, TIV, manufacturer and scanner strength were included as nuisance covariates to reduce possible confounding effects, and absolute threshold masking was applied as 0.15. The significance of statistical analysis was determined at an uncorrected voxel-wise level threshold of $p < 0.005$ with a cluster threshold of $k > 1265$, which corresponded to a cluster-level corrected familywise error rate of $p < 0.05$.

The cluster extents for multiple comparisons correction were estimated via a Monte Carlo simulation via AFNI's 3dClustSim-ACF option.[8]

In addition, we later performed the additional non-parametric analysis using the statistical non-parametric mapping toolbox, SnPM13,[9] to investigate the validity of the result performed in SPM. SnPM uses the general linear model to estimate the pseudo t-statistics based on 5000 permutations testing without variance smoothing. The same statistical threshold criterion was applied as in the SPM analysis.

[8] http://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html

[9] http://warwick.ac.uk/snpm

5.2.6 Statistical analysis

Due to the non-normal distribution of the sample, descriptive and non-parametric statistics (Mann–Whitney *U* test) were run to analyze the demographic, clinical and imaging data. Further, the *p*-values of the cognitive and imaging data were adjusted for age. Categorical variables were compared using Fisher's exact test since in some variables, the number of observations was less than five. Then, Spearman's rank correlations were performed to study the association between cognition (as assessed by MoCA, LNS and SDMT), dopaminergic deficiency and amyloid depositions in the frontostriatal circuit (caudate, putamen and frontal areas). Statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for Windows. Statistical significance was set at a five percent level.

5.3 Results

5.3.1 Study cohort characteristics

In total, we analyzed the data of 33 subjects enrolled in the PPMI study and with a diagnosis of idiopathic PD. Six PD (18%) were defined as PDAβ+ (SUVR≥1.45) and were compared to the PDAβ- group. The clinical and demographic data of the two groups are reported in Table 3.1.

The groups did not differ in age, education or sex distribution; although there was a trend for the PDAβ+ group to be older (according to the median scores). No significant differences were noted in disease duration, motor impairments and functional autonomy — but LEDD was significantly higher in the PDAβ- group (p=0.04).

From the cognitive point of view, we found no differences between these two groups, although there was a tendency for a lower MoCA score in the PDAβ+ vs. PDAβ- group (median scores: 25 vs. 27, respectively) as well as on executive functions performance (LNS and SDMT).

This sample did not include PD with dementia, as all patients were independent in functional autonomy (as assessed by ADL score > 80/100).

There were no between-groups differences in onset of motor symptoms as well as CSF markers at baseline.

Table 5.1 Demographic, clinical and cognitive features according to amyloid status

	PD Aβ− (n = 27)		PD Aβ+ (n = 6)		*p*-value
	Mean (SD)	Median (min− max)	Mean (SD)	Median (min− max)	
Age, y	64.26 (8.58)	61.00 (51− 84)	66.00 (7.80)	68.50 (55− 75)	0.69
Sex (m/f)	20/7		6/0		0.30
Education, y	15.74 (2.38)	16.00 (12− 21)	16.33 (2.25)	16.50 (14− 20)	0.52
Disease duration, y	4.79 (1.44)	4.60 (3.20− 9.00)	5.68 (1.51)	5.30 (3.60− 7.90)	0.12
LEDD	430.33 (252.08)	400 (0−1000)	209.17 (139.16)	150 (100− 450)	**0.04**
MoCA score	26.93 (2.50)	27 (21− 30)	25.33 (2.94)	25 (22− 29)	0.20#
LNS	10.78 (2.67)	10 (7− 17)	8.83 (3.19)	9 (5− 12)	0.14#
SDMT	43.59 (8.44)	47 (30− 59)	38.67 (12.86)	43 (21− 51)	0.28#
GDS score	5.04 (1.16)	5 (1−7)	5.33 (1.37)	5 (4− 7)	0.85
MDS-UPDRS III	25.00 (9.75)	27 (8− 41)	24.83 (12.00)	20 (17− 48)	0.62
ADL	85.93 (7.08)	80 (80− 100)	87.50 (6.12)	90 (80− 95)	0.49
Motor symptoms at onset					
Tremor	20 (74.1%)		5.0 (83.3%)		0.99
Rigidity	21 (77.8%)		4.5 (66.7%)		0.62
Bradykinesia	22 (81.5%)		4.0 (66.7%)		0.58
CSF markers, pg/mL					
$A\beta_{42}$	386.33 (96.13)	368.00 (238− 621)	353.33 (66.48)	335.50 (295− 460)	0.41
α-synuclein	1717.19 (766.73)	1588.0 (333− 3540)	1735.83 (318.87)	1805.5 (1344− 2103)	0.67
Total tau	42.04 (17.38)	38 (22− 92)	42.00 (10.37)	38 (33−59)	0.62
Phosphorylated tau	16.00 (11.22)	11 (6− 45)	11.17 (2.40)	11 (8−15)	0.76
$A\beta_{42}$:tau ratio	10.03 (3.02)	9.41 (4.95− 16.79)	8.80 (2.61)	8.33 (5.90−12.78)	0.29

Note. PD, Parkinson's disease; Aβ, Amyloid-β; FBB, [^{18}F]florbetaben; SD, standard deviation; LEDD, levodopa equivalent daily dose; MoCA, Montreal Cognitive Assessment; LNS, Letter Number Sequencing test; SDMT, Symbol Digit Modalities Test; GDS, Geriatric Depression Scale; MDS-UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale; ADL, Activity of Daily Living; CSF, cerebrospinal fluid. #: Age adjusted *p*-value. Significant value is in bold type.

5.3.2 Regional amyloid depositions and DAT binding in PDAβ+ versus PDAβ-

[^{18}F]florbetaben regional SUVRs and [^{123}I]FP-CIT-SPECT SBRs as a function of the amyloid status (Aβ+ or Aβ-) with *p*-value adjusted for age are reported in Table 3.2.

[^{18}F]florbetaben regional SUVRs were consistently more elevated in PDAβ+ compared to the PDAβ- group. These differences were highly significant in several regions, particularly in cortical areas (i.e., frontal, orbitofrontal, temporal, mesial and lateral temporal, parietal, occipital regions, posterior and anterior cingulate cortex regions), subcortical nuclei (i.e., caudate, putamen, thalamus), pons, rectus, and cerebellar white matter regions.

We found also that [^{123}I]FP-CIT-SPECT binding differed between the two groups: namely, in the PDAβ- group DAT binding ratios were bilaterally lower in the caudate nucleus as well as in the right putamen compared to PDAβ+. Interestingly, after age correction, this difference was even more significant. This suggests the PDAβ+ group had a less prominent striatal dopaminergic deficit as expressed by higher DAT binding compared to the PDAβ- group, although it was characterized by a widespread distribution of amyloid-β depositions in cortical and subcortical regions.

Table 5.2 Between-group comparisons according to the amyloid status (PDAβ- vs. PDAβ+) on regional [^{18}F]florbetaben SUVRs and [^{123}I]FP-CIT-SPECT SBRs uptake

Ligand	Region	PDAβ− (n = 27)		PDAβ+ (n = 6)		*p*-value	Age adjusted *p*-value
		Mean (SD)	Median (min− max)	Mean (SD)	Median (min− max)		
[^{18}F]florbetaben	Mesial temporal cortex R	1.21 (0.07)	1.21 (1.08− 1.38)	1.39 (0.08)	1.38 (1.31− 1.49)	0.0006	**0.0001**
	Mesial temporal cortex L	1.21 (0.09)	1.19 (1.07− 1.48)	1.35 (0.06)	1.34 (1.28− 1.43)	0.0019	0.0020
	Pons	1.74 (0.18)	1.77 (1.30− 2.04)	1.96 (0.17)	1.91 (1.80− 2.22)	0.0320	0.0175
	Subcortical white matter	2.00 (0.18)	2.00 (1.63− 2.32)	2.40 (0.25)	2.36 (2.13− 2.81)	0.0011	**0.0001**
	Cerebellar white matter	2.08 (0.17)	2.11 (1.66− 2.39)	2.49 (0.22)	2.50 (2.18− 2.74)	0.0012	**0.0001**
	Rectus R	1.20 (0.11)	1.20 (0.94− 1.41)	1.49 (0.19)	1.45 (1.28− 1.82)	0.0008	0.0002
	Rectus L	1.24 (0.10)	1.24 (1.05− 1.47)	1.46 (0.14)	1.44 (1.34− 1.73)	0.0012	0.0002
	Cingulum anterior R	1.25 (0.10)	1.23 (1.04− 1.44)	1.54 (0.24)	1.46 (1.32− 1.93)	0.0015	0.0002
	Cingulum anterior L	1.30 (0.12)	1.30 (1.06− 1.52)	1.63 (0.22)	1.57 (1.40− 1.96)	0.0010	**0.0001**
	Cingulum posterior R	1.32 (0.14)	1.35 (1.08− 1.58)	1.71 (0.11)	1.73 (1.51− 1.83)	0.0002	**<0.0001**
	Cingulum posterior L	1.38 (0.13)	1.41 (1.10− 1.60)	1.77 (0.06)	1.78 (1.71− 1.87)	0.0002	**<0.0001**
	Caudate R	1.42 (0.10)	1.41 (1.29− 1.68)	1.65 (0.21)	1.61 (1.47− 2.05)	0.0028	0.0010
	Caudate L	1.36 (0.11)	1.34 (1.20− 1.61)	1.55 (0.22)	1.49 (1.36− 1.95)	0.0200	0.0074
	Putamen R	1.46 (0.10)	1.46 (1.19− 1.69)	1.75 (0.16)	1.82 (1.51− 1.89)	0.0010	**<0.0001**
	Putamen L	1.42 (0.08)	1.41 (1.22− 1.63)	1.65 (0.14)	1.70 (1.45− 1.80)	0.0012	**<0.0001**
	Thalamus R	1.47 (0.13)	1.44 (1.28− 1.87)	1.66 (0.08)	1.67 (1.52− 1.75)	0.0032	0.0042
	Thalamus L	1.56 (0.14)	1.56 (1.33− 1.96)	1.73 (0.13)	1.68 (1.60− 1.92)	0.0063	0.0129
	Occipital cortex R	1.30 (0.09)	1.30 (1.16− 1.52)	1.51 (0.09)	1.49 (1.41− 1.65)	0.0005	**0.0001**
	Occipital cortex L	1.32 (0.09)	1.31 (1.18− 1.52)	1.55 (0.09)	1.52 (1.44− 1.68)	0.0004	**<0.0001**
	Parietal cortex R	1.26 (0.09)	1.25 (1.09− 1.44)	1.56 (0.13)	1.53 (1.42− 1.77)	0.0002	**<0.0001**
	Parietal cortex L	1.26 (0.09)	1.25 (1.08− 1.43)	1.56 (0.15)	1.56 (1.38− 1.79)	0.0004	**<0.0001**
	Lateral temporal cortex R	1.24 (0.07)	1.23 (1.10− 1.38)	1.45 (0.10)	1.47 (1.33− 1.57)	0.0005	**<0.0001**
	Lateral temporal cortex L	1.23 (0.08)	1.22 (1.12− 1.45)	1.42 (0.08)	1.44 (1.30− 1.50)	0.0005	0.0002
	Orbitofrontal cortex R	1.23 (0.09)	1.23 (1.08− 1.44)	1.48 (0.18)	1.43 (1.32− 1.76)	0.0008	0.0002
	Orbitofrontal cortex L	1.24 (0.08)	1.22 (1.10− 1.43)	1.44 (0.15)	1.38 (1.32− 1.70)	0.0007	0.0003
	Frontal cortex R	1.29 (0.09)	1.28 (1.16− 1.49)	1.59 (0.14)	1.58 (1.43− 1.79)	0.0003	**<0.0001**
	Frontal cortex L	1.30 (0.08)	1.30 (1.16− 1.49)	1.58 (0.13)	1.58 (1.41− 1.75)	0.0004	**<0.0001**
	Temporal cortex R	1.23 (0.07)	1.23 (1.10− 1.38)	1.44 (0.09)	1.44 (1.33− 1.55)	0.0005	**<0.0001**
	Temporal cortex L	1.23 (0.08)	1.21 (1.11− 1.46)	1.40 (0.07)	1.41 (1.29− 1.48)	0.0009	0.0003
[^{123}I]FP-CIT-SPECT (DaTSCAN)	Caudate R	1.52 (0.44)	1.50 (0.78− 2.37)	2.34 (0.82)	2.28 (1.27− 3.33)	0.0180	0.0030
	Caudate L	1.49 (0.44)	1.57 (0.63− 2.16)	2.16 (0.43)	2.15 (1.62− 2.67)	0.0059	0.0038
	Putamen R	0.57 (0.23)	0.49 (0.28− 1.43)	1.09 (0.57)	1.01 (0.27− 1.91)	0.0240	0.0018
	Putamen L	0.54 (0.18)	0.53 (0.17− 1.03)	0.75 (0.40)	0.65 (0.36− 1.52)	0.1100	0.0536

Note. SUVR, Standard Uptake Value ratio; PD, Parkinson's disease; Aβ, Amyloid-β; SD, standard deviation; R, right; L, left. Highly significant results ($p \leq 0.0001$) are reported in bold type.

