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STRUCTURAL AND FUNCTIONAL BRAIN ALTERATIONS IN PARKINSON'S DISEASE AND POSSIBLE NON-PHARMACOLOGICAL APPROACHES

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1) Chapter 2: patient recruitment has been performed by Professor Kostic and collaborators, Clinic of Neurology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia. Chapters 3-5: patient recruitment has been performed by Dr. Maria Antoniettà Volonté and her collaborators from the Neurology Unit at San Raffaele Hospital, Milan, Italy.
2) Chapter 2: MRI acquisition of data reported in Chapter 2 has been performed by Professor Kostic and collaborators at Clinic of Neurology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia. Chapters 3-5: MRI acquisition was performed in collaboration with Prof. Falini and his group, Department of Neuroradiology, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy.
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DEDICATION

ABSTRACT

Parkinson's disease (PD) is one of the most common neurodegenerative conditions, characterized by the presence of motor symptoms due to a progressive loss of dopaminergic neurons in the substantia nigra pars compacta. This thesis aims: (1) to focus on the distinct disease progression profiles between idiopathic PD and patients with glucocerebrosidase (GBA) gene mutation (GBA-positive), and (2) to demonstrate the importance of non-pharmacological interventions to rehabilitate gait, balance and upper limb movements in PD patients. We examined the longitudinal disease course of PD GBA-positive compared to PD non-carriers (GBA-negative) along 5 years of observation, evaluating changes in clinical and cognitive outcomes, cortical thickness, and gray matter volumes: a more widespread temporo-parietal-occipital pattern of cortical damage was observed in GBA-positive individuals compared to the other group at baseline, which progressed to posterior, frontal and orbito-frontal cortices; additionally, they worsened significantly in motor and cognitive impairment. These findings suggest the importance of the early detection of genetic cases for intervention purposes. We further investigated whether a 6-week dual-task gait/balance training combined with Action Observation Training and Motor Imagery (DUAL-TASK+AOT-MI) could improve mobility, balance, cognition and brain functional reorganization compared to dual-task training alone (DUAL-TASK). We observed that DUAL-TASK+AOT-MI induced reduced recruitment of frontal areas and increased activity in the cerebellum, which correlated with balance/turning velocity and executive improvements, respectively. Lastly, we propose a new 8-week physiotherapy training with Virtual Reality (VR) for rehabilitation of bradykinesia of the upper limb in PD patients. We aim to assess improvements in speed and amplitude of movements, in handwriting and touch screen technology usage, and the relationship between brain activity modifications and patient clinical outcomes. Finally, we intend to explore the effects of VR-training on bodily sense of agency and PD-related cognitive functions. This thesis sheds light on the contribution of advanced magnetic resonance imaging techniques to study structural and functional correlates of disease evolution in PD patients, and the possibility to combine innovative non-pharmacological interventions with antiparkinsonian medication to improve patients' quality of life and independence.

TABLE OF CONTENTS

ACRONYMS AND ABBREVIATIONS

10MWT=10 meters walking test; ABC=activities balance confidence scale; ACE-R= Addenbrooke's cognitive examination-revised; AOT-MI=action observation training-motor imagery; AST=attention switching task; BDI=Beck Depression Inventory; CANTAB=Cambridge Neuropsychological Test Automated Battery; COMT= catechol-O-methyl transferase; DAT=dopamine transporter; DTC=Dual-task cost; FDR=False Discovery Rate; FIRST=FMRIB's Integrated Registration and Segmentation Tool; fMRI=functional magnetic resonance imaging; FoG=freezing of gait; GBA=glucocerebrosidase; GLM=general linear model; GM=gray matter; HAMA=Hamilton Anxiety Rating Scale; HDRS=Hamilton Depression Rating Scale; HY=Hoehn & Yahr; IC=independent components; ICA=independent component analysis; LEDD=Levodopa equivalent daily dose; LRRK2=leucine-rich repeat kinase 2; MAO-B= monoamine oxidase B; MCI=mild cognitive impairment; MDS=Movement Disorder Society; MiniBESTest=Mini Balance Evaluation Systems Test; MMSE=Mini Mental State Examination; MoCA=Montreal Cognitive Assessment Test; MNI=Montreal Neurological Institute; MNS=mirror neuron system; MRI=magnetic resonance imaging; NFoG-Q=New freezing of gait questionnaire; NMS-Q=Nonmotor symptoms questionnaire; NPI=Neuropsychiatric Inventory; PD=Parkinson's disease; PDQ-39=Parkinson's disease Questionnaire-39 items; PIGD=postural instability and gait disorders; PRM=Pattern Recognition Memory; QUIP-RS=Questionnaire for Impulsive-Compulsive in Parkinson's disease rating scale; RAVLT=Rey's Auditory Verbal Learning Test; RBD=REM sleep behavioural disorder; RBDSQ=REM sleep behavioural disorder screening questionnaire; RHI=rubber hand illusion; ROI=region of interest; RS-FC=resting-state functional connectivity; SHAPS=Snaith-Hamilton Pleasure Scale; SMA=supplementary motor area; SNpc=substantia nigra pars compacta; SnPM=Statistical non Parametric Mapping; SPM=Spatial Parametric Mapping; TD=tremor dominant; TMT=Trail Making Test; TUG=Timed Up and Go test; UPDRS=Unified Parkinson's Disease Rating Scale; VR=virtual reality; WM=white matter.

Chapter 1 – Introduction

1.1 Parkinson's Disease

1.1.1 Definition and epidemiology

Parkinson's disease (PD) is one of the most common neurodegenerative conditions, firstly described by James Parkinson in his 1817's work "Essay on the Shaking Palsy", which reported: *"there appears to be sufficient reason for hoping that some remedial process may ere long be discovered, by which, at least, the progress of the disease may be stopped"* (Parkinson, 2002). By definition, PD is distinguished from other neurodegenerative conditions by a progressive loss of dopaminergic neurons in the nigrostriatal system (specifically, in the substantia nigra pars compacta [SNpc]) and by abnormal accumulation of alfa-synuclein or Lewy body inclusions in the neurons (Lew, 2007). PD incidence is estimated to range from 5 every 100.000 cases to 35 every 100.000 new cases every year, and to increase from 5 to 10 fold after the sixth decade of life (Twelves, Perkins et al., 2003). In general, in the last 30 years, the incidence of PD has reached 1% of affected individuals over 60 years of age, which counts almost 6 million affected individuals worldwide (Collaborators, 2018). Mortality risk is not increased when patients are diagnosed with PD and within the first decade after diagnosis compared to healthy people, but it significantly increases afterward (Pinter, Diem-Zangerl et al., 2015). Due to the progressive ageing of the general population worldwide, PD prevalence is estimated to increase substantially, almost doubling in the next decades, which will cause an escalation of economic and societal burden for most of the high-income countries (Dorsey, Sherer et al., 2018).

PD etiopathology is still partially unknown, but it has a multifactorial nature. Most of PD risk is attributable to environmental and behavioural factors, while genetic heritability seems to play a crucial role in only 30% of affected cases (Goldman, Marek et al., 2019). Environmental factors include the exposure to toxicant chemicals (e.g., pesticides, chlorinated solvents, trichloroethylene, manganese, or iron) (Tanner, Goldman et al., 2014), while behavioural factors comprise, for example, dietary intake of dairy products. On the other hand, there is evidence that some factors seem to reduce the risk of

developing PD, namely cigarette smoking, tobacco usage, coffee drinking, and intense and frequent physical activity (Simon, Tanner et al., 2020).

In addition, biological sex differences are determined in PD: epidemiological observation suggests older age at onset and in general lower incidence in women compared to men, probably due to their higher physiological striatal dopamine levels induced by oestrogens activity, therefore suggesting a more "benign" PD phenotype in women (Haaxma, Bloem et al., 2007).

1.1.2 Pathophysiology

As reported in the previous paragraph, PD is characterized by a progressive loss of dopaminergic neurons in the SNpc in the midbrain and is associated to the presence of Lewy bodies, cytoplasmic inclusions which contain insoluble alpha-synuclein aggregates. Nonetheless, there is evidence that a more widespread pathology is present in other brain regions, involving also non-dopaminergic neurons (Simon et al., 2020). At diagnosis, patients have already lost a large amount of dopaminergic neurons in the SNpc, and neurodegeneration has reached other regions of the central nervous system. In fact, PD manifests itself clinically when pathology has already reached an advanced stage (Fearnley, 1991). In the case of sporadic PD, a staging of brain pathology has been proposed in 2003 by Braak and colleagues (Braak, Del Tredici et al., 2003), which comprises six stages based on temporal and spatial progression of pathological processes.

In stages I and II, alpha-synuclein inclusions are present in the lower brainstem. Firstly, lesions appear in the dorsal IX/X motor nucleus, intermediate reticular zone, and olfactory system; secondly, in the caudal raphe nuclei, gigantocellular reticular nucleus, and coeruleus–subcoeruleus complex. These first two stages are considered pre-symptomatic stages, where motor abilities are still spared, but early non-motor symptoms can already be present. In stages III and IV, intraneuronal pathology progresses rostrally involving the upper brainstem (specifically, the SNpc) and the temporal mesocortex. In this phase, patients have clinical motor manifestations of the disease. In stages V and VI the pathology has spread from the temporal mesocortex into the adjoining neocortex and limbic structures. The extensive damage of the brain may lead to declining intellectual abilities and impaired cognition of late PD. In these stages, the brain is strongly compromised by the spreading of the pathology (Braak et al., 2003). Figure 1 represents

a scheme of the progression of neuropathology in sporadic PD patients according to Braak's staging. The progressive loss of dopaminergic neurons causes a reduced dopaminergic transmission to the basal ganglia system (in particular, the striatum), which is close to the caudate. Because of the deposition of altered misfolded proteins, the whole basal ganglia system is progressively altered both functionally and structurally, therefore leading to important deficits in movements control and goal-directed behaviours (Poewe, Seppi et al., 2017).

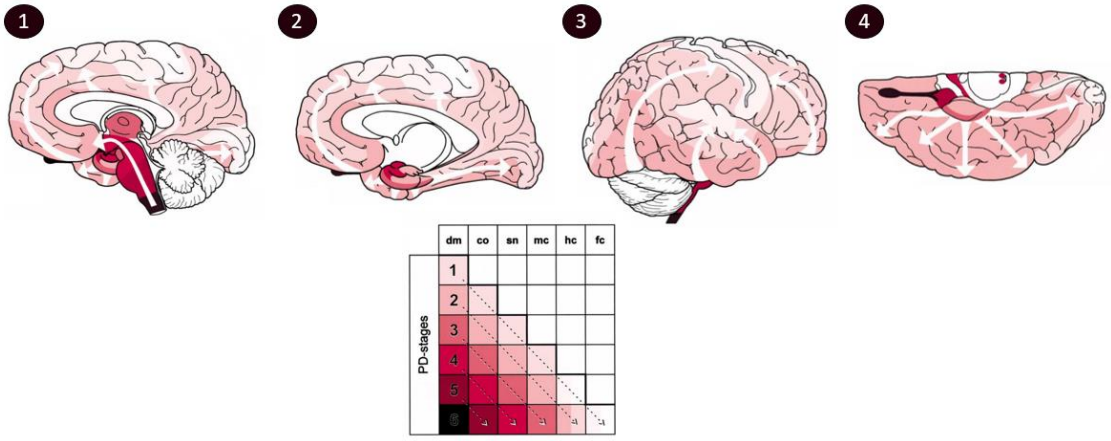

Figure 1. Progression of intraneuronal pathology in sporadic PD patients. (Above) *Pathological spreading in PD.* ***(Bottom)*** *Topographic lesions expansion (from left to right) and growing severity of overall pathology (from top to bottom: stages 1–6). Abbreviations: co, coeruleus–subcoeruleus complex; dm, dorsal motor nucleus of the glossopharyngeal and vagal nerves; fc, first order sensory association areas, premotor areas, as well as primary sensory and motor fields; hc, high order sensory association areas and prefrontal fields; mc, anteromedial temporal mesocortex. Figure adaptation from Braak et al., Neurobiol Aging 2003.*

Since the SN is one of the largest sources of dopamine in the central nervous system, PD motor features seem to occur due to a deficiency in dopamine supply to the motor circuit of the basal ganglia. The cortex communicates with the basal ganglia via two major parallel projection systems, namely the direct and the indirect pathways. These systems originate from distinct populations of striatal neurons with opposite effects upon the basal ganglia output (Alexander, DeLong et al., 1986). The indirect pathway tends to inhibit actions by competing with the direct pathway, which allows movement to occur by liberating the motor thalamus from constant inhibition (Schmidt, Leventhal et al., 2013). The basal ganglia, once the cortical input is received and processed, return it via the

thalamus back to the cortex itself and back to the striatum via a direct thalamo-striatal pathway. Overall, the basal ganglia function is to select desired actions and to inhibit competing unwanted actions, resembling a "breaking system" (Mink, 1996). Accordingly, the outputs of the basal ganglia arise from globus pallidus internus and SN pars reticulata and are inhibitory to the thalamus, superior colliculi, and the pedunculopontine nucleus (Poewe et al., 2017). As these inhibitory outputs release tonic inhibition on the desired motor pattern generator by decreasing their discharge, they do the opposite to other competing motor pattern generators. In PD, bradykinesia results from the loss of nigrostriatal dopaminergic neurons: this loss depends on striatal dopamine depletion, which produces an imbalance between direct (facilitatory) and indirect (inhibitory) pathways through the basal ganglia.

1.1.3 Genetics

As reported previously, most PD cases are sporadic without a family history of the disease. The initial identification of genetic forms of PD was possible with twin studies, and the population-based Swedish Twin Registry reported the presence of a concordance rate for PD of 4% for monozygotic twins, and a modest heritability of PD longitudinally (Wirdefeldt, Gatz et al., 2011). Almost 5-10% of all PD cases is caused by monogenes, ranging from rare variants with very large effects to genetic variants with only modest effects and quite common in the general population (Cherian & Divya, 2020). In general, clinical manifestations of PD genetic forms are similar to idiopathic PD, but these patients usually present earlier disease onset, more rapid progression, and higher risk of cognitive impairment (Balestrino & Schapira, 2020).

The first discovered monogenic form associated to PD was SNCA, a gene which function is to encode alpha-synuclein proteins; apart from SNCA, the most common genes linked to PD are PARK1/4 alpha-synuclein and PRKN PARK2 Parkin. A recent review showed that more than 20 gene mutations are associated with PD (please refer to Figure 2) (Balestrino & Schapira, 2020). On the other hand, mutations in LRRK2 and parkin are the most common causes of dominant- and recessive-inherited PD.

A recent meta-analysis revealed that 24 loci appear to be linked to increased risk to develop PD; apart from well-known genes such as LRRK2 and SNCA, these loci include also the glucocerebrosidase (GBA) gene. This gene encodes for the lysosomal enzyme β-

GBA and is associated to the greatest genetic risk factor for developing PD pathology (Nalls, Pankratz et al., 2014).

Gene	Locus name	Protein name	Chromosome	Inheritance	Clinics	Frequency in PD	Protein function
SNCA	PARK1/4	α-synuclein	4q21–23	AD	EOPD	<1%	Synaptic
PRKN	PARK2	Parkin	6q25–27	AR	EOPD, slow progression, + dystonia	1%–5% (up to 44% in EOPD)	Ubiquitin-ligase
UCHL1	PARK5	UCHL-1	4p14	AD	EOPD, LOPD	<1%	Uncertain
PINK1	PARK6	PTEN-induced putative kinase I	1p35–37	AR	EOPD, slow progression	2%–5%	Mitochondrial kinase
DJ-1	PARK7	Protein DJ-1	1p36	AR	EOPD, slow progression	1%	Cellular sensor of oxidative stress
LRRK2	PARK8	Leucine-rich repeat serine/ threonine-protein kinase 2	12p11–q13	AD	LOPD, slow progression	1%–5% (up to 40% in North African Berber Arab patients)	Multiple functions domain dependent
ATP13A2	PARK9	ATPase type 13A2	1p36	AR	Atypical parkinsonism, Kufor Rakeb syndrome	<1%	Lysosomal protein
PLA2G6	PARK14	A2 phospholipase	22q13	AR	EOPD, dystonia-parkinsonism	<1%	Unknown
FOXB7	PARK15	F-box protein 7	22q12–13	AR	EOPD, atypical parkinsonism	<1%	Unknown
VPS35	PARK17	Vacuolar protein sorting-associated protein 35	16q11	AD or risk	LOPD	<1%	Unknown
GBA					Glucocerebrosidase	1q21	Risk factor
Earlier onset +	dementia	5%–25% (10%–30% in Ashkenazi Jewish patients)	Lysosomal protein				

AD, autosomal dominant; AR, autosomal recessive; EOPD, early onset PD; LOPD, late onset PD.

Figure 2. The most common gene mutations associated with PD. *Figure adapted from Balestrino and Shapira, Eur J Neurol 2020. Abbreviations: AD=autosomal dominance; AR=autosomal recessive; EOPD=early onset Parkinson's disease; LOPD=late onset Parkinson's disease.*

1.1.4 Diagnosis

According to the Movement Disorder Society (MDS) Clinical Diagnostic Criteria for PD, diagnosis of parkinsonism is mostly based on clinical features, and it refers to the presence of motor and non-motor symptoms (Postuma, Berg et al., 2015). Diagnosis requires the presence of bradykinesia, in combination with either rest tremor or rigidity, or both, and with at least two supportive criteria (such as beneficial response to dopaminergic treatment, presence of levodopa-induced dyskinesia, rest tremor of a limb, positive result from at least one ancillary diagnostic test having a specificity greater than 80% for differential diagnosis of PD from other parkinsonian conditions, and the presence of either olfactory loss or cardiac sympathetic denervation), absence of absolute exclusion criteria (e.g., cerebellar abnormalities, downward vertical supranuclear gaze palsy,

diagnosis of probable behavioural variant of frontotemporal dementia or primary progressive aphasia, parkinsonian features restricted to the lower limbs for more than 3 years, etc.) and red flags (e.g., rapid progression of gait impairment requiring wheelchair within 5 years from disease onset, early bulbar dysfunction, etc.) (Postuma et al., 2015). However, several non-motor features, such as constipation, anosmia, REM sleep behaviour disorder (RBD), cognitive dysfunction, apathy and depression can develop years prior to the onset of motor impairment. Diagnostic accuracy can vary according to patient disease duration, age, or expertise of the clinician. However, definitive diagnosis of PD can only be made based on post-mortem identification of hallmark neuropathological changes in the brain, namely accumulations of alpha-synuclein in Lewy bodies and Lewy neurites. Two possible levels of diagnostic certainty have been devised according to the MDS criteria for PD: clinically established PD (Figure 3B) or clinically probable PD (Figure 3C-D) (Bloem, Okun et al., 2021).

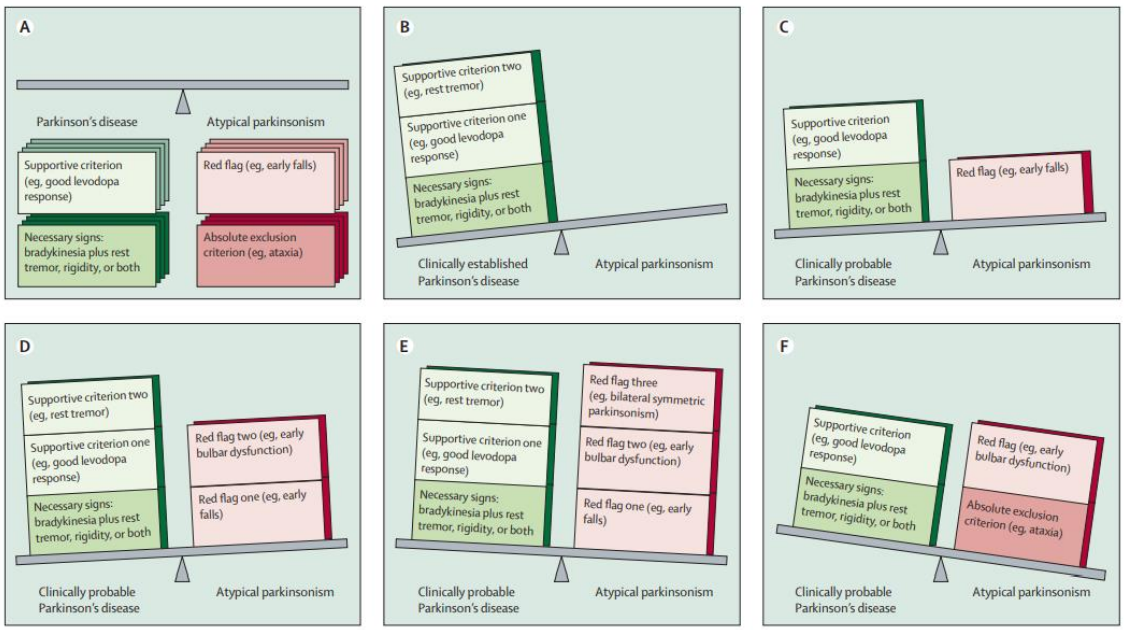

Figure 3. Diagnostic weighting process (International Parkinson and Movement Disorder Society diagnostic criteria for Parkinson's disease). *(A) PD diagnosis is established from a combination of symptoms/signs which should be present, and exclusion of absolute exclusion criteria and red flags; (B-D) Ways to establish "clinically established" and "clinically probable" PD. Figure adapted from Bloem et al., Lancet 2021.*

To date, the gold standard clinical rating scale adopted to evaluate PD severity is the MDS Unified PD Rating Scale (MDS-UPDRS) (Goetz, Tilley et al., 2008), which is a revision of the classic UPDRS, which lacked consistent anchors among subscales, and

the inclusion of attention to non-motor features of PD. MDS-UPDRS comprises 50 five-level Likert items (from 0=normal to 4=severe), and it is divided as follows:

- Part I: non-motor experiences of daily living (13 questions);
- Part II: motor experiences of daily living (13 questions);
- Part III: motor examination (18 questions)
- Part IV: motor complications (6 questions).

Furthermore, PD severity is also evaluated through the Hoehn & Yahr (H&Y) staging scale, which combines functional deficits (disability) and objective signs (impairment) (Goetz, Poewe et al., 2004). H&Y scale is composed by five stages:

- Stage 1: unilateral involvement;
- Stage 2: bilateral involvement without balance difficulties;
- Stage 3: presence of postural instability;
- Stage 4: loss of physical independence;
- Stage 5: being wheelchair- or bed-bound

More advanced stages correspond to worse quality of life.

1.1.5 Motor and non-motor features

As previously reported in paragraph 1.1.4, PD has three main motor characteristics, namely rest tremor, bradykinesia/hypokinesia/akinesia, and rigidity, which worsen over time with disease progression (Berardelli, Wenning et al., 2013, Postuma, Aarsland et al., 2012). PD can be distinguished from other parkinsonisms since motor symptoms appear insidiously and asymmetrically at disease onset (usually involving one limb segment), with bilateral involvement occurring only with disease progression; furthermore, this disease has a slow progression and good response to levodopa administration (Lew, 2007). Several PD clinical subtypes have been described based on motor signs and symptoms, cognitive impairment, non-motor features and behavioural disturbances; regarding predominant motor features and based on the MDS-UPDRS, patients can be divided in 'tremor-dominant' (TD), 'postural instability and gait difficulty' (PIGD), or 'indeterminate' phenotypes (Jankovic, McDermott et al., 1990), each showing a distinctive clinical progression and prognosis. TD patients usually present a slower progression and less disability compared to PIGD patients, who in turn show a more rapid clinical progression, increased risk to develop disability and dementia, and poorer

response to treatment (Fereshtehnejad & Postuma, 2017). Here follows a brief description of motor features:

- Tremor: usually one of the first motor signs, it is a 4-6 Hz rhythmical contraction of agonist-antagonist muscles, which usually worsens with emotional and cognitive stress (Greenland & Barker, 2018). Generally, tremor has unilateral onset, even though it later involves both limbs. Furthermore, tremor disappears with action-based movements (Greenland & Barker, 2018).
- Bradykinesia: slowness and decrement in amplitude of repeated movements, which can lead to akinesia. The principal signs of bradykinesia are hypomimia, eye blinking reduction, lower and monotone voice, sialorrhea and progressive micrographia (Hayes, 2019). Usually, bradykinesia is assessed through specific movements: finger tapping, repetitive hand movements, pronation-supination, toe tapping and foot tapping (Jankovic, 2008).
- Rigidity: increased tone, described as "lead-pipe" resistance and associated to "cogwheel" phenomenon (Greenland & Barker, 2018). It can increase during voluntary movement and during cognitive tasks (Leenders & Oertel, 2001).
- Postural instability: it is defined as an impairment in balance which can affect patient posture, usually being a consequence of less flexible postural responses to perturbations (Kim, Allen et al., 2013). It is one of the most disabling features of PD: being the first cause of falling, it significantly increases falling risk with disease progression (Blaszczyk, Orawiec et al., 2007).
- Freezing: described as "motor block", it is a form of akinesia usually associated with gait impairment (or 'freezing of gait', [FoG]), which causes falls in these patients. Typically, patients describe this phenomenon as having their "feet glued to the floor" (Ebersbach, Moreau et al., 2013), and it can happen at the initiation of gait, while transitioning through a narrow space, or immediately after reaching a destination (Amano, Roemmich et al., 2013). Freezing can also involve the upper limbs, for example during activities such as handwriting.

Apart from motor symptoms, approximately 90% of PD patients develop non-motor symptoms with disease progression (Jankovic, 2008). Even though non-motor features are disabling for patients with PD, they are generally under-reported and under-investigated by clinicians, and they impact significantly patients' quality of life (Schapira,

Chaudhuri et al., 2017). Non-motor symptoms comprehend hyposmia, psychiatric symptoms (e.g., depression, anxiety, apathy, hallucinations, psychosis), cognitive impairment (please refer to paragraph 1.1.6), genitourinary features (e.g., constipation, reduced stomach emptying), dysphagia, sialorrhea, dysarthria, hypophonia, sleep disturbances (e.g., REM sleep behaviour disorder [RBD]), and cardiovascular issues (e.g., blood pression variations) (Balestrino & Schapira, 2020). Some of these symptoms have been recognized as 'prodromal/premotor' symptoms, occurring even ten years prior to the onset of motor symptoms; hyposmia, depression, constipation and RBD are the most common prodromal features, but others such as visual changes, anxiety and autonomic disturbances might co-occur with disease onset (Postuma et al., 2012). Figure 4 depicts a scheme relative to the progression of both motor and non-motor features.

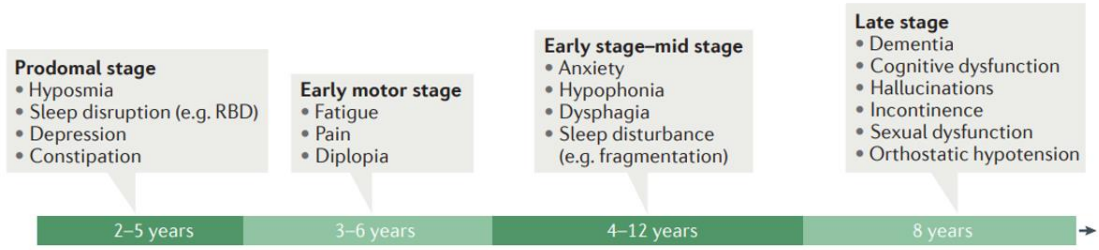

Figure 4: Onset of motor and non-motor features in PD. *Symptoms are reported from prodromal stage (left) to late stage (right). From Shapira et al., Nat Rev Neurosci 2017. Abbreviations: RBD=REM sleep behaviour disorder.*

In general, non-motor features can occur insidiously several years before the onset of motor symptoms, and the duration of this prodromal stage varies considerably between patients (Schapira et al., 2017).

1.1.6 Cognitive impairment in Parkinson's disease

Cognitive impairment is one of the most common and disabling non-motor features of PD patients, being up to six times more frequent in these patients than in the general population (Aarsland, Andersen et al., 2001). Cognitive decline can occur years or decades after the PD diagnosis; in general, it has high variability in terms of both severity, progression and which cognitive domains are involved (Fengler, Liepelt-Scarfone et al., 2017). PD individuals might display the full spectrum of cognitive impairment (Figure 5), from subjective cognitive decline (SCD) and mild cognitive impairment (MCI) to dementia (PDD).

SCD can be defined as a self-perceived decline in cognitive performance which is not detected through standardized neuropsychological cognitive tests (Jessen, Amariglio et al., 2014). On the other hand, MCI is considered a progressive decline in cognitive performance reported by both patients and their clinicians and caregivers, with relatively spared functional independence in the activities of daily living. Based on the number of affected cognitive domains, MCI patients can be divided in single-domain MCI or multiple-domain MCI (Litvan, Goldman et al., 2012). In addition, patients with PDD show cognitive deficits in at least two of four cognitive domains (among attention, visuospatial abilities, memory, and executive functioning) (Emre, Aarsland et al., 2007), displaying functional impairment which affects normal performance in daily life. Among *de novo* PD patients who complain subjective memory complaints, almost 30% is likely to develop MCI within 2 years compared to those patients without memory complaints (Erro, Santangelo et al., 2014). Regarding PD-MCI, it is estimated that almost 20% of PD patients have MCI at time of diagnosis which increases to 40-50% after 5 years of follow-up, while the estimate in the general population ranges from 16% to 20% (Roberts & Knopman, 2013). However, longitudinal assessments demonstrated that the course of MCI is very variable, and it is not infrequent to observe a stabilization of cognitive function (or even reversion from PD-MCI to normal cognition), which is estimated to occur in 25% of cases (Pedersen, Larsen et al., 2017). While a global prevalence of 5-7% of dementia is observed in the general older population, almost 17% of PD patients develop dementia after 5 years from diagnosis, becoming 46% after 10 years, and approximately 83% 20 years later (Buter, van den Hout et al., 2008, Hely, Reid et al., 2008, Prince, Bryce et al., 2013). These data suggest that the identification of early predictors of cognitive decline becomes of outmost importance for prevention and ameliorating disease prognosis. A lot of factors might contribute to increase the risk of developing cognitive difficulties or dementia: hallucinations, advanced age, worsening of motor symptoms severity, speech impairment, higher H&Y stage, PIGD, fewer years of education, depression co-occurrence and male sex (Marinus, Zhu et al., 2018). Interestingly, difficulties in distinct cognitive domains might have different predicting power: specifically, frontal and executive dysfunction and frontal atrophy of the brain are associated with higher risk to convert to PDD (Chung, Lee et al., 2020).

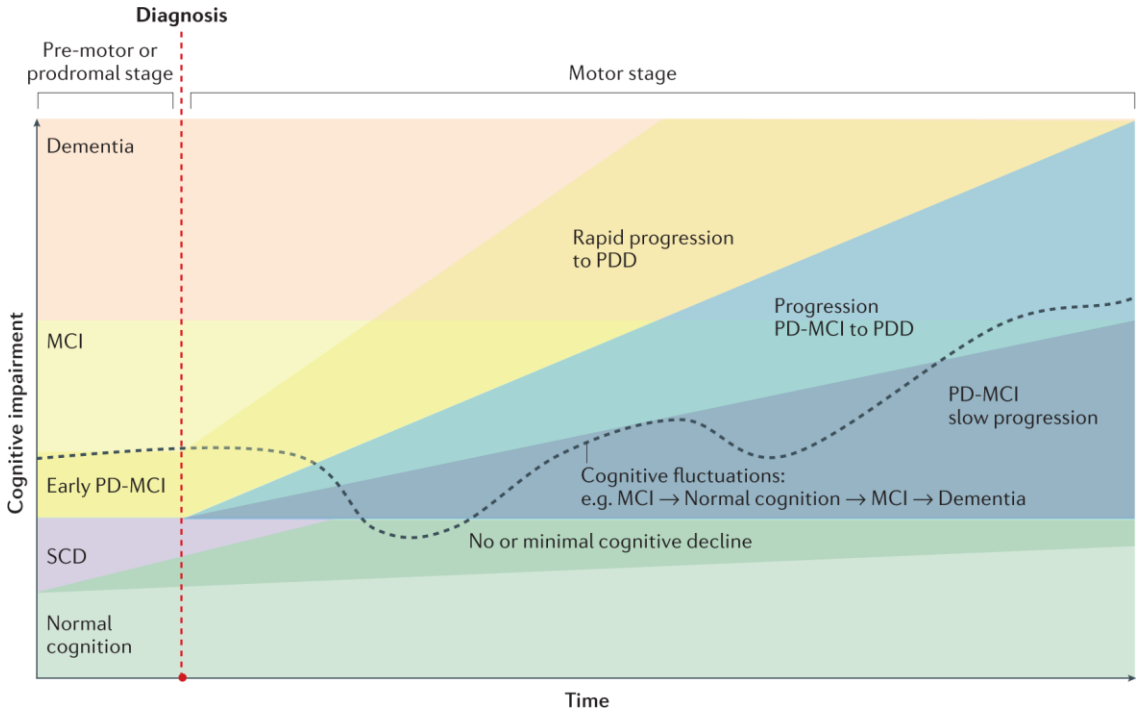

Figure 5. Cognitive impairment progression in patients with PD. *From Aarsland et al., Nat Rev Dis 2021. Abbreviations: MCI=Mild Cognitive Impairment; PD=Parkinson's disease; PDD=Parkinson's disease with dementia; SCD=subjective cognitive decline.*

The diagnosis of cognitive status in patients with PD can be performed through the evaluation of global cognitive functioning with neuropsychological assessment, which allows the evaluation of several cognitive domains, namely attention, language, memory, executive functions, and visuospatial skills. Neuropsychological tests are validated standardized tests with population norms; raw scores are influenced by education level and patient age and, based on the norms, are transformed into z-scores or equivalents. To date, SCD is not easily detected with neuropsychological testing and no validated instruments are able to detect this condition in PD (Aarsland, Batzu et al., 2021). However, criteria to diagnose PD-MCI have been proposed by the MDS in 2012, and include the presence of cognitive decline as reported by the patient, caregiver or clinician, and impairment in neuropsychological testing, with preserved functional impendence in the activities of daily living (Litvan et al., 2012). Figure 6 reports the MDS PD-MCI diagnostic criteria, which are characterised by two levels of assessment (Level I and II). These criteria appeared to have prognostic validity for the progression to dementia with both Level I and Level II assessments; however, specificity and sensitivity of Level I assessment are lower than Level II (Baiano, Barone et al., 2020).

Movement Disorder Society PD-MCI diagnostic criteria

Level I – Abbreviated assessment

- Impairment on Parkinson disease (PD)-appropriate global cognitive ability scale (such as Montreal Cognitive Assessment (MoCA), Parkinson's Disease – Cognitive Rating Scale (PD-CRS), Mattis Dementia Rating Scale Second Edition (MDRS-2))
- Impairment on at least two neuropsychological tests when a limited set of tests is used (less than two tests per domain or less than five cognitive domains assessed)

Level II – Comprehensive assessment

✓ Neuropsychological testing includes two tests per domain:
- Attention and working memory
- Executive functions
- Language
- Memory
- Visuospatial skills

✓ Impairment on two tests in one domain or impairment on one test in two different domains
✓ Impairment shown by:
- Score 1–2 SD below norm
- Significant decline on serial testing
- Significant decline from estimated premorbid functioning

PD with mild cognitive impairment (PD-MCI) subtype classification (comprehensive level II assessment required)

- Single domain: impairment on two or more tests in one domain
- Multiple domain: impairment on at least one test in each of two or more domains

Figure 6. Movement Disorder Society PD-MCI diagnostic criteria. *Adapted from Litvan et al., Mov Disord 2012. Level I refers to an abbreviated assessment with a limited battery of neuropsychological tests, while Level II consists on comprehensive neuropsychological evaluation testing five cognitive domains. Abbreviations: SD=standard deviation.*

Similar to PD-MCI criteria, PDD criteria contain a two-level operational scheme (refer to Figure 7). The main feature describing PDD is an insidious decline in more than one cognitive domain lasting for at least six months and impairing daily life. Importantly, behavioural aspects (e.g., mood alterations, apathy, sleepiness, hallucinations) may be present, and functional impairment due to cognitive impairment is essential for diagnosis.

Movement Disorder Society PDD diagnostic criteria

Level I – Parkinson disease dementia (PDD)

- A diagnosis of Parkinson disease (PD) based on the UK Brain Bank criteria for PD
- PD developed prior to the onset of dementia
- MMSE < 26
- Cognitive deficits severe enough to impact daily living (caregiver interview or Pill Questionnaire) independent of motor symptoms
- Impairment in more than one cognitive domain, that is, at least two of the following aspects:
  - ✓ Months Reversed or Seven Backward
  - ✓ Lexical Fluency or Clock Drawing
  - ✓ MMSE Pentagons
  - ✓ Three-Word Recall
- Absence of major depression, delirium and other abnormalities that obscure diagnosis

Level II – Comprehensive assessment for characterizing PDD

This evaluation assesses four domains:

- Decreased global cognitive effiency
- Subcorticofrontal features of PDD
- Instrumental (cortically mediated) functions:
  - ✓ Language
  - ✓ Visuoconstructive
  - ✓ Visuospatial
  - ✓ Visuoperceptive
- Neuropsychiatric features:
  - ✓ Apathy
  - ✓ Depression
  - ✓ Visual hallucinations
  - ✓ Psychosis

Figure 7. Movement Disorder Society PDD diagnostic criteria. *Adapted from Litvan et al., Mov Disord 2012. Abbreviations: MMSE=Mini Mental State Examination.*

With the advent of new technologies, computerized cognitive testing has become far more common in clinical settings, enabling also patients' evaluations remotely from their homes through the use of computers or tablets; benefits of these new instruments include the possibility of frequent testing without learning effects for the patients, which in turn ameliorates the sensitivity of detecting cognitive decline (Brooker, Williams et al., 2020).

However, many challenges in tele-neuropsychology remain, such as the access to stable internet connection, copyright issues, and feasibility of using internet and digital platforms in older populations (who might present sensory loss or impairment). Given these limitations, face-to-face examination is routine in clinical settings, but future studies should understand how computerized testing can support health care professionals.

1.1.7 Pharmacological treatment

Even though disease-modifying therapies to treat PD patients are currently unavailable, pharmacological treatment of PD is focused on the dopaminergic pathway. Levodopa is the most common treatment for PD, it converts to dopamine in the

dopaminergic neurons of the SNpc and is the most effective therapy against motor symptoms (Fox, Katzenschlager et al., 2011). However, this treatment might cause side effects in the patients, such as sleepiness, hallucinations, nausea, hypotension, or compulsive behaviours. Furthermore, motor complications might occur with disease progression and therefore dopaminergic degeneration (e.g., dyskinesia, wearing-off, fluctuations); to reduce motor fluctuations in advanced PD cases, gastrostomy catheters might be required for the administration of levodopa directly into the duodenum (Fernandez & Odin, 2011).

Dopamine agonists are another therapeutic possibility, consisting on the direct stimulation of postsynaptic dopamine D1-D3 receptors in the striatum. Although they are not as effective as levodopa administration for treating motor symptoms, they are associated to lower risk of dyskinesia. Usually, they are administered in the early disease phases, or together with levodopa administration (Blandini & Armentero, 2014).

In early/mild cases, other compounds such as monoamine oxidase B (MAO-B) inhibitors might be employed, due to their ability to reduce dopamine metabolism and boost dopaminergic stimulation, and their less complications compared to levodopa. Unfortunately, these compounds do not modify PD natural history (Robakis & Fahn, 2015).

Another possibility relies on the inhibition of catechol-O-methyl transferase (COMT) enzymes, which have a role in levodopa metabolism. Usually, this treatment is used together with levodopa administration to increase its half-life (Muller, 2015).

To date, several new compounds are under active investigation, but the heterogeneity of PD clinical phenotypes and progression of the disease reflects different underlying pathogenic mechanisms, leading to different therapeutic response for patients. Clinical trials might benefit from the early identification of pre-clinical populations, who constitute the perfect target for disease-modifying compounds. However, dopaminergic degeneration in the SNpc is already advanced when a clinical diagnosis of PD is reached, therefore complicating and excluding the possibility of early therapeutic possibilities (Balestrino & Schapira, 2020).

Despite the beneficial effect of pharmacological options to treat motor symptoms in PD, non-pharmacological possibilities have been proposed in recent years in conjunction to pharmacological therapy, with the aim to reduce functional impairment and improve

patients' quality of life (Van de Weijer, Hommel et al., 2018). This aspect will be further discussed in the following section and in Chapter 5 (paragraph 5.1)

1.2 Neurorehabilitation In Parkinson's Disease

1.2.1 Neuroplasticity

Neuroplasticity is the capacity of our central nervous system to adjust in response to internal and external stimuli by altering its structure and function in response to environmental requirements (Kleim, 2011, Petzinger, Fisher et al., 2013). It is widely reported in the literature that physical practice may lead to changes in terms of neuroplasticity, inducing brain structure and function alterations in a positive direction for the patient. However, it is important to remember that this field of research is still in its infancy: the majority of published studies investigating exercise-induced neuroplasticity in PD are based on small cohorts, and usually lack of information regarding generalizability of findings, feasibility aspects, or lack of transparency on which population the patients were enrolled from (Johansson, Hagstromer et al., 2020).

A primary focus of neurorehabilitation in PD has regarded motor deficits, which occur since the early disease phases: the delivery of exercises incorporating goal-based motor skill learning has proved to ameliorate motor skill performance, which can be pursued also with cognitive engagement (Petzinger et al., 2013). There is evidence that the combination of goal-based behaviour with aerobic training might reduce the amount of attentional demand of consciously processing behaviours (e.g., during walking) (Yogev, Giladi et al., 2005). Most of the reported studies which focused on motor exercises in PD pointed out the neuroplasticity effect of restoring to some extent basal ganglia circuitry, which might improve motor learning and behaviour in PD patients (Petzinger et al., 2013).

1.2.2 Action Observation Training and Motor Imagery

As reported previously, patients with PD exhibit alterations in balance and gait, which limit consistently patients' independence in daily living. The use of external cues in association to physical exercise has been widely employed in the past years, thus

improving active and automatic movements, which are usually affected since the early disease stages and with disease progression (Santiago, de Oliveira et al., 2015).

Mental practice techniques are rehabilitative cognitive practices aimed to imagining a motor action without its physical execution, performed repeatedly, in order to improve motor accuracy and control (Guillot, Collet et al., 2009). At the neural level, these techniques contribute to the activation of sensory-motor networks, facilitating motor preparation, learning, planning, and movement execution (Schuster, Hilfiker et al., 2011). Studies on PD patients combined mental practices to physical exercises; however, no consensus has been reached regarding its results, probably due to the variability of rehabilitation protocols and training time.

Action Observation Training (AOT) and Motor Imagery (MI) are two mental practice techniques aimed to improve motor learning by relying on the mirror neuron system (MNS) of the brain. The MNS comprehends brain regions such as the inferior parietal lobule and the premotor cortex and, in humans, this brain system plays an important role in understanding the intentions of others. The involvement of the MNS in motor learning has led to the development of a rehabilitation approach called AOT. This type of training consists in asking a patient to observe actions performed by an operator, then try to imitate them after observation (Sarasso, Gemma et al., 2015); there is evidence that this type of training induces reorganization changes in the primary motor cortex, thus reinforcing intact cortical networks and facilitating the activation of the impaired ones, and then boosting the formation of motor memories (Wang, Collinger et al., 2010). Previous studies highlighted how AOT recruits areas belonging to the motor network and the MNS (e.g., ventral premotor cortex, inferior frontal gyrus and inferior parietal lobule), which are activated both during the observation of actions and while acquiring new motor skills (Buccino, Vogt et al., 2004, Stefan, Cohen et al., 2005).

MI is another mental practice technique based on the patient imagining himself while performing an action, and subsequently executing that action. This technique favours motor learning through the MNS, and neuroplasticity is ideal when time of imagination and that of gesture execution is similar (Di Rienzo, Collet et al., 2012). The neural pathways used during MI are the same of motor execution and involve supplementary motor area, premotor cortex, primary visual cortex, posterior parietal regions, and the cerebellum (Moran & O'Shea, 2020). MI has shown to induce beneficial effects on gait

re-education and creation of new attentional strategies in patients with PD (Mirelman, Maidan et al., 2013).

Both AOT and MI have important advantages, such as being non-invasive, safe, low-cost, and very practical (they can be performed even at patients' home) (Caligiore, Mustile et al., 2017). Furthermore, a very recent systematic review pointed out that interventions with AOT and MI might improve disease severity, balance, and gait in PD patients. Group interventions with AOT have also showed improvement in balance, spatiotemporal gait parameters, and freezing of gait; furthermore, in the short- and long-term, the combination of both AOT and MI is considered as the most effective compared to adopting AOT and MI alone (Lahuerta-Martin, Llamas-Ramos et al., 2022).

1.2.3 Upper limb rehabilitation using Virtual Reality

Physiotherapy is a useful and mandatory approach for the management of PD; in fact, it has the potential to improve motor and non-motor performance through the modulation of cerebral function and structure. To achieve these goals, physiotherapists usually employ cueing strategies, cognitive movements strategies and exercise to maintain and increase independence and safety in the patients. In general, neurorehabilitation strategies aim to ameliorate motor learning, defined as the ability to learn, improve, and retain performance through practice thanks to the possibility to modify connectivity and activation of motor and cognitive networks during training (Paul, Dibble et al., 2018).

The main aim of rehabilitation of the upper limb in PD patients is to ameliorate fine motor abilities, precision, and ability to segment manual movements, with the final goal to improve functional activities of daily living. To achieve these goals, physiotherapist can improve motor learning abilities through goal-oriented approaches, in which the patients are driven by motivation to perform the activities.

In the last years, the introduction of new neurorehabilitation technologies, such as virtual reality (VR), opens up new perspective in the rehabilitation of function and activity in several neurological disorders, including PD (Picelli, Tamburin et al., 2014). VR is a system consisting of the interaction with an artificial reality that emulates the real world, where characteristics of movements are controlled, measurable and modifiable (Arias, Robles-Garcia et al., 2012). VR could optimize motor learning facilitating the execution of a greater number of repetitions, increasing the sensory feedback on the task

performance, enhancing the challenge of the proposed tasks, and augmenting the engagement and motivation of patients through a game-like setup. Indeed, using VR, patients can modify their position, make movements, and perform goal-oriented actions through the interaction with the virtual environment and receiving an immediate and on-line feedback on their performance. Depending on the degree to which the participant is separated from the physical surroundings, VR technology can vary from non-immersive to fully immersive. Non-immersive VR allows interaction with the environment through monitors, mouse, or joysticks, while immersive VR uses tools connected to the human body to perform the same motor task, resulting in a higher level of embodiment of the VR system. Few studies suggested VR as a possible way to train upper limb abilities in patients with PD, and specifically to improve functional outcomes, such as movement speed, amplitude, resistance, and consequently quality of life (van Beek, van Wegen et al., 2019). Furthermore, VR-based games can also improve coordination and fine manual dexterity in PD patients, and it seems to be more effective than conventional physiotherapy alone (Fernandez-Gonzalez, Carratala-Tejada et al., 2019, van Beek et al., 2019). VR is also frequently used for assessing hand and upper limbs movements because it offers the opportunity to obtain objective quantifications of motor dysfunctions (Bank, Cidota et al., 2018). In addition, VR can be useful as a complementary treatment to stimulate patients' motivation and adherence to treatment (Fernandez-Gonzalez et al., 2019). This aspect is particularly true for immersive VR, where the patients are more engaged with the training (Fernandez-Gonzalez et al., 2019). However, optimal input devices and personalized exercises development seems to be crucial to obtain significant results in neurorehabilitation (Pazzaglia, Imbimbo et al., 2020).

1.3 Magnetic Resonance Imaging In Parkinson's Disease

Brain magnetic resonance imaging (MRI) is commonly used in the clinical practice to evaluate structural brain anatomy and pathology. In neurodegenerative conditions, MRI examination can help the clinician in studying the pattern of brain degeneration and reach a correct diagnosis (Meijer & Goraj, 2014). In the case of PD, MRI examination is recommended for diagnostic purposes (Berardelli et al., 2013), especially for excluding cerebrovascular damage or the presence of atypical parkinsonian disorders, which is very difficult also for the most experienced movement disorder specialists. In fact, modern and

unconventional MRI techniques have improved diagnostic accuracy for differential diagnosis of neurodegenerative parkinsonism, which is crucial for determining disease prognosis and targeting the most suitable therapeutic approaches (Mahlknecht, Hotter et al., 2010). Furthermore, recent advances in MRI techniques facilitate early diagnosis of these patients and contribute to monitor disease progression over time. Compared to unconventional MRI, conventional MRI examination usually do not show abnormalities in patients with PD outside of the SNpc, and also the basal ganglia usually appear normal at MRI examination or only show subtle changes in terms of volume, diffusion measurements or iron deposition (Chougar, Pyatigorskaya et al., 2020). Therefore, advanced neuroimaging techniques have become increasingly promising for the identification of early biomarkers of disease onset and progression. In the following paragraphs, the importance of using structural and functional MRI assessments will be investigated and discussed.

1.3.1 Structural MRI

Structural MRI enables to evaluate grey matter (GM) and white matter (WM) integrity. GM alterations are usually studied through probabilistic (i.e., voxel-based morphometry [VBM]) or quantitative tools (i.e., cortical thickness) (De Micco, Russo et al., 2018). Usually, the available studies which focused on GM changes in PD patients stratified their sample based on different criteria, such as disease duration and severity, medication intake, and the presence of cognitive alterations. In accordance with pathological evolution of the disease as suggested by Braak and colleagues (Braak et al., 2003) and considering disease duration, *de novo* PD cases might show no atrophy at baseline (Caspell-Garcia, Simuni et al., 2017). However, GM atrophy and cortical thinning involving fronto-parietal and temporal/hippocampal structures might occur only 2-3 years from disease onset, and it is usually associated to visuospatial and executive-attentive impairments (Tessa, Lucetti et al., 2014). Basal ganglia atrophy (especially in the caudate and putamen) is usually observed in the middle stages of the disease, even though an early involvement of these structures has been observed in a few studies (Campabadal, Uribe et al., 2017, Lewis, Du et al., 2016).

Considering patient cognitive status, subtle cortical thinning has been observed in frontal, temporal, parietal and occipital lobes in cognitively normal patients (Duncan,

Firbank et al., 2013), while development of subsequent PD-MCI status and PDD are associated to progressive worsening of cortical atrophy (Filippi, Canu et al., 2020). Several cross-sectional and longitudinal structural MRI studies have reported both cortical and subcortical damage underlying cognitive impairment in PD patients: MCI development is related to increased cortical thinning of fronto-temporo-parietal areas, while progressive atrophy in frontotemporal areas, hippocampus, thalamus and caudate nucleus is related to progression to PDD (Chung, Shin et al., 2017, Chung, Yoo et al., 2019, Filippi et al., 2020, Gasca-Salas, Garcia-Lorenzo et al., 2019, Gee, Dukart et al., 2017, Gorges, Kunz et al., 2020).

A recent 4-year longitudinal study from our research group showed that cortical damage is evident since the initial stages of cognitive decline: compared to healthy subjects, posterior brain regions (parietal and occipital cortices) are the first regions to be hit along cognitive decline course (especially in patients who are likely to convert to PD-MCI), while involvement of fronto-temporo-parietal regions is associated to conversion to more severe stages of cognitive impairment (Filippi et al., 2020). Furthermore, progressive volume loss in the thalamus and hippocampus was retrieved in severely cognitively impaired individuals, suggesting a possible role of these regions in the development of cognitive deficits and dementia. These findings suggested that, in PD, cortical alterations reached a sort of "plateau" in the early phases of the disease without further progressing with disease course. An interesting hypothesis raised from this work is related to the possible different cognitive trajectories: in fact, cognitive evolution of PD patients might depend on the brain regions hit by the disease, rather than by the total amount of cortical damage. This might in turn mean that patients with early posterior cortical deficits have higher risk of developing subsequent dementia.

1.3.2 Functional MRI

Functional MRI (fMRI) enables the study of brain function, neural activation, and those mechanisms associated with brain plasticity. Measurement is performed by exploiting the inherent blood paramagnetic properties which enable to identify modifications of the transverse magnetization relaxation time associated to the blood flow fluctuations and blood oxygenation level-dependent (BOLD) mechanism. When a brain region is activated, cerebral metabolism increases, with consequent increased

vasodilatation and blood flow. Extra blood supply exceeds the request and results in a reduction of deoxyhaemoglobin; this reduction causes a change of relative levels of oxygenated blood (oxyhaemoglobin) and deoxygenated blood (deoxyhaemoglobin) that can be detected through differential magnetic susceptibility (oxyhaemoglobin is diamagnetic, deoxyhaemoglobin is paramagnetic). When a diamagnetic substance is exposed to the magnetic field, the field decreases; on the contrary, when a paramagnetic substance is exposed to the magnetic field, the field increases (Ogawa, Lee et al., 1990). Thus, high concentration of deoxyhaemoglobin causes a decrease of MRI signal, and low concentration causes an increase of MRI signal (Haller & Bartsch, 2009). These changes are used to generate T2 weighted MRI echo-planar sequences. fMRI can be used to study the brain activity at rest (resting-state fMRI [RS-fMRI], see paragraph 1.3.2.1) or to capture stimulus evoked-changes in network organization while executing a task (task-based functional MRI, see paragraph 1.3.2.2).

1.3.2.1 Resting-state functional MRI

RS-fMRI measures interrelations between regional spontaneous dynamics at rest, while awake, and allows to study the brain functional reorganization in several neurodegenerative diseases, including PD condition. During MRI examination and specifically RS condition, a co-activation of different brain regions delineates RS functional networks. Typically, brain networks which are recognized during RS are: Default Mode Network (DMN), Executive Control Network (ECN), Sensorimotor Network (SN), Salience Network (SAL), Dorsal Attention Network (DAN), Visual Processing Network (VPN), and Auditory Network (AN) (Filippi, Sarasso et al., 2019). These RS brain networks and their functions are depicted and described in Figure 8. FMRI is considered as a useful tool to identify early brain functional connectivity alterations, which are likely to occur before structural damage, and possibly as a crucial biomarker to detect brain plasticity mechanisms in neurodegenerative conditions induced by pharmacological and neurorehabilitation treatment effects.