5.3.3 Cognition and its association with frontostriatal amyloid load and DAT binding

In this analysis, we considered the whole PD cohort (PDAβ+ and PDAβ-) and tested the hypothesis that amyloid accumulations can possibly disrupt cognitive performance. Thus, we calculated the correlation between amyloid SUVRs in frontostriatal regions, the performance at MoCA scale and fronto-executive tests (LNS and SDMT).

We found a moderate negative association between amyloid burden (in dorsal striatum and frontal areas) and MoCA. Similar correlation was noted also between amyloid accumulations in frontal regions and SDMT (Table 5.3). These correlations suggest greater amyloid load in the frontostriatal network is associated with a worse performance on global cognition and executive tasks.

Further, we analyzed the relationship between cognitive tests and DAT binding, where we found an inverse correlation between DAT binding and cognitive performance. Namely, there was a negative correlation between the LNS test performance and dorsal striatum DAT binding, indicating better cognitive performance was associated with reduced DAT binding. We also observed a similar inverse association between MoCA scale and DAT binding in the right caudate nucleus.

5.3.4 Model for cognition in PD: effect of amyloid depositions, age and DAT binding

Stepwise regression analysis using MoCA score as the dependent variable showed that increasing amyloid depositions in frontal areas (i.e., [^{18}F]florbetaben SUVR) accounted for 23.1 percent of the variance of the cognitive scale. Instead, using LNS total score as the dependent variable, 33.3 percent of the variance was explained by striatal DAT binding (Table 3.4).

In addition, using SDMT score as the dependent variable, 38.5 percent of the variance was accounted for by increasing age and amyloid depositions in frontal areas (as measured by [^{18}F]florbetaben SUVR).

Thus, in summary, the variance in some cognitive scales seems to be better explained by amyloid depositions rather than dopaminergic deficiency.

Table 5.3 Spearman's rank correlation between cognitive tests versus amyloid burden and SBRs

			MoCA	LNS	SDMT
[^{18}F]florbetaben	SUVR amyloid caudate nucleus (bilateral)	r_s	-0.35	-0.11	-0.20
		p-value	**0.0449**	0.5354	0.2705
	SUVR amyloid putamen (bilateral)	r_s	-0.35	-0.14	-0.17
		p-value	**0.0459**	0.4536	0.3566
	SUVR amyloid frontal area R	r_s	-0.44	-0.05	-0.39
		p-value	**0.0099**	0.7763	**0.0268**
	SUVR amyloid frontal area L	r_s	-0.39	-0.11	-0.41
		p-value	**0.0230**	0.5365	**0.0177**
[^{123}I]FP-CIT-SPECT (DaTSCAN)	Caudate R	r_s	-0.40	-0.55	-0.21
		p-value	**0.0215**	**0.0009**	0.2516
	Caudate L	r_s	-0.28	-0.48	-0.28
		p-value	0.1121	**0.0043**	0.1180
	Putamen R	r_s	-0.26	-0.39	-0.05
		p-value	0.1421	**0.0269**	0.7965
	Putamen L	r_s	-0.01	-0.37	-0.09
		p-value	0.9390	**0.0362**	0.6179

Note. SBRs, specific binding ratios; caudate, caudate nucleus, R, right; L, left; MoCA, Montreal Cognitive Assessment; LNS, Letter Number Sequencing test; SDMT, Symbol Digit Modalities Test; SUVR, standardize uptake value ratio. Significant values are in bold type.

Table 5.4 Stepwise regression analyses for MoCA and fronto-executive tests (LNS and SDMT)

Variable	Coefficient b	SE (b)	Beta	*t*	*p*-value
MoCA					
Constant	38.382	3.869		9.919	
SUVR amyloid frontal area (bilateral)	-8.693	2.848	-0.481	-3.052	0.005
Letter Number Sequencing test					
Constant	15.301	1.306		11.718	
Mean striatal SBR	-4.321	1.099	-0.577	-3.931	<0.0001
Symbol Digit Modalities Test					
Constant	103.864	14.347		7.239	
SUVR amyloid frontal area (bilateral)	-24.254	9.77	-0.374	-2.483	0.019
Age	-0.44	0.169	-0.393	-2.606	0.014

Note. MoCA model fit: F = 9.32; R^2 = 0.231; excluded variables age and mean striatal SBR. LNS model fit: F = 15.45; R^2 = 0.333; excluded variables age and amyloid in frontal areas; SDMT model fit: F = 6.16; R^2 = 0.385; excluded variable mean striatal SBR. The mean striatal SBR scores were calculated as the mean of the left and right caudate and putamen SBR scores. PD, Parkinson's disease; MoCA, Montreal Cognitive Assessment; SBR, specific binding ratio; SUVR, standardized uptake value ratio; LNS, Letter Number Sequencing test; SDMT, Symbol Digit Modalities Test.

5.3.5 Effect of amyloid on gray matter volume

As reported in Figure 3.1 and Table 3.5, whole brain direct comparison between PDAβ+ and PDAβ- reveals decreased gray matter volume in the PDAβ+ group in the middle cingulate cortex (MCC), including right medial superior frontal gyrus and right superior frontal gyrus, as well as the left fusiform gyrus extending to bilateral calcarine cortex. Notably, as the effect of age was controlled in this analysis, it is more probable the observed gray matter atrophies are related to the presence of amyloid in the PDAβ+, rather than age-related degeneration.

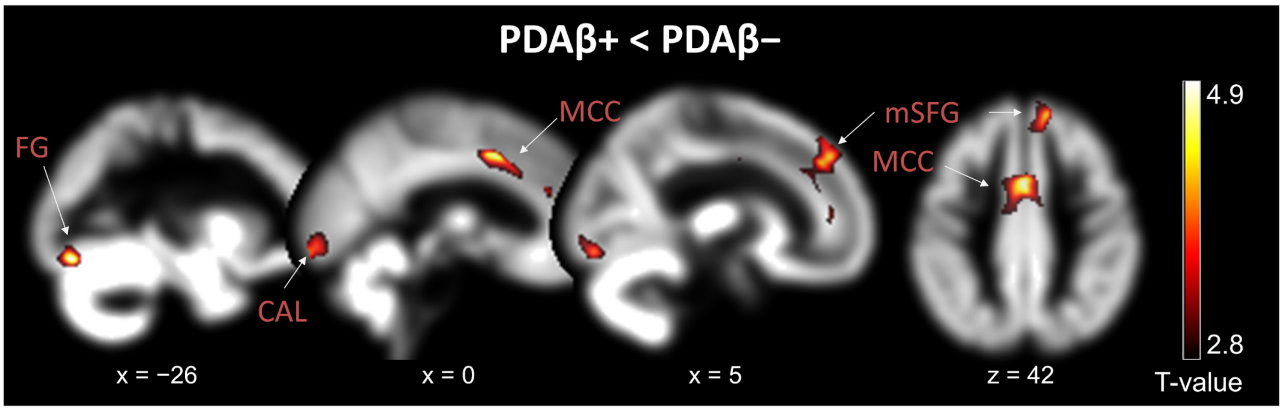


Figure 5.1 Structural MRI analysis: regions of reduced gray matter in the amyloid positive group (cluster level corrected family-wise error $p < 0.05$). Aβ, Amyloid-β; FG, fusiform gyrus; CAL, calcarine cortex; MCC, middle cingulate cortex; mSFG, medial superior frontal gyrus.

Table 5.5 Gray matter volume comparisons between PDAβ+ and PDAβ− groups

			MNI (mm)			
	Brain region (Brodmann area)	**Cluster size**	**X**	**Y**	**Z**	***T* value**
PDAβ+ < PDAβ−	**Middle Cingulate (BA 24)**	2293	0	9	42	4.99
	R Superior Frontal (BA 9)		12	47	42	4.22
	R Medial Superior Frontal (BA 32)		5	33	33	3.75
	L Fusiform (BA 18)	2308	-26	-81	-15	4.91
	R Calcarine (BA 18)		5	-89	-11	4.21
	L Calcarine (BA 17)		-3	-98	-12	4.21

Note. Reported clusters are corrected at cluster level p < 0.05 familywise error. PD, Parkinson's disease; Aβ, Amyloid-β, L, left; R, right; MNI, Montreal Neurological Institute and Hospital, BA, Broadmann Area.

5.4 Discussion

The neuropathology underlying cognitive impairment in PD is heterogeneous, and the contribution of β-amyloid to synuclein pathology is still under investigation (Irwin et al., 2013). Even less is known about the contribution of these mechanisms in the early stages of PD.

The underpinning hypothesis of this study was that amyloid depositions can possibly serve as a vulnerability factor to facilitate the spread of synuclein aggregates; hence, we focused particularly on frontostriatal circuit and executive dysfunctions, which are known to be altered since the early stages of the disease (Lewis et al., 2003).

To the best of our knowledge, this is the first multimodal study in which dopamine SPECT imaging, PET-amyloid, and MRI-based measures of atrophy were used in conjunction to evaluate a cohort of early-PD.

Two major findings characterize this study: first, high amyloid levels in early-PD, detected by [^{18}F]florbetaben PET imaging, were associated with reduced striatal dopaminergic deficits (as expressed by higher DAT uptake); second, high amyloid levels were associated with a tendency to show more frequent cognitive dysfunctions and increased brain atrophy progression rates.

These results add to the view that PD patients in the early phase of the disease, with concomitant amyloid pathology, have higher brain atrophy rates and can experience more cognitive deficits, but they are likely to show less prominent dopaminergic defects. While PD patients without amyloid pathology can possibly have less frequent cognitive impairment, lower brain atrophy rates, and can potentially show a greater dopaminergic deficit.

Taken together, our findings support our initial hypothesis that amyloid depositions increase vulnerability to dopaminergic deficits in PD. Indeed, amyloid can possibly act in synergy with synuclein pathology in the dopaminergic circuit, leading to progressive cognitive alterations in patients with amyloid-β comorbidity.

Our findings agree with a recent neuropathological hypothesis that considers synaptic axonal damage and dysfunction as PD's key feature (Tagliaferro & Burke, 2016). Indeed, dopaminergic system neurons are particularly vulnerable to synuclein pathology due to their axonal features — long, thin and unmyelinated.

This is also aligned with evidence from human imaging with DAT-binding PET (Caminiti et al., 2017), FDG-PET (Eidelberg, 2009; Pappata et al., 2011), and animal models (Walsh & Selkoe, 2016); suggesting synuclein aggregations in PD can affect synaptic function, and thus signal transmission from very early stages.

Further, it has been suggested an interaction between synuclein and the coincident amyloid pathology, wherein amyloid burden may facilitate the spread of synuclein (i.e., LB) (Toledo et al., 2016), and we speculate that this interaction can further contribute to axonal vulnerability.

Noteworthy, axonal damage has also been suggested as a source of amyloid-β, as axonal damage may lead to protein accumulation (e.g., amyloid precursor protein), which can be cleaved to form amyloid-β (Johnson et al., 2010; Stokin et al., 2005), and in turn, if there is presence of amyloid facilitates synuclein spreading, this would further lead to additional synaptic axonal damage, leading to a vicious cycle. In addition, we think it also is conceivable a contribution from neuroinflammation, since amyloid depositions are surrounded by reactive microglia and in turn, synuclein aggregates attract microglia, then potentially resulting in neuronal death and disease progression (Edison et al., 2013; Zhang et al., 2005).

To summarize, our results agree with a model of PD, where amyloid load amplifies dopaminergic dysfunction, leading to neuronal disconnection and consequently cognitive dysfunctions. Indeed, when both synuclein and amyloid pathologies coexist, brain functional and structural connectivity are maximally altered, leading to a breakdown of anatomical connections between brain areas (Jacquemont et al., 2017). Hence, the extent of cognitive alterations can be possibly due to the concomitance of dopaminergic pathology in conjunction to amyloid-β deposits; although amyloid seems to be the best predictor of cognitive defects.

Indeed, a recent biomarker study reveals that in healthy subjects, the incidence of dementia is increased by 3-fold (between ages 65 and 85) in presence of neurodegenerative disease and an absence of amyloid aggregations; while this incidence increases up to 9-fold when both neurodegeneration and amyloidosis are present; and this again underlines the 'clinically malignant nature' of this state (Jack et al., 2016).

Our finding of an association between increased amyloid as well as brain atrophy rates in PD suggests that amyloid deposition can possibly lead to a progressive loss of gray matter over time. In this regard, we found a broader atrophy in occipital areas (calcarine

cortex and fusiform gyrus) as well as frontal regions (superior frontal gyrus and MCC). This is aligned with our amyloid-PET results, since these regions were also associated with a significantly elevated amyloid burden; even though evidence from patients with MCI showed that brain tissue loss is not necessarily overlapping with the areas of high amyloid distribution (Tosun, Joshi, & Weiner, 2013). Furthermore, metabolic studies in PD identified cingulate and visual association cortex as vulnerable regions to cognitive alterations and dementia (Bohnen et al., 2011), and in turn this agreed with our hypothesis that amyloid burden contributes to brain atrophy and cognitive dysfunction.

It is worth noting that in the present study, MRI-imaging scans were performed at baseline (approximately 3-year before the PET-amyloid assessment), thus we can suppose that MRI-structural changes could possibly show an even broader distribution at time of PET scanning assessment. Indeed, our results are aligned with a recent study, based on the PPMI cohort, reporting that PD patients with lower CSF-amyloid levels showed a widespread cortical atrophy, especially in frontal regions, rather than in areas typically associated with AD (McMillan & Wolk, 2016). However, larger studies are required to confirm this pattern of atrophy, especially to better explain the possible relationship between amyloid pathology and brain atrophy rates in PD.