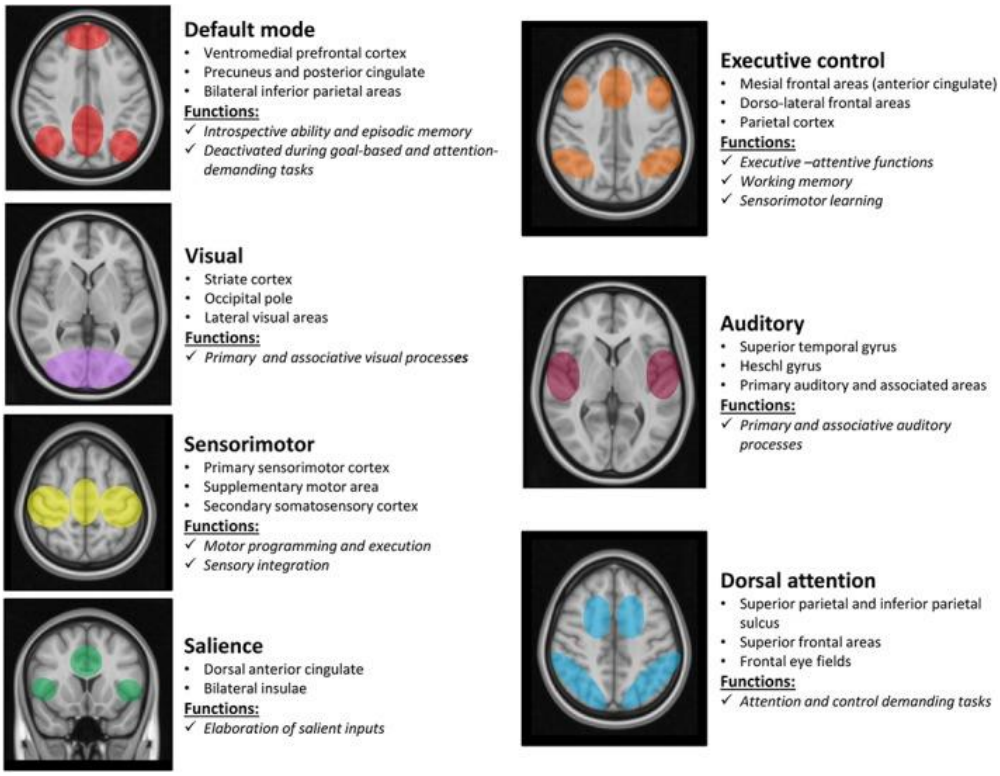

Figure 8. Resting-state brain networks with respective functions. *Filippi et al., Mov Disord Clin Pract 2019.*

1.3.2.2 Task-based functional MRI

Task-based fMRI commonly compares rest condition versus activation. However, due to the small changes in amplitude of the BOLD signal (which is approximately 3-4%), it is necessary to perform long acquisition to increase reliability (Filippi, Sarasso et al., 2018). Two experimental designs are usually employed for task-based fMRI studies (Norris, 2006):

1. Event related design: each task is presented individually for a short time;
2. Block design: the action of interest is repeated multiple times within a block of several seconds that alternate with block at rest (or control activity).

Dual-task conditions, especially during motor performance, have been extensively studied in fMRI settings in PD patients. A few studies showed altered cortical and subcortical brain activity in PD patients while performing gait-mimicking tasks, and this was especially observed in patients who presented FoG (Gilat, Dijkstra et al., 2019, Piramide, Agosta et al., 2020). Another study employing VR to imitate gait turning showed increased reliance on frontal areas in PD patients with FoG, which became even

greater when they experienced dual-task conditions. Worse dual-task performance has also been correlated with greater activation of cerebellar and cortico-subcortical circuits (Gao, Zhang et al., 2017).

FMRI is undoubtedly a useful tool to investigate and define brain functional abnormalities in neurodegenerative conditions, including PD. However, it is clear that the identification of sensitive and early biomarkers in the prodromal phases of the disease is crucial to detect those patients at risk to develop cognitive impairment and to accurately and promptly treat patients in the best way as possible (Filippi et al., 2019)

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1.4 Thesis Aims

In the context of highlighting the importance of using both structural and functional advanced MRI techniques to study disease progression and predict disability status in patients with PD, and to report the lack of resolutive and long-lasting antiparkinsonian treatments for PD patients, the experimental chapters of the present thesis had the following broad aims:

- In *Chapter 2,* I focused on studying the longitudinal clinical, cognitive and neuroanatomical changes in patients with PD and GBA mutation compared to idiopathic PD, trying to elucidate whether these genetic cases have a distinct disease evolution compared to nongenetic cases;
- In *Chapters 3 and 4,* I studied the clinical, motor, cognitive and brain fMRI features in PD patients who underwent a 6-week rehabilitation training which combined dual-task gait/balance training with AOT and MI, trying to evaluate how PD patients can benefit from neurorehabilitation approaches both in the short- and long-term;
- In *Chapter 5,* I reported the study design of a project currently ongoing, which intend to improve upper limb movements and sense of agency in patients with PD with the use of VR.

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Chapter 2 – Longitudinal clinical, cognitive, and neuroanatomical changes over 5 years in GBA-positive Parkinson's disease

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ORIGINAL COMMUNICATION

Longitudinal clinical, cognitive, and neuroanatomical changes over 5 years in GBA-positive Parkinson's disease patients

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Abstract

Objective To study the longitudinal disease course of Parkinson's disease (PD) patients with glucocerebrosidase (GBA) mutation (GBA-positive) compared to PD non-carriers (GBA-negative) along a 5-year follow-up, evaluating changes in clinical and cognitive outcomes, cortical thickness, and gray-matter (GM) volumes.

Methods Ten GBA-positive and 20 GBA-negative PD patients underwent clinical, neuropsychological, and MRI assessments (cortical thickness and subcortical, hippocampal, and amygdala volumes) at study entry and once a year for 5 years. At baseline and at the last visit, each group of patients was compared with 22 age-matched healthy controls. Clinical, cognitive, and MRI features were compared between groups at baseline and over time.

Results At baseline, GBA-positive and GBA-negative PD patients had similar clinical and cognitive profiles. Compared to GBA-negative and controls, GBA-positive patients showed cortical thinning of left temporal, parietal, and occipital gyri. Over time, compared to GBA-negative, GBA-positive PD patients progressed significantly in motor and cognitive symptoms, and showed a greater pattern of cortical thinning of posterior regions, and frontal and orbito-frontal cortices. After 5 years, compared to controls, GBA-negative PD patients showed a pattern of cortical thinning similar to that showed by GBA-positive cases at baseline. The two groups of patients showed similar patterns of subcortical, hippocampal, and amygdala volume loss over time.

Conclusions Compared to GBA-negative PD, GBA-positive patients experienced a more rapid motor and cognitive decline together with a greater, earlier and faster cortical thinning. Cortical thickness measures may be a useful tool for monitoring and predicting PD progression in accordance with the genetic background.

Keywords Glucocerebrosidase gene · GBA · Parkinson's disease · Cortical thickness · Magnetic resonance imaging

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2.1 Introduction

The GBA gene encodes for the lysosomal enzyme glucocerebrosidase (GCase), which catalyses the cleavage of a major glycolipid glucosylceramide into glucose and ceramide (Romero, Ramanathan et al., 2019). The absence of a fully functional enzyme leads to the accumulation of its lipid substrates in lysosomes, causing the rare Gaucher's disease, which demonstrates a wide phenotypic variability, from an asymptomatic form to disease with severe organ damage (Grabowski, 2008, Romero et al., 2019). GBA mutations, both homo- and heterozygotic, are also the most common known genetic risk factor for developing PD (Neudorfer, Giladi et al., 1996, Tayebi, Callahan et al., 2001, Tayebi, Walker et al., 2003).

Large multicentre studies in PD showed that the risk for GBA mutation carriers (GBA-positive) to develop PD varies from 7 to 12% (Aflaki, Westbroek et al., 2017, Sidransky, Nalls et al., 2009). Moreover, several cross-sectional and longitudinal clinical studies reported that GBA-positive PD patients are characterized by an earlier disease onset, worse motor impairment, more rapid disease progression, higher risk of cognitive decline and depression, more severe autonomic dysfunction and reduced survival rate compared with idiopathic PD (Brockmann, Srulijes et al., 2011, Brockmann, Srulijes et al., 2015, Cilia, Tunesi et al., 2016, Stoker, Camacho et al., 2020, Zhang, Shu et al., 2018). Only few studies so far have investigated cross-sectional neuroimaging characteristics in GBA-positive PD patients (Agosta, Kostic et al., 2013, Greuel, Trezzi et al., 2020, Kono, Ouchi et al., 2010, Saunders-Pullman, Hagenah et al., 2010, Thaler, Kliper et al., 2018). Molecular imaging showed that, compared to non-carriers, GBA-positive PD patients have hypometabolism in the striatum, anteromedial frontal cortex, supplemental motor area and parieto-occipital cortices (Kono et al., 2010, Saunders-Pullman et al., 2010). A dopamine transporter (DAT) imaging study reported a more severe reduction in both putamen and caudate nuclei uptake in GBA-carriers with parkinsonism compared to GBA-negative patients(Kono et al., 2010). Compared to idiopathic PD, GBA-positive patients showed also WM microstructural MRI abnormalities in inter- and intra-hemispheric bundles including the corpus callosum, olfactory tract, cingulum, internal and external capsule, while no GM volume differences between groups were detected (Agosta et al., 2013). Another study found neither cortical thickness nor subcortical volume differences between GBA-positive PD and patients with LRRK2 mutations

(Thaler et al., 2018). In GBA-positive PD compared to GBA-negative patients, a reduced resting state functional connectivity between the bilateral caudate nuclei and the occipital cortex, and between the right nucleus accumbens and the left superior parietal and the right occipital fusiform cortex was observed (Greuel et al., 2020).

Against this background, assessing structural brain changes over time in GBA-positive PD patients may help elucidating why these patients show a distinct disease evolution compared to idiopathic PD. The present work aims to describe the longitudinal disease course of GBA-positive compared to GBA-negative PD patients enrolled in their very early disease stage (i.e., H&Y $\leq$ 1.5) along a 5-year follow-up, evaluating clinical, cognitive and structural MRI outcomes.

2.2 Materials and methods

2.2.1 Patients

Eighty-six patients with a diagnosis of PD at a very early disease stage (H&Y $\leq$1.5) were prospectively recruited at the Movement Disorders Department of the Neurology Clinic, Clinical Center of Serbia, within the framework of an ongoing longitudinal project, as previously described (Filippi, Canu et al., 2020a, Filippi, Sarasso et al., 2020b). All patients fulfilled the UK PD Society Brain Bank diagnostic criteria (Hughes, Daniel et al., 1992). At study entry, the sample included both naïve patients and patients with a stable dopaminergic treatment. Patients were evaluated at study entry and every year or every two years for at least two and a maximum of five follow-up visits within five years of observation (Supplementary Figure 1). Patients were excluded if they had moderate/severe head tremor at rest, dementia at study entry according to the MDS diagnostic criteria for PD dementia (Emre, Aarsland et al., 2007), cerebrovascular disorders or intracranial masses on routine MRI, a history of traumatic brain injury, and any other neurological and medical conditions. The cohort is still under active follow-up.

At baseline, all patients underwent a genetic screening to retrieve the presence of any GBA mutations. Among the 86 PD patients, the screening reported the presence of a GBA mutation in 10 patients (see Supplementary Table 1 for specific mutations and type). Among the 76 GBA-negative patients, a sub-sample of 20 cases matched to the GBA-positive cohort in terms of age, age at onset, sex, H&Y stage and disease severity (i.e., UPDRS-III total score) was selected for the analysis. Twenty-two age-matched healthy

controls without any neurological, psychiatric, or other disorders, were recruited among nonconsanguineous relatives, institute personnel and by word of mouth for baseline comparison with PD patients. Healthy controls performed clinical, cognitive/behavioural and MRI assessments only at baseline. Demographic features of the cohort are reported in Table 1.

Approval was received from the local ethical standards committees on human experimentation and written informed consent was obtained from all subjects prior to study participation.

2.2.2 Clinical evaluation

At study entry and each follow-up visit, an experienced neurologist blinded to MRI results and GBA status performed clinical assessments. Patients were examined in ON state (i.e., period when the dopaminergic medication is working and symptoms are well controlled). Demographic, general clinical and family data (sex, education, age, age at onset, side of onset, PD duration) were obtained using a semi-structured interview. Levodopa equivalent daily dose (LEDD) (Tomlinson, Stowe et al., 2010) was calculated, and disease severity was defined using the H&Y stage score (Hoehn & Yahr, 1967) and the UPDRS (Movement Disorder Society Task Force on Rating Scales for Parkinson's, 2003). UPDRS was used to evaluate non-motor symptoms (UPDRS I), motor symptoms (UPDRS II), and motor signs (UPDRS III). The presence of other non-motor symptoms (i.e., gastrointestinal, urinary, olfactory, orthostatic and sexual dysfunctions) was assessed according to the NonMotor Symptoms questionnaire (NMS-Q) (Chaudhuri, Martinez-Martin et al., 2006). Sleep disorders were investigated using the REM Sleep Behaviour Disorder Screening Questionnaire (RBDSQ) (Stiasny-Kolster, Mayer et al., 2007). All these variables were obtained at each time point except for NMS-Q, UPDRS I (presence of hallucinations and psychosis), and RBDSQ scores, which were acquired until year 3. Clinical features are reported in Table 1.

2.2.3 Genetic screening

Sequence analysis was performed for exons 8-11 of the GBA gene. Primers used for the amplification of exons 8 and 9 were specific to the functional gene (GBA) rather than the pseudogene (GBAP). Exons 10 and 11 were amplified using nested PCR with partially

mismatched primers to avoid co-amplification of the GBAP. Subjects identified with the D409H mutation were also sequenced for H255Q. A part of these results has been previously published with a detailed methodological description (Kumar, Ramirez et al., 2013).

2.2.4 Neuropsychological and behavioural evaluations

At study entry and each follow-up visit, patients performed neuropsychological and behavioural evaluations within 48 hours from MRI. The same test battery was administered to healthy controls at study entry. Evaluations were performed by expert neuropsychologists, blinded to clinical, GBA status and MRI results, as previously described (Filippi et al., 2020a). All the neuropsychological and behavioural variables were acquired at each time point. Cognitive and behavioural characteristics are reported in Table 2.

Neuropsychological assessment evaluated: global cognition with the Mini Mental State Examination (MMSE) and the Addenbrooke's Cognitive Examination-revised (ACE-R); memory with the Rey Auditory Verbal Learning Test, pattern recognition memory (PRM) tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) and the Digit Span forward; executive functions with the digit span backward, the Clock Drawing Test, Intra/Extra Dimensional Set Shift test from the CANTAB, and the Stroop colour-word test; attention and working memory with the Trial Making Test, the digit ordering test and the letter cancellation test; language with the Boston Naming Test and the language subtest of ACE-R; fluency with semantic and phonemic fluencies; visuospatial abilities with the Hooper Visual Organization test and the visuospatial subtest of ACE-R. Mood was evaluated with the Hamilton Depression Rating Scale score, Hamilton Anxiety Rating scale score, Beck Depression Inventory (BDI) and Apathy Evaluation Scale.

2.2.5 MRI acquisition

Brain MRI scans were acquired at baseline and each follow up visit on the same 1.5 Tesla Philips Medical System Achieva machine at the Clinic of Neurology in Belgrade, Serbia. The following MR sequences were obtained: (i) dual-echo (DE) turbo spin-echo

(SE); and (ii) 3D sagittal T1-weighted Turbo Field Echo (TFE) (See Supplementary materials for details).

2.2.6 MRI analysis

MRI analysis was performed at the Neuroimaging Research Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy, by experienced observers, blinded to subjects' identity.

2.2.6.1 Cortical thickness measurement

Cortical reconstruction and estimation of cortical thickness were performed on the 3D T1-weighted TFE images using the FreeSurfer image analysis suite, version 5.3 (http://surfer.nmr.mgh.harvard.edu/) (Fischl & Dale, 2000). Further details are reported in the Supplemental Materials.

2.2.6.2 Gray matter volumetry

FMRIB's Integrated Registration and Segmentation Tool (FIRST) in FSL (http://www.fmrib.ox.ac.uk/fsl/first/index.html) was applied to TFE images of each subject at each visit and used to automatically segment GM regions, i.e., caudate, pallidum, putamen, thalamus and nucleus accumbens, amygdala and hippocampus, bilaterally. Mean GM volumes were calculated and multiplied by the normalization factor derived from SIENAx to correct for subject head size (http://www. fmrib.ox.ac.uk/fsl/sienax/index.html).

2.2.7 Statistical analysis

2.2.7.1 Demographic, clinical and cognitive data

Demographic, clinical (motor and non-motor) and cognitive data were compared between groups using the Kruskal-Wallis test, followed by Dunn's post-hoc test with Bonferroni p-value adjustment or Fisher's exact test. Test for linear trend was estimated in both PD groups and group-by-time interaction was assessed to evaluate longitudinal between-group differences using time as a continuous variable. Random effect of subject (ID) for each model has been considered. P values were adjusted for multiple comparisons controlling the False Discovery Rate (FDR) at level 0.05 using Benjamini-Hochberg step-up procedure. Two-sided p value <0.05 was considered for statistical significance. All

statistical analyses were performed using R Statistical Software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria).

2.2.7.2 Cross-sectional MRI: cortical thickness (vertex-by-vertex and regional) and GM volumes

A cross-sectional vertex-by-vertex analysis was performed to assess differences of cortical thickness between groups at baseline and at last visit, using a general linear model in FreeSurfer. Maps showing baseline comparisons were obtained by thresholding the t-statistic at $p < 0.05$, Monte Carlo corrected for multiple comparisons. Furthermore, the mean cortical thickness of 34 ROIs per hemisphere and the mean GM volumes were compared between groups at baseline and at last visit using the Kruskal-Wallis test, followed by Dunn's post-hoc test with Bonferroni p-value adjustment. The same analyses were performed to compare PD groups at the last visit with baseline MRI data of healthy controls. All statistical analyses were performed using R Statistical Software.

2.2.7.3 Longitudinal MRI: cortical thickness (regional) and GM volumetry

Changes over time in the mean cortical thickness of the 34 ROIs and in the mean GM volumes were assessed with general linear models using time as a continuous variable. Group-by-time interaction was assessed to evaluate longitudinal between-group differences. Random effect of subject (ID) for each model has been considered. P values were adjusted for multiple comparisons at $p < 0.05$ (R Statistical Software).

According to the careful matching and observed similarity among patient groups, and between patients and healthy controls, in terms of several sociodemographic and clinical variables, the statistical analyses did not include any covariate.

2.3 Results

2.3.1 Baseline clinical and cognitive findings

Compared to healthy controls, at study entry, both groups of PD patients were matched for age and similar for sex and education (Table 1). In terms of cognitive and psychopathological functioning, both groups of patients performed worse than controls in global cognition, memory and attention, and had higher scores in questionnaires

assessing depression and apathy (Table 2). Furthermore, GBA-positive patients performed worse than controls also in the language domain (Table 2).

At study entry, the two PD groups were matched for age, sex, age at onset, disease staging and severity and were also similar for other demographic, motor and non-motor clinical features (Table 1). In terms of cognitive functioning, no significant differences between the two groups were found for all cognitive and behavioural domains (Table 2).

2.3.2 Longitudinal clinical and cognitive findings

Clinical and cognitive changes in PD groups are reported in Tables 1 and 2. Over time, both GBA-positive and GBA-negative patients showed progressive worsening in all clinical motor features and increased LEDD. However, compared to GBA-negative, GBA-positive patients showed a greater disease severity progression (i.e., HY and UPDRS total and sub-scores, see Figure 1). In terms of cognitive and behavioural features, GBA-positive patients worsened over time in terms of attentive and visuospatial skills, and in their ability to inhibit cognitive interference (assessed with the Stroop Test). On the other hand, GBA-negative patients worsened over time in memory, verbal fluency, attention, and showed more depressive symptoms. Group x Time interactions also showed that GBA-positive patients progressed in visuospatial deficits more than GBA-negative cases (Figure 1). Raw results of the linear mixed effects modelling for each time point are shown in Supplementary Table 2.

2.3.3 Baseline MRI findings

2.3.3.1 Cortical thickness: vertex-by-vertex analysis

At study entry, compared to controls, GBA-positive PD patients showed cortical thinning in the left supramarginal gyrus, lateral occipital, middle and inferior temporal gyri (Figure 2a). Compared to GBA-negative, GBA-positive PD patients showed cortical thinning in the left precentral, postcentral and lateral occipital gyri (Figure 2b). No differences were observed between GBA-negative PD patients and controls.

2.3.3.2 Cortical thickness: regional analysis

At study entry, compared to controls, GBA-positive PD patients showed a greater cortical thinning in the left caudal anterior cingulate, inferior temporal, inferior parietal

and lateral occipital gyri, and in the right inferior frontal gyrus (pars opercularis) (Table 3). Compared to controls, GBA-negative patients showed greater cortical thickness in the right paracentral region (Table 3). Comparing the two PD groups, GBA-positive patients showed greater cortical thinning in the left superior parietal and supramarginal gyri, and in the right inferior frontal gyrus (pars opercularis) (Table 3).

2.3.3.3 Gray matter volumes

At baseline, compared to controls, both PD groups did not show any significant difference in terms of grey matter volumes. No significant differences were retrieved between the two PD groups. (Table 4).

2.3.4 Last visit findings and longitudinal MRI changes

2.3.4.1 Cortical thickness: vertex-by-vertex analysis

At the last visit (after 5 years of follow-up), compared to controls at baseline, GBA-positive PD patients accumulated a widespread bilateral pattern of cortical damage involving the bilateral supramarginal and superior frontal gyri, left precentral, rostral middle frontal and inferior temporal regions, precuneus, and right inferior frontal (pars opercularis), superior temporal and inferior parietal gyri (Figure 2c). On the other hand, compared to controls, GBA-negative patients showed cortical thinning in the left lingual gyrus, middle temporal and inferior parietal regions (Figure 2d). When comparing the two PD groups, GBA-positive patients showed more cortical damage than GBA-negative patients in the bilateral superior frontal gyri, in the left caudal and rostral middle frontal gyri, postcentral gyrus, lateral occipital and middle temporal regions, and in the right fusiform gyrus (Figure 2e).

2.3.4.2 Cortical thickness: regional analysis

Within-group analyses showed that GBA-positive patients accumulated significant cortical thinning in the bilateral middle and inferior temporal gyri, temporal pole and isthmus cingulate, left medial orbitofrontal gyri, superior parietal and postcentral gyri, banks of the superior temporal sulcus, inferior parietal gyrus, and right entorhinal, fusiform and lingual gyri (Table 3). On the other hand, GBA-negative patients accumulated a widespread pattern of significant cortical thinning in almost all considered

brain regions. Group x Time interactions indicated that, over time, no significant differences were retrieved between GBA-positive and GBA-negative patients (Table 3).

At the last follow-up visit, compared to controls at baseline, each group of PD patients showed a widespread pattern of cortical thinning involving mainly temporal, parietal and occipital brain regions. Compared to controls, the GBA-positive patients showed further cortical thinning in the inferior frontal and isthmus of the cingulate gyrus (Supplementary Table 3). At the last follow-up visit, no significant differences were retrieved between the two PD groups (Supplementary Table 3).

2.3.4.3 Gray matter volumes

Over the five years of follow-up, both PD groups showed significant atrophy in the caudate, hippocampus, putamen and thalamus bilaterally, and in the left amygdala and pallidum. GBA-negative PD patients showed also significant changes in the right amygdala and pallidum. The Group x Time interaction showed that GBA-positive patients accumulated more damage in the right caudate region, while GBA-negative patients had more severe cortical thinning in the right amygdala (Table 4).

At the last follow-up visit, compared to controls at baseline, each group of PD patients showed GM volume loss in all considered brain regions (Supplementary Table 3). On the contrary, no significant differences were found between GBA-positive and GBA-negative patients (Supplementary Table 3).

2.4 Discussion

In this study, we followed the longitudinal disease course of 10 GBA-positive and 20 GBA-negative PD patients from the very early disease stage (H&Y ≤1.5) over five years, evaluating changes in clinical and cognitive outcomes, cortical thickness and GM volumes. Longitudinal studies have the advantage to provide a characterization of disease progression, and specifically in this work, where we combined clinical and cognitive manifestations with MRI findings, they might improve our understanding of the underlying neurodegenerative process. At the study entry, we purposely matched the two groups of early PD patients for sociodemographic and clinical features, such as age at disease onset, disease staging and motor severity, and we observed that GBA-positive and GBA-negative early PD patients were also similar for LEDD and non-motor features,

including cognition. Despite their clinical similarity, we observed that GBA-positive patients showed a left-sided prevalent pattern of cortical thinning involving mainly temporal (middle and inferior gyri), parietal (supramarginal and postcentral gyri) and occipital (lateral gyrus) regions compared to the GBA-negative patients and healthy subjects. Consistently with pathological findings (Braak, Bohl et al., 2006), PD patients with posterior cortical alterations usually present a worse clinical profile (Agosta et al., 2013, Alcalay, Caccappolo et al., 2012, Lewis, Du et al., 2016, Mata, Leverenz et al., 2016, Winder-Rhodes, Evans et al., 2013), might show cognitive dysfunctions mainly involving visuospatial abilities (Williams-Gray, Evans et al., 2009), and have a higher risk of developing subsequent dementia (Williams-Gray et al., 2009). In line with these studies, over time, GBA-positive PD patients showed a more rapid trajectory of disease progression with higher disease severity and motor impairment (as measured with H&Y scale and UPDRS total and sub-scores), and greater worsening on visuospatial functions compared to GBA-negative patients. Our findings also confirmed previous longitudinal reports observing that, compared to GBA-negative individuals, GBA-positive patients have more severe motor manifestations with rapid disease progression (Pal, Robertson et al., 2016) and a greater cognitive decline characterized by visuospatial dysfunctions (Avenali, Toffoli et al., 2019, Brockmann et al., 2015, Mata et al., 2016, Winder-Rhodes et al., 2013).

Our longitudinal neuroimaging findings are coherent with GBA-patients' clinical and cognitive trajectories. In fact, after five years of observation, either when compared to controls and to GBA-negative patients, GBA-positive patients still showed a greater cortical thinning of posterior regions and additional greater involvement of frontal (superior, inferior, and middle) and orbitofrontal lobes.

At study entry, no significant differences were detected between GBA-negative patients and healthy subjects. Over time, GBA-negative group significantly accumulated cortical damage. However, it is important to notice that the pattern of damage showed by GBA-positive patients at the first visit is reached by the GBA-negative patients when compared to healthy controls only after five years of follow-up. This observation highlights that the two PD groups likely follow similar topographic trajectories of brain damage but with very distinct progression speed. Furthermore, it underlies the importance

of the early detection of GBA-positive individuals for defining prompt disease prognosis and, possibly, targeted interventions.

Another interesting finding of our study is the subcortical progression of damage in our cohort. The pattern of subcortical GM atrophy was similar in the two PD groups: it was nearly absent at the study entry, while it significantly worsened in each group over time in mostly all subcortical ROIs. However, compared to GBA-negative cases, GBA-positive patients showed smaller volumes of the right caudate in the Group X time interaction analysis. These latter findings well reflect the greater progression of the GBA group on motor impairment, despite the motor similarity with the GBA-negative patients at the study entry. An interesting finding of our study is also the slight discrepancy between the larger amount of cortical compared to the subcortical progression of damage in the PD-GBA cohort. The cortical thinning in GBA-positive patients affected motor and, mainly, extra-motor brain regions. Several longitudinal studies reported the presence of an early cognitive decline in GBA-positive PD patients compared to non-carriers (Riboldi & Di Fonzo, 2019, Thaler, Gurevich et al., 2017), especially in individuals with more severe GBA mutations (Cilia et al., 2016). However, to our knowledge, there is no evidence of a possible different time course for cognitive and motor impairments in these populations. Our speculation is that cortical and subcortical damage in GBA-positive patients might follow a different timeline, with the subcortical trajectories resembling more that of idiopathic PD. Further studies are needed to confirm our hypothesis.

This study is not without limitations. Firstly, we did not acquire longitudinal MRI data of healthy subjects, thus we could not compare normal and pathological brain changes over time. Second, we used a 1.5 Tesla MRI scanner, which is characterized by a lower spatial resolution compared with higher field strength scanners. Third, given that GBA mutations are not very common in the overall PD population, our cohort is small; therefore, our results did not take into account the heterogeneity of the GBA mutations (in terms of type and clinical impact) and should be interpreted with caution even though our analyses were corrected for multiple comparisons. Fourth, although our longitudinal linear mixed effects models took into account all possible visits for each patient, we need to acknowledge that several patients missed the visit at 48 months and returned at month 60. In addition, even though our PD groups were matched in terms of age at disease onset, given the fact that establishing with precision the exact disease onset of genetic

syndromes is challenging, we cannot exclude that GBA-positive patients could be at a slightly more advanced stage in their disease course. Lastly, our study focused only on GM changes. The combination of studying both structural GM and WM characteristics is pivotal to provide a complete framework of the underlying pathological processes in these populations.

In conclusion, our data suggest that, compared to GBA-negative, GBA-positive PD patients showed an earlier and greater cortical thinning, which worsened over 5 years of observation. GBA-negative PD patients reached the pattern of cortical thinning of GBA-positive at baseline only after five years, reflecting a slower disease progression. We can conclude that cortical thickness may be a useful tool for monitoring and predicting PD disease progression in accordance with the genetic background.

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2.6 Appendix

Table 1. Demographic and clinical features of patients and healthy controls at baseline and changes over 5 years in PD patients.

Variables	PD GBA-positive	PD GBA-negative	Healthy controls	P: PD GBA-positive vs healthy controls	P: PD GBA-negative vs healthy controls	P: PD GBA-positive vs PD GBA-negative	P for linear trend PD GBA-positive	PD for linear trend PD GBA-negative	P for linear trend PD GBA-positive vs PD GBA-negative
Number	10	20	22	-	-	-	-	-	-
Sex [Men/women] (men, %)	3/7 (30%)	6/14 (30%)	9/13 (41%)	0.56	0.46	1.00	-	-	-
Age at MRI [years]	62.1 ± 4.9 (54.0-68.0) 64.1 (58.3-65.9)	62.4 ± 5.2 (56.0-76.0) 62.4 (58.6-65.0)	62.1 ± 5.6 (52.0-73.0) 62.0 (58.8-65.9)	1.00	1.00	1.00	-	-	-
Education [years]	13.0 ± 3.0 (8.0-17.0) 12.0 (11.2-16.0)	12.6 ± 2.1 (8.0-16.0) 12.0 (12.0-13.2)	13.3 ± 2.4 (8.0-16.0) 12.0 (12.0-16.0)	1.00	1.00	1.00	-	-	-
Age at onset [years]	59.7 ± 5.1 (52.0-66.0) 61.5 (55.0-63.5)	60.0 ± 5.6 (52.0-73.0) 59.5 (55.0-63.0)	-	-	-	0.96	-	-	-
PD duration [years]	2.4 ± 0.7 (1.3-3.8) 2.4	2.4 ± 2.0 (0.1-6.6) 1.7	-	-	-	0.48	**<0.001 (261.30)**	**<0.001 (13238.26)**	0.24

	(1.9-2.7)	(0.9-3.1)							
Levodopa equivalent dose [mg]	343.0 ± 209.0 (0.0-580.0) 350.0 (300.0-510.0)	272.4 ± 186.6 (0.0-560.0) 331.2 (112.5-400.0)	-	-	-	0.36	**<0.001 (7.90)**	**<0.001 (21.67)**	0.18
Side of onset [right/left]	7/3	12/8	-	-	-	0.70	-	-	-
Clinical motor variables									
Hoehn & Yahr	1.1 ± 0.2 (1.0-1.5) 1.0 (1.0-1.0)	1.0 ± 0.1 (1.0-1.5) 1.0 (1.0-1.0)	-	-	-	0.20	**<0.001 (23.29)**	**<0.001 (33.74)**	**0.004 (4.53)**
UPDRS Total	30.2 ± 15.5 (7.0-49.0) 33.5 (19.2-43.0)	26.5 ± 8.3 (11.0-40.0) 27.5 (20.0-32.5)	-	-	-	0.40	**<0.001 (36.95)**	**<0.001 (23.09)**	**<0.001 (5.76)**
UPDRS-I Total	6.9 ± 4.6 (1.0-16.0) 6.5 (4.0-9.2)	5.6 ± 3.4 (0.0-12.0) 5.5 (2.7-7.2)	-	-	-	0.54	**<0.001 (8.16)**	**<0.001 (14.14)**	0.69
UPDRS-II Total	7.6 ± 5.8 (0.0-19.0) 9.0 (3.0-10.7)	5.4 ± 2.9 (0.0-11.0) 5.0 (3.7-7.0)	-	-	-	0.34	**<0.001 (26.61)**	**<0.001 (17.16)**	**<0.001 (7.00)**
UPDRS-III Total	15.4 ± 6.5 (5.0-26.0) 17.5 (10.7-19.5)	15.6 ± 4.3 (10.0-23.0) 14.5 (12.7-19.0)	-	-	-	0.98	**<0.001 (24.55)**	**<0.001 (13.92)**	**0.001 (5.08)**
FoG-Q	1.0 ± 0.7 (0.0-2.0) 1.0 (1.0-1.0)	1.0 ± 1.2 (0.0-4.0) 1.0 (0.0-1.2)	-	-	-	0.43	**0.002 (6.21)**	**<0.001 (11.74)**	0.69

Clinical non-motor variables									
RBDSQ	3.7 ± 3.6 (1.0-10.0) 1.0 (1.0-6.7)	2.3 ± 2.2 (1.0-9.0) 1.0 (1.0-3.2)	-	-	-	0.43	-	-	-
UPDRS-I hallucinations and psychosis [No/Yes] (%)	8/2 (80%)	19/1 (95%)	-	-	-	0.25	-	-	-
***NMS-Q* Gastrointestinal symptoms [No/Yes] (%)**	4/6 (40%)	6/14 (30%)	-	-	-	0.78	-	-	-
***NMS-Q* Urinary symptoms [No/Yes] (%)**	2/8 (20%)	10/10 (50%)	-	-	-	0.11	-	-	-
***NMS-Q* Olfactory dysfunction [No/Yes] (%)**	7/3 (70%)	14/6 (70%)	-	-	-	1.00	-	-	-
***NMS-Q* Sexual dysfunction [No/Yes] (%)**	6/4 (60%)	13/7 (65%)	-	-	-	0.94	-	-	-
***NMS-Q* Orthostatic symptoms [No/Yes] (%)**	8/2 (80%)	16/4 (80%)	-	-	-	1.00	-	-	-

Values are reported as means ± standard deviations (range) – first and second rows –, medians and interquartile ranges (first and third quartiles) – third and fourth rows – or absolute and percentage frequency (%) for continuous and categorical variables, respectively. Differences between groups at baseline were assessed using Kruskal-Wallis test (for continuous demographic and general clinical variables), followed by post-hoc pairwise comparisons (Dunn test, p values are corrected for Bonferroni test) or Fisher's exact test. Test for linear trend was estimated in both PD groups and Group X Time interaction was assessed to evaluate longitudinal between-group differences. Values in bold indicate statistically significant results. Beta values are reported only for

significant results. ***Abbreviations:*** *FoG-Q=freezing of gait questionnaire; GBA=glucocerebrosidase; mg=milligram; MRI=magnetic resonance imaging; NMS-Q=Non-Motor Symptoms Questionnaire; PD=Parkinson's Disease; RBDSQ=REM Sleep Behaviour Disorder Screening Questionnaire; UPDRS=Unified Parkinson's Disease Rating Scale.*

Table 2. Cognitive and behavioural features of patients and healthy controls at baseline and changes over 5 years in PD patients.

Variables	PD GBA-positive	PD GBA-negative	Healthy controls	p PD GBA-positive vs healthy controls	p PD GBA-negative vs healthy controls	p PD GBA-positive vs PD GBA-negative	P for linear trend PD GBA-positive	P for linear trend PD GBA-negative	P for linear trend PD GBA-positive vs PD GBA-negative
Psychopathological features									
HDRS	7.2 ± 4.9 (0.0-17.0) 8.0 (4.0-9.0)	7.4 ± 7.0 (0.0-22.0) 6.5 (1.0-10.7)	1.6 ± 2.9 (0.0-13.0) 1.0 (0.0-2.0)	**0.01**	**<0.01**	1.00	0.35	0.37	1.00
BDI	10.5 ± 9.4 (1.0-32.0) 8.5 (4.5-12.7)	7.7 ± 6.9 (0.0-27.0) 5.5 (3.7-12.0)	1.2 ± 3.0 (0.0-12.0) 0.0 (0.0-0.0)	**<0.001**	**0.001**	0.99	0.34	**0.03 (3.36)**	0.45
HAMA	5.9 ± 4.9 (0.0-15.0) 5.5 (2.5-9.0)	6.3 ± 7.2 (0.0-23.0) 4.0 (0.7-9.2)	2.0 ± 2.5 (0.0-9.0) 1.0 (0.0-3.0)	0.09	0.13	1.00	0.93	0.35	1.00
Apathy Scale	10.8 ± 7.8 (2.0-27.0) 11.0 (4.2-13.7)	11.5 ± 7.9 (0.0-28.0) 10.5 (5.5-17.5)	1.0 ± 2.0 (0.0-8.0) 0.0 (0.0-1.0)	**<0.001**	**<0.001**	1.00	0.06	0.18	1.00
Global cognition									
MMSE	28.1 ± 1.7 (25.0-30.0) 28.5	28.9 ± 1.2 (26.0-30.0) 29.0	29.8 ± 0.5 (28.0-30.0) 30.0	**0.001**	**0.01**	0.58	0.06	0.29	0.39

	(27.2-29.0)	(28.0-30.0)	(30.0-30.0)						
ACE-R, Total	89.5 ± 8.3 (72.0-99.0) 90.5 (86.0-96.0)	92.1 ± 6.5 (70.0-98.0) 93.0 (90.5-97.0)	97.0 ± 2.3 (93.0-100.0) 97.0 (96.0-99.0)	**0.01**	**0.01**	1.00	0.30	0.70	1.00
Memory									
ACE-R, memory	22.5 ± 3.6 (14.0-26.0) 23.0 (21.0-25.0)	23.1± 3.1 (14.0-26.0) 24.0 (22.0-25.2)	25.6 ± 0.9 (23.0-26.0) 26.0 (25.2-26.0)	**<0.01**	**<0.01**	1.00	0.41	**0.045 (3.03)**	0.41
Digit span, forward	8.8 ± 1.9 (6.0-12.0) 8.5 (8.0-9.0)	7.8 ± 2.1 (5.0-12.0) 7.0 (6.5-8.5)	9.0 ± 2.1 (5.0-12.0) 9.0 (8.0-10.0)	1.00	0.13	0.53	0.43	1.00	0.69
RAVLT, immediate recall	37.7 ± 10.7 (26.0-59.0) 34.0 (29.0-44.5)	40.4 ± 9.5 (21.0-54.0) 40.5 (35.0-47.5)	47.2 ± 10.3 (24.0-64.0) 48.5 (41.2-54.7)	0.055	0.10	1.00	0.33	0.08	1.00
RAVLT, delayed recall	6.8 ± 2.3 (3.0-10.0) 7.0 (5.0-8.7)	7.3 ± 2.4 (4.0-11.0) 7.0 (5.0-9.2)	9.6 ± 2.3 (5.0-13.0) 9.0 (8.0-12.0)	**0.02**	**0.02**	1.00	1.00	0.26	1.00
RAVLT, recognition	12.5 ± 2.0 (9.0-15.0) 12.5 (12.0-13.7)	13.4 ± 1.7 (9.0-15.0) 14.0 (12.7-15.0)	14.4 ± 1.1 (12.0-15.0) 15.0 (14.0-15.0)	**0.01**	0.10	0.70	0.52	0.81	1.00
PRM [% correct]	72.9 ± 12.6 (45.8-87.5) 72.9 (70.8-80.2)	75.4 ± 11.4 (50.0-91.7) 75.0 (66.7-83.3)	82.9 ± 8.2 (62.5-95.8) 83.3 (83.3-87.5)	0.09	0.09	1.00	0.48	0.54	1.00
Language									

ACE-R, language	24.0 ± 2.1 (21.0-26.0) 24.5 (22.2-26.0)	25.0 ± 2.1 (17.0-26.0) 26.0 (25.0-26.0)	25.9 ± 0.3 (25.0-26.0) 26.0 (26.0-26.0)	**0.01**	0.11	0.41	1.00	0.27	1.00
Boston Naming Test	53.3 ± 6.2 (44.0-60.0) 54.0 (49.0-58.7)	56.6 ± 3.3 (50.0-60.0) 57.0 (55.7-59.2)	57.9 ± 1.5 (54.0-60.0) 58.0 (57.2-59.0)	0.21	1.00	0.85	0.72	0.26	0.74
Executive functions									
ACE-R, fluency	10.0 ± 2.9 (6.0-14.0) 9.5 (8.0-12.7)	10.8 ± 1.9 (7.0-14.0) 11.0 (9.7-12.0)	11.6 ± 1.7 (9.0-14.0) 11.5 (10.2-13.0)	0.37	0.59	1.00	0.71	**0.01 (4.38)**	1.00
Digit backward	6.2 ± 2.8 (2.0-11.0) 5.5 (5.0-8.0)	5.3 ± 1.9 (2.0-10.0) 5.0 (4.0-6.0)	7.3 ± 2.0 (5.0-11.0) 7.0 (6.0-8.7)	0.49	**0.01**	0.87	0.39	1.00	1.00
Digit ordering [max span]	6.7 ± 2.0 (5.0-12.0) 6.5 (5.2-7.0)	6.9 ± 2.1 (4.0-12.0) 6.5 (5.0-8.0)	8.2 ± 2.4 (4.0-12.0) 8.0 (6.2-10.0)	0.17	0.22	1.00	0.74	0.84	1.00
Phonemic fluency	36.6 ± 12.4 (23.0-63.0) 35.0 (26.2-43.7)	35.3 ± 11.8 (14.0-62.0) 36.5 (28.2-42.5)	39.7 ± 8.0 (28.0-56.0) 40.5 (33.0-43.0)	1.00	0.91	1.00	1.00	0.43	1.00
Semantic fluency	17.3 ± 6.0 (8.0-26.0) 17.0 (12.7-21.5)	18.5 ± 4.7 (11.0-29.0) 18.0 (16.0-21.0)	21.4 ± 4.7 (12.0-30.0) 21.5 (18.2-25.0)	0.16	0.15	1.00	0.18	**0.03 (3.33)**	1.00
Stroop, interference [total correct]	45.4 ± 14.8 (22.0-73.0) 47.0	42.1 ± 9.0 (27.0-65.0) 41.0	38.9 ± 10.4 (19.0-60.0) 40.0	0.56	1.00	1.00	**<0.001 (8.41)**	0.08	0.06

	(34.7-54.0)	(36.0-48.0)	(33.0-45.0)						
IED [total errors]	55.2 ± 46.8 (15.0-161.0) 53.5 (23.2-58.5)	52.7 ± 15.8 (22.0-72.0) 58.0 (54.0-62.0)	32.3 ± 30.4 (7.0-146.0) 20.5 (15.2-40.5)	0.29	**0.001**	0.83	1.00	1.00	1.00
Attention									
ACE-R, attention/orientation	17.3 ± 1.0 (16.0-18.0) 18.0 (16.2-18.0)	17.6 ± 0.9 (14.0-18.0) 18.0 (18.0-18.0)	18.0 ± 0.2 (17.0-18.0) 18.0 (18.0-18.0)	**0.03**	0.58	0.42	**0.002 (5.96)**	1.00	0.21
TMT-A	61.7 ± 39.4 (23.0-158.0) 50.5 (41.0-67.5)	50.6 ± 18.1 (22.0-90.0) 46.5 (37.5-60.0)	34.1 ± 13.4 (18.0-78.0) 33.0 (25.0-38.7)	**0.01**	**<0.01**	1.00	0.46	**0.02 (3.76)**	0.15
Letter cancellation correct	29.5 ± 6.7 (20.0-39.0) 28.0 (24.7-36.0)	27.5 ± 5.6 (20.0-39.0) 28.5 (22.7-30.0)	28.5 ± 6.1 (20.0-40.0) 27.0 (25.0-31.5)	1.00	1.00	1.00	0.18	0.56	0.39
Visuospatial abilities									
ACE-R, visuospatial	15.7 ± 0.5 (15.0-16.0) 16.0 (15.2-16.0)	15.6 ± 0.8 (13.0-16.0) 16.0 (15.0-16.0)	15.9 ± 0.3 (15.0-16.0) 16.0 (16.0-16.0)	0.61	0.25	1.00	**0.01 (4.92)**	0.33	0.07
Clock Drawing Test	5.0 ± 0.0 (5.0-5.0) 5.0 (5.0-5.0)	4.9 ± 0.5 (3.0-5.0) 5.0 (5.0-5.0)	5.0 ± 0.2 (4.0-5.0) 5.0 (5.0-5.0)	1.00	1.00	1.00	**0.049 (3.24)**	0.75	0.89
Hooper	23.1 ± 4.7 (15.0-29.0) 23.7 (20.0-26.7)	22.5 ± 4.9 (10.0-28.0) 24.5 (18.7-26.0)	23.3 ± 3.1 (17.0-30.0) 23.7 (21.6-25.0)	1.00	1.00	1.00	**0.002 (6.39)**	1.00	**0.04 (2.95)**

Values are reported as mean ± standard deviation (range) – first and second rows –, medians and interquartile ranges (first and third quartiles) – third and fourth rows. Differences between groups at baseline were assessed using the Kruskal-Wallis test, followed by Dunn's post-hoc test (for continuous demographic and general clinical variables). P values are corrected with Bonferroni test. Test for linear trend was estimated in both PD groups and Group X Time interaction was assessed to evaluate longitudinal between-group differences. Values in bold indicate statistically significant results. Beta values are reported only for significant results. ***Abbreviations:*** *ACE-R=Addenbrooke's Cognitive Examination-Revised; BDI=Beck Depression Inventory; GBA=glucocerebrosidase; HAMA=Hamilton Anxiety Rating Scale; HDRS=Hamilton Depression Rating Scale; IED=Intra-Extra Dimensional Set Shift; MMSE= Mini Mental State Examination; PD=Parkinson's Disease; PRM=Pattern Recognition Memory; RAVLT=Rey Auditory Verbal Learning Test; TMT=Trial Making Test.*

Table 3. Cortical thickness measures in patients and healthy controls at baseline and changes over 5 years in PD patients.

Variable	Side	PD GBA-positive	PD GBA-negative	Healthy controls	p: PD GBA-positive *vs* HC	p: PD GBA-negative *vs* HC	p: PD GBA-positive vs PD GBA-negative	p for linear trend PD GBA-positive	p for linear trend PD GBA-negative	p for differential trend PD GBA-positive *vs* PD GBA-negative
Banks of the superior temporal sulcus	**L**	2.3 ± 0.1 (2.0-2.5)	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.2-2.6)	0.09	0.17	1.00	**0.01 (4.89)**	**0.002 (3.72)**	0.74
	R	2.3 ± 0.2 (1.9-2.6)	2.4 ± 0.1 (2.0-2.5)	2.4 ± 0.1 (2.2-2.8)	1.00	1.00	0.69	0.74	**0.01 (4.58)**	0.84
Caudal anterior cingulate	**L**	2.6 ± 0.2 (2.2-2.9)	2.5 ± 0.2 (2.1-3.2)	2.4 ± 0.2 (1.9-2.8)	**0.049**	0.14	1.00	0.85	0.32	1.00
	R	2.3 ± 0.2 (1.9-2.5)	2.4 ± 0.2 (2.1-2.8)	2.3 ± 0.2 (1.8-2.7)	0.99	1.00	0.98	0.14	**0.02 (3.45)**	0.87
Caudal middle frontal	**L**	2.3 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.2-2.6)	1.00	0.98	0.52	0.08	**<0.001 (8.33)**	1.00
	R	2.4 ± 0.1 (2.3-2.6)	2.4 ± 0.1 (2.3-2.6)	2.3 ± 0.1 (2.2-2.6)	0.92	0.09	1.00	1.00	**0.001 (5.64)**	1.00
Entorhinal	**L**	3.2 ± 0.3 (2.9-3.7)	3.2 ± 0.2 (2.7-3.7)	3.2 ± 0.2 (2.7-3.8)	1.00	0.76	1.00	1.0	**0.01 (3.89)**	1.00
	R	3.3 ± 0.3 (2.9-3.9)	3.3 ± 0.3 (2.8-3.9)	3.3 ± 0.3 (2.7-3.9)	1.00	1.00	1.00	**0.01 (4.36)**	**0.003 (4.93)**	0.66
Fusiform	**L**	2.4 ± 0.1 (2.2-2.6)	2.5 ± 0.1 (2.3-2.7)	2.4 ± 0.1 (2.3-2.7)	1.00	1.00	0.86	0.28	**0.003 (4.80)**	0.72
	R	2.4 ± 0.1 (2.2-2.6)	2.5 ± 0.1 (2.3-2.6)	2.4 ± 0.1 (2.2-2.7)	1.00	1.00	1.00	**0.04 (3.46)**	**0.004 (4.66)**	0.70

Inferior parietal	**L**	2.2 ± 0.1 (2.1-2.4)	2.3 ± 0.1 (2.1-2.5)	2.3 ± 0.1 (2.2-2.5)	**0.01**	0.54	0.15	**0.005 (5.15)**	**<0.001 (8.48)**	1.00
	R	2.2 ± 0.1 (2.0-2.4)	2.3 ± 0.1 (2.1-2.5)	2.3 ± 0.1 (2.2-2.5)	0.33	1.00	0.10	0.33	**<0.001 (6.85)**	0.55
Inferior temporal	**L**	2.5 ± 0.1 (2.4-2.7)	2.6 ± 0.1 (2.4-2.7)	2.6 ± 0.1 (2.5-2.9)	**0.048**	0.39	0.74	**0.01 (4.88)**	**<0.001 (7.11)**	0.75
	R	2.5 ± 0.1 (2.4-2.8)	2.6 ± 0.1 (2.4-2.9)	2.6 ± 0.1 (2.4-2.9)	0.56	1.00	0.61	**0.05 (3.21)**	**0.002 (5.27)**	0.48
Isthmus cingulate	**L**	2.0 ± 0.1 (1.8-2.2)	2.1 ± 0.2 (1.8-2.4)	2.1 ± 0.1 (1.9-2.3)	0.70	1.00	0.63	**0.01 (5.12)**	0.91	0.77
	R	1.9 ± 0.1 (1.8-2.1)	2.0 ± 0.2 (1.8-2.3)	2.1 ± 0.2 (1.9-2.2)	0.22	1.00	0.78	**0.04 (3.42)**	0.22	0.14
Lateral occipital	**L**	1.9 ± 0.1 (1.8-2.2)	2.0 ± 0.1 (1.9-2.2)	2.1 ± 0.1 (1.9-2.4)	**0.01**	0.85	0.08	0.14	**<0.001 (10.74)**	1.00
	R	1.9 ± 0.1 (1.8-2.1)	2.1 ± 0.1 (1.9-2.3)	2.1 ± 0.2 (1.8-2.4)	0.17	1.00	0.54	0.72	**<0.001 (6.13)**	1.00
Lateral orbito-frontal	**L**	2.4 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.1-2.6)	2.4 ± 0.1 (2.2-2.6)	1.00	0.84	1.00	0.1	1.00	0.91
	R	2.3 ± 0.1 (2.1-2.6)	2.4 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.2-2.6)	1.00	1.00	1.00	0.15	**0.003 (4.94)**	0.80
Lingual	**L**	1.8 ± 0.1 (1.7-1.9)	1.8 ± 0.1 (1.6-2.0)	1.9 ± 0.1 (1.6-2.2)	0.34	1.00	0.46	0.10	**<0.001 (7.10)**	0.16
	R	1.8 ± 0.1 (1.7-1.9)	1.9 ± 0.1 (1.7-2.0)	1.9 ± 0.1 (1.7-2.1)	1.00	1.00	1.00	**0.005 (5.22)**	**0.03 (3.40)**	0.72
Medial orbito-frontal	**L**	2.2 ± 0.1 (2.1-2.4)	2.2 ± 0.1 (2.0-2.5)	2.2 ± 0.1 (2.0-2.4)	1.00	1.00	1.00	**0.03 (3.59)**	**0.02 (3.62)**	1.00
	R	2.2 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (2.0-2.6)	2.3 ± 0.1 (2.0-2.6)	0.12	0.62	0.92	0.10	0.22	0.39
Middle temporal	**L**	2.6 ± 0.1 (2.3-2.8)	2.6 ± 0.1 (2.4-2.8)	2.6 ± 0.1 (2.4-2.9)	0.15	1.00	0.65	**0.03 (3.68)**	**<0.001 (5.76)**	0.36

	R	2.6 ± 0.1 (2.3-2.8)	2.7 ± 0.1 (2.4-2.8)	2.7 ± 0.1 (2.4-2.9)	0.32	1.00	0.59	**0.03 (3.73)**	**<0.001 (8.55)**	1.00
Paracentral	**L**	2.3 ± 0.1 (1.9-2.4)	2.3 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (1.9-2.5)	0.26	0.15	1.00	0.18	0.29	0.67
	R	2.3 ± 0.1 (1.9-2.4)	2.3 ± 0.1 (2.1-2.4)	2.2 ± 0.1 (1.8-2.5)	0.08	**0.02**	1.00	0.52	0.10	1.00
Parahippocampal	**L**	2.6 ± 0.2 (2.2-2.9)	2.5 ± 0.3 (2.0-3.2)	2.6 ± 0.3 (2.2-3.1)	1.00	1.00	1.00	0.26	**0.03 (3.25)**	0.36
	R	2.5 ± 0.2 (2.1-2.9)	2.5 ± 0.2 (2.1-2.8)	2.5 ± 0.2 (2.1-2.9)	1.00	1.00	1.00	0.14	0.36	0.51
Pars opercularis	**L**	2.3 ± 0.1 (2.2-2.5)	2.4 ± 0.1 (2.3-2.6)	2.4 ± 0.1 (2.2-2.6)	0.09	1.00	0.21	0.23	**0.01 (4.24)**	0.34
	R	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.3-2.7)	2.4 ± 0.1 (2.3-2.6)	**0.047**	0.88	**0.01**	0.67	**0.01 (4.44)**	1.00
Pars orbitalis	**L**	2.4 ± 0.2 (2.2-2.8)	2.5 ± 0.1 (2.2-2.7)	2.5 ± 0.2 (2.2-2.8)	1.00	1.00	1.00	0.67	**0.04 (3.03)**	1.00
	R	2.5 ± 0.2 (2.2-2.8)	2.5 ± 0.1 (2.3-2.9)	2.5 ± 0.1 (2.3-2.8)	1.00	1.00	1.00	0.77	**0.002 (5.12)**	1.00
Pars triangularis	**L**	2.2 ± 0.2 (1.9-2.5)	2.3 ± 0.1 (1.9-2.5)	2.3 ± 0.1 (2.0-2.6)	0.52	1.00	0.83	0.12	**0.002 (5.01)**	1.00
	R	2.2 ± 0.2 (1.9-2.5)	2.3 ± 0.1 (2.1-2.5)	2.3 ± 0.1 (2.2-2.5)	0.75	1.00	1.00	0.56	**0.001 (5.62)**	1.00
Pericalcarine	**L**	1.5 ± 0.1 (1.4-1.6)	1.6 ± 0.1 (1.4-1.8)	1.6 ± 0.1 (1.4-1.8)	0.51	1.00	0.51	0.14	**0.01 (3.84)**	0.07
	R	1.6 ± 0.1 (1.5-1.7)	1.6 ± 0.1 (1.4-2.0)	1.6 ± 0.1 (1.3-1.8)	0.86	1.00	1.00	0.44	0.15	0.20
Postcentral	**L**	1.9 ± 0.1 (1.8-2.1)	2.0 ± 0.1 (1.9-2.2)	2.0 ± 0.1 (1.8-2.3)	0.39	1.00	0.14	**0.02 (3.99)**	**<0.001 (8.14)**	1.00
	R	1.9 ± 0.1 (1.8-2.1)	1.9 ± 0.1 (1.8-2.1)	2.0 ± 0.1 (1.8-2.2)	0.86	0.68	1.00	0.34	**0.02 (3.67)**	1.00