Another important observation is that we did not observe differences in disease severity between PDAβ- and PDAβ+ groups, although the PDAβ- group showed more elevated dopaminergic loss. In other words, DAT binding was significantly lower in the right putamen and bilateral caudate nuclei. This finding supports our hypothesis that amyloid can possibly affect motor functions as well, since in presence of comorbid amyloid pathology, a minimal dopaminergic deficit is sufficient to trigger motor symptoms and thus PDAß+ patients would be also more vulnerable to motor impairments. Similarly, in AD, motor dysfunctions are reported even though there is no presence of dopaminergic degeneration (Thomas et al., 2017).

We recognize that our PDAβ- group has a higher intake of dopaminergic medications (as assessed by LEDD) than the PDAβ+ patients; however, in our cohort we did not find an association between motor dysfunctions and LEDD (r_s=-0.059, p=0.7458), and adjusting MDS-UPDRS motor score for LEDD, this difference remains not significant (p=0.7722).

Another important finding of the present study is the correlation between high amyloid depositions in frontal regions, worse performance on global cognition and

attentive/executive functions. Results from the stepwise linear regression analysis showed that the 23 percent of MoCA variability was better explained by amyloid burden in frontal areas, while 39 percent of SDMT variance was explained mostly by age and amyloid depositions. Previous studies did not find these results in non-demented PD patients and this is possibly due to different cognitive scales being used (i.e., DRS-2 and MMSE) (Akhtar et al., 2017; Siderowf et al., 2014). Indeed, our finding is aligned with the recent literature where MoCA showed a high sensitivity in detecting early cognitive deficits in non-demented PD (Biundo et al., 2016a).

Coversely, we obtained inconsistent correlations between cognitive measures and DAT binding. Namely, we found an inverse association between dorsal striatum uptake and executive/working-memory performance (i.e., LNS test), and between right caudate nucleus and MoCA scores. Our results are in disagreement with a recent study (based on the PPMI cohort), in which a positive correlation between DAT binding and executive functions was observed (Siepel et al., 2014); but notably, in that study the authors recognized the correlation was relatively weak, even in a very large cohort, suggesting that most of the variance in fronto-executive tasks cannot be attributed solely to dopamine dysfunction. In this regard, there are so far, discordant results in the literature about dopaminergic depletion and cognition, suggesting that dopaminergic deficiency, and hence synuclein, is not the only pathological determinant of cognitive alterations in PD (Delgado-Alvarado et al., 2016).

Amyloid deposits seem to have a crucial role in PD, and prevalence of concomitant AD pathology is up to 50 percent in postmortem cases (Irwin et al., 2012; Mattila et al., 2000). In the present study, we assessed amyloid aggregates by means of [^{18}F]florbetaben imaging, a ligand widely used in AD, and we observed that only 18 percent of our early-PD population was identified as amyloid positive. Previous evidence on PD patients found similar rates: 15 percent of amyloid positivity (i.e., using [^{11}C] Pittsburgh compound B) (Petrou et al., 2012), and 16.5 percent (i.e., using CSF Aβ_{42}) (McMillan & Wolk, 2016); suggesting different approaches have a high correspondence in detecting presence of amyloid aggregates.

CSF Aβ_{42} has been recently identified as a reliable biomarker of cognitive decline (after two years) in PD patients (Fereshtehnejad, Zeighami, Dagher, & Postuma, 2017; Schrag, Weintraub, & Schott, 2017). However, we found no significant difference in the CSF Aβ_{42}

level between our groups, and this is possibly because CSF $A\beta_{42}$ was collected at the baseline visit (2 or 3 years before amyloid PET assessment).

A main strength of our study is that we focused on PD in the early disease phase and without dementia, while the majority of previous PET-amyloid studies focused on patients with dementia and at advanced disease stage (Petrou et al., 2015). However, our study has also several limitations that should be considered. First, the small sample size, as only a minority of patients in the PPMI dataset had PET-amyloid imaging. In addition, PET-imaging was analyzed only by SUVRs, while it is possible to use more sophisticated quantification methods (Cecchin et al., 2017). Then the cognitive battery used was limited, and not all executive functions were assessed. Lastly, the neuroimaging assessment and CSF measure were not assessed at the same time, but it is worth noting that all these data were collected from highly specialized movement disorders centers, and thus we expect a high reliability of these data.

In summary, the present study suggests a possible synergy between synuclein and amyloid pathology in early-PD, and this is consistent with the hypothesis that presence of amyloid increases axonal vulnerability.

Future longitudinal studies are necessary i) to elucidate the prognostic features of PD with comorbid amyloid pathology, as this could have key implications for patient care as well as life expectancy, and ii) to understand the disease's underlying mechanisms and possibly translate these findings into new treatments. Further, targeting amyloid depositions early may represent a valuable strategy to slow the disease's progression including cognitive decline and ideally to prevent dementia in PD.

CHAPTER 6

BRAIN STRUCTURAL PROFILE OF MULTIPLE SYSTEM ATROPHY PATIENTS WITH COGNITIVE IMPAIRMENT[10]

6.1 Introduction

MSA is characterized by a variable combination of progressive parkinsonism, cerebellar ataxia, autonomic failure, and pyramidal symptoms (Gilman et al., 2008) (see also Chapter 1 for further details). Regarding the pathological substrate of MSA, diagnosis is currently based on the presence of glial cytoplasmic inclusions, the hallmark of MSA, in the cerebellum, pontine nuclei, inferior olivary nucleus, striatum and substantia nigra (Quinn, 1989). According to the clinical manifestations, MSA patients can be classified as MSA-P or MSA-C. However, during the course of the disease, the clinical and pahological features of MSA subtypes frequently overlap (Antonini et al., 1998; Ciolli, Krismer, Nicoletti, & Wenning, 2014; Colosimo et al., 2001; Wenning, Ben-Shlomo, Magalhaes, Daniel, & Quinn, 1995).

In contrast to other synucleinopathies (e.g., PD and DLB), presence of dementia is considered a non-supporting feature for the MSA diagnosis (Gilman et al., 2008) — however, there is growing evidence that MSA patients can experience cognitive impairment ranging from executive dysfunctions to multiple-domain cognitive deficits (Gerstenecker, 2017).

In addition, a recent 'Position statement by the Neuropsychology Task Force of the Movement Disorder Society MSA study group' posited that cognitive alterations in MSA could be under-recognized (Stankovic et al., 2014); indeed, frequently, MSA patients presenting with parkinsonism and cognitive deficits tended to be misdiagnosed as other neurodegenerative diseases — namely, other synucleinopathies or PSP (PSP) (Koga et al., 2015). The frequency of cognitive impairment differs according to applied methodologies

[10] *Published as: Fiorenzato, E., Weis, L., Seppi, K., Onofrj, M., Cortelli, P., Zanigni, S., . . . Imaging Study, G. (2017). Brain structural profile of multiple system atrophy patients with cognitive impairment. Journal of Neural Transmission (Vienna), 124(3), 293-302.*

and sample size; however, it varies approximately between 14 percent and 37 percent (Koga & Dickson, 2017).

Although, we demonstrated that MoCA is more sensitive than the commonly used MMSE in detecting cognitive impairment in MSA (for further details see Chapter 3); recently a large multicenter study has suggested using a cut-off score <27 of MMSE to increase its sensitivity in detecting cognitive dysfunctions in MSA (Auzou et al., 2015).

The underlying mechanisms of cognitive impairment in MSA are still not understood, and in this regard evidence from MRI studies suggested a discrete cortical and subcortical contribution to explain cognitive deficits in MSA (Kim et al., 2015; Lee et al., 2016a), even though these studies were based on a relatively small number of patients at various disease stages as well as being single-center.

The main objective of the present multicenter study was to better characterize structural abnormalities underpinning cognitive impairment in MSA. Thus, we investigated brain morphology changes using voxel-based morphometry (VBM) analysis of the gray and white-matter regions of a cohort of MSA with cognitive alterations, defined as MMSE < 27. Further, since VBM analysis is not sensitive in detecting subcortical structures changes, we also applied a fully-automated segmentation of gray matter nuclei in conjunction to our VBM analysis.

6.2 Methods

6.2.1 Study population

Seventy-two probable MSA patients were retrospectively collected from five international movement disorders institutions: namely, IRCCS San Camillo Hospital Foundation, Venice-Lido, Italy (n=34); Clinical Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria (n = 8); IRCCS Institute of Neurological Sciences of Bologna, Bologna, Italy (n=7); Department of Neuroscience, Imaging and Clinical Sciences, Gabriele d'Annunzio University, Pescara, Italy (n=7); Dysautonomia Center, Department of Neurology, New York University School of Medicine, New York, USA (n=6). MRI and clinical data were compared with a control sample of 36 healthy controls (HC), collected at the institution in Venice, who volunteered to take part in this study. HC were matched for age and education to the MSA group. All participants were scanned between 2010 and 2015. This study received ethical approval from the Venice

Research Ethics Committee, Venice, Italy; and the research was completed in accordance with the Declaration of Helsinki. All patients gave their written informed consent before study enrolment.

6.2.2 Clinical and cognitive assessment

Clinical, cognitive and MRI-data were available for all the participants. MSA patients fulfilled the MSA clinical established diagnostic criteria of a probable diagnosis, which was made by expert neurologists and based on clinical history as well as neurological examination (Gilman et al., 2008). Exclusion criteria were presence of: i) deep brain stimulation, ii) psychiatric or other neurological comorbidity, iii) motion artifacts, and iv) significant cortical or white matter vascular lesions of grades 2 and 3 (as seen on T2-weighted axial and T2-weighted fluid attenuation inversion recovery) (Schmidt, Enzinger, Ropele, Schmidt, & Fazekas, 2003).

The clinical features assessed were: age, sex, education, age at onset, disease duration, and motor severity, that was measured by means of the motor part of the MDS-UPDRS III. Global cognition was assessed using the MMSE (Folstein et al., 1975), performed within four weeks from the MRI assessment. According to the published MMSE threshold specific for MSA patients (Auzou et al., 2015), we defined MSA with cognitive impairment when the MMSE total score was below 27. Thus, we identified two subgroups: MSA with normal cognition (MSA-NC) and MSA cognitively impaired MSA-CI.

Since our MSA sample consisted of patients in moderate to advanced disease stages, which at time of assessment showed a combination of clinical manifestations (i.e., parkinsonism and cerebellar features) in addition to autonomic failure, we did not analyze MSA-C and MSA-P separately.

6.2.3 MRI imaging protocols

In 66 MSA patients and 36 HC, brain MRI was acquired on a 1.5T scanner, while the remaining six MSA were assessed on a 3T scanner according to the routinely applied protocols at each institution. Further details on T1-weighted 3D volumetric parameters are reported in Table 6.1.

Table 6.1 Scanner characteristics and acquisition parameters at each institution

Centers	Manufac-turer	Scanner	Sequence	Voxel size (mm^3)	FOV	Pixel band width (Hz/Px)	TR (ms)	TE (ms)	Inversion time (ms)	Flip angle (°)
1	Philips	Philips Achieva 1.5T	T1-TFE	0.9x0.9x0.9	288x288	173	8.3	4.2	974	8
2	Siemens	Avanto 1.5T	T1-MPRAGE	0.9x0.9x1.2	256x192		1600.0	3.4	800	15
3	Siemens	TrioTim 3T	T1-MPRAGE	1.0x1.0x1.0	256x256	238	2300.0	3.4	900	
4	GE	Signa Horizon LX	T1-FSPGR	1.0x1.0x1.0	256x256		1250.0	5.1	600	10
5	Philips	Philips Achieva 1.5T	T1-FFE	1.0x1.0x0.8	256x256	191	19.0	3.7	/	30

Note. Centers: 1 = Venice, Italy; 2 = Innsbruck, Austria 3 = New York, USA; 4 = Bologna, Italy; 5 = Chieti, Italy; FOV, field-of-view; TR, repetition time; TE, echo time.

6.2.4 Voxel-based morphometry analysis

Structural data were analyzed with FSL-VBM pipeline, carried out with FSL tools (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). First, structural images were brain-extracted using the Brain Extraction Tool, after cropping the image at the medulla level and after automatically removing hyperintense non-brain tissue (e.g., fat or muscle) by means of the MRIcro tool (Smith, 2002).[11] Then, segmentation was performed using the FSL tool FAST.

Both gray and white-matter partial volumes were aligned to MNI152 standard space using FSL tool FLIRT, followed by non-linear registration using FNIRT, which uses a b-spline representation of registration warp field. The resulting images were averaged to create a specific template, based on a randomly selected subgroup of patients and then the original images were non-linearly re-recorded using the template. To minimize T1-sequence variability across institutions and scanners (i.e., 3T and 1.5T), spatial noise patterns associated with a field's inhomogeneity were corrected by means of FSL-SUSAN pipeline, which reduces noise using nonlinear filtering (Smith & Brady, 1997). The recorded partial volume images were then modulated (to correct for local expansion or contraction), by

[11] http://www.mricro.com

dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of four mm. Sample homogeneity, implemented in CAT12 within SPM,[12] was checked using covariance matrixes to identify potential outliers. Partial correlations analysis was run including age, sex and estimated total intracranial volume (eTIV) as nuisance variables. Participants were defined as outliers if the covariance was below two SD from the sample mean.

In the following non-parametric analyses, the masks used were applied, but trying carefully to define the brainstem structure, and then avoiding gray and white-matter misassignment associated with the partial-volume effect.