Precentral	**L**	2.3 ± 0.1 (2.0-2.6)	2.5 ± 0.1 (2.3-2.7)	2.4 ± 0.1 (2.2-2.7)	1.00	0.18	0.09	0.73	**<0.001 (7.07)**	0.59
	R	2.4 ± 0.2 (1.9-2.6)	2.4 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.1-2.6)	1.00	0.74	1.00	0.23	**0.02 (3.73)**	1.00
Precuneus	**L**	2.2 ± 0.1 (2.1-2.3)	2.2 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (1.9-2.5)	0.67	1.00	0.65	0.57	**0.001 (5.65)**	1.00
	R	2.2 ± 0.1 (2.0-2.3)	2.2 ± 0.1 (2.0-2.4)	2.2 ± 0.1 (2.1-2.5)	0.58	1.00	0.26	0.09	**<0.001 (8.94)**	0.44
Rostral middle frontal	**L**	2.2 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (2.1-2.5)	0.56	1.00	0.79	0.57	**0.001 (5.44)**	1.00
	R	2.2 ± 0.1 (2.0-2.3)	2.2 ± 0.1 (2.1-2.5)	2.2 ± 0.1 (2.1-2.4)	0.94	1.00	0.48	1.00	**0.001 (5.42)**	0.66
Superior frontal	**L**	2.5 ± 0.1 (2.3-2.6)	2.5 ± 0.1 (2.4-2.7)	2.5 ± 0.1 (2.4-2.7)	1.00	0.54	0.87	0.32	**<0.001 (7.02)**	1.00
	R	2.5 ± 0.1 (2.1-2.7)	2.5 ± 0.1 (2.3-2.7)	2.5 ± 0.1 (2.3-2.7)	1.00	0.53	0.42	0.07	**<0.001 (8.15)**	1.00
Superior parietal	**L**	2.0 ± 0.1 (1.9-2.2)	2.1 ± 0.1 (1.9-2.3)	2.1 ± 0.1 (2.0-2.3)	0.058	1.00	**0.01**	**0.049 (3.25)**	**<0.001 (9.70)**	1.00
	R	2.0 ± 0.1 1.9-2.2	2.1 ± 0.1 (2.0-2.2)	2.1 ± 0.1 (1.9-2.3)	0.38	1.00	0.12	0.08	**0.001 (5.54)**	0.79
Superior temporal	**L**	2.5 ± 0.1 (2.4-2.8)	2.6 ± 0.1 (2.4-2.8)	2.6 ± 0.1 (2.3-2.8)	0.29	1.00	0.28	0.06	**<0.001 (8.96)**	0.45
	R	2.5 ± 0.2 (2.3-2.8)	2.6 ± 0.1 (2.5-2.9)	2.6 ± 0.1 (2.4-2.8)	0.41	1.00	0.33	0.13	**<0.001 (6.53)**	1.00
Supramarginal	**L**	2.3 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.3-2.5)	2.4 ± 0.1 (2.3-2.6)	0.12	1.00	**0.03**	0.31	**<0.001 (12.05)**	1.00
	R	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.2-2.6)	0.56	1.00	0.32	1.00	**<0.001 (7.45)**	0.93
Temporal pole	**L**	3.5 ± 0.2 (3.1-3.9)	3.6 ± 0.3 (3.0-4.0)	3.4 ± 0.2 (2.9-3.8)	1.00	0.28	0.76	**0.03 (3.76)**	**<0.001 (9.57)**	1.00

	R	3.6 ± 0.3 (3.2-4.1)	3.6 ± 0.3 (3.2-4.3)	3.6 ± 0.3 (3.2-4.2)	1.00	1.00	1.00	**0.01 (4.64)**	**<0.001 (10.67)**	0.13
Transverse temporal	**L**	2.2 ± 0.2 (1.9-2.5)	2.3 ± 0.1 (1.9-2.5)	2.3 ± 0.1 (2.0-2.5)	1.00	1.00	1.00	0.60	**0.001 (5.63)**	0.78
	R	2.3 ± 0.2 (1.8-2.6)	2.3 ± 0.1 (2.1-2.7)	2.3 ± 0.2 (2.0-2.7)	1.00	0.22	1.00	0.85	**0.045 (3.03)**	0.79

Values (mm) are reported as mean ± standard deviation (range). Cortical thickness values are in mm. Differences between groups at baseline were assessed using the Kruskal-Wallis test, followed by Dunn's post-hoc test. P values are corrected with Bonferroni test. Test for linear trend was estimated in both PD groups and Group X Time interaction was assessed to evaluate longitudinal between-group differences. Analyses were corrected for time between scans. Values in bold indicate statistically significant results; only regions with at least one significant contrast were reported. Beta values are reported only for significant results. ***Abbreviations:*** *GBA=glucocerebrosidase; HC=healthy controls; L=left; PD=Parkinson's disease; R= right.*

Table 4. Grey matter volumes in patients and healthy controls at baseline and changes over time in PD patients.

Variables	Side	PD GBA-positive	PD GBA-negative	Healthy controls	p: PD GBA-positive *vs* HC	p: PD GBA-negative *vs* HC	p: PD GBA-positive vs PD GBA-negative	p for linear trend PD GBA-positive	p for linear trend PD GBA-negative	p for differential trend PD GBA-positive vs PD GBA-negative
Caudate	**L**	4158.3 ± 457.2 (3615.0-4947.1)	4241.1 ± 403.5 (3594.5-5213.6)	4254.0 ± 608.7 (2948.7-5576.8)	1.00	1.00	1.00	**<0.001 (24.42)**	**<0.001 (144.32)**	0.22
	R	4320.1 ± 603.9 (3349.9-5182.4)	4443.2 ± 431.4 (3763.2-5552.9)	4500.9 ± 483.8 (3515.5-5783.4)	1.00	1.00	1.00	**<0.001 (14.82)**	**<0.001 (133.42)**	**0.01 (4.23)**
Pallidum	**L**	2201.3 ± 156.0 (1935.6-2449.0)	2359.9 ± 229.4 (2000.8-2995.8)	2314.1 ± 261.1 (1938.9-2897.6)	1.00	0.95	0.24	**<0.001 (11.49)**	**<0.001 (81.99)**	0.40
	R	2252.0 ± 147.1 (2054.1-2513.6)	2379.8 ± 156.1 (2121.3-2711.0)	2380.0 ± 274.6 (1973.2-3020.9)	0.68	1.00	0.24	0.34	**<0.001 (94.58)**	0.27
Putamen	**L**	5855.6 ± 577.2 (4873.4-6813.9)	6151.5 ± 569.9 (4959.0-6993.4)	6377.7 ± 564.7 (5143.8-7601.4)	0.06	0.75	0.49	**<0.001 (23.37)**	**<0.001 (179.88)**	0.79
	R	5758.5 ± 566.1 (4816.9-6625.9)	6192.2 ± 516.4 (5189.2-6980.6)	6199.6 ± 493.3 (5164.8-6915.7)	0.15	1.00	0.20	**<0.001 (26.97)**	**<0.001 (146.14)**	0.86
Thalamus	**L**	9613.2 ± 865.6 (7524.0-10429.9)	9992.6 ± 719.5 (9031.6-11154.2)	9898.5 ± 735.6 (8141.0-11091.0)	1.00	1.00	1.00	**<0.001 (23.70)**	**<0.001 (198.06)**	1.00
	R	9403.5 ± 870.7 (7278.1-10254.7)	9604.4 ± 641.8 (8699.3-10856.1)	9539.9 ± 644.8 (8070.3-11014.0)	1.00	1.00	1.00	**<0.001 (16.77)**	**<0.001 (182.34)**	0.13
Hippocampus	**L**	4935.8 ± 393.5 (4520.0-5620.4)	4873.1 ± 527.9 (3875.4-6120.9)	4869.0 ± 515.8 (4098.9-5778.2)	1.00	1.00	1.00	**<0.001 (15.83)**	**<0.001 (90.35)**	1.00

	R	5043.7 ± 449.8 (4352.3-5601.8)	5194.6 ± 377.5 (4602.0-6123.4)	5055.5 ± 572.6 (4104.7-6206.9)	1.00	1.00	1.00	**<0.001 (15.99)**	**<0.001 (125.58)**	0.57
Amygdala	**L**	1853.4 ± 229.8 (1308.7-2098.4)	1957.7 ± 207.4 (1523.5-2507.3)	1791.6 ± 292.2 (1039.5-2296.3)	1.00	0.17	1.00	**<0.001 (7.32)**	**<0.001 (53.34)**	0.38
	R	1863.5 ± 192.5 (1489.8-2147.5)	1796.5 ± 344.8 (820.6-2326.2)	1844.2 ± 335.3 (858.1-2356.7)	1.00	1.00	1.00	1.00	**<0.001 (24.46)**	**0.02 (3.60)**

Values (mm^3) are reported as mean ± standard deviation (range). Differences between PD patients and HC and between PD groups at baseline were assessed using the Kruskal-Wallis test, followed by Dunn's post-hoc test. P values are corrected with Bonferroni test. Test for linear trend was estimated in both PD groups and Group X Time interaction was assessed to evaluate longitudinal between-group differences. Analyses were corrected for time between scans. Values in bold indicate statistically significant results. Beta values are reported only for significant results. ***Abbreviations:*** *GBA=glucocerebrosidase; HC=healthy controls; L=left; PD=Parkinson's disease; R=right.*

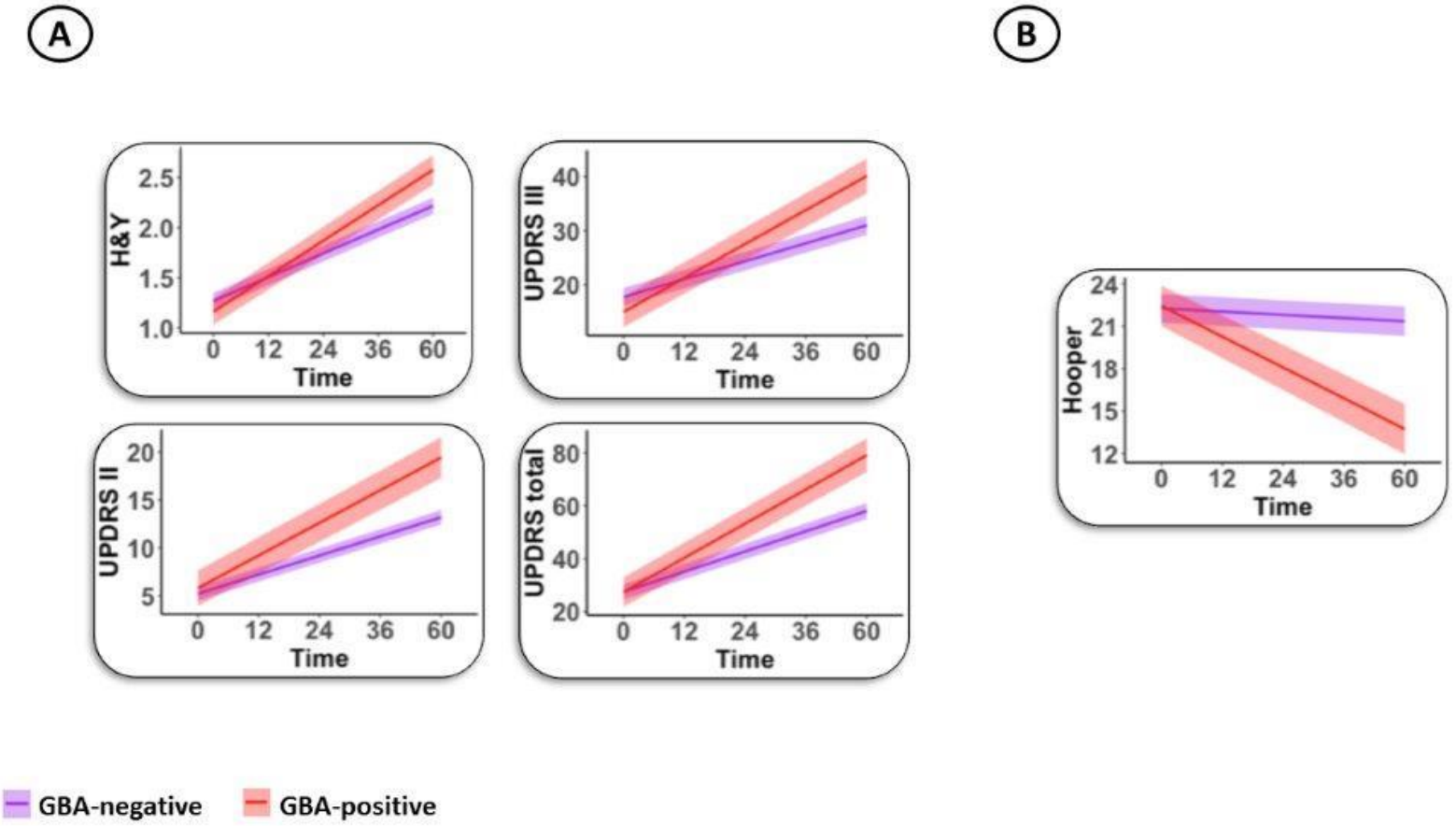


Figure 1. *Group x Time interaction: clinical and cognitive worsening in GBA-positive compared to GBA-negative PD patients over 5 years of evaluation. Time is reported on the x axis, while clinical (A) and cognitive (B) measures are reported on the y axis. Only significant results are reported. Red lines refer to GBA-positive patients, violet lines to GBA-negative patients. GBA =glucocerebrosidase; H&Y=Hoehn and Yahr; UPDRS =Unified Parkinson's Disease Rating Scale.*

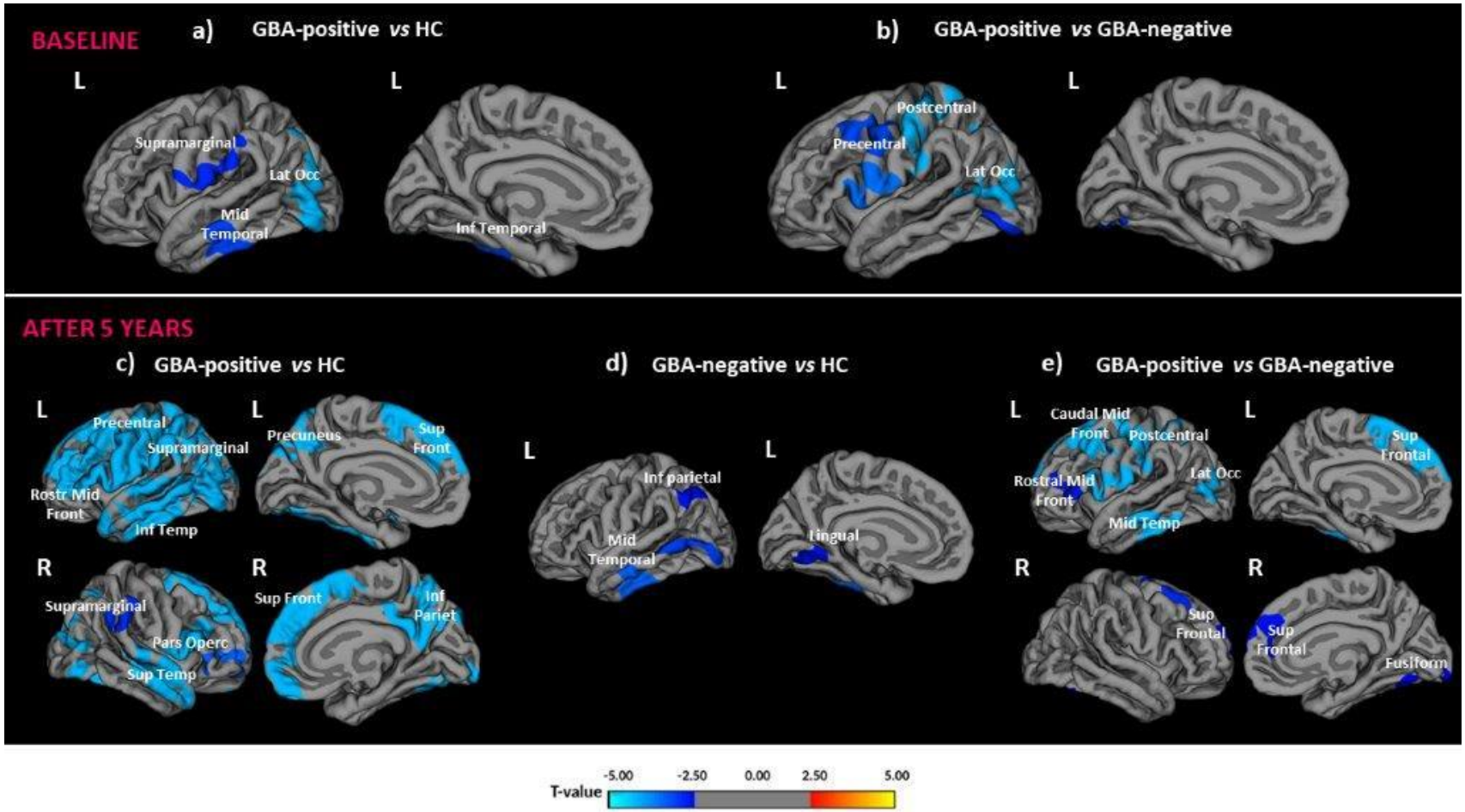


Figure 2. *Cortical thinning (vertex by vertex) patterns at baseline (upper row) and at the last follow-up visit (bottom row) of: (a) GBA-positive PD compared to healthy controls at study entry; (b) GBA positive PD compared to GBA-negative at study entry; c) GBA-positive PD compared to healthy controls at the last follow-up visit; d) GBA-negative PD compared to healthy controls at the last follow-up visit; e) GBA-positive compared to GBA-negative at the last follow-up visit. Patterns of cortical thinning are indicated in blue colours. Only significant comparisons are shown. The p values are*

Monte Carlo corrected for multiple comparisons, $p < 0.05$. Analyses were corrected for time between scans. Colour bar represents t-values. L=left; R=right; GBA =glucocerebrosidase.

2.7 Supplementary material

2.7.1 MRI acquisition parameters

Brain MRI scans were acquired at baseline and each follow up visit on the same 1.5 Tesla Philips Medical System Achieva machine at the Clinic of Neurology in Belgrade, Serbia. The following MR sequences were obtained: (i) dual-echo (DE) turbo spin-echo (SE) (repetition time [TR]=3125 ms, echo time [TEs]=20/100 ms, echo train length [ETL]=6, 44 axial slices, thickness=3.0 mm, matrix size =256×247, field of view [FOV]=240×232 mm^2; voxel size, 0.94×0.94×3 mm, in-plane sensitivity encoding [SENSE] parallel reduction factor, 1.5); (ii) 3D sagittal T1-weighted Turbo Field Echo (TFE) (frequency direction=anterior-posterior, TR=7.1 ms, TE=3.3 ms, inversion time= 1000 ms, flip angle=8°, matrix size=256×256×180 [inferior-superior, anterior-posterior], FOV=256×256mm^2, section thickness= 1 mm; voxel size= 1×1×1 mm, out-of-plane SENSE parallel reduction factor= 1.5, sagittal orientation).

2.7.2 Cortical thickness measurement

Cortical reconstruction and estimation of cortical thickness were performed on the 3D T1-weighted TFE images using the FreeSurfer image analysis suite, version 5.3 (http://surfer.nmr.mgh.harvard.edu/). On 3D TFE images acquired at baseline, the contrast between GM and WM was enhanced by nulling out all image values below the mean intensity of the cerebrospinal fluid (CSF), and by performing a rescaling of all image intensities above threshold to the new null value. After registration to Talairach space and intensity normalization, the process involved an automatic skull stripping, which removes extra-cerebral structures, cerebellum and brainstem, by using a hybrid method combining watershed algorithms and deformable surface models. Images were carefully checked for skull stripping errors. After this step, images were segmented into GM, WM, and CSF, cerebral hemispheres were separated, and subcortical structures divided from cortical components. The WM/GM boundary was tessellated, and the surface was deformed following intensity gradients to optimally place WM/GM and GM/CSF borders, thus obtaining the WM and pial surfaces (Dale, Fischl et al., 1999). Afterwards, surface inflation and registration to a spherical atlas were performed (Dale et al., 1999) and the cerebral cortex parcellated into 34 regions per hemisphere, based on gyral and sulcal structures, as described by Desikan and colleagues (Desikan, Segonne et

al., 2006). Finally, cortical thickness was estimated as the average shortest distance between the WM boundary and the pial surface. Surface maps were generated following registration of all subjects' cortical reconstructions to a common average surface and then smoothed using a surface-based Gaussian kernel of 10 mm full width half-maximum. To evaluate longitudinal cortical changes in PD patients, T1-weighted images of each subject at each of five time points were processed with the Freesurfer longitudinal stream (Reuter, Rosas et al., 2010). Specifically, an unbiased within-subject template space and image was created from the five T1-weighted scans using a robust, inverse consistent registration. Several processing steps (including skull stripping, Talairach transforms, atlas registration, as well as spherical surface maps and parcellations) were then initialized on the timepoint scans, with common information from the within-subject template. This allowed to create surface maps of the five time points with a significantly increased reliability and statistical power compared to those produced by the cross-sectional Freesurfer pipeline (Reuter et al., 2010). Individual surface maps were registered to a common average surface and then smoothed using a Gaussian kernel of 10 mm full width half-maximum.

Supplementary Table 1. List of GBA-positive PD patients with the relative GBA mutation and type.

Patients	Mutation	Severity*
Patient 1	D409H	severe
Patient 2	N370S	mild
Patient 3	N370S	mild
Patient 4	RecNciI	severe
Patient 5	D409H	severe
Patient 6	RecNciI	severe
Patient 7	N392S	unknown
Patient 8	R463H	severe
Patient 9	N370S	mild
Patient 10	R463H	severe

**The 'severity' referred to the clinical effect of each mutation (when available) and has been defined according to the classification provided by (Beutler, Gelbart et al., 2005).*

Supplementary Table 2. Raw results of the linear mixed effects modelling for each time point (please refer also to Figure 1).

	BASELINE		12 MONTHS		24 MONTHS		36 MONTHS		60 MONTHS	
	GBA-positive	**GBA-negative**	**GBA-positive**	**GBA-negative**	**GBA-positive**	**GBA-negative**	**GBA-positive**	**GBA-negative**	**GBA-positive**	**GBA-negative**
Hoehn & Yahr	1.1 ± 0.2 (1.0-1.5)	1.0 ± 0.1 (1.0-1.5)	1.7 ± 0.4 (1.0-2.0)	1.7 ± 0.4 (1.0-2.5)	1.7 ± 0.4 (1.0-2.0)	1.9 ± 0.3 (1.0-2.5)	2.0 ± 0.3 (1.5-2.5)	1.9 ± 0.4 (1.0-2.5)	2.8 ± 0.7 (2.0-4.0)	2.1 ± 0.5 (1.0-3.0)
UPDRS-II Total	7.6 ± 5.8 (0.0-19.0)	5.4 ± 2.9 (0.0-11.0)	10.0 ± 5.4 (3.0-19.0)	7.5 ± 2.8 (3.0-11.0)	9.5 ± 4.0 (5.0-16.0)	8.7 ± 3.4 (2.0-16.0)	12.3 ± 6.3 (1.0-20.0)	10.6 ± 4.2 (2.0-18.0)	25.8 ± 6.2 (15.0-31.0)	13.9 ± 4.6 (6.0-25.0)
UPDRS-III Total	15.4 ± 6.5 (5.0-26.0)	15.5 ± 4.3 (10.0-23.0)	25.1 ± 10.5 (8.0-38.0)	23.8 ± 6.3 (13.0-35.0)	22.8 ± 9.3 (11.0-35.0)	25.3 ± 8.7 (7.0-39.0)	30.0 ± 11.2 (10.0-45.0)	26.5 ± 9.7 (12.0-46.0)	46.8 ± 3.7 (43.0-53.0)	30.7 ± 11.0 (11.0-52.0)
UPDRS Total	30.2 ± 15.5 (7.0-49.0)	26.0 ± 9.6 (0.0-40.0)	44.5 ± 18.7 (15.0-70.0)	37.7 ± 9.7 (20.0-52.0)	46.1 ± 18.8 (20.0-67.0)	43.5 ± 15.2 (14.0-66.0)	55.3 ± 20.1 (17.0-78.0)	46.7 ± 15.4 (26.0-74.0)	95.5 ± 10.4 (84.0-113.0)	59.9 ± 20.3 (26.0-106.0)
Hooper	23.0 ± 4.7 (15.0-29.0)	22.5 ± 4.9 (10.0-28.0)	19.9 ± 5.6 (7.0-27.0)	21.5 ± 3.7 (14.0-27.0)	17.3 ± 5.3 (8.5-25.0)	21.5 ± 3.9 (12.0-27.0)	16.2 ± 4.3 (11.5-23.5)	22.8 ± 7.2 (11.0-48.0)	14.4 ± 5.0 (9.0-21.0)	20.6 ± 4.8 (11.0-26.0)

Values are reported as mean ± standard deviation (range). ***Abbreviations:*** *GBA=glucocerebrosidase; UPDRS=Unified Parkinson's disease Rating Scale.*

Supplementary Table 3. Cortical thickness measures and grey matter volumes of PD patients at the last visit compared with each other and baseline MRI variables of healthy controls.

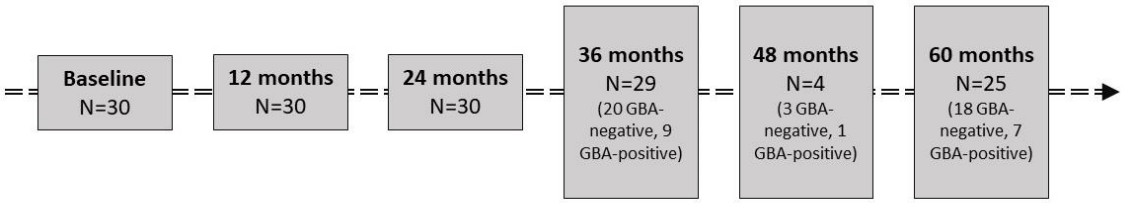
Region	Side	GBA-positive PD	GBA-negative PD	Healthy controls	p: GBA-positive PD vs healthy controls	p: GBA-negative PD vs healthy controls	p: GBA-positive vs GBA-negative PD
Cortical thickness regions							
Banks of the superior temporal sulcus	**L**	2.1 ± 0.1 (1.9-2.2)	2.2 ± 0.1 (1.8-2.4)	2.4 ± 0.1 (2.2-2.6)	**<0.001**	**0.02**	0.22
	R	2.3 ± 0.1 (2.2-2.4)	2.3 ± 0.1 (2.0-2.6)	2.4 ± 0.1 (2.2-2.8)	0.19	0.45	1.00
Caudal anterior cingulate	**L**	2.6 ± 0.2 (2.5-3.0)	2.5 ± 0.2 (2.1-3.1)	2.4 ± 0.2 (1.9-2.8)	0.10	0.21	1.00
	R	2.2 ± 0.1 (1.9-2.4)	2.3 ± 0.2 (1.9-2.7)	2.3 ± 0.2 (1.8-2.7)	0.18	1.00	0.22
Caudal middle frontal	**L**	2.3 ± 0.1 (2.2-2.4)	2.3 ± 0.1 (2.1-2.6)	2.4 ± 0.1 (2.2-2.6)	0.07	0.29	0.91
	R	2.4 ± 0.1 (2.2-2.5)	2.3 ± 0.1 (2.1-2.5)	2.3 ± 0.1 (2.2-2.6)	1.00	1.00	1.00
Cuneus	**L**	1.8 ± 0.1 (1.7-1.8)	1.7 ± 0.1 (1.5-1.9)	1.8 ± 0.1 (1.5-2.1)	1.00	0.87	0.47
	R	1.7 ± 0.1 (1.5-1.8)	1.7 ± 0.1 (1.5-2.0)	1.8 ± 0.1 (1.5-1.9)	1.00	0.56	1.00
Entorhinal	**L**	3.2 ± 0.2 (2.9-3.6)	3.1 ± 0.3 (2.3-3.5)	3.2 ± 0.2 (2.7-3.8)	1.00	0.25	1.00
	R	3.2 ± 0.3 (2.7-3.6)	3.2 ± 0.2 (2.8-3.6)	3.3 ± 0.3 (2.7-3.9)	1.00	1.00	1.00
Fusiform	**L**	2.3 ± 0.0 (2.3-2.4)	2.4 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.3-2.7)	**0.01**	1.00	0.09
	R	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.2-2.6)	2.4 ± 0.1 (2.2-2.7)	0.27	1.00	0.90
Inferior parietal	**L**	2.1 ± 0.1 (2.0-2.2)	2.2 ± 0.1 (2.0-2.5)	2.3 ± 0.1 (2.2-2.5)	**<0.001**	**<0.01**	0.26
	R	2.2 ± 0.1 (2.0-2.3)	2.2 ± 0.1 (2.0-2.4)	2.3 ± 0.1 (2.2-2.5)	**0.04**	0.26	0.76
Inferior temporal	**L**	2.4 ± 0.0 (2.3-2.5)	2.5 ± 0.1 (2.3-2.8)	2.6 ± 0.1 (2.5-2.9)	**<0.001**	**0.02**	0.25
	R	2.5 ± 0.1 (2.4-2.6)	2.5 ± 0.1 (2.3-2.8)	2.6 ± 0.1 (2.4-2.9)	**0.04**	0.48	0.51
Isthmus cingulate	**L**	1.9 ± 0.1 (1.8-2.2)	2.1 ± 0.2 (1.8-2.4)	2.1 ± 0.1 (1.9-2.3)	0.23	1.00	0.54
	R	1.9 ± 0.1	2.0 ± 0.2	2.1 ± 0.2	**0.01**	0.69	0.14

		(1.8-1.9)	(1.7-2.3)	(1.9-2.2)			
Lateral occipital	**L**	1.9 ± 0.1 (1.8-2.0)	1.9 ± 0.1 (1.8-2.2)	2.1 ± 0.1 (1.9-2.4)	**<0.01**	0.06	0.37
	R	1.9 ± 0.1 (1.9-2.1)	2.0 ± 0.1 (1.8-2.2)	2.1 ± 0.2 (1.8-2.4)	0.09	0.26	1.00
Lateral orbito-frontal	**L**	2.3 ± 0.1 (2.2-2.4)	2.3 ± 0.1 (2.2-2.5)	2.4 ± 0.1 (2.2-2.6)	0.79	0.33	1.00
	R	2.4 ± 0.1 (2.2-2.5)	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.2-2.6)	1.00	0.15	1.00
Lingual	**L**	1.8 ± 0.1 (1.7-1.9)	1.8 ± 0.1 (1.5-1.9)	1.9 ± 0.1 (1.6-2.2)	0.39	0.28	1.00
	R	1.8 ± 0.1 (1.6-1.9)	1.8 ± 0.1 (1.6-2.0)	1.9 ± 0.1 (1.7-2.1)	0.46	1.00	1.00
Medial orbito-frontal	**L**	2.1 ± 0.1 (2.1-2.2)	2.2 ± 0.1 (2.0-2.4)	2.2 ± 0.1 (2.0-2.4)	0.35	0.32	1.00
	R	2.1 ± 0.2 (2.0-2.5)	2.2 ± 0.1 (2.0-2.4)	2.3 ± 0.1 (2.0-2.6)	0.07	0.11	1.00
Middle temporal	**L**	2.4 ± 0.1 (2.2-2.5)	2.5 ± 0.1 (2.3-2.8)	2.6 ± 0.1 (2.4-2.9)	**<0.001**	**0.03**	0.10
	R	2.5 ± 0.1 (2.4-2.6)	2.6 ± 0.1 (2.3-2.8)	2.7 ± 0.1 (2.4-2.9)	**<0.01**	**0.02**	0.96
Paracentral	**L**	2.2 ± 0.1 (2.1-2.3)	2.2 ± 0.1 (2.0-2.5)	2.2 ± 0.1 (1.9-2.5)	1.00	1.00	1.00
	R	2.2 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (2.0-2.4)	2.2 ± 0.1 (1.8-2.5)	0.87	0.74	1.00
Parahippocampal	**L**	2.4 ± 0.2 (2.2-2.7)	2.5 ± 0.3 (1.9-3.1)	2.6 ± 0.3 (2.2-3.1)	1.00	1.00	1.00
	R	2.5 ± 0.1 (2.4-2.7)	2.4 ± 0.2 (2.0-2.8)	2.5 ± 0.2 (2.1-2.9)	1.00	1.00	1.00
Pars opercularis	**L**	2.3 ± 0.1 (2.1-2.5)	2.3 ± 0.1 (2.1-2.6)	2.4 ± 0.1 (2.2-2.6)	**0.02**	0.17	0.62
	R	2.3 ± 0.1 (2.1-2.4)	2.4 ± 0.1 (2.3-2.6)	2.4 ± 0.1 (2.3-2.6)	**0.02**	0.48	0.26
Pars orbitalis	**L**	2.4 ± 0.2 (2.1-2.7)	2.4 ± 0.1 (2.1-2.6)	2.5 ± 0.2 (2.2-2.8)	0.95	1.00	1.00
	R	2.4 ± 0.2 (2.2-2.9)	2.4 ± 0.2 (2.2-2.8)	2.5 ± 0.1 (2.3-2.8)	0.84	0.23	1.00
Pars triangularis	**L**	2.2 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (1.9-2.5)	2.3 ± 0.1 (2.0-2.6)	0.09	0.21	1.00
	R	2.2 ± 0.2 (1.9-2.4)	2.2 ± 0.1 (2.1-2.4)	2.3 ± 0.1 (2.2-2.5)	0.41	**0.03**	1.00
Pericalcarine	**L**	1.5 ± 0.1 (1.4-1.6)	1.5 ± 0.1 (1.4-1.8)	1.6 ± 0.1 (1.4-1.8)	1.00	0.46	1.00
	R	1.5 ± 0.1 (1.5-1.7)	1.6 ± 0.1 (1.4-1.9)	1.6 ± 0.1 (1.3-1.8)	0.61	1.00	1.00
Postcentral	**L**	1.8 ± 0.1 (1.7-1.9)	1.9 ± 0.1 (1.8-2.0)	2.0 ± 0.1 (1.8-2.3)	**0.001**	0.07	0.19

	R	1.9 ± 0.0 (1.8-1.9)	1.9 ± 0.1 (1.7-1.9)	2.0 ± 0.1 (1.8-2.2)	**0.02**	**<0.01**	1.00
Posterior cingulate	**L**	2.3 ± 0.1 (2.1-2.4)	2.3 ± 0.1 (2.1-2.5)	2.3 ± 0.1 (2.0-2.6)	1.00	1.00	1.00
	R	2.2 ± 0.1 (2.0-2.3)	2.2 ± 0.1 (2.0-2.5)	2.2 ± 0.1 (1.9-2.5)	0.92	1.00	1.00
Precentral	**L**	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.2 (2.1-2.6)	2.4 ± 0.1 (2.2-2.7)	0.70	1.00	1.00
	R	2.3 ± 0.1 (2.2-2.4)	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.1-2.6)	0.58	1.00	1.00
Precuneus	**L**	2.1 ± 0.1 (2.0-2.2)	2.1 ± 0.1 (2.0-2.4)	2.2 ± 0.1 (1.9-2.5)	0.09	0.14	1.00
	R	2.0 ± 0.1 (1.8-2.2)	2.1 ± 0.1 (1.9-2.3)	2.2 ± 0.1 (2.1-2.5)	**<0.01**	0. 37	0.13
Rostral middle frontal	**L**	2.2 ± 0.1 (2.0-2.4)	2.1 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (2.1-2.5)	0.26	**0.03**	1.00
	R	2.2 ± 0.1 (2.0-2.4)	2.2 ± 0.1 (1.9-2.4)	2.2 ± 0.1 (2.1-2.4)	0.47	0.16	1.00
Superior frontal	**L**	2.4 ± 0.1 (2.4-2.5)	2.4 ± 0.1 (2.2-2.7)	2.5 ± 0.1 (2.4-2.7)	0.76	0.41	1.00
	R	2.4 ± 0.0 (2.4-2.5)	2.4 ± 0.1 (2.2-2.7)	2.5 ± 0.1 (2.3-2.7)	0.45	0.86	1.00
Superior parietal	**L**	1.9 ± 0.0 (1.9-2.1)	2.1 ± 0.1 (1.9-2.3)	2.1 ± 0.1 (2.0-2.3)	**<0.001**	0.15	0.08
	R	1.9 ± 0.1 (1.8-2.1)	2.0 ± 0.1 (1.8-2.2)	2.1 ± 0.1 (1.9-2.3)	**0.01**	0.34	0.11
Superior temporal	**L**	2.4 ± 0.1 (2.2-2.5)	2.5 ± 0.1 (2.1-2.7)	2.6 ± 0.1 (2.3-2.8)	**<0.01**	0.14	0.25
	R	2.5 ± 0.1 (2.3-2.6)	2.5 ± 0.1 (2.2-2.7)	2.6 ± 0.1 (2.4-2.8)	0.11	0.16	1.00
Supramarginal	**L**	2.2 ± 0.0 (2.1-2.3)	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.3-2.6)	**<0.001**	0.11	0.08
	R	2.3 ± 0.0 (2.2-2.3)	2.3 ± 0.1 (2.1-2.5)	2.4 ± 0.1 (2.2-2.6)	**0.03**	0.12	0.83
Temporal pole	**L**	3.3 ± 0.3 (2.8-3.8)	3.4 ± 0.3 (2.6-3.8)	3.4 ± 0.2 (2.9-3.8)	1.00	1.00	1.00
	R	3.4 ± 0.4 (2.9-3.9)	3.5 ±0.3 (2.8-4.2)	3.6 ± 0.3 (3.2-4.2)	0.24	0.83	1.00
Transverse temporal	**L**	2.1 ± 0.2 (1.9-2.3)	2.2 ± 0.1 (1.9-2.4)	2.3 ± 0.1 (2.0-2.5)	**0.049**	0.21	0.88
	R	2.2 ± 0.3 (1.7-2.5)	2.2 ± 0.2 (1.8-2.5)	2.3 ± 0.2 (2.0-2.7)	1.00	1.00	1.00
Gray matter volumes							
Caudate	**L**	2951.7 ± 573.8 (2183.9-3744.9)	3013.9 ± 348.3 (2332.0-3468.0)	4254.0 ± 608.7 (2948.7-5576.8)	**<0.001**	**<0.001**	1.00

	R	2648.9 ± 1209.1 (292.9-4117.9)	3192.9 ± 383.4 (2528.9-4069.0)	4500.9 ± 483.8 (3515.5-5783.4)	**<0.001**	**<0.001**	1.00
Pallidum	**L**	1639.1 ± 192.9 (1378.9-1959.9)	1754.3 ± 299.4 (1478.0-2701.0)	2314.1 ± 261.1 (1938.9-2897.6)	**<0.001**	**<0.001**	1.00
	R	1886.1 ± 682.9 (1298.9-3380.9)	1775.1 ± 189.7 (1521.9-2236.0)	2380.0 ± 274.6 (1973.2-3020.9)	**<0.01**	**<0.001**	1.00
Putamen	**L**	4092.5 ± 489.8 (3226.9-4610.9)	4311.2 ± 490.7 (3402.9-5059.0)	6377.7 ± 564.7 (5143.8-7601.4)	**<0.001**	**<0.001**	1.00
	R	3856.8 ± 637.7 (2808.9-4795.9)	4345.2 ± 509.7 (3657.9-5551.0)	6199.6 ± 493.3 (5164.8-6915.7)	**<0.001**	**<0.001**	1.00
Thalamus	**L**	6898.8 ± 544.1 (6284.9-7777.9)	7231.0 ± 762.0 (5985.0-8632.9)	9898.5 ± 735.6 (8141.0-11091.0)	**<0.001**	**<0.001**	1.00
	R	6075.1 ± 2092.5 (1518.9-7949.9)	7003.5 ± 710.5 (5978.0-8217.9)	9539.9 ± 644.8 (8070.3-11014.0)	**<0.001**	**<0.001**	1.00
Hippocampus	**L**	3504.9 ± 290.0 (3084.9-3959.0)	3513.2 ± 439.8 (2752.9-4809.9)	4869.0 ± 515.8 (4098.9-5778.2)	**<0.001**	**<0.001**	1.00
	R	3469.5 ± 1047.7 (1197.9-4278.0)	3722.9 ± 454.4 (3007.0-5135.9)	5055.5 ± 572.6 (4104.7-6206.9)	**<0.001**	**<0.001**	1.00
Amygdala	**L**	1342.6 ± 229.2 (989.9-1712.9)	1417.5 ± 192.1 (1137.9-1754.0)	1791.6 ± 292.2 (1039.5-2296.3)	**<0.01**	**<0.001**	1.00
	R	2106.3 ± 2012.6 (1055.9-6632.9)	1345.6 ± 175.1 (1062.0-1667.9)	1844.2 ± 335.3 (858.1-2356.7)	0.06	**<0.001**	1.00

Values are reported as mean ± standard deviation (range). Cortical thickness regions are in mm, volumes in mm^3. Differences between groups were assessed using the Kruskal-Wallis test, followed by Dunn's post-hoc test. P values are corrected with Bonferroni test. Values in bold indicate statistically significant results; only regions with at least one significant contrast were reported. ***Abbreviations:*** *GBA=glucocerebrosidase; L=left; PD=Parkinson's disease; R= right.*



Supplementary Figure 1. *Number of participants who remained in the study at each time point.*

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Chapter 3 – Action Observation and Motor Imagery improve dual-task in Parkinson's disease: a clinical/fMRI study

RESEARCH ARTICLE

Action Observation and Motor Imagery Improve Dual Task in Parkinson's Disease: A Clinical/fMRI Study

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ABSTRACT: Background: Action observation training and motor imagery may improve motor learning in Parkinson's disease (PD).
Objectives: The objectives of this study were to assess mobility and balance (performing motor and dual tasks) and brain functional reorganization following 6 weeks of action observation training and motor imagery associated with dual-task gait/balance exercises in PD patients with postural instability and gait disorders relative to dual-task training alone.
Methods: Twenty-five PD–postural instability and gait disorder patients were randomized into 2 groups: the DUAL-TASK+AOT-MI group performed a 6-week gait/balance training consisting of action observation training–motor imagery combined with practicing the observed-imagined exercises; the DUAL-TASK group performed the same exercises combined with watching landscape videos. Exercises were increasingly difficult to include the dual task. At baseline and at 6 weeks, patients underwent: mobility, gait, and balance evaluations (also repeated 2 months after training), cognitive assessment, and functional MRI, including motor and dual tasks.
Results: Dual-task gait/balance training enhanced mobility, during both single- and dual-task conditions, and executive functions in PD–postural instability and gait disorders, with a long-lasting effect at 14 weeks. When exercises were preceded by action observation training–motor imagery, PD–postural instability and gait disorders showed greater improvement of balance and gait velocity both with and without the dual task, particularly during the turning phase. After training, the DUAL-TASK+AOT-MI group showed reduced recruitment of frontal areas and increased activity of cerebellum during functional-MRI motor and dual task, correlating with balance/turning velocity and executive improvements, respectively. The DUAL-TASK group showed reduced activity of supplementary motor area and increased recruitment of temporo-parietal areas during the dual task and decreased cerebellar activity during the motor task correlating with faster turning velocity. Functional MRI results were not corrected for multiple comparisons and should be interpreted carefully.
Conclusions: Adding action observation training–motor imagery to dual-task gait/balance training promotes specific functional reorganization of brain areas involved in motor control and executive-attentive abilities and more long-lasting effects on dual-task mobility and balance in PD–postural instability and gait disorders. © 2021 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; action observation; motor imagery; dual task; fMRI

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3.1 Introduction

PD is a disabling neurological disorder with heterogeneous clinical manifestations due to altered cortico-subcortical loops (Filippi, Basaia et al., 2020, Filippi, Sarasso et al., 2020, Poewe, Seppi et al., 2017). PIGD are common features in PD, which appear with disease progression as a result of loss of automaticity and progressive failure of compensatory movement strategies relying on cognitive resources (de Souza Fortaleza, Mancini et al., 2017, Piramide, Agosta et al., 2020). PD-PIGD patients usually become less responsive to pharmacological treatment, perform worse in executive-attentive functions, and have more rapid progression of the disease relative to PD-TD cases (Thenganatt & Jankovic, 2014). PD-PIGD patients show particular difficulties in managing with dual-task situations (Strouwen, Molenaar et al., 2015a). Such a cognitive overload could explain FoG or gait imbalance, particularly during specific gait phases such as turning or obstacle negotiation that require higher executive-attentive involvement relative to straight walking to adapt the locomotor pattern (Curtze, Nutt et al., 2016, de Souza Fortaleza et al., 2017, Plotnik, Giladi et al., 2011). Reduced gait speed and stride length, and increased gait variability and asymmetry were reported in PD patients during dual-task gait (Curtze et al., 2016, de Souza Fortaleza et al., 2017). Noteworthy, a reduced turning peak velocity has been highly correlated with lower balance confidence leading to a reduced gait safety, increased risk of falls and lower quality of life (Crenna, Carpinella et al., 2007, Curtze et al., 2016, Grimbergen, Munneke et al., 2004).

Despite the past controversies about the potential usefulness and risks of dual-task training in PD patients (Strouwen et al., 2015a), recent evidence suggests dual-task training as a safe method to improve dual-task gait/mobility and to reduce falls in PD (De Freitas Tb Ms, Leite et al., 2020, Maidan, Rosenberg-Katz et al., 2017, Strouwen, Molenaar et al., 2017a), by optimizing brain efficiency and motor learning (Maidan et al., 2017). Other recent emerging approaches targeted to improve motor learning in PD patients are mental practice techniques such as AOT and MI (Gatti, Sarasso et al., 2019, Sarasso, Gemma et al., 2015). AOT and MI exploit the MNS activity, creating an inner knowledge of the actions and having the potential to consolidate motor learning and improve motor recovery by facilitating motor and cognitive pathways (Agosta, Gatti et al., 2017, Buccino, 2014, Rizzolatti, Fogassi et al., 2001). In PD patients, several studies

demonstrated that AOT not only improves bradykinesia, balance, gait abilities, FoG and quality of life (Agosta et al., 2017, Pelosin, Avanzino et al., 2010, Pelosin, Bove et al., 2013), but also working memory and executive/attentive abilities (Agosta et al., 2017, Buccino, 2014). These effects are retained for longer than after physiotherapy training "alone" (Agosta et al., 2017, Pelosin et al., 2010, Pelosin et al., 2013) probably because mental practice techniques have the potential to modulate brain plasticity as demonstrated by preliminary fMRI findings (Agosta et al., 2017). Our group showed that four weeks of AOT combined with balance and mobility exercises promotes a functional reorganization of the fronto-parietal MNS that correlates with clinical changes in PD patients with FoG (Agosta et al., 2017). A recent pilot study suggested the potential benefits of combining AOT with dual-task training in PD rehabilitation by enhancing working memory and attention (Caligiore, Mustile et al., 2017).

To date, few studies concentrated on the effect of MI on motor learning in PD reporting preliminary encouraging findings on bradykinesia and mobility improvement (Abbruzzese, Avanzino et al., 2015, Caligiore et al., 2017, Mirelman, Maidan et al., 2013, Tamir, Dickstein et al., 2007). Similarly to AOT, MI can stimulate the activity of areas that are recruited also during movement execution (i.e., premotor cortex, anterior cingulate, inferior parietal lobule, and cerebellum), and thus it can be combined with the motor practice to potentiate the effects of physiotherapy (Abbruzzese et al., 2015, Caligiore et al., 2017, Mirelman et al., 2013, Tamir et al., 2007). Recent reviews suggested that AOT and MI can be considered complimentary approaches to improve motor learning by promoting an enhanced activation of an overlapping cortical-subcortical network (Abbruzzese et al., 2015, Caligiore et al., 2017).

Against this background, we hypothesized that combining both AOT and MI with physiotherapy focused on dual-task balance/gait exercises in PD-PIGD patients has the potential to boost the motor learning effects, reducing the need to control movement not only in single tasks, but also during dual-task conditions of daily life. The aim of our study was to assess brain functional reorganization and gait/mobility changes performing dual-task after six weeks of AOT and MI associated with dual-task gait/balance exercises in PD-PIGD patients.

3.2 Materials and methods

3.2.1 Subjects and study design

Twenty-five right-handed, idiopathic PD (Hughes, Daniel et al., 1992) outpatients were recruited at the Movement Disorders Unit, Unit of Neurology, IRCCS Ospedale San Raffaele, Milan, Italy according to the following inclusion criteria: H&Y score ≤ 4 (Hoehn & Yahr, 1967); PIGD phenotype (Stebbins, Goetz et al., 2013); stable dopaminergic medication for at least four weeks and without any changes during the observation period (14 weeks); no dementia (Litvan, Goldman et al., 2012) and MMSE ≥24 (Folstein, Folstein et al., 1975); no significant head tremor. At study entry, patients underwent neurological, motor functional and neuropsychological evaluations and MRI scan. Twenty-three age- and sex-matched, right-handed, healthy controls were recruited by word of mouth among non-consanguineous relatives and institute personnel, and performed neuropsychological and MRI assessments at baseline. Participants were excluded if they had: medical illnesses or substance abuse that could interfere with cognition; any (other) major systemic, psychiatric, neurological, visual and musculoskeletal disturbances or other causes of walking inability; contraindications to undergo MRI examination; brain damage at routine MRI, including lacunae and extensive cerebrovascular disorders.

After baseline evaluation (T0), patients were randomized into two training groups: the DUAL-TASK+AOT-MI group and the DUAL-TASK group. Randomization was performed through minimization method in order to balance the following variables between the two groups: sex; presence/absence of MCI (Geurtsen, Hoogland et al., 2014); risk of falling (low, moderate or high) (Paul, Canning et al., 2013) (Table 1). Allocation was concealed by using sequentially numbered, sealed and opaque envelopes prepared by an individual not involved in the study. After six weeks of training (W6) patients repeated the neurological, motor functional, neuropsychological, and MRI evaluations. Clinical assessments were also repeated at 14-week follow-up (W14). All the clinical evaluations and the treatment were performed in ON condition (under regular dopaminergic medication); the neurological assessment was also performed in OFF. The same blinded assessors performed evaluations at each time-point.

Local ethical standards committee on human experimentation approved the study protocol and all subjects provided written informed consent prior to study participation.

3.2.2 Physiotherapy

The DUAL-TASK+AOT-MI group performed a gait/balance training consisting of AOT-MI combined with practicing the observed-imagined exercises (four gait/balance exercises each session were proposed with the following modality: two minutes of task observation → five minutes of task execution → two minutes of task imagination → five minutes of task execution). DUAL-TASK group performed the same amount of exercises combined with watching landscape videos instead of observation/imagination. The training lasted six weeks, three times a week, about one hour each session and exercises were increasingly difficult up to include dual-task. Supplementary material reports a detailed description of the training according to the TIDieR checklist (https://www.equator-network.org/reporting-guidelines/tidier/).

3.2.3 Clinical evaluation

A blinded and experienced neurologist performed the following evaluations: H&Y scale (Goetz, Poewe et al., 2004), UPDRS-II (Goetz, Tilley et al., 2008), and UPDRS-III (Goetz et al., 2008).

A blinded, experienced physiotherapist performed the following motor functional evaluations: Pre-assessment Information Form (Paul et al., 2013); Mini Balance Evaluation Systems Test (MiniBESTest) (King & Horak, 2013); Timed Up and Go Test (TUG) (Morris, Morris et al., 2001); TUG with cognitive (TUG-COG) and manual dual-task (TUG-MAN), consisting respectively of TUG while counting backwards by seven starting from 100 and holding in the right hand a glass full of water (Hofheinz & Schusterschitz, 2010, Lundin-Olsson, Nyberg et al., 1998); 10 meters walking test (10MWT) (Johnston, de Morton et al., 2013); Activities Balance Confidence Scale (ABC) (Powell & Myers, 1995); Parkinson's Disease Questionnaire-39items (PDQ-39) (Peto, Jenkinson et al., 1995); New Freezing of Gait Questionnaire (NFoG-Q) (Shine, Moore et al., 2012). We also obtained turning velocity parameters during the execution of TUG, TUG-COG, and TUG-MAN using an optoelectronic system. Details on gait analysis are reported in supplementary materials. Dual-task cost (DTC) was calculated as follows (Bertoli, Croce et al., 2019):

$$\text{DTC} = \frac{\text{dual-task} - \text{single-task}}{\text{dual-task}} \times 100$$

where single task is TUG, and dual-task is TUG-COG or TUG-MAN.

A blinded and experienced neuropsychologist performed a comprehensive cognitive evaluation including a screening battery in order to detect the presence of MCI (Geurtsen et al., 2014). A sub-sample of 10 patients per group performed also the Attention Switching Task (AST) subtest of the computerized CANTAB battery, which assessed changes in executive functions (supplementary materials).