The gray-matter VBM mean-template was binarized using a threshold equal to 0.2 of the fractional intensity value to include gray-matter. Whereas, a white-matter VBM mask was obtained based on fractional anisotropy (FA) mean-template. Namely, in a subsample of MSA patients, FA was calculated with FreeSurfer's dt_recon software tool, after motion and eddy current correction. Then a specific FA template was created, using the FA images from all participants (Abe et al., 2010). All participants' FA images were corecorded to the standard template, provided by FSL (FMRIB58_1mm), using an affine 12-parameter transformation followed by a non-linear transformation. The resulting normalized FA images were then smoothed with an eight mm isotropic Gaussian kernel, and a mean image was created. Individual participants' FA images were then recorded to the customized FA template, using the FSL registration tool (Tract-Based Spatial Statistics preprocessing). The FA mean-template was then binarized using a 0.3 FA value, as the conservative threshold for white-matter inclusion.

6.2.5 Full-automated subcortical volumes segmentation

Subcortical brain volumes were calculated from MRI T1-3D images using the software package FreeSurfer (version 6.00b)[13] that has a specialized tool for automated parcellation of the neocortical gray-matter and subcortical volumes (Dale, Fischl, & Sereno, 1999; Fischl, Sereno, & Dale, 1999). Mapping between subjects and the atlas was executed using a non-rigid registration on the inflated surface. The outcome is the human cortex parcellation into 34 cortical regions of interest (in each hemisphere) and into 19 subcortical

[12] http://dbm.neuro.uni-jena.de/cat/

[13] http://surfer.nmr.mgh.harvard.edu/

white-matter and deep gray matter volumetric structures (Desikan et al., 2006). Furthermore, the Bayesian based brainstem segmentation was used to obtain volumes of the superior cerebellum peduncles, pons, midbrain and medulla (Iglesias et al., 2015). For study purposes, only subcortical volumes were included. An overall mean of the left and right hemisphere indexes was calculated after pairwise *t* test, in order to verify the absence of significant between-hemisphere differences for each subcortical structure.

6.2.6 Statistical analyses

Chi-squared analyses were run to assess between-group differences in the distribution of categorical variables, namely to compare the whole MSA group and HC, as well as the MSA-NC group and MSA-CI. Instead, within-group comparisons of continuous variables (i.e., clinical and demographic data) were analyzed using the Mann–Whitney *U* test adjusted with bootstrap (1000 iterations to reduce false positives).

VBM general linear model (GLM) analysis was run by means of FSL's 'randomize' tool to compare MSA and HC using the following covariates: age, education, sex, and eTIV. While in the direct comparison of MSA-CI versus MSA-NC: age, education, MDS-UPDRS III, scanning sites and eTIV were used as covariates. Lastly, non-parametric statistics were performed using the FSL 'randomize' tool with 15,000 permutations, and then correcting for multiple comparisons across space using the threshold free cluster enhancement option either enabled or disabled (i.e., voxel-based thresholding are uncorrected for multiple comparisons), using previously calculated gray and white-matter masks. To obtain anatomical localization of statistical results of gray and white-matter, the Automated Anatomical Labeling template and the Johns Hopkins University white-matter tractography atlas were applied, respectively (Mori, Wakana, Van Zijl, & Nagae-Poetscher, 2005; Rolls, Joliot, & Tzourio-Mazoyer, 2015).

Subcortical volumes obtained with full-automated segmentation were compared between groups (i.e., MSA vs. HC, and MSA-CI vs. MSA-NC) using GLM multivariate analyses, taking into account the same covariates of the previous analysis. The Partial Eta-Squared (η_p^2) value was calculated as an estimate of effect size. Statistical analyses were performed using SPSS 20, release version 20.0 (Armonk, NY). Statistical significance threshold was set at $p < 0.05$.

6.3 Results

6.3.1 Demographic and clinical differences between groups

Table 6.2 shows demographic and clinical details of MSA compared to HC, and MSA subgroups (MSA-NC and MSA-CI), while Table 6.3 reported the comparisons between MSA clinical variants (MSA-P vs. MSA-C).

MSA and HC groups agreed in each variable, except for MMSE that was significantly lower in MSA patients. In the direct comparison between MSA-NC and MSA-CI group, the latter were older, had less education and showed a tendency to be older at age of onset. Regarding the comparisons between the two MSA clinical phenotypes, we found no significant difference between MSA-C and MSA-P variants in all the clinical variables, even though there was a trend for the MSA-P subgroup to be older than the MSA-C group ($p = 0.0605$).

Further, after checking the sample homogeneity, we did not exclude any participants from the analyses.

6.3.2 Voxel-based morphometry

Results from the VBM analysis revealed gray matter volume thinning in several cortical regions, when comparing the MSA group with HC, especially in the bilateral cerebellum, and bilaterally in cortical regions (i.e., frontal, parietal, and occipital lobes, middle cingulate gyrus, and partially in the temporal lobe). Gray matter volume reductions were found in subcortical regions (i.e., bilateral putamen). Further, we found volume increases bilaterally in the occipital gray matter (i.e., anterior lingual gyrus and calcarine cortex), in right amygdala, periaqueductal gray (PAG) and in the posterior thalamus, caudate nucleus, and olfactory cortex (Figure 6.1; Table 6.4a). White-matter volume thinning was observed mainly in the anterior thalamic radiation, cingulum, cerebellum, and corpus callosum (Figure 6.1; Table 6.4c).

The direct comparison of MSA-CI versus MSA-NC groups showed a focal gray matter thinning only in the left dorsolateral prefrontal cortex (DLPFC) of the MSA-CI group (Figure 6.2, Table 6.4b).

Table 6.2 Demographic and clinical features of HC and MSA, and their subgroups

	MSA (n = 72)		**HC (n = 36)**		**MSA-NC (n = 50)**		**MSA-CI (n = 22)**		**MSA vs. HC**	**MSA-NC vs. MSA-CI**
	Mean (SD)	Mdn	Mean (SD)	Mdn	Mean (SD)	Mdn	Mean (SD)	Mdn	*p*	*p*
Age, y	63.8 (6.8)	64	61.6 (7.4)	62.0	62.6 (6.6)	62.0	66.4 (6.5)	67.5		*
Education, y	11.2 (4.7)	11	12.5 (4.5)	13.0	12.4 (4.5)	12.0	8.4 (4.1)	8.0		**
Sex (m/f)	29/43		21/15		22/28		7/15			
Age of onset, y	59.0 (7.2)	60			58.0 (7.0)	58.0	61.3 (7.2)	62.0		*
Disease duration, y	4.6 (3.0)	4			4.4 (2.9)	4.0	5.1 (3.3)	4.0		
MMSE	26.7 (3.1)	28	29.1 (1.0)	29.0	28.4 (1.1)	28.0	22.8 (2.7)	23.0	***	***
MDS-UPDRS III	41.3 (14.9)	41			39.7 (16.1)	41.0	44.9 (11.4)	42.0		
MSA-C/ MSA-P	25/47				18/32		7/15			
Center (1/2/3/4/5)	34/18/6/7/7		36/0/0/0/0		21/14/6/7/2		13/4/0/0/5		***	*
eTIV	1463.7 (160.0)	1436.8	1439.8 (178.7)	1441.4	1477.0 (170.7)	1447.3	1433.7 (131.2)	1423.6		

Note. Centers: 1 = Venice, Italy; 2 = Innsbruck, Austria 3 = New York, USA; 4 = Bologna, Italy; 5 = Chieti, Italy; MSA, multiple system atrophy; HC, healthy controls; MSA-NC, MSA with normal cognition; MSA-CI, MSA with cognitive impairment; SD, standard deviation; Mdn, median; y, year; MMSE, Mini-Mental State Examination; MDS- UPDRS III, Movement Disorder Society Unified Parkinson Disease Rating Scale; eTIV, estimated total intracranial volume. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ corrected for multiple comparisons.

Table 6.3 Demographic and clinical features between MSA clinical subtypes

	MSA-C (n = 25)				**MSA-P (n = 47)**			
	Mean (SD)	Mdn	min	max	Mean (SD)	Mdn	min	max
Age, y	61.2 (7.3)	61	49	76	65.13 (6.1)	66	51	78
Education, y	11.3 (4.3)	11	5	20	11.1 (5.0)	11	5	23
Sex (m/f)	11/14				18/29			
MMSE	26.7 (2.7)	28	21	30	26.7 (3.4)	29	27	30
Center (1/2/3/4/5)	9/7/5/2/2				25/11/1/5/5			
eTIV	1430.6 (161.5)				1481.4 (158.1)			

Note. No significant differences in the MSA-P vs. MSA-C group comparison. Centers: 1 = Venice, Italy; 2 = Innsbruck, Austria 3 = New York, USA; 4 = Bologna, Italy; 5 = Chieti, Italy; MSA-C, multiple system atrophy of the cerebellar type; MSA-P, multiple system atrophy of the parkinsonian type; HC, healthy controls; SD, standard deviation; Mdn, median; y, year; MMSE, Mini-Mental State Examination; eTIV, estimated total intracranial volume.

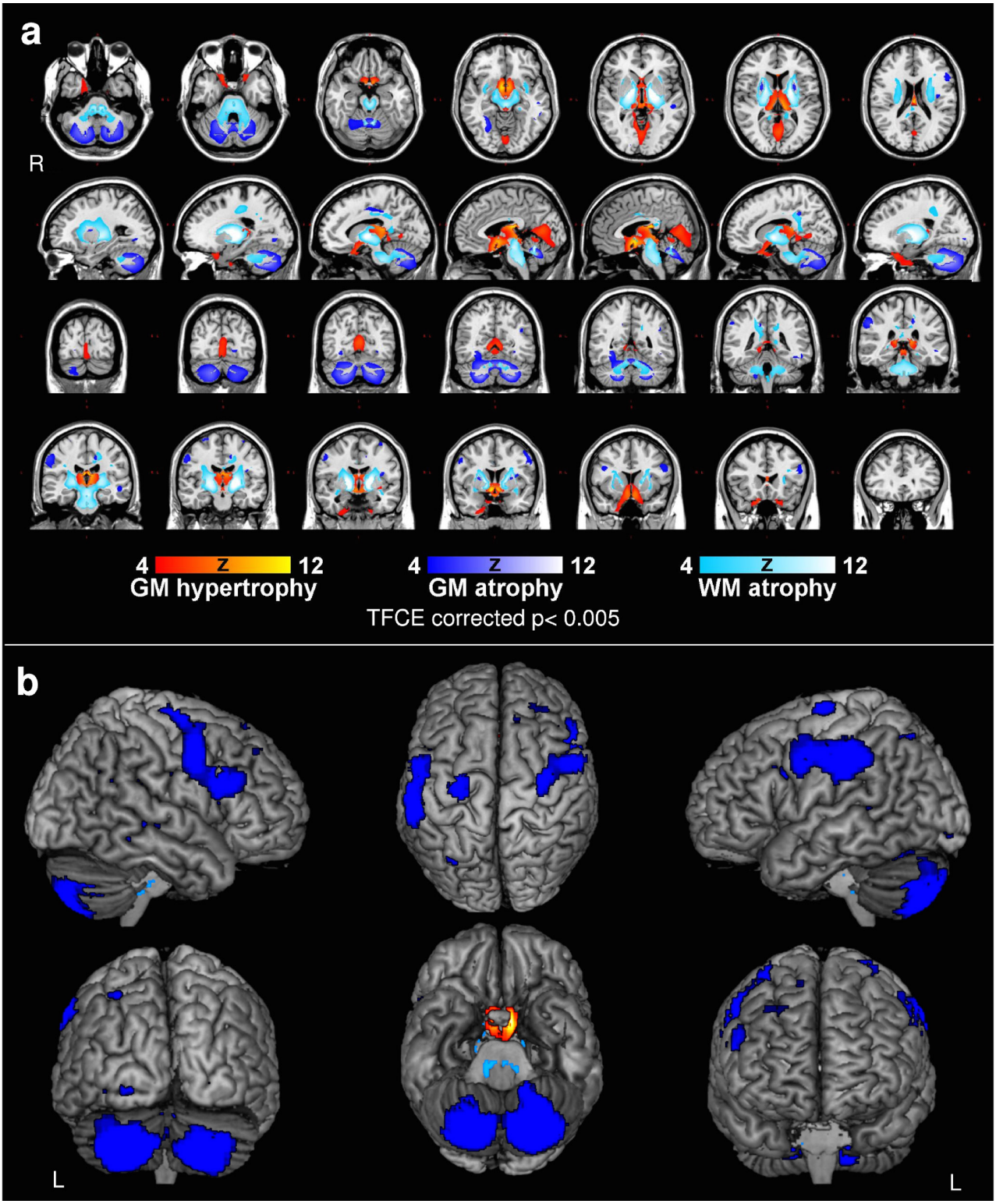


Figure 6.1 Voxel-based morphometry comparison between MSA and HC group. (a): axial view; (b): 3D view. A statistical threshold Z<4 was used for visualization purpose. MSA: multiple system atrophy; HC: healthy control; GM: gray matter; WM: white matter; TFCE: Threshold-Free Cluster Enhancement.