3.2.4 Neuropsychological assessment

A blinded and experienced neuropsychologist performed the following assessments: MMSE (Folstein et al., 1975); Digit span forward (Orsini, Grossi et al., 1987) and Rey Auditory Verbal Learning Test (RAVLT) immediate and delayed recall (Carlesimo, Caltagirone et al., 1996) and recognition (Rey, 1964); the recall of the Rey-Osterrieth Complex Figure (Carlesimo et al., 1996); Ten-point Clock Drawing Test (Manos, 1999); Modified Card Sorting Test (Caffarra, Vezzadini et al., 2004); phonemic and semantic verbal fluency tests (Novelli, Papagno et al., 1986); Attentive Matrices Test (1987); Trail Making Test (TMT) (Giovagnoli, Del Pesce et al., 1996) and digit span backward (Monaco, Costa et al., 2013); Copy of the Rey-Osterrieth Complex Figure (Carlesimo et al., 1996); Freehand copying of drawings with and without landmarks (Carlesimo et al., 1996); Benton Judgment of Line Orientation Test (Qualls, Bliwise et al., 2000); the visuospatial subtests of the Addenbrooke's Cognitive Examination Revised (ACE-R) (Mioshi, Dawson et al., 2006); Confrontation naming battery of BADA (Miceli, Laudanna et al., 1994); Token Test (De Renzi & Vignolo, 1962); BDI (Beck, Ward et al., 1961); Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960); Hamilton anxiety rating scale (HAMA) (Hamilton, 1959); Apathy Rating Scale (Starkstein, Merello et al., 2009); Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith, Hamilton et al., 2018); Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease–Rating Scale (QUIP-RS) (Weintraub, Mamikonyan et al., 2012). The NPI (Cummings, Mega et al., 1994) was used to perform a behavioural assessment of the caregiver.

In addition, to assess executive function changes over time we used the percent of correct trials during the Attention Switching Test (AST) incongruent condition, as implemented within the Cambridge Neuropsychological Test Automated Battery (CANTAB). AST is a test of executive functions, which provides a measure of cued

attentional set-shifting (www.cambridgecognition.com). Ten subjects for each group completed the AST (supplementary figure 1).

3.2.5 Description of the rehabilitative program

Two physiotherapists with a wide experience in movement disorders managed the rehabilitation program. The training was performed in a rehabilitative gym and was provided individually. All patients underwent about 60 minutes of balance and gait training each session (during ON time), three times a week, for 6 weeks (for a total of 18 sessions). During each session, subjects belonging to the DUAL-TASK+AOT-MI group were asked to observe a 2-minute video clip showing a balance or gait task; to perform the same task for 5 minutes; to imagine the same exercise for 2 minutes; and finally to repeat again the task for 5 minutes. This approach allowed to alternate observation or imagination of the task with the execution of the task itself, according to the modality: observation → execution → imagination → execution. This method was used for all the 4 balance/gait exercises proposed in each session. The DUAL-TASK group performed exactly the same exercises, but both observation and imagination of the motor task were replaced by the observation of landscapes without animated subjects. All the patients were encouraged to do their best and to concentrate during balance and gait performances. During exercise execution, both the DUAL-TASK+AOT-MI group and the DUAL-TASK group were trained with the same posology, similar explanation methods and corrections. Patients of the DUAL-TASK+AOT-MI group were explicitly asked to concentrate on how the actions were performed in the videos and to carefully use both kinaesthetic and visual imagination in order to improve their motor performance as a consequence of the AOT and MI practice. Patients were not allowed to perform any movement while watching videos or during imagination.

Exercises were shaped on the abilities of patients in order to be always feasible but challenging. Exercises were studied to train the major gait and balance components: anticipatory postural adjustments, feet-in-place and change-in-support reactive balance strategies, sensory re-weighting and walking speed. Exercises became increasingly challenging by adding proprioceptive, vestibular and visual difficulties and because of the inclusion of dual-task adding motor and cognitive interferences to the gait/balance tasks (i.e. reciting the alphabet backward; or listing days of the week/month backwards;

or enumerating words belonging to different categories such as crafts, colours, animals, names; or making lists of words with a specific initial letter; or changing pattern/direction of movement according to a specific associative cues, i.e. “Red!= Go!”, “Green!=Stop!”, “Yellow!=Right!”, “Blue!=Left!”; or moving objects in space with the upper limbs, i.e. walking holding a tray with a glass or throwing and catching a ball; or maintaining balance while picking up an object from the floor/reaching a distant object). All the patients received the same amount of dual-task training. The materials used for training are easy to retrieve and included a chair, a step, different foams/balance pads, a volleyball, a tennis ball, a tray, obstacles and agility discs/cones. All patients had a positive coping to the rehabilitation program (please refer to Supplementary Table 8).

3.2.6 MRI acquisition

3.0T MRI scans were obtained between 12 noon and 1 PM during OFF time. Patients performed two fMRI tasks: the “motor-task” consisted in alternated self-paced dorsal and plantar flexion movements of the feet with eyes closed; the “dual-task” consisted in the same foot anti-phase movement executed while mentally counting backwards by threes starting from 100. See supplementary materials for further details.

3.2.7 Gait analysis

A six-camera SMART-DX7000 (BTS Bioengineering, Italy) optoelectronic system acquiring at 500 Hz sample frequency was used to obtain turning velocity parameters during TUG; TUG-COG and manual dual-task (TUG-MAN). To analyse gait data, the software SMART Analyzer v.1.10.465.0 (BTS Bioengineering, Italy) was used. The signal was filtered with a Butterworth low-pass filter below 4Hz. The turning phase was defined as the timeframe in which the angle between the vector connecting the acromions and the transversal reference system of the laboratory varied more than during the physiological oscillations of the straight gait. Peak turning velocity was defined as the maximum value of the angular velocity within the timeframe of turning (the angular velocity was obtained calculating the first derivate of the angle covered by the vector between the acromions on the transversal plane). Mean turning velocity was calculated as the mean of the right and left velocities obtained dividing the length of the track of the malleolus by the duration of the turning phase.

3.2.8 Statistical analysis

Based on previous evidence (Wong-Yu & Mak, 2015), we carried out an a-priori power analysis assuming that the total execution time of TUG-COG (primary outcome) after balance training would decrease by 3.1 sec (with a standard deviation of 4.5). Assuming a reduction of 8.2 sec in the experimental group after six weeks of training based on our clinical experience, 24 participants (12 in each group) would be needed for 80% power to detect significant differences (α=0.05) between the treatment groups.

Data distribution was assessed using Shapiro-Wilk test and non-parametric tests were used to analyse data. The socio-demographic and clinical variables at T0 were compared between groups using Mann Whitney test. Longitudinal changes (T0-W6 and T0-W6-W14) were assessed in both PD groups using linear mixed-effect models. Using the same models, a group-by-time interaction was performed to evaluate longitudinal between-group differences (T0-W6 and T0-W6-W14). Such models were adjusted for the baseline value of each considered variable and for the baseline variable-by-time interaction. P values were Bonferroni-corrected for multiple comparison at $p<0.05$. All statistical analyses were performed using R Statistical Software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria).

3.2.9 fMRI analysis

fMRI data were analyzed using SPM12 software (http://www.fil.ion.ucl.ac.uk/spm, Wellcome Trust Center for Neuroimaging, London). Prior to statistical analysis, all images were realigned to the first one to correct for subject motion (all study participants showed maximal head movements lower than 3 mm in each direction), spatially normalized into the standard MNI space (Montreal Neurological Institute), and smoothed applying a 10-mm, 3D-Gaussian filter. The signal variations of the BOLD effect associated with the execution of each task (considering movement parameters as confounds) were evaluated voxel by voxel using the General Linear Model (GLM) and the Gaussian field theory. Specific effects were tested applying appropriate linear contrasts. Significant hemodynamic changes for each contrast were evaluated using a nonparametric permutation-based approach, i.e., Statistical nonParametric Mapping (SnPM), a toolbox for SPM (Nichols & Holmes, 2002).

One-sample t test in SnPM was used to evaluate significant mean brain activations of each group during each task; differences between groups (healthy controls *vs* PD-PIGD patients, DUAL-TASK vs DUAL-TASK+AOT-MI) was assessed using a two-sample t-test. Differences between dual-task and motor-task in PD-PIGD at baseline and changes over time in each task for each group were evaluated using paired t-test. Differences between the two groups of the study over time were evaluated using a GLM model, in which group and time were included as distinct factors (2 × 2 factorial design). Multiple linear regression models were used to assess the correlation between changes in fMRI results and changes in clinical and gait analysis data after training. Given the relatively small sample, in order to reduce multiple comparisons, a hypothesis-driven mask from the AAL brain atlas was created including areas involved in motor and executive processing, which are expected to change after training (Agosta et al., 2017, Maidan et al., 2017, Wager & Smith, 2003, Wu & Hallett, 2008): frontal and parietal areas, MNS, basal ganglia and cerebellum. The mask was applied to the SnPM dataset using WFU Pickatlas. For all permutation-based contrasts, non-parametric testing was performed with 5000 random permutations. All findings are shown at $p<0.001$ uncorrected and only clusters greater than 5 voxels were considered.

3.3 Results

3.3.1 Participants

Forty-one PD patients were screened and 25 of them, meeting inclusion criteria, accepted to participate and were randomly allocated to one of the training groups (12 to DUAL-TASK, 13 to DUAL-TASK+AOT-MI group). In the DUAL-TASK+AOT-MI group, one patient was lost at W6 and one at W14 due to medical issues not related to the treatment (Figure 1). Twenty-three healthy controls were recruited.

3.3.2 Clinical results at baseline

PD-PIGD patients and healthy controls, and both groups of patients were similar for socio-demographic variables (Table 1). The two groups of patients were similar also for all neurological, motor and neuropsychological variables at baseline (Table 2; supplementary table 1). PD-PIGD patients showed significantly lower MMSE score relative to healthy controls and performed worse on verbal memory, executive/attentive

and visuospatial abilities tests (supplementary table 1). For detailed description of neuropsychological results see supplementary materials.

3.3.3 Neuropsychological results at baseline

PD-PIGD patients showed significantly lower MMSE score relative to healthy controls and performed worse in verbal memory (RAVLT and digit span foreword), executive/attentive (TMT and semantic and phonemic fluency) and visuospatial (copy of drawing with landmarks) tests. The two groups of patients were similar for neuropsychological variables at baseline (Supplementary table 1).

3.3.4 Longitudinal changes of neurological, motor functional and cognitive variables

Results are summarized in Table 2 and in Supplementary Figure 1. At W6, the DUAL-TASK group showed a significant change of TUG, TUG-COG, TUG-MAN, DTC on mean turning velocity during TUG-COG, UPDRS-III OFF and AST percent of correct trials (incongruent condition) and a trend toward a better UPDRS-III ON and an increased mean turning velocity during TUG-COG. TUG, TUG-COG, TUG-MAN, UPDRS-III ON and turning velocity during TUG-COG changes were maintained at W14 in the DUAL-TASK group. The DUAL-TASK+AOT-MI group showed improvement both at W6 and W14 of TUG, TUG-COG, TUG-MAN and their relative peak and mean turning velocity, DTC during TUG-COG and DTC on mean TUG-COG turning velocity, MiniBESTest, ABC, 10MWT (maximal and comfortable speed), NFoG-Q and PDQ-39. UPDRS-III OFF, H&Y OFF and AST percent of correct trials (incongruent condition) improved at W6 only.

The DUAL-TASK+AOT-MI relative to the DUAL-TASK group showed greater improvement of TUG-COG, mean and peak of turning velocity during TUG-COG, peak turning velocity during TUG and TUG-MAN, DTC during TUG-COG, MiniBESTest, 10MWT (comfortable speed) and ABC both at W6 and W14. Moreover DUAL-TASK+AOT-MI group showed greater improvement of mean turning velocity during TUG and DTC on mean turning velocity during TUG-COG at W6 and of 10MWT (maximum speed) at W14 relative to DUAL-TASK cases.

3.3.5 fMRI results

3.3.5.1 fMRI motor-task at baseline

During the fMRI motor-task, both PD-PIGD patients and healthy controls showed the recruitment of frontal, parietal and motor areas and cerebellum (Figure 2a). PD-PIGD patients showed reduced activity of bilateral inferior frontal gyri pars triangularis, and increased recruitment of right cerebellum crus 1 and bilateral cerebellum crus 2 relative to healthy controls (Figure 2c; supplementary table 2). No significant differences between DUAL-TASK and DUAL-TASK+AOT-MI groups were observed.

3.3.5.2 fMRI dual-task at baseline

During the fMRI dual-task, both PD-PIGD patients and healthy controls showed the recruitment of frontal, parietal and motor areas and cerebellum (Figure 2b). PD-PIGD patients relative to controls showed reduced activity of left caudate and increased recruitment of bilateral medial superior frontal gyri and supplementary motor areas (SMA) and left cerebellum crus 2 (Figure 2d; supplementary table 3). No differences between DUAL-TASK and DUAL-TASK+AOT-MI groups were observed.

3.3.5.3 fMRI dual-task vs motor-task at baseline

Areas specifically related to dual-task interference are presented in supplementary table 4 and supplementary figure 3.

3.3.5.4 fMRI motor-task after training (T0-W6)

At W6 compared with baseline, the DUAL-TASK group showed reduced recruitment of left cerebellum lobules VIII and IX during the motor-task. The DUAL-TASK+AOT-MI group showed reduced left medial superior frontal gyrus activity and increased right cerebellum lobules IV-V recruitment. The DUAL-TASK+AOT-MI relative to the DUAL- TASK group showed increased recruitment of the right cerebellum lobule VIII and vermis IV-V and reduced left middle/inferior frontal gyrus activity at W6 relative to baseline (Figure 3a; supplementary table 5).

3.3.5.5 fMRI dual-task after training (T0-W6)

At W6 relative to baseline, the DUAL-TASK+AOT-MI group showed reduced right inferior frontal gyrus activity during the fMRI dual-task and increased left cerebellum lobule VI activity, while the DUAL-TASK group showed reduced SMA activity and increased recruitment of right temporal and parietal areas during the same task. DUAL-TASK+AOT-MI relative to DUAL-TASK group showed reduced right superior/middle frontal gyrus activity at W6 relative to baseline (Figure 3b; supplementary table 6).

3.3.6 Correlation analysis

Correlations between fMRI and clinical changes at W6 are shown in supplementary figure 2 and supplementary table 7.

In the DUAL-TASK+AOT-MI group, during the fMRI motor-task, a reduced activity of left inferior frontal gyrus correlated with an increased ABC, a reduced recruitment of left middle frontal gyrus with an increased MiniBESTest, and a reduced recruitment of left superior/middle frontal gyrus with an increased TUG peak turning velocity. In the DUAL-TASK group, an increased peak turning velocity during TUG correlated with a decreased activity of left cerebellum lobule IX after training. During the fMRI dual-task, in the DUAL-TASK+AOT-MI group increased left cerebellum lobule VI recruitment and reduced right middle frontal gyrus recruitment correlated with MiniBESTest improvement after training, while an increased recruitment of right cerebellum IV-VI correlated with higher percentage of correct trials at the AST (incongruent condition).

3.4 Discussion

The primary aim of this study was to assess if a dual-task gait/balance training combined with AOT and MI of the proposed exercises was more effective than a dual-task balance/gait training alone to improve mobility during dual-task situations in PD-PIGD patients. We expected that AOT and MI together with dual-task training could facilitate motor learning of complex movement sequences by enhancing the (re)activation of circuits involved in the action motor representation and by improving executive-attentive abilities (Abbruzzese et al., 2015, Agosta et al., 2017, Caligiore et al., 2017, Tamir et al., 2007).

Clinical findings suggested that both groups after training showed an improvement of mobility during TUG-COG, TUG-MAN and TUG, which was maintained two months after training. These results are in line with the literature (Conradsson, Löfgren et al., 2015, Strouwen, Molenaar et al., 2015b, Strouwen, Molenaar et al., 2017b), suggesting that dual-task training in PD stimulates task automatization and more efficient integration of task-related brain networks, leading to gait speed and balance amelioration under dual-task conditions.

DUAL-TASK+AOT-MI relative to DUAL-TASK group showed a greater change of TUG-COG, mean and peak of turning velocity during TUG and TUG-COG, MiniBESTest and ABC, which were maintained at follow-up. Moreover, only DUAL-TASK+AOT-MI patients showed a decrease of DTC during TUG-COG, suggesting a lower dual-task interference on motor ability. Previous findings supported the long-term effect of AOT on walking speed (Agosta et al., 2017), but the most interesting finding is probably the higher velocity during dual-task turning (TUG-COG). Turning and dual-task are considered among the most challenging situations for PD-PIGD patients and a reduced turning velocity, particularly under a dual-task condition, has been correlated to lower balance, balance confidence and quality of life (Creaby & Cole, 2018, Curtze et al., 2016). Noteworthy, together with the turning velocity increase, the DUAL-TASK+AOT-MI group also showed a greater improvement of balance and balance confidence and an enhanced quality of life and FoG. These findings are supported by previous results in independent samples (Agosta et al., 2017, Pelosin et al., 2013), showing that AOT has the potential to boost the effect of a balance/gait training and to obtain better and more long-lasting effects on balance, FoG and consequently quality of life in PD patients with FoG. Our sample included several FoG patients that were equally distributed between the two groups. In addition, three subjects (one in the DUAL-TASK group, two in the DUAL-TASK+AOT-MI group) experienced FoG during TUG, especially during TUG-COG. As shown in the spaghetti plot (supplementary figure 1), subjects with lower TUG and TUG-COG performance at baseline showed clinically relevant improvement after training. However, also the majority of the other patients showed a significant change. We replicated the analysis without the three freezers subjects and results did not change, suggesting that our findings are likely to be independent of FoG improvement. Moreover, the majority of our patients presented mild to moderate cognitive deficits up to include

MCI, indicating that both DUAL-TASK and DUAL-TASK+AOT-MI can be successfully administered in these subjects. Interestingly, both groups showed improvement of AST after training, suggesting that dual-task exercise might improve not only motor but also executive functions. Future larger studies should stratify subjects according to FoG or MCI in order to test the effect of training in specific PD populations.

It has been suggested that both motor learning facilitation techniques (AOT and MI) and dual-task training might stimulate the activation of brain areas responsible for executive-attentive functions that are usually altered in patients with PD (Agosta et al., 2017, Caligiore, Mustile et al., 2019, Filippi, Elisabetta et al., 2018, Piramide et al., 2020, Sarasso, Agosta et al., 2020, Strouwen et al., 2015b). Indeed, AOT and MI activated the MNS that can be useful during the early phases of motor learning processes, while a subsequent dual-task practice could be important to train working memory and to achieve a persistent goal maintenance. Our fMRI findings supported these hypotheses, showing both similar and different brain functional mechanisms of reorganization after training in DUAL-TASK+AOT-MI and DUAL-TASK groups. During the fMRI motor-task and dual-task, patients receiving AOT and MI showed a reduced recruitment of frontal areas relative to the DUAL-TASK group. The reduced activity of frontal lobe after training in the DUAL-TASK+AOT-MI group correlated with balance, balance confidence and turning velocity improvement. As shown in previous studies (Gilat, Shine et al., 2015, Maidan et al., 2017, Thumm, Maidan et al., 2018), the reduced activity of frontal areas can be interpreted as a more efficient and optimal motor control of movement and a lower reliance on executive-attentive resources (Maidan et al., 2017). Considering that at baseline PD-PIGD subjects showed higher frontal activity relative to healthy subjects performing the fMRI dual-task, the reduced frontal activity can be considered as a brain reorganization during dual-task performance. As expected, also the DUAL-TASK group showed a pattern of partial “normalization” of baseline fMRI findings after training, showing a reduced activity of cognitive-motor cerebellar areas (lobule VIII-IX) during the motor task and of SMA during the fMRI dual-task, which was found to be hyperactivated at baseline in PD-PIGD relative to healthy subjects. This is in line with previous imaging studies showing that the increased premotor and cerebellar activity in PD patients can be modulated through dual-task practice (Strouwen et al., 2015b, Wu & Hallett, 2008). The correlation between the reduced activity of cerebellum IX and the

increased turning velocity supports this finding was likely a positive brain functional adaptation.

Both groups of patients showed brain functional changes of areas involved in sensorimotor integration (Sarasso, Agosta et al., 2018), which can be interpreted as a strengthening of compensatory mechanisms: the DUAL-TASK group after training showed an increased recruitment of temporal and parietal areas that are usually hyper-activated during a dual-task (Hartley, Jonides et al., 2011, Herz, Eickhoff et al., 2014, Maidan, Rosenberg-Katz et al., 2016, Otomune, Mihara et al., 2019, Strouwen et al., 2015b, Wager & Smith, 2003, Wu & Hallett, 2008), while the DUAL-TASK+AOT-MI group had an increased activity of the cerebellum during both the fMRI motor- and dual-task that was already higher in PD-PIGD patients relative to healthy controls at baseline (Wu & Hallett, 2008, Wu & Hallett, 2013, Wu, Hallett et al., 2015, Yu, Sternad et al., 2007). The increased activity of cerebellum IV-VI was correlated with a better balance and AST performance in the DUAL-TASK+AOT-MI group, suggesting that this area could contribute both to motor and executive abilities, as previously proposed (Guell & Schmahmann, 2020). Noteworthy, the fMRI dual-task changes we observed after training in both PD groups suggest a successful brain activity reorganization in areas specifically related to dual-task interference (supplementary figure 3). Considering clinical, fMRI and correlation findings, we can hypothesize that dual-task improvement are the consequence of both motor and cognitive abilities changes.

This study is not without limitations. The sample size is relatively small, but the difficulty to recruit a sample of PD-PIGD patients able to perform fMRI should be considered. Because of the small sample we restricted fMRI analysis to hypothesis-driven regions of interest and future studies should extend analysis to the whole-brain. fMRI results should be interpreted carefully as we did not obtain significant findings with a family-wise-error correction. Our protocol, including the sample size, has been set according to the personnel experience and current knowledge on this topic. Future studies should contribute to define standardized procedures, assessing the most appropriate training duration and frequency, time of follow-up, and clinical/fMRI evaluations. Future studies should also register the behavioural performance during fMRI and include a counting task in order to test specific dual-task interference effects.

Both DUAL-TASK and DUAL-TASK+AOT-MI trainings showed effects on clinical improvement and brain functional reorganization during dual-task in PD-PIGD patients. Adding AOT-MI to a dual-task gait/balance training could be useful to obtain specific functional reorganization of brain areas involved in motor control and executive-attentive abilities and to obtain more specific and long-lasting effects on dual-task mobility and balance in PD-PIGD.

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3.6 Appendix

Table 1. Socio-demographic and clinical variables in healthy controls and PD-PIGD patients at baseline.

Socio-demographic variables	HC	All PD-PIGD	DUAL-TASK group	DUAL-TASK+ AOT-MI group	*p* all PD-PIGD *vs* HC	*p* PD DUAL-TASK *vs* PD DUAL-TASK+AOT-MI
N	**23**	**25**	12	13		
Age [years]	63.75 ± 8.69 60.45 (55.49 – 72.38)	63.86± 13.82 66.41 (60.85 – 69.83)	63.81 ± 9.23 63.14 (57.27 – 69.56)	67.51 ± 6.12 66.76 (63.43 – 70.87)	0.34	0.23
Sex [M/F]	10/13	16/9	8/4	8/5	0.35	1.00
Education [years]	12.14 ± 3.80 12.5 (9.5 – 14)	11.36 ± 4.45 13 (8 – 14.5)	11.08 ± 4.87 9 (8 – 15.25)	11.62 ± 4.21 13 (7.5 – 14.5)	0.55	0.73
PD duration [years]	/	8.00 ± 3.76 7 (5.5 – 11)	7.92 ± 3.53 7.5 (6 - 11)	8.08 ± 4.13 7 (4.5 - 12)	/	0.94
LEDD [mg]	/	669.20 ± 380.52 610 (500 – 777.5)	555 ± 217.25 535.5 (425 – 703.75)	757.23 ± 480.49 652 (555 – 815)	/	0.29
H&Y ON (2/2.5/3)	/	11/8/6	5/4/3	6/4/3	/	1.00
H&Y OFF (2/2.5/3)	/	9/8/8	4/4/4	5/4/4	/	1.00
Risk of fall [low/medium/high]	/	8/7/10	4/4/4	4/3/6	/	0.78
FoG [yes/no]	/	18/7	8/4	10/3	/	0.67

UPDRS-II	/	11.44 ± 5.36 11 (7 - 14)	12.58 ± 5.14 11.5 (9.25 – 13.75)	10.38 ± 5.55 10 (6.5 – 15.5)	/	0.32
MCI [yes/no]	/	6/19	3/9	3/10	/	1.00

Values are mean ± standard deviation in the first row, and median (1st quartile – 3rd quartile) in the second row. Categorical variables are reported as frequency. p values refer to Mann Whitney test or Chi squared test for categorical variables. P values were Bonferroni-corrected for multiple comparison at $p<0.05$. ***Abbreviations:*** *AOT-MI= action observation and motor-imagery; FoG= freezing of gait; HC= healthy controls, H&Y= Hoehn and Yahr score; LEDD= levodopa equivalent daily dose; MCI= mild cognitive impairment; M/F= male/female; mg= milligrams; N= number; PD=Parkinson's disease; PIGD= postural instability and gait disorders phenotype; UPDRS-II= Unified Parkinson's Disease Rating Scale part II.*

Table 2. Neurological, motor functional and gait analysis changes in the two PD-PIGD patient groups at different time points.