Table 6.4 Voxel-based morphometry results of gray and white matter differences between MSA vs. HC and between MSA subgroups (MSA-CI vs. MSA-NC)

	GM VBM	AAL2 atlas	Voxels§	Z Score	*P* Value	X (mm) (MNI)	Y (mm) (MNI)	Z (mm) (MNI)	Side
a	**MSA < HC**								
		Cerebellum VIII	5614	9.5	< 0.00001	-22	-54	-46	left
		Cerebellum VIII		9.3		22	-58	-46	right
		Cerebellum IX		7.7		-18	-50	-46	left
		Cerebellum IX		5.3		18	-46	-46	right
		Cerebellum VIIb		6.4		-14	-76	-46	left
		Cerebellum VIIb		6.5		18	-74	-46	right
		Cerebellum Crus II		6.3		-30	-74	-46	left
		Cerebellum Crus II		5.5		30	-78	-42	right
		Cerebellum VI		5.7		-30	-60	-26	left
		Cerebellum VI		5.9		26	-60	-30	right
		Cerebellum (Vermis)		6.8		0	-62	-26	midline
		Fusiform		5.3		-30	-58	-8	left
		Supramarginal	452	6.1	< 0.00001	-64	-20	38	left
		Precentral	59	5.2	< 0.00001	-26	-20	68	left
		Postcentral	55	5.0	< 0.00001	-58	-2	40	left
		Middle Cingulate	33	5.5	< 0.00001	-14	-42	36	left
		Putamen	40	6.2	< 0.00001	-26	2	12	left
		Inferior Frontal	20	4.8	< 0.00001	-40	10	24	left
		Middle Occipital	3	4.6	< 0.00001	-20	-90	-2	left
		Lingual	1	4.6	< 0.00001	-16	-82	0	left
		Inferior Occipital	1	4.6	< 0.00001	-24	-90	-6	left
		Lingual	28	5.3	< 0.00001	20	-76	-2	right
		Inferior Temporal	27	5.4	< 0.00001	50	-44	-14	right
		Middle Temporal	25	5.0	< 0.00001	52	-30	-10	right
		Putamen	112	6.0	< 0.00001	28	0	12	right
		Middle Cingulate	67	5.4	< 0.00001	16	-34	38	right
		Precentral	63	5.1	< 0.00001	56	2	42	right
		Inferior Frontal	60	4.8	< 0.00001	52	14	26	right
		Superior Frontal	17	4.9	< 0.00001	32	-8	66	right
		Fusiform	9	4.9	< 0.00001	32	-66	-6	right
		Angular	3	4.7	< 0.00001	44	-50	30	right
	MSA > HC								
		Thalamus		8.6	< 0.00001	-6	-28	0	left
		Amygdala	10	4.9	< 0.00001	20	-2	-14	right
		Thalamus	4081	11.7	< 0.00001	6	-24	16	right
		Thalamus		6.9		-8	-18	10	left
		Olfactory		8.3		6	10	-14	right
		Olfactory		5.2		-8	16	-14	left
		Periaqueductal Gray		4.3	< 0.00001	2	-22	-16	right
		Calcarine	1173	6.7	< 0.00001	2	-70	10	right
		Calcarine		6.0		-5	-72	10	left
		Lingual		5.9		6	-60	2	right
		Lingual	19	5.0	< 0.00001	-10	-40	-4	left
b	**MSA-CI < MSA-NC**								
		Middle Frontal	572	3.80	0.00007	-32	26	44	left

(continued on the next page)

Table 6.4 (continued)

WM VBM	JHU white-matter atlas	Voxels[§]	Z score	*P* Value	X (mm) (MNI)	Y (mm) (MNI)	Z (mm) (MNI)	Side
c	**MSA < HC**							
	Anterior thalamic radiation	10143	15.9	< 0.00001	-18	-2	0	left
	Anterior thalamic radiation		16.3		18	-4	0	right
	Cerebellum white matter		12.0		-4	-56	-16	left
	Cerebellum white matter		13.4		4	-56	-16	right
	Corticospinal tract		10.3		4	-30	-40	right
	Superior longitudinal fasciculus		9.5		32	-8	6	right
	Cingulum	1565	6.9	< 0.00001	-6	-38	30	left
	Splenium of corpus callosum		6.0		-6	-40	24	left
	Body of corpus callosum		4.8		-4	-28	18	left
	Cingulum		6.2		14	-16	36	right
	Cingulum (cingulate gyrus)	22	5.3	< 0.00001	-4	2	34	left
	Inferior longitudinal fasciculus	12	3.9	0.00005	-48	-16	-16	left
	Cingulum (hippocampus)	55	7.6	< 0.00001	24	-30	-12	right
	Body of corpus callosum	40	5.0	< 0.00001	4	-16	28	right
	Inferior fronto-occipital fasciculus	17	4.3	< 0.00001	18	34	-16	right
	Uncinate fasciculus	2	4.5	< 0.00001	18	10	-14	right
	Fornix	1	5.2	< 0.00001	4	-2	-8	left
	Callosal body posterior pars	1	3.6	0.0002	-2	-24	20	left
	Callosal body posterior pars	1	3.5	0.0002	2	-24	20	right

Note. Images were overlaid into MNI 2x2x2 mm^3 template. GM: gray matter; WM: white matter; VBM: voxel-based morphometry; MNI, Montreal Neurological Institute and Hospital; AAL2, new Anatomical Automatic Labeling; JHU, Johns Hopkins University; MSA, multiple system atrophy; HC, healthy controls; MSA-NC, MSA with normal cognition; MSA-CI, MSA with cognitive impairment.

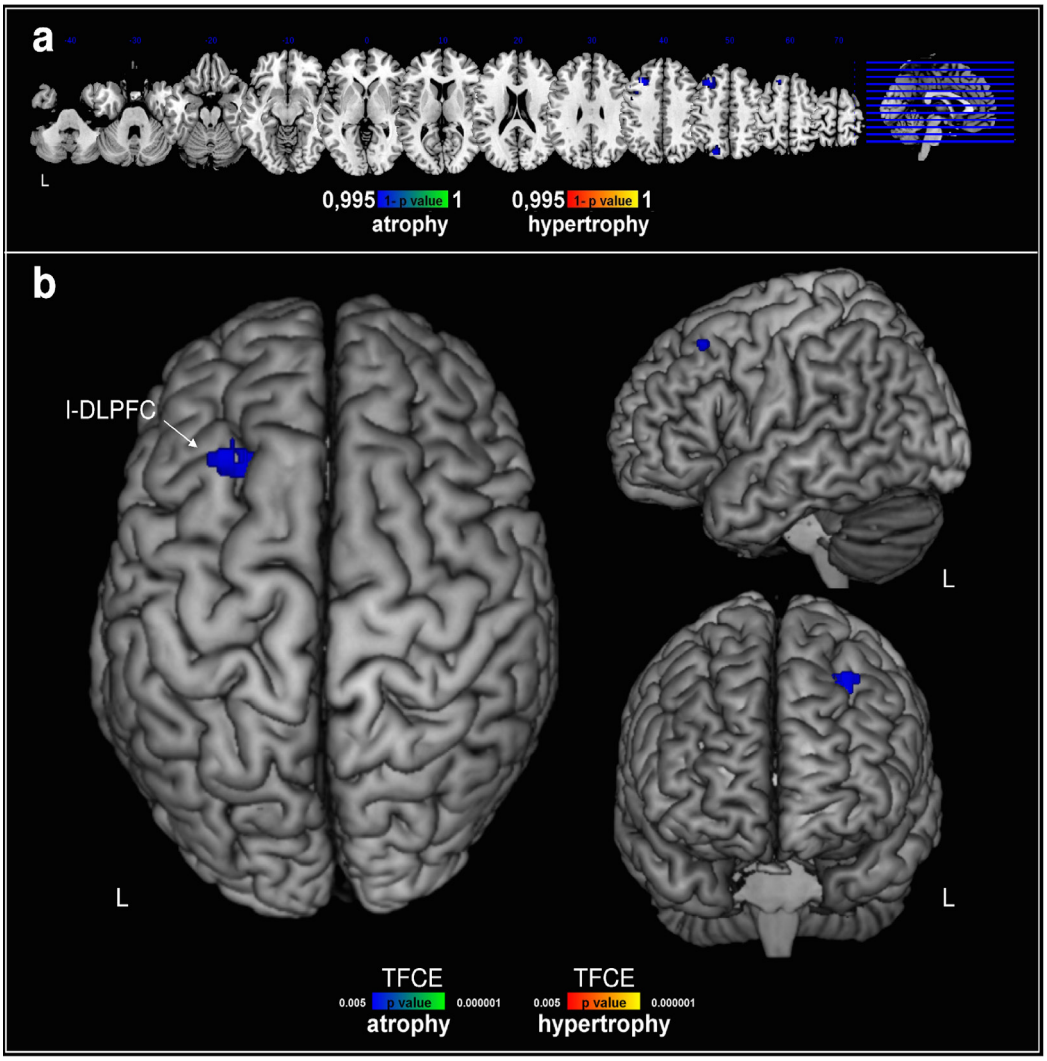


Figure 6.2 Voxel-based morphometry comparison between MSA-CI and MSA-NC. (a) uncorrected (*P* value<0.005) maps; (b) axial and 3D views. MSA: multiple system atrophy; MSA-CI: MSA with cognitive impairment; MSA-NC: MSA with normal cognition; TFCE: Threshold-Free Cluster Enhancement; l-DLPFC: left dorsolateral prefrontal cortex.

6.3.3 Subcortical volumetric segmentation

Analysis of subcortical volumes segmentation showed numerous volume reductions in MSA versus HC comparison, particularly in medulla, superior cerebellar peduncles, pons, midbrain, middle posterior corpus callosum, cerebellar white and gray matter, putamen, globus pallidus, nucleus accumbens, thalamus and ventral diencephalon (Table 6.5). The direct comparison of MSA-CI versus MSA-NC groups showed no significant volumetric differences in subcortical structures.

Table 6.5 Full-automated subcortical segmentation analyses: significant volumes

	Subcortical regions	HC	MSA	F	*p*	Effect size
		Mean (SD)	Mean (SD)			
MSA vs. HC	Medulla	4759.3 (496.1)	4397.8 (644.3)	11.16	0.001	0.099
	Pons	15378.4 (1884.1)	12296.2 (3080.0)	37.88	<0.0001	0.271
	Superior cerebellum peduncle	247.2 (38.8)	220.1 (55.0)	8.56	0.004	0.077
	Midbrain	6438.2 (666.7)	5894.7 (807.2)	16.70	<0.0001	0.141
	Middle posterior corpus callosum	405.6 (89.9)	333.5 (87.2)	10.02	0.002	0.089
	WM Cerebellum LH+RH	13791.2 (1880.9)	10243.2 (3765.5)	33.87	<0.0001	0.249
	GM Cerebellum LH+RH	97527.8 (9972.3)	84899.9 (16706.4)	23.58	<0.0001	0.188
	Putamen LH+RH	9637.5 (958.3)	7248.3 (1751.0)	59.78	<0.0001	0.370
	Globus pallidus LH+RH	2947.1 (403.5)	2437.5 (547.1)	23.97	<0.0001	0.190
	Nucleus accumbens LH+RH	992.5 (172.3)	804.8 (173.6)	20.26	<0.0001	0.166
	Hippocampus LH+RH	7938.3 (702.2)	7365.2 (962.4)	9.16	0.003	0.082
	Thalamus LH+RH	11251.3 (946.3)	10193.6 (1294.6)	21.28	<0.0001	0.173
	Ventral Diencephalon LH+RH	7663.3 (739.6)	6862.9 (875.8)	24.14	<0.0001	0.191

Note. MSA, multiple system atrophy; HC, healthy control; SD, standard deviation; WM, white matter; GM, gray matter; LH, left hemisphere; RH, right hemisphere.

6.4 Discussion

Patients with MSA showed a broader thinning in cortical brain regions (bilateral frontal, occipito-temporal and parietal areas), subcortical alterations, and white matter thinning compared to the HC group. Further, a focal reduction in the left DLPFC region was associated with the presence of cognitive alterations in MSA.

This finding is aligned with previous evidence showing neuronal loss in the frontal region (Salvesen et al., 2015), as well as hypometabolism and hypoperfusion in MSA patients with cognitive impairment (Kawai et al., 2008; Kitayama et al., 2009; Lyoo et al., 2008). In this regard, the focal alteration in the left DLPFC agrees also with the neuropsychological profile of MSA patients, wherein the fronto-executive domain is the most frequently altered (Gerstenecker, 2017; Stankovic et al., 2014).

Overall, our results revealed a different pattern underlying cognitive manifestations in MSA as compared to the other synucleinopathies — in PD patients, cognitive deficits are associated with widespread cortical atrophies, involving not only frontal regions but also posterior regions (Biundo et al., 2016a); suggesting that MSA and PD cognitive

dysfunctions are related to distinct underpinning mechanisms. Indeed, MSA-CI patients are characterized by focal frontal alterations (i.e., DLPFC), probably secondary to striato-pallido-thalamo-cortical circuits. This structural pattern underlying cognitive manifestation in MSA is possibly associated with the concept of 'subcortical dementia' that is characterized by executive dysfunctions, memory-retrieval type deficits, slow information processing as well as impairment in cognitive tasks based on frontal network functioning (Brown & Marsden, 1988; Cummings, 1986; Stankovic et al., 2014; Tekin & Cummings, 2002).

Noteworthy, our previous findings are aligned with these 'subcortical alterations' and support our observations regarding MSA performance on MMSE subitems (Fiorenzato et al., 2016). Particularly, the MSA-CI group showed a worse performance especially in the executive (i.e., calculation), memory retrieval (i.e., retrieval of three words) and visuoconstructive subitems (i.e., copy of pentagons). The 'copy of pentagons' activity should be interpreted as dependent on executive control, since previous evidence showed frontal involvement in the execution of this task (Filoteo, Reed, Litvan, & Harrington, 2014).

Another salient observation is that the natural motor course of MSA usually severely affects the functional independence of these patients; thus, it is challenging to identify if the 'subcortical cognitive dysfunctions' are per se sufficient to significantly impact functional autonomy. For this reason, we propose to use the term 'subcortical dementia' with caution, since in this disease involving subcortical lesions, severe motor impairment is part of the clinical picture and may hamper cognitive assessment, leading to an overestimation of the cognitive deficits (Pillon et al., 1996).

Another key finding is the widespread brain alteration in MSA patients compared to controls, which expand previous neuroimaging evidence especially due to the large sample size of our cohort (Brenneis et al., 2003; Chang et al., 2009; Minnerop et al., 2010; Minnerop et al., 2007; Shigemoto et al., 2013). In addition, we applied a fully-automated segmentation method, to more precisely segment the subcortical regions; and we found atrophies in the superior cerebellar peduncle, pons, medulla oblongata, and midbrain. We identified also significant volume reductions in the cerebellum and in the putamen — but interestingly not in the caudate nucleus (Messina et al., 2011; Scherfler et al., 2016). This result is aligned with recent neuropathological and neuroimaging studies, reporting that the degeneration in the caudate nucleus can possibly be absent or mild also in advanced disease

stages (Barbagallo et al., 2016; Wenning et al., 1997), and notably is less prominent compared to the putamen, whose role in motor functions is extensively recognized (Alexander, 1986).

Regarding the white matter analyses, in agreement with previous studies, we noted reductions in the anterior thalamic radiation, cerebellum, corticospinal tract and corpus callosum (Minnerop et al., 2010; Shigemoto et al., 2013; Worker et al., 2014).

Furthermore, volume increases in the bilateral occipital lobe (anterior lingual gyrus and calcarine cortex), posterior thalamus, nucleus caudate, olfactory cortex, right amygdala and PAG were found; and these probably reflect microstructural changes and remodeling, related to the pathological and neurodegenerative processes. Indeed, cortical and subcortical gray matter increases have been extensively described also in other neurodegenerative diseases (i.e., AD and HD); and previous studies posited that in the early phase of the disease, volume increases can possibly precede atrophies, which characterize more advanced patients (Fortea et al., 2011; Rosas et al., 2008). In this regard, volume increases can be related to local inflammation and/or neuronal hypertrophy (Fortea et al., 2010).

On the other hand, we found a conflicting result regarding the thalamus volume, as we observed a volume reduction in the full-segmented analysis, but a volume increase in the VBM analysis (namely, in the posterior region of thalamus). However, previous evidence showed that full-segmented analyses have higher accuracy than VBM analyses, whose accuracy is even lower in the subcortical structures located near the ventricles, as the thalamus (Schwarz et al., 2014). Thus, we support the finding of a volume reduction in the thalamus in MSA patients, compared to HC.

The present retrospective multicenter study has some limitations: first, our MSA cohort was based on clinical diagnoses and not autopsy-based; however, the diagnoses were made by movement disorders specialists; second, we applied a MMSE threshold below 27 to identify the MSA subgroup with cognitive impairment. The MMSE cut-off score has been recently validated and is based on a neuropsychological extensive battery. However, to better identify the magnitude of cognitive impairments using a comprehensive neuropsychological battery is strongly recommended, and in the present study due to its retrospective design, we could only obtain the MMSE total score as the cognitive measure for the whole sample. Then, MRI data were collected with both 1.5T and 3T scanners and we could enroll the control group only at one institution (Venice, Italy). Although we

checked our sample homogeneity and found no outliers in both the subgroups, and in addition, we performed a field homogeneity correction including the institutions as covariates in the VBM analysis.

In sum, our findings agree with previous evidence showing widespread cortical and subcortical alterations in MSA compared to healthy subjects, and in the present study we underlined also the crucial involvement of white matter in this disease.

Indeed, clinicians should consider the heterogeneous nature of this pathology, characterized by neurodegenerative processes involving white and gray cortical matter as well as subcortical structures.

Presence of significant cognitive alterations in MSA, as assessed by MMSE, is associated with focal volume reduction in the left DLPFC and suggests only a marginal contribution of cortical pathology to cognitive manifestations. The neuropathology underpinning MSA seems to be very different from other neurodegenerative diseases, such as PD, AD and PSP.

Thus, we proposed that cognitive alterations in MSA could be possibly associated with the disruption of the striato-pallido-thalamo-cortical circuit, wherein cortical deficits are focal and only secondary to subcortical alterations. This again supports the evidence that cognitive impairment in MSA is less prominent than in other parkinsonian disorders. Further, our findings should encourage revising the consensus criteria for MSA diagnosis and listing the presence of specific frontostriatal cognitive deficits among the supporting features (Gilman et al., 2008), namely MSA patients with mild cognitive deficits should not be excluded in the differential diagnostic process in order to avoid possible misdiagnosis (Koga et al., 2015).

CHAPTER 7

GENERAL CONCLUSIONS

The experimental work included in the present thesis aims at characterizing the cognitive profile of patients with parkinsonian disorders as well as at investigating the neuroanatomical changes underlying cognitive impairment particularly in PD and MSA.

The studies reported in the second part of the thesis have been designed to explore which one is the most sensitive cognitive screening instrument to detect cognitive alterations in atypical parkinsonisms (Chapter 3), and to assess prospectively cognitive deterioration (Chapter 4).

Indeed, the results from the multicenter study presented in Chapter 3 provided evidence that MoCA is more sensitive than the commonly used MMSE in detecting cognitive dysfunctions, especially in patients with PSP. The superiority of MoCA was determined by subitems assessing attentive and executive domains, and also by the lack of ceiling effect compared to MMSE. In this regard, executive and attention/working-memory dysfunctions are common in MSA and PSP (Gerstenecker, 2017), but PSP patients were markedly impaired on phonemic fluency subitem compared to PD and MSA. Another compelling finding was that seven or fewer words per minute distinguish PSP with a high sensitivity and specificity, from both PD and MSA. In conclusion, MoCA is more sensitive than MMSE in detecting cognitive impairment in atypical parkinsonisms, and together with its verbal fluency subitem is a useful test to support PSP diagnosis.

Interestingly, results of the longitudinal study in Chapter 4 strengthened these previous observations — PSP showed a more pronounced cognitive decline than MSA and PD particularly in the executive and language domains with more cases developing dementia. Verbal fluencies tasks and particularly phonemic fluency were severely impaired.

MoCA was more sensitive in detecting cognitive changes in PSP, while MMSE was better in PD.

Taken together these findings mirror the different distribution of the underlying pathology among the parkinsonian disorders. In PSP, tau-pathology involves both cortical and subcortical structures (from the frontal cortex to the dentate nucleus of the cerebellum) (Hauw et al., 1994; Williams et al., 2007). By contrast, MSA is characterized by alterations primarily in subcortical structures, and cortical pathology is not considered as a

predominant feature (Papp & Lantos, 1994), indeed, the cortical involvement can possibly be secondary to subcortical abnormalities.

Our findings show similar cognitive profiles in MSA and PD consistent with previous evidence (Lee et al., 2012). Our PD cohort had relatively short disease duration (approximately 8 years) and the proportion of PDD is in line with the literature (Hely et al., 2008). In MSA, dementia is infrequent, and it is reported only in patients with disease duration longer than 13 years (Petrovic et al., 2012).

In conclusion, the findings of the first two studies recommend the use of cognitive assessments to support differential diagnosis in atypical parkinsonisms, and to better understand the underlying pathology and its progression.

In the third part of the thesis (chapters 5 and 6), I investigated structural changes in synucleinopathies and the relationship with cognitive impairment. Several neuroimaging studies investigated the basis of cognitive deficits in PD (see Section 2.3.1), but recent evidence suggests synergistic contribution of amyloid to synuclein pathology (see Section 2.2.1).

Hence, the study presented in Chapter 5 was driven by the hypothesis that amyloid depositions in PD is a vulnerability factor and may facilitate the spread of synuclein aggregates.

Our analyses focused on the frontostriatal circuit and executive dysfunctions that are known to be altered since the disease's early stages (Lewis et al., 2003).

High amyloid levels in early-PD, measured by [^{18}F]florbetaben PET, were associated with: a) reduced striatal dopaminergic deficits (as expressed by higher striatal DAT binding). b) a tendency to show more frequent cognitive dysfunctions and increased brain atrophy rates (i.e., in frontal and occipital regions).

Notably, our results are consistent with recent neuropathological findings suggesting synaptic axonal damage and dysfunction in PD (Tagliaferro & Burke, 2016). Thus, we speculate that the interaction between synuclein and the coincident amyloid pathology, can further contribute to axonal vulnerability leading to a more 'malignant prognosis' (Irwin et al., 2013).

A growing body of literature also suggests MSA − like the other synucleinopathies − frequently experience cognitive deficits, although dementia is still considered a non-supporting feature for the diagnosis of MSA. Thus, the experimental study reported in

Chapter 6 was aimed at investigating the neuroanatomical basis underpinning cognitive dysfunction in MSA (see Section 2.3.2).

Our results showed that in MSA, focal reduction in the left DLPFC region is associated with the presence of cognitive alterations. Interestingly, the pattern underlying cognitive dysfunctions is focal and in the frontal region, which is different from PD, where cognitive deficits are associated with widespread cortical atrophies involving both frontal and posterior regions (Biundo et al., 2016a). Taken together, these observations suggest only a marginal contribution of cortical pathology to cognitive dysfunctions in MSA, revealing that cognitive alterations are driven by focal frontostriatal degeneration in line with the concept of 'subcortical cognitive impairment'.

In conclusion, the findings of the last two-neuroimaging studies in the synucleinopathies suggest that cognitive dysfunctions in MSA and PD are related to distinct underlying mechanisms. Cognitive deficits in MSA are associated with focal frontal atrophy, probably secondary to striato-pallido-thalamo-cortical circuits, while in PD the broader involvement of the frontal as well as more posterior regions may reflect the synergistic effect between synuclein and amyloid pathology.

In MSA, Kim and others (2013) showed limited contribution of amyloid pathology on cognition function — but further studies will be necessary to better investigate this issue.

In summary, all the experiments proposed in the present thesis highlight that cognitive alterations have a distinct pattern among the parkinsonian disorders, and together with clinical and neuroimaging evaluation, provides an essential contribution in the diagnostic process and in predicting clinical outcome.

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APPENDICES

- **APPENDIX I:** Diagnostic criteria for Parkinson's disease
- **APPENDIX II:** Diagnostic criteria for multiple system atrophy
- **APPENDIX III:** Diagnostic criteria for Progressive supranuclear palsy
- **APPENDIX IV:** Criteria for the Diagnosis of PD-MCI
- **APPENDIX V:** Criteria for the Diagnosis of PDD
- **APPENDIX VI:** Algorithm for diagnosing PDD at Level I

APPENDIX I Diagnostic criteria for Parkinson's disease. From Gelb et al, 1999.

Grouping of clinical features of Parkinson's disease according to diagnostic utility

GROUP A: Features characteristic of Parkinson's disease
Resting tremor
Bradykinesia
Rigidity
Asymmetric onset

GROUP B: Features suggestive of alternative diagnoses
Features unusual early in the clinical course
Prominent postural instability in the first 3 years after symptom onset
Freezing phenomena in the first 3 years
Hallucinations unrelated to medications in the first 3 years
Dementia preceding motor symptoms or in the first year
Supranuclear gaze palsy (other than restriction of upward gaze) or slowing of vertical saccades
Severe, symptomatic dysautonomia unrelated to medications
Documentation of a condition known to produce parkinsonism and plausibly connected to the patient's symptoms (such as suitably located focal brain lesions or neuroleptic use within the past 6 months)

Criteria for POSSIBLE diagnosis of Parkinson's disease

At least 2 of the 4 features in Group A are present; at least 1 of these is tremor or bradykinesia
And either:
None of the features in Group B is present
or symptoms have been present for less than 3-year and none of the features in Group B is present to date
And either:
Substantial and sustained response to levodopa or a dopamine agonist has been documented
or patient has not had an adequate trial of levodopa or dopamine agonist

Criteria for PROBABLE diagnosis of Parkinson's disease

At least 3 of the 4 features in Group A are present
and
None of the features in Group B is present (note: symptom duration of at least 3 years is needed to meet this requirement)
and
Substantial and sustained response to levodopa or a dopamine agonist has been documented

Criteria for DEFINITE diagnosis of Parkinson's disease

All criteria for POSSIBLE Parkinson's disease are met
and
Histopathological confirmation of the diagnosis is obtained at autopsy

Proposed criteria for histopathological confirmation of Parkinson disease

- Substantial nerve cell depletion with accompanying gliosis in the substantia nigra
- At least 1 Lewy body in the substantia nigra or in the locus coeruleus (note: it may be necessary to examine up to 4 non-overlapping sections in each of these areas before concluding that Lewy bodies are absent)
- No pathological evidence for other diseases that produce parkinsonism (progressive supranuclear palsy, multiple system atrophy, corticalbasal ganglionic degeneration) (Note: in excluding other diseases that produce Parkinsonism, published consensus criteria should be used when available)

APPENDIX II Diagnostic criteria for multiple system atrophy**.** From Gilman et al. 2008.

Criteria for POSSIBLE MSA

A sporadic, progressive, adult (>30 y)–onset disease characterized by:

- Parkinsonism (bradykinesia with rigidity, tremor, or postural instability) or
- A cerebellar syndrome (gait ataxia with cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction) and
- At least one feature suggesting autonomic dysfunction (otherwise unexplained urinary urgency, frequency or incomplete bladder emptying, erectile dysfunction in males, or significant orthostatic blood pressure decline that does not meet the level required in probable MSA) and
- At least one of the additional features:

Criteria for the diagnosis of PROBABLE MSA

A sporadic, progressive, adult (>30 y)–onset disease characterized by:

- Autonomic failure involving urinary incontinence (inability to control the release of urine from the bladder, with erectile dysfunction in males) or an orthostatic decrease of blood pressure within 3 min of standing by at least 30 mmHg systolic or 15 mmHg diastolic *and*
- Poorly levodopa-responsive parkinsonism (bradykinesia with rigidity, tremor, or postural instability) *or*
- A cerebellar syndrome (gait ataxia with cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction)

Additional features of POSSIBLE MSA

Possible MSA-P or MSA-C

● Babinski sign with hyperreflexia
● Stridor

Possible MSA-P

● Rapidly progressive parkinsonism
● Poor response to levodopa
● Postural instability within 3 y of motor onset
● Gait ataxia, cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction
● Dysphagia within 5 y of motor onset
● Atrophy on MRI of putamen, middle cerebellar peduncle, pons, or cerebellum
● Hypometabolism on FDG-PET in putamen, brainstem, or cerebellum

Possible MSA-C

● Parkinsonism (bradykinesia and rigidity)
● Atrophy on MRI of putamen, middle cerebellar peduncle, or pons
● Hypometabolism on FDG-PET in putamen
● Presynaptic nigrostriatal dopaminergic denervation on SPECT or PET

Criteria for DEFINITE MSA

Neuropathologic findings of widespread and abundant CNS synuclein–positive glial cytoplasmic inclusions (Papp– Lantos inclusions) in association with neurodegenerative changes in striatonigral or olivopontocerebellar structures

APPENDIX III Diagnostic criteria for Progressive supranuclear palsy. From Höglinger et al. 2017.

	Basic features
B1: Mandatory inclusion criteria	1. Sporadic occurrence 2. Age 40 or older at onset of first PSP-related symptom 3. Gradual progression of PSP-related symptoms
B2: Mandatory exclusion criteria	**Clinical findings** 1. Predominant, otherwise unexplained impairment of episodic memory, suggestive of AD 2. Predominant, otherwise unexplained autonomic failure, e.g., orthostatic hypotension (orthostatic reduction in blood pressure after 3 minutes standing 30mmHg systolic or 15mmHg diastolic), suggestive of multiple system atrophy or Lewy body disease 3. Predominant, otherwise unexplained visual hallucinations or fluctuations in alertness, suggestive of dementia with Lewy bodies 4. Predominant, otherwise unexplained multisegmental upper and lower motor neuron signs, suggestive of motor neuron disease (pure upper motor neuron signs are not an exclusion criterion) 5. Sudden onset or step-wise or rapid progression of symptoms, in conjunction with corresponding imaging or laboratory findings, suggestive of vascular etiology, autoimmune encephalitis, metabolic encephalopathies, or prion disease 6. History of encephalitis 7. Prominent appendicular ataxia 8. Identifiable cause of postural instability, e.g., primary sensory deficit, vestibular dysfunction, severe spasticity, or lower motor neuron syndrome **Imaging findings** 1. Severe leukoencephalopathy, evidenced by cerebral imaging 2. Relevant structural abnormality, e.g., normal pressure or obstructive hydrocephalus; basal ganglia, diencephalic, mesencephalic, pontine or medullary infarctions, hemorrhages, hypoxic-ischemic lesions, tumors, or malformations
B3: Context dependent exclusion criteria	**Imaging findings** 1. In syndromes with sudden onset or step-wise progression, exclude stroke, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) or severe cerebral amyloid angiopathy, evidenced by diffusion-weighted imaging (DWI), fluid attenuated inversion recovery, or T2*-MRI 2. In cases with very rapid progression, exclude cortical and subcortical hyperintensities on DWI-MRI suggestive of prion disease **Laboratory findings** 1. In patients with PSP-CBS, exclude primary AD pathology (typical CSF constellation [i.e., both elevated total tau/phospho-tau protein and reduced beta-amyloid 42] or pathological beta-amyloid PET imaging) 2. In patients aged<45 years, exclude a. Wilson's disease (e.g., reduced serum ceruloplasmin, reduced total serum copper, increased copper in 24-hour urine, and Kayser-Fleischer corneal ring) b. Niemann-Pick disease, type C (e.g., plasma cholestan-3ß,5a,6ß-triol level, filipin test on skin ibroblasts) c. Hypoparathyroidism d. Neuroacanthocytosis (e.g., Bassen-Kornzweig, Levine Critchley, McLeod disease) e. Neurosyphilis 3. In rapidly progressive patients, exclude a. Prion disease (e.g., elevated 14-3-3, neuron-specific enolase, very high total tau protein [>1,200 g/mL], or positive real-time quaking-induced conversion in CSF) b. Paraneoplastic encephalitis (e.g., anti-Ma1, Ma2 antibodies) 4. In patients with suggestive features (i.e., gastrointestinal symptoms, arthralgias, fever, younger age, and atypical neurological features such as myorhythmia), exclude Whipple's disease (e.g., T. Whipplei DNA polymerase chain reaction in CSF) **Genetic findings** 1. MAPT rare variants (mutations) are no exclusion criterion, but their presence defines inherited, as opposed to sporadic PSP. 2. MAPT H2 haplotype homozygosity is not an exclusion criterion, but renders the diagnosis unlikely. 3. LRRK2 and Parkin rare variants have been observed in patients with autopsy confirmed PSP, but their causal relationship is unclear so far. 4. Known rare variants in other genes are exclusion criteria, because they may mimic aspects of PSP clinically, but differ neuropathologically; these include: a. Non-MAPT associated frontotemporal dementia (e.g., C9orf72, GRN, FUS, TARDBP, VCP, CHMP2B); b. PD (e.g., SYNJ1, GBA); c. AD (APP, PSEN1, PSEN2); d. Niemann-Pick disease, type C (NPC1, NPC2); e. Kufor-Rakeb syndrome (ATP13A2); f. Perry syndrome (DCTN1); g. Mitochondrial diseases (POLG, mitochondrial rare variants); h. Dentatorubral pallidoluysian atrophy (ATN1); i. Prion-related diseases (PRNP); j. Huntington's disease (HTT); k. Spinocerebellar ataxia (ATXN1, 2, 3, 7, 17).

APPENDIX III *Continued*

Core clinical features				
	Functional Domain			
Levels of Certainty	**Ocular Motor Dysfunction**	**Postural Instability**	**Akinesia**	**Cognitive Dysfunction**
Level 1	O1: Vertical supranuclear gaze palsy	P1: Repeated unprovoked falls within 3 years	A1: Progressive gait freezing within 3 years	C1: Speech/language disorder, i.e., nonfluent/agrammatic variant of primary progressive aphasia or progressive apraxia of speech
Level 2	O2: Slow velocity of vertical saccades	P2: Tendency to fall on the pull-test within 3 years	A2: Parkinsonism, akinetic-rigid, predominantly axial, and levodopa resistant	C2: Frontal cognitive/behavioral presentation
Level 3	O3: Frequent macro square wave jerks or 'eyelid opening apraxia'	P3: More than two steps backward on the pull-test within 3 years	A3: Parkinsonism, with tremor and/or symmetric and/or levodopa responsive	C3: Corticobasal syndrome

APPENDIX III *Continued*

Operationalized definitions of core clinical features, supportive clinical clues, and supportive imaging findings		
	Ocular motor dysfunction	
O1	Vertical supranuclear gaze palsy	A clear limitation of the range of voluntary gaze in the vertical more than in the horizontal plane, affecting both up- and downgaze, more than expected for age, which is overcome by activation with the vestibulo-ocular reflex; at later stages, the vestibulo-ocular reflex may be lost, or the maneuver prevented by nuchal rigidity.
O2	Slow velocity of vertical saccades	Decreased velocity (and amplitude) of vertical greater than horizontal saccadic eye movements; this may be established by quantitative measurements of saccades, such as infrared oculography, or by bedside testing; gaze should be assessed by command ('Look at the flicking finger') rather than by pursuit ('Follow my finger'), with the target >20 degrees from the position of primary gaze; to be diagnostic, saccadic movements are slow enough for the examiner to see their movement (eye rotation), rather than just initial and final eye positions in normal subjects; a delay in saccade initiation is not considered slowing; findings are supported by slowed or absent fast components of vertical optokinetic nystagmus (i.e., only the slow following component may be retained).
O3	Frequent macro square wave jerks or 'eyelid opening apraxia'	Macro square wave jerks are rapid involuntary saccadic intrusions during fixation, displacing the eye horizontally from the primary position, and returning it to the target after 200 to 300 milliseconds; most square wave jerks are <1 degree in amplitude and rare in healthy controls, but up to 3 to 4 degrees and more frequent (>10/min) in PSP. 'Eyelid opening apraxia' is an inability to voluntarily initiate eyelid opening after a period of lid closure in the absence of involuntary forced eyelid closure (i.e., blepharospasm); the term is written in quotation marks because the inability to initiate eyelid opening is often attributed to activation of the pretarsal component of the orbicularis oculi (i.e., pretarsal blepharospasm) rather than failure to activate the levator lpebrae.
	Postural instability	
P1	Repeated unprovoked falls within 3 years	Spontaneous loss of balance while standing, or history of more than one unprovoked fall, within 3 years after onset of PSP-related features.
P2	Tendency to fall on the pull-test within 3 years	Tendency to fall on the pull-test if not caught by examiner, within 3 years after onset of PSP-related features. The test examines the response to a quick, forceful pull on the shoulders with the examiner standing behind the patient and the patient standing erect with eyes open and feet comfortably apart and parallel, as described in the MDS-UPDRS item 3.12.
P3	More than two steps backward on the pull-test within 3 years	More than two steps backward, but unaided recovery, on the pull-test, within 3 years after onset of PSP-related features.
	Akinesia	
A1	Progressive gait freezing within 3 years	Sudden and transient motor blocks or start hesitation are predominant within 3 years after onset of PSP-related symptoms, progressive and not responsive to levodopa; in the early disease course, akinesia may be present, but limb rigidity, tremor, and dementia are absent or mild.
A2	Parkinsonism, akinetic-rigid, predominantly axial and levodopa resistant	Bradykinesia and rigidity with axial predominance, and levodopa resistance (see in next page, Clinical Clue CC1 for operationalized definition).

APPENDIX III *Continued*

	Cognitive dysfunction	
C1	Speech/language disorder	Defined as at least one of the following features, which has to be persistent (rather than transient): 1. Nonfluent/agrammatic variant of primary progressive aphasia (nfaPPA) or Loss of grammar and/or telegraphic speech or writing 2. Progressive apraxia of speech (AOS) Effortful, halting speech with inconsistent speech sound errors and distortions or slow syllabically segmented prosodic speech patterns with spared single-word comprehension, object knowledge, and word retrieval during sentence repetition.
C2	Frontal cognitive/behavioral presentation	Defined as at least three of the following features, which have to be persistent (rather than transient): 1. Apathy Reduced level of interest, initiative, and spontaneous activity; clearly apparent to informant or patient. 2. Bradyphrenia Slowed thinking; clearly apparent to informant or patient. 3. Dysexecutive syndrome E.g., reverse digit span, Trails B or Stroop test, Luria sequence (at least 1.5 standard deviations below mean of age- and education-adjusted norms). 4. Reduced phonemic verbal fluency E.g., 'D, F, A, or S' words per minute (at least 1.5 standard deviations below mean of age- and education-adjusted norms). 5. Impulsivity, disinhibition, or perseveration E.g., socially inappropriate behaviors, overstuffing the mouth when eating, motor recklessness, applause sign, palilalia, echolalia.
C2	CBS	Defined as at least one sign each from the following two groups (may be asymmetric or symmetric): 1. Cortical signs a. Orobuccal or limb apraxia. b. Cortical sensory deficit. c. Alien limb phenomena. (more than simple levitation). 2. Movement disorder signs a. Limb rigidity. b. Limb akinesia. c. Limb myoclonus.
	Clinical clues	
CC1	Levodopa resistance	Levodopa resistance is defined as improvement of the MDS-UPDRS motor scale by _30%; to fulfill this criterion patients should be assessed having been given at least 1,000 mg (if tolerated) at least 1 month OR once patients have received this treatment they could be formally assessed following a challenge dose of at least 200 mg.
CC2	Hypokinetic, spastic dysarthria	Slow, low volume and pitch, harsh voice.
CC3	Dysphagia	Otherwise unexplained difficulty in swallowing, severe enough to request dietary adaptations.
CC4	Photophobia	Intolerance to visual perception of light attributed to adaptative dysfunction.
	Imaging findings	
IF1	Predominant midbrain atrophy or hypometabolism	Atrophy or hypometabolism predominant in midbrain relative to pons, as demonstrated, e.g., by MRI or [18F]DG-PET.
IF2	Postsynaptic striatal dopaminergic degeneration	Postsynaptic striatal dopaminergic degeneration, as demonstrated, e.g., by [123I]IBZM-SPECT or [18F]-DMFP-PET.

APPENDIX III *Continued*

Degrees of diagnostic certainty, obtained by combinations of clinical features and clinical clues

Diagnostic Certainty	*Definition*	*Combinations*	*Predominance Type*	*Abbreviation*
Definite PSP	Gold standard defining the disease entity	Neuropathological diagnosis	Any clinical presentation	def. PSP
Probable PSP	Highly specific, but not very sensitive for PSP Suitable for therapeutic and biological studies	(O1 or O2) + (P1 or P2)	PSP with Richardson's Syndrome	prob. PSP-RS
		(O1 or O2) + A1	PSP with progressive gait freezing	prob. PSP-PGF
		(O1 or O2) + (A2 or A3)	PSP with predominant parkinsonism	prob. PSP-P
		(O1 or O2) + C2	PSP with predominant frontal presentation	prob. PSP-F
Possible PSP	Substantially more sensitive, but less specific for PSP Suitable for descriptive epidemiological studies and clinical care	O1	PSP with predominant ocular motor dysfunction	poss. PSP-OM
		O2 + P3	PSP with Richardson's syndrome	poss. PSP-RS
		A1	PSP with progressive gait freezing	poss. PSP-PGF
		(O1 or O2) + C1	PSP with predominant speech/ language disorders	poss. PSP-SL
		(O1 or O2) + C3	PSP with predominant CBS	poss. PSP-CBS
Suggestive of PSP	Suggestive of PSP, but not passing the threshold for possible or probable PSP Suitable for early identification	O2 or O3	PSP with predominant ocular motor dysfunction	s.o. PSP-OM
		P1 or P2	PSP with predominant postural instability	s.o. PSP-PI
		O3 + (P2 or P3)	PSP with Richardson's syndrome	s.o. PSP-RS
		(A2 or A3) + (O3, P1, P2, C1, C2, CC1, CC2, CC3, or CC4)	PSP with predominant parkinsonism	s.o. PSP-P
		C1	PSP with predominant speech/ language disorder	s.o. PSP-SL
		C2 + (O3 or P3)	PSP with predominant frontal presentation	s.o. PSP-F
		C3	PSP with predominant CBS	s.o. PSP-CBS

APPENDIX IV Criteria for the Diagnosis of PD-MCI. From (Litvan et al., 2012)

I. Inclusion criteria

- Diagnosis of Parkinson's disease as based on the UK PD Brain Bank Criteria
- Gradual decline, in the context of established PD, in cognitive ability reported by either the patient or informant, or observed by the clinician
- Cognitive deficits on either formal neuropsychological testing or a scale of global cognitive abilities (detailed in section III)
- Cognitive deficits are not sufficient to interfere significantly with functional independence, although subtle difficulties on complex functional tasks may be present

II. Exclusion criteria

- Diagnosis of PD dementia based on MDS Task Force proposed criteria18
- Other primary explanations for cognitive impairment (e.g., delirium, stroke, major depression, metabolic abnormalities, adverse effects of medication, or head trauma)
- Other PD-associated comorbid conditions (e.g., motor impairment or severe anxiety, depression, excessive daytime sleepiness, or psychosis) that, in the opinion of the clinician, significantly influence cognitive testing

III. Specific guidelines for PD-MCI Level I and Level II categories

A. Level I (abbreviated assessment)

- Impairment on a scale of global cognitive abilities validated for use in PD or
- Impairment on at least two tests, when a limited battery of neuropsychological tests is performed (i.e., the battery includes less than two tests within each of the five cognitive domains, or less than five cognitive domains are assessed)

B. Level II (comprehensive assessment)

- Neuropsychological testing that includes two tests within each of the five cognitive domains (i.e., attention and working memory, executive, language, memory, and visuospatial)
- Impairment on at least two neuropsychological tests, represented by either two impaired tests in one cognitive domain or one impaired test in two different cognitive domains
- Impairment on neuropsychological tests may be demonstrated by:
    - Performance approximately 1 to 2 SDs below appropriate norms *or*
    - Significant decline demonstrated on serial cognitive testing *or*
    - Significant decline from estimated premorbid levels

IV. Subtype classification for PD-MCI (optional, requires two tests for each of the five cognitive domains assessed and is strongly suggested for research purposes)

- PD-MCI single-domain—abnormalities on two tests within a single cognitive domain (specify the domain), with other domains unimpaired *or*
- PD-MCI multiple-domain—abnormalities on at least one test in two or more cognitive domains (specify the domains)

APPENDIX V Criteria for the Diagnosis of PDD. Emre at al. 2007

Features of dementia associated with Parkinson's disease

I. Core features

1. Diagnosis of Parkinson's disease according to Queen Square Brain Bank criteria
2. A dementia syndrome with insidious onset and slow progression, developing within the context of established Parkinson's disease and diagnosed by history, clinical, and mental examination, defined as:
   - Impairment in more than one cognitive domain
   - Representing a decline from premorbid level
   - Deficits severe enough to impair daily life (social, occupational, or personal care), independent of the impairment ascribable to motor or autonomic symptoms

II. Associated clinical features

1. Cognitive features:
   - Attention: Impaired. Impairment in spontaneous and focused attention, poor performance in attentional tasks; performance may fluctuate during the day and from day to day
   - Executive functions: Impaired. Impairment in tasks requiring initiation, planning, concept formation, rule finding, set shifting or set maintenance; impaired mental speed (bradyphrenia)
   - Visuo-spatial functions: Impaired. Impairment in tasks requiring visual-spatial orientation, perception, or construction
   - Memory: Impaired. Impairment in free recall of recent events or in tasks requiring learning new material, memory usually improves with cueing, recognition is usually better than free recall
   - Language: Core functions largely preserved. Word finding difficulties and impaired comprehension of complex sentences may be present
2. Behavioral features:
   - Apathy: decreased spontaneity; loss of motivation, interest, and effortful behavior
   - Changes in personality and mood including depressive features and anxiety
   - Hallucinations: mostly visual, usually complex, formed visions of people, animals or objects
   - Delusions: usually paranoid, such as infidelity, or phantom boarder (unwelcome guests living in the home) delusions
   - Excessive daytime sleepiness

III. Features which do not exclude PD-D, but make the diagnosis uncertain

- Co-existence of any other abnormality which may by itself cause cognitive impairment, but judged not to be the cause of dementia, e.g. presence of relevant vascular disease in imaging
- Time interval between the development of motor and cognitive symptoms not known

IV. Features suggesting other conditions or diseases as cause of mental impairment, which, when present make it impossible to reliably diagnose PD-D

- Cognitive and behavioral symptoms appearing solely in the context of other conditions such as:

Acute confusion due to

a. Systemic diseases or abnormalities
b. Drug intoxication

Major Depression according to DSM IV

- Features compatible with 'Probable Vascular dementia' criteria according to NINDS-AIREN (dementia in the context of cerebrovascular disease as indicated by focal signs in neurological exam such as hemiparesis, sensory deficits, and evidence of relevant cerebrovascular disease by brain imaging AND a relationship between the two as indicated by the presence of one or more of the following: onset of dementia within 3 months after a recognized stroke, abrupt deterioration in cognitive functions, and fluctuating, stepwise progression of cognitive deficits)

Criteria for the diagnosis of probable and possible PDD

Probable PDD

A. Core features: Both must be present
B. Associated clinical features:
- Typical profile of cognitive deficits including impairment in at least two of the four core cognitive domains (impaired attention which may fluctuate, impaired executive functions, impairment in visuo-spatial functions, and impaired free recall memory which usually improves with cueing)
- The presence of at least one behavioral symptom (apathy, depressed or anxious mood, hallucinations, delusions, excessive daytime sleepiness) supports the diagnosis of Probable PD-D, lack of behavioral symptoms, however, does not exclude the diagnosis

C. None of the group III features present
D. None of the group IV features present

Possible PD-D

A. Core features: Both must be present
B. Associated clinical features:
- Atypical profile of cognitive impairment in one or more domains, such as prominent or receptive-type (fluent) aphasia, or pure storage-failure type amnesia (memory does not improve with cueing or in recognition tasks) with preserved attention
- Behavioral symptoms may or may not be present

OR

C. One or more of the group III features present
D. None of the group IV features present

APPENDIX VI Algorithm for diagnosing PDD at Level I. From Dubois et al., 2007

1	A diagnosis of Parkinson's disease based on the Queen's Square Brain Bank criteria for PD
2	PD developed prior to the onset of dementia
3	MMSE below 26
4	Cognitive deficits severe enough to impact daily living (Caregiver interview or Pill Questionnaire)
5	Impairment in at least two of the following tests: - Months reversed or Seven backward - Lexical fluency or Clock drawing - MMSE Pentagons - 3-Word recall

The presence of one of the following behavioral symptoms: apathy or depressed mood or elusions16 or excessive daytime sleepiness may support the diagnosis of probable PD-D.
The presence of major depression or delirium or any other abnormality, which may by itself cause significant cognitive impairment makes the diagnosis uncertain.

PUBLICATIONS AND PRESENTATIONS ASSOCIATED WITH THE THESIS

Full peer review journal articles

Fiorenzato, E., Weis, L., Falup-Pecurariu, C., Diaconu, S., Siri, C., Reali, E., . . . Biundo, R. (2016). Montreal Cognitive Assessment (MoCA) and Mini-Mental State Examination (MMSE) performance in progressive supranuclear palsy and multiple system atrophy. *Journal of Neural Transmission (Vienna), 123*(12), 1435−1442.

Fiorenzato, E., Weis, L., Seppi, K., Onofrj, M., Cortelli, P., Zanigni, S., . . . Imaging Study, G. (2017). Brain structural profile of multiple system atrophy patients with cognitive impairment. *Journal of Neural Transmission (Vienna), 124*(3), 293−302.

Fiorenzato, E., Antonini, A., Wenning, G., & Biundo, R. (2017). Cognitive impairment in multiple system atrophy. *Movement Disorders, 32*(9), 1338−1339.

Under review

Fiorenzato, E., Biundo, R., Cecchin, D., Frigo, A.C., Kim, J., Strafella, A.P., Antonini, A. Amyloid deposition affects cognitive and motor manifestations in Parkinson disease: the PPMI dataset.

Proceedings

Fiorenzato, E., Biundo, R., Weis, L., Seppi, K., Onofrj, M., Cortelli, P., Kaufmann, H., Poewe, W., Krismer, F., Wenning, G., Antonini, A. (2016). Brain structural abnormalities in multiple system atrophy patients with cognitive impairment [abstract]. Movement Disorders, 31 Suppl 2.

Fiorenzato, E., Biundo, R., Weis, L., Seppi, K., Onofrj, M., Cortelli, P., Kaufmann, H., Krismer, F., Wenning, G., Antonini, A. on behalf of the MODIMSA neuropsychology and imaging working groups (2016). Anatomical profile of cognitive impairment in MSA [abstract]. Parkinsonism & Related Disorders, 22:e118.

Fiorenzato, E., Weis, L., Falup-Pecurariu C., Antonini, A., Biundo, R. (2016). MoCA vs. MMSE sensitivity as screening instruments of cognitive impairment in PD, MSA and PSP patients [abstract]. Parkinsonism & Related Disorders, 22: e59-e60.

Fiorenzato, E., Weis, L., Antonini, A., Biundo, R. (2017). Cognitive profiling in patients with Parkinson's disease, multiple system atrophy and progressive supranuclear palsy: a 15-month longitudinal study [abstract]. Movement Disorders. 2017; 32 (suppl 2).

International academic conference presentations

Fiorenzato, E., Biundo, R., Weis, L., Seppi, K., Onofrj, M., Cortelli, P., Kaufmann, H., Poewe, W., Krismer, F., Wenning, G., Antonini, A. Brain structural abnormalities in multiple system atrophy patients with cognitive impairment.
Poster at XX International Congress of Parkinson's Disease and Movement Disorders, Berlin, June 19th – 23rd, 2016 and IV International Congress on Multiple System Atrophy, Salerno, April 22nd – 23rd, 2016.

Fiorenzato, E., Biundo, R., Weis, L., Seppi, K., Onofrj, M., Cortelli, P., Kaufmann, H., Krismer, F., Wenning, G., Antonini, A. on behalf of the MODIMSA neuropsychology and imaging working groups. Anatomical profile of cognitive impairment in MSA.
Poster at IAPRD-XXI World Congress on Parkinson's Disease and Movement Disorders, Milan, Italy, December 6th – 9th, 2015.

Fiorenzato, E., Weis, L., Falup-Pecurariu C., Antonini, A., Biundo, R. (2016). MoCA vs. MMSE sensitivity as screening instruments of cognitive impairment in PD, MSA and PSP patients.
Poster at IAPRD-XXI World Congress on Parkinson's Disease and Movement Disorders. Milan, Italy, December 6th – 9th, 2015 and IV International Congress on Multiple System Atrophy. Salerno, April 22nd – 23rd, 2016.

Fiorenzato, E., Weis, L., Antonini, A., Biundo, R. (2017). Cognitive profiling in patients with Parkinson's disease, multiple system atrophy and progressive supranuclear palsy: a 15-month longitudinal study.
Poster at XXI International Congress of Parkinson's Disease and Movement Disorders, Vancouver, Canada, June 4th – 8th, 2017.

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