		DUAL-TASK group (N=12)				DUAL-TASK+AOT-MI group (N=13)				DUAL-TASK+ AOT-MI group *vs* DUAL-TASK group		
		Mean ± SD	**Median (1st – 3rd quartiles)**	***p*** T0-W6*	***p*** T0-W14*	**Mean ± SD**	**Median (1st – 3rd quartiles)**	***p*** T0-W6*	***p*** T0-W14*	***p*# T0**	***p*^ T0-W6**	***p*^ T0-W14**
TUG-COG [s]	T0	15.79 ± 10.36	11.72 (10.29 – 17.46)	**<0.001**	**<0.001**	17.79 ± 13.75	12.13(9.76 – 18.85)	**<0.001**	**<0.001**	1.00	**<0.001**	**0.02**
	Delta T0-W6	-3.68 ± 7.18	-1.78 (-4.93 – 0.50)			-8.17 ± 12.75	-3.5 (-6.16 – -0.44)					
	Delta T0-W14	-4.24 ± 7.94	-1.86 (-4.01 – -0.97)			-6.29 ± 9.94	-2.85 (-7.11 – -0.16)					
TUG-MAN [s]	T0	14.86 ± 9.53	11.80 (10.25 – 13.59)	**<0.001**	**<0.001**	11.66 ± 3.66	10.15(9.29 – 12.84)	**0.02**	**<0.001**	0.21	0.21	0.15
	Delta T0-W6	-3.42 ± 6.67	-0.72 (-2.98 – -0.09)			-2.11 ± 2.61	-1.65 (-4.65 – 0.02)					
	Delta T0-W14	-3.45 ± 5.75	-2.27 (-3.01 – -0.29)			-1.70 ± 2.18	-1.30 (-3.50 – -0.04)					
TUG [s]	T0	12.94 ± 5.04	11.20 (9.75 – 13.32)	**0.002**	**<0.001**	11.23 ± 2.68	10.55(9.56 – 13.24)	**<0.001**	**<0.001**	0.44	1.00	1.00
	Delta T0-W6	-2.08 ± 2.64	-0.99 (-3.05 – -0.31)			-2.11 ± 1.69	-2.62 (-3.26 – -0.90)					
	Delta T0-W14	-2.82 ± 2.92	-1.58 (-3.43 – -1.16)			-2.04 ± 1.69	-2.39 (-2.98 – -0.62)					
DTC TUG-COG [%]	T0	15.94 ± 25.66	8.44 (-0.88 – 31.11)	0.39	0.92	49.59 ± 89.02	14.97 (2.46 – 35.68)	**<0.001**	**<0.001**	0.35	**<0.001**	**<0.001**
	Delta T0-W6	-4.61 ± 23.00	1.15 (-19.78 – 12.51)			-43.51 ± 80.59	-2.75 (-33.30 – 3.43)					
	Delta T0-W14	-1.16 ± 25.71	6.24 (-13.30 – 14.32)			-28.46 ± 64.71	-0.43 (-47.92 – 10.04)					
DTC TUG-MAN [%]	T0	9.14 ± 17.50	2.51 (-0.13 – 10.78)	0.45	0.47	5.20 ± 12.18	6.03 (-7.77 – 12.61)	1.00	0.18	0.65	0.65	0.86
	Delta T0-W6	-3.26 ± 17.95	2.11 (-12.33 – 7.29)			0.82 ± 14.89	-0.21 (-10.11 – 16.91)					
	Delta T0-W14	2.28 ± 12.64	6.17 (-13.65 – 12.54)			9.51 ± 19.67	12.89 (-10.38 – 22.05)					
Mean turning velocity TUG-COG [m/s]	T0	0.82 ± 0.26	0.91 (0.59 – 1.02)	0.05	**0.03**	0.72 ± 0.37	0.86 (0.34 – 0.97)	**0.001**	**<0.001**	0.65	**0.03**	**0.04**
	Delta T0-W6	0.11 ± 0.16	0.11 (-0.01 – 0.21)			0.30 ± 0.25	0.31 (0.10 – 0.47)					
	Delta T0-W14	0.10 ± 0.16	0.11 (0.00 – 0.21)			0.23 ± 0.17	0.21 (0.11 – 0.31)					
Mean turning velocity TUG-MAN [m/s]	T0	0.82 ± 0.20	0.79 (0.65 – 1.03)	0.24	0.19	0.88 ± 0.22	0.93 (0.68 – 1.07)	0.06	**0.02**	0.61	0.53	0.95
	Delta T0-W6	0.07 ± 0.17	0.03 (-0.07 – 0.16)			0.14 ± 0.19	0.10 (0.01 – 0.24)					
	Delta T0-W14	0.10 ± 0.14	0.10 (-0.02 – 0.21)			0.13 ± 0.17	0.09 (0.00 – 0.22)					
Mean turning velocity TUG [m/s]	T0	0.89 ± 0.16	0.89 (0.73 – 1.04)	0.35	0.65	0.84 ± 0.28	0.92 (0.67 – 1.00)	**<0.001**	**<0.001**	0.77	**0.04**	0.05
	Delta T0-W6	0.06 ± 0.14	0.02 (-0.05 – 0.16)			0.18 ± 0.14	0.18 (0.06 – 0.28)					
	Delta T0-W14	0.06 ± 0.16	0.04 (-0.08 – 0.23)			0.22 ± 0.17	0.24 (0.13 – 0.34)					
DTC mean turning velocity TUG-COG[%]	T0	-10.21 ± 20.76	-7.11 (-18.66 – 1.93)	**0.02**	0.06	-19.77 ± 27.69	-7.72 (-25.45 – -3.23)	**0.002**	**<0.001**	0.44	**0.03**	0.05
	Delta T0-W6	7.31 ± 12.44	5.79 (0.10 – 12.44)			20.21 ± 25.18	15.19 (-0.17 – 37.54)					

	Delta T0-W14	7.36 ± 24.05	1.11 (-7.86 – 19.79)			6.28 ± 12.77	2.81 (-3.64 – 11.13)					
DTC mean turning velocity TUG-MAN [%]	T0	-7.59 ± 10.15	-5.56 (-17.83 – -3.14)	0.57	0.51	-5.45 ± 14.59	-7.25 (-20.18 – 4.73)	1.00	0.27	0.92	1.00	0.09
	Delta T0-W6	3.28 ± 12.34	-0.18 (-4.38 – 19.88)			0.03 ± 10.01	-0.27 (-4.74 – 7.26)					
	Delta T0-W14	5.98 ± 15.36	4.40 (-6.55 – 19.53)			-8.27 ± 19.80	-10.88 (-24.78 – 10.93)					
Peak turning velocity TUG-COG [deg/s]	T0	108.38 ± 24.57	115.86 (82.32 – 125.93)	0.36	0.32	107.04 ± 39.30	112.41 (80.34 – 127.93)	**0.001**	**<0.001**	0.81	**0.02**	**0.01**
	Delta T0-W6	10.58 ± 24.77	7.99 (-7.43 – 24.66)			41.53 ± 31.76	39.06 (16.04 – 61.69)					
	Delta T0-W14	9.58 ± 18.90	4.34 (-7.22 – 25.54)			35.59 ± 24.37	30.86 (22.14 – 50.17)					
Peak turning velocity TUG-MAN [deg/s]	T0	96.35 ± 15.59	91.70 (83.88 – 114.93)	0.94	0.07	106.56 ± 23.11	102.44 (86.34 – 122.08)	**0.01**	**0.004**	0.31	**0.01**	**0.046**
	Delta T0-W6	2.64 ± 14.11	1.62 (-7.27 – 14.25)			21.68 ± 19.93	26.93 (4.59 – 36.56)					
	Delta T0-W14	12.32 ± 20.02	9.37 (-6.19 – 21.40)			15.19 ± 17.42	12.65 (-1.45 – 32.85)					
Peak turning velocity TUG [deg/s]	T0	125.40 ± 23.85	118.99 (110.31 – 145.50)	1.00	1.00	119.76 ± 28.67	127.35 (105.80 – 141.74)	**<0.001**	**<0.001**	0.73	**0.01**	**0.01**
	Delta T0-W6	3.76 ± 19.32	0.30 (-11.31 – 14.28)			29.74 ± 20.15	31.15 (12.56 – 40.92)					
	Delta T0-W14	2.16 ± 25.44	1.75 (-18.12 – 22.72)			29.79 ± 22.46	34.74 (14.54 – 51.73)					
DTC peak turning velocity TUG-COG [%]	T0	-13.37 ± 13.62	-14.86 (-24.32 – -1.12)	0.17	0.35	-10.33 ± 22.10	-6.31 (-17.70 – 4.11)	0.18	0.43	0.35	0.81	0.88
	Delta T0-W6	5.44 ± 12.88	7.68 (-3.72 – 14.63)			10.95 ± 27.10	8.19 (-9.78 – 11.51)					
	Delta T0-W14	8.22 ± 18.95	8.45 (-0.63 – 20.01)			4.90 ± 19.78	3.75 (-16.96 – 23.77)					
DTC peak turning velocity TUG-MAN [%]	T0	-21.07 ± 7.39	-21.83 (-27.33 – -14.70)	1.00	0.27	-17.94 ± 14.31	-20.48 (-28.09 – -10.80)	1.00	0.96	1.00	1.00	0.15
	Delta T0-W6	0.71 ± 12.64	1.24 (-11.69 – 7.29)			2.07 ± 16.69	3.51 (-14.41 – 19.29)					
	Delta T0-W14	7.28 ± 13.98	6.11 (-1.24 – 11.13)			-5.55 ± 16.02	-6.71 (-21.83 – 6.25)					
MiniBESTest	T0	21.75 ± 5.66	24 (19.00 – 24.75)	1.00	1.00	19.46 ± 5.71	22.00 (17.50 – 23.00)	**<0.001**	**<0.001**	0.09	**0.01**	**0.02**
	Delta T0-W6	0.33 ± 2.53	-0.50 (-1.00 – 1.75)			2.92 ± 2.02	3.00 (1.00 – 4.75)					
	Delta T0-W14	0.67 ± 3.55	1.0 (-1.75 – 3.75)			3.27 ± 2.72	3.00 (1.00 – 6.00)					
10MWT_MS [s]	T0	6.99 ± 1.43	6.39 (5.82 - 8.00)	0.14	0.20	7.51 ± 2.96	6.32 (5.59 – 9.36)	**<0.001**	**<0.001**	0.98	0.22	**0.01**
	Delta T0-W6	-0.34 ± 0.64	-0.27 (-1.04 – 0.24)			-0.70 ± 1.21	-0.12 (-1.65 – 0.13)					
	Delta T0-W14	-0.10 ± 0.38	-0.09 (-0.48 – 0.17)			-0.83 ± 1.69	-019 (-0.50 – 0.04)					
10MWT_CS [s]	T0	9.16 ± 1.53	9.10 (8.10 – 10.01)	1.00	0.81	9.94 ± 3.41	9.06 (8.10 – 10.66)	**<0.001**	**<0.001**	0.98	**0.045**	**0.002**
	Delta T0-W6	-0.18 ± 0.97	-0.11 (-0.78 – 0.49)			-1.01 ± 1.11	-0.68 (-2.09 – -0.29)					
	Delta T0-W14	-0.33 ± 0.73	-0.36 (-1.06 – 0.07)			-1.65 ± 2.01	-1.17 (-1.57 – -0.79)					
ABC scale	T0	78.10 ± 18.50	78.13 (71.33 – 94.53)	0.57	1.00	71.01 ± 21.11	79.38 (57.50 – 88.44)	**<0.001**	**<0.001**	0.41	**0.01**	**0.03**
	Delta T0-W6	2.53 ± 8.78	-0.31 (-4.06 – 7.58)			11.43 ± 9.11	8.13 (4.30 – 17.50)					
	Delta T0-W14	0.76 ± 9.76	2.81 (-6.56 – 4.69)			11.53 ± 11.78	9.38 (3.75 – 16.25)					
NFoG-Q	T0	4.75 ± 5.23	3.00 (0.00 – 8.50)	0.26	0.72	6.77 ± 5.02	7.00 (2.00 – 11.00)	**0.04**	**0.02**	0.32	1.00	1.00

	Delta T0-W6	-1.67 ± 3.96	-0.50 (-4.50 – 0.00)			-2.25 ± 3.11	-0.50 (-5.50 – 0.00)					
	Delta T0-W14	-0.92 ± 5.55	0.0 (-3.00 – 1.75)			-2.18 ± 2.96	-1.00 (-5.00 – 0.00)					
PDQ-39	T0	18.95 ± 12.16	13.30 (10.19 – 26.19)	1.00	0.12	18.19 ± 7.52	18.83 (13.52 – 20.45)	**0.01**	**0.003**	0.61	0.38	0.41
	Delta T0-W6	-0.62 ± 8.44	0.07 (-6.87 – 4.05)			-4.61 ± 5.70	-3.22 (-9.06 – 0.61)					
	Delta T0-W14	-4.28 ± 5.72	-4.91 (-7.62 – 0.83)			-4.14 ± 6.77	-5.16 (-9.22 – 1.00)					
	Delta T0-W6	-0.21 ± 0.58	0.0 (-0.88 – 0.00)			-0.25 ± 0.40	0.0 (-0.50 – 0.00)					
	Delta T0-W14	-	-			-	-					
UPDRS III ON	T0	28.83 ± 8.47	28.00 (24.25 – 35.00)	0.06	**0.02**	26.27 ± 9.88	26.00 (18.25 – 30.50)	1.00	1.00	0.32	0.35	0.33
	Delta T0-W6	-3.58 ± 7.76	-2.50 (-10.50 – 2.75)			1.79 ± 10.44	1.0 (-5.25 – 5.00)					
	Delta T0-W14	1.00 ± 7.19	2.00 (-6.50 – 6.75)			1.59 ± 5.89	3.00 (-3.00 – 7.00)					
UPDRS III OFF	T0	34.00 ± 10.94	33.00 (27.25 – 41.00)	**0.004**	**-**	34.77 ± 12.87	32.00 (25.50 – 39.50)	**0.01**	-	0.94	1.00	-
	Delta T0-W6	-5.79 ± 6.53	-4.50 (-10.25 – -0.75)			-4.29 ± 5.27	-4.50 (-9.50 – 0.75)					
	Delta T0-W14	-	-			-	-					
AST percent correct trials (incongruent)	T0	77.75 ± 14.93	80.00 (75.63 – 87.81)	**<0.001**	-	73.98 ± 22.04	80.00 (45.00 – 91.25)	**0.02**	-	0.86	0.69	-
	Delta T0-W6	8.25 ± 2.90	9.38 (5.94 – 10.31)			6.5 ± 6.87	4.38 (1.88 – 9.69)					
	Delta T0-W14	-	-			-	-					

Values are mean ± standard deviation in the first row and median (1st quartile – 3rd quartile) in the second row. All the variables at T0 were compared between groups using Mann Whitney test (p#). Longitudinal changes (T0-W6 and T0-W6-W14) were assessed in both PD groups using linear mixed-effect models (*p**). Using the same models, a group-by-time interaction was assessed to evaluate longitudinal between-group differences (T0-W6 and T0-W6-W14) (p^). P values were Bonferroni-corrected for multiple comparison at p<0.05. ***Abbreviations:*** *ABC= Activities-specific Balance Confidence scale; AOT-MI= action observation and motor-imagery; AST= attention switching task; deg= degrees; m= meters; DTC= dual-task cost; MiniBESTest= Mini Balance Evaluation Systems Test; N= number; NFoG-Q= New Freezing of Gait Questionnaire; PD= Parkinson's disease; PDQ-39= Parkinson's Disease Questionnaire; PIGD= postural instability and gait disorders phenotype; TUG= Timed Up and Go; s= seconds; TUG-COG= TUG with cognitive dual-task; TUG-MAN= TUG with manual dual-task; T0= baseline; UPDRSIII= Unified Parkinson's Disease Rating Scale part III; W6= six weeks (post-treatment); W14= 14 weeks (follow-up); 10MWT_MS= 10 Meters Walking Test maximum speed; 10MWT_CS= 10 Meters Walking Test comfortable speed.*

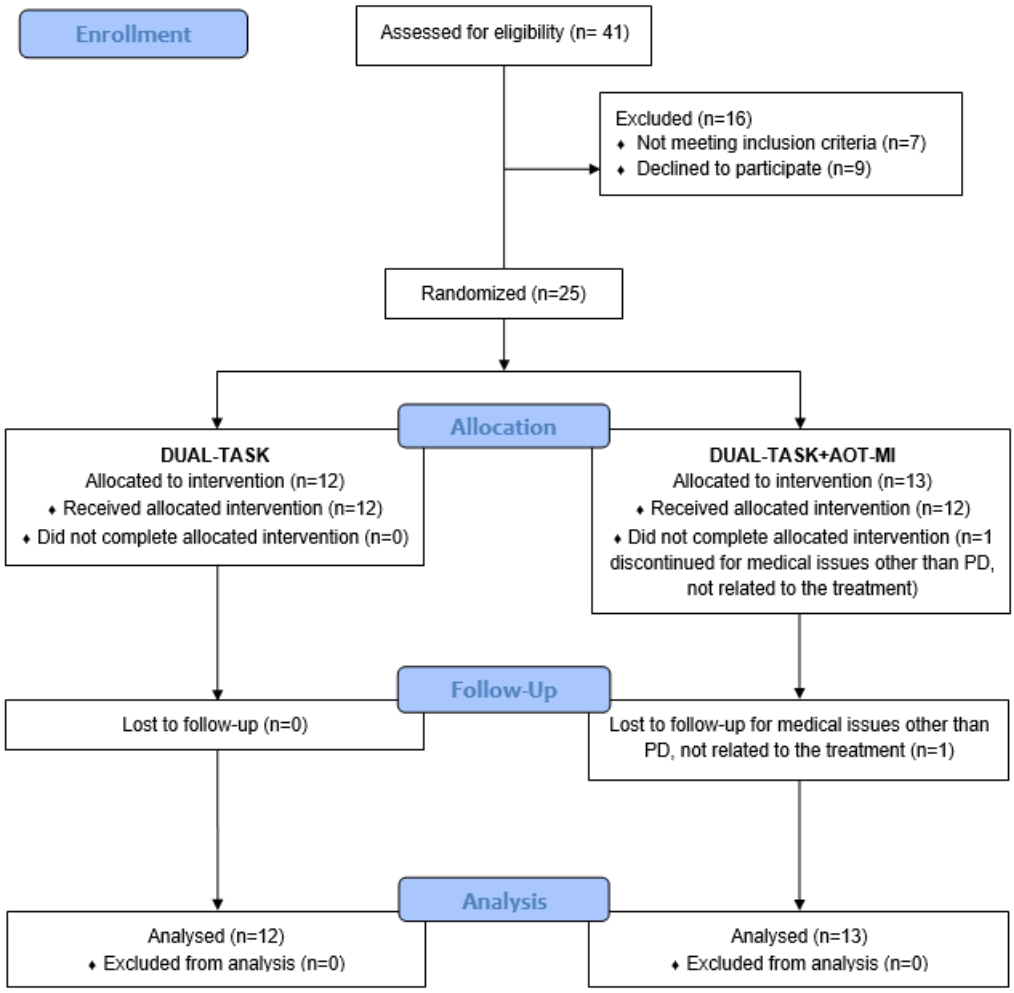


Figure 1. *Flow diagram of the study.*

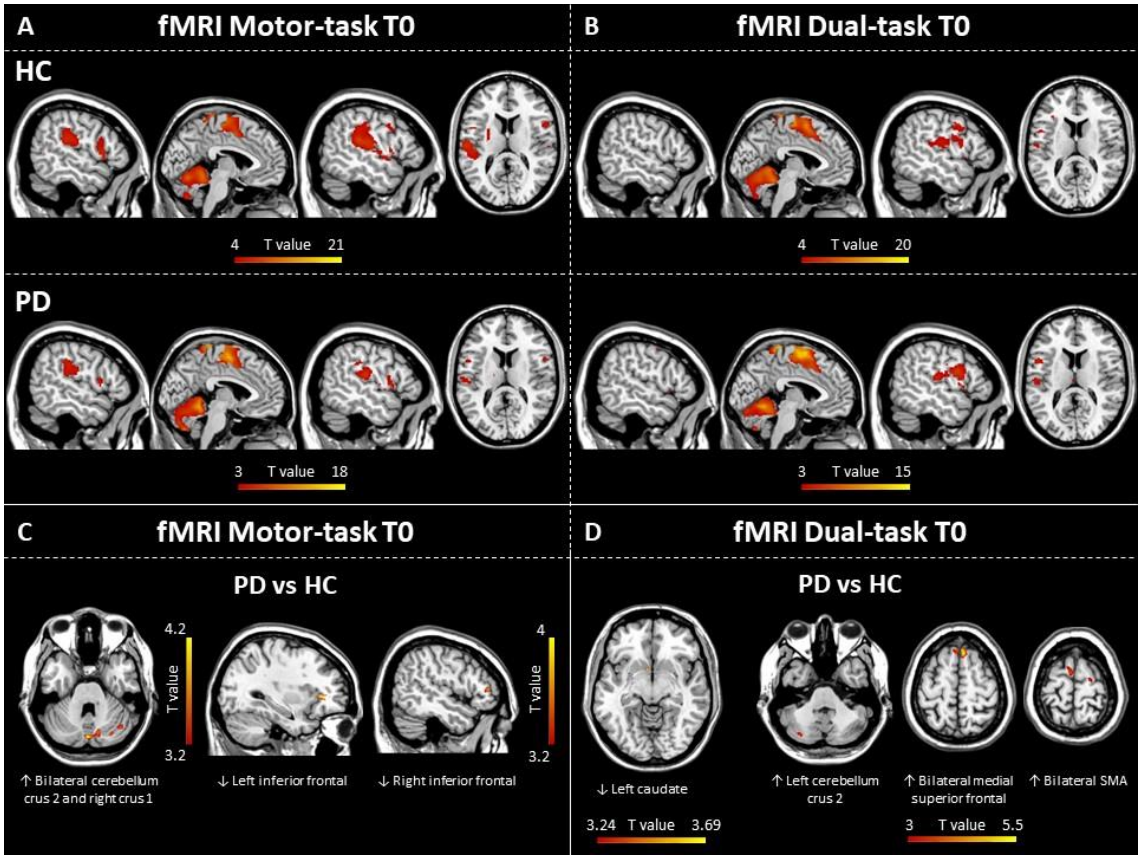


Figure 2. *fMRI patterns of activations on a rendered brain in Parkinson's disease (PD) patients with postural instability and gait disorders (PIGD) and healthy controls (HC) at baseline (T0).* ***A)*** *Brain activity during fMRI motor-task in PD-PIGD patients and HC;* ***B)*** *Brain activity during fMRI dual-task in PD-PIGD patients and HC;* ***C)*** *fMRI differences in PD-PIGD patients relative to HC during fMRI motor-task;* ***D)*** *fMRI differences in PD-PIGD patients relative to HC during fMRI dual-task. Arrows represent increased (↑) or decreased (↓) brain activity in PD-PIGD patients relative to HC. All findings are shown at p<0.001 uncorrected (5000 permutations) and only clusters greater than 5 voxels are reported. Results are shown on axial, sagittal and coronal sections of the Montreal Neurological Institute standard brain. Colour bars denote T values.*

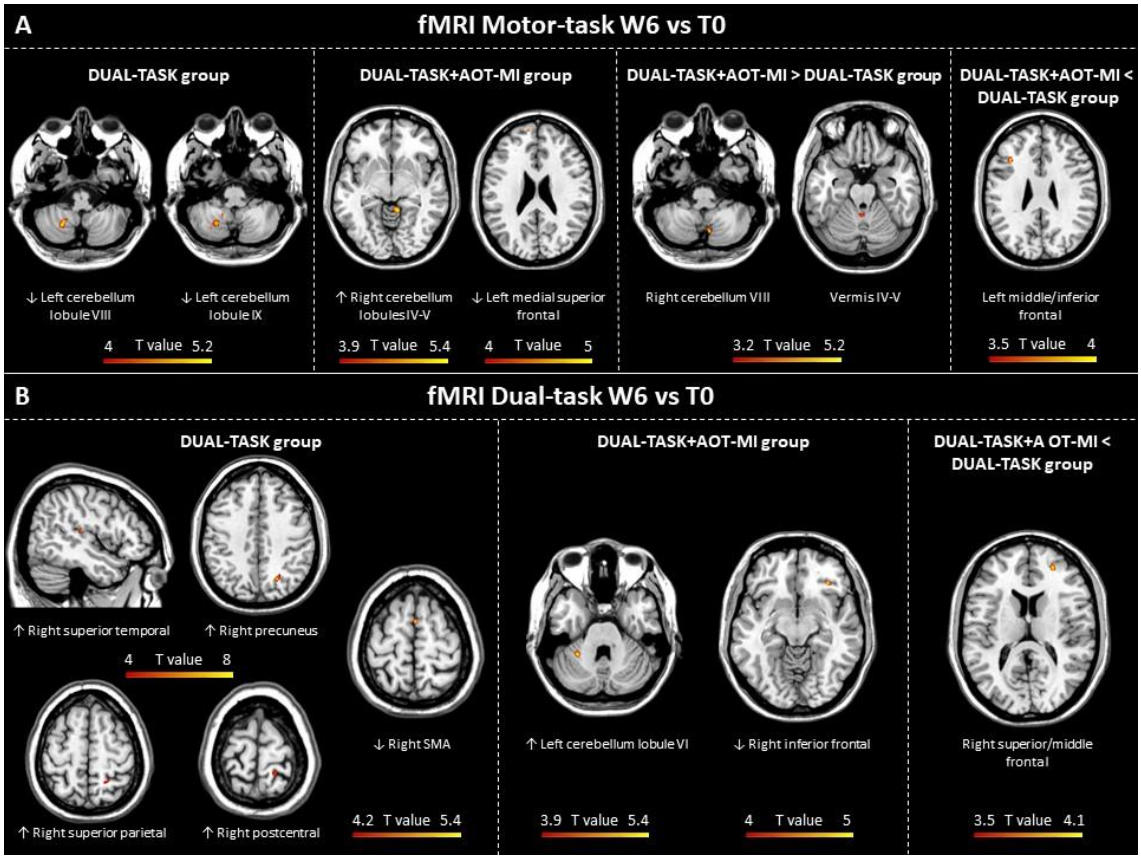


Figure 3. *fMRI patterns of changes after training (T0 vs W6).* ***A)*** *fMRI motor-task: brain activity changes in the DUAL-TASK and DUAL-TASK+AOT-MI group, and differences between the two groups;* ***B)*** *fMRI dual-task: brain activity changes in DUAL-TASK and DUAL-TASK+AOT-MI groups, and differences between the two groups. All findings are shown at p<0.001 uncorrected (5000 permutations) and only clusters greater than 5 voxels are reported. Results are shown on axial and sagittal sections of the Montreal Neurological Institute standard brain. Color bars denote T values. Color bars denote T values.*

3.7 Supplementary materials

3.7.1 MRI acquisition

Using a 3.0 Tesla Philips Intera scanner (Ingenia CX, Philips Medical Systems, Best, The Netherlands) MRI scans were obtained between 12 noon and 1 PM during OFF time i.e., at least 12 hours after their regular evening dopaminergic therapy administration, to mitigate the pharmacological effects on neural activity. fMRI scans were obtained at baseline (T9) and the day after the end of training (W6), with a tolerance of 3 days. FMRI was obtained using a T2* weighted echo planar imaging sequence with the following parameters: echo time (TE)= 35 ms, repetition time (TR)= 1572 ms, flip angle= 70°, field of view (FOV)= 240×240 mm, matrix= 96×94, 48 contiguous axial sections, thickness= 3 mm, acquisition time= 3 min and 57 sec, voxel reconstruction 2.5 x 2.5 x 3 mm. Patients

performed two fMRI tasks: 1) the “motor-task” consisted in alternated self-paced dorsal and plantar flexion movements of the feet with eyes closed, with the knees supported by a soft wedge and flexed of about 30°. To standardize the amplitude of movements, subjects were asked to reach a fixed wood bar with their insteps so that the dorsal flexion was about 90°; 2) the “dual-task” consisted in the same foot anti-phase movement executed while mentally counting backwards by threes starting from 100. A block design (ABAB) was used, in which the activation A (lasting about 19 seconds) corresponded to the dorsal and plantar flexion, while during the resting period B (lasting about 19 seconds) subjects were asked to maintain their eyes closed, and each period was repeated 6 times (total duration 3’47”). Subjects performed the task according to auditory stimuli (“go” and “stop”) at the beginning and at the end of the movement. A visual signal (green/red light), visible to the operators outside the MRI scanner, was projected in order to monitor the correct time of task execution. Subjects were trained to perform the task outside the scanner and were carefully monitored visually by an observer inside the scanner room during scanning to ensure a correct performance. Tasks were performed equally well by all the subjects.

The following structural MRI sequences were acquired at baseline to exclude subjects with eventual structural brain alterations: i) 3D T2-weighted sequence: TR = 2500 ms, TE = 330 ms, flip angle = 90°, 192 contiguous sagittal sections, thickness = 1 mm, field of view (FOV) = 256 mm x 256 mm, matrix = 256 x 258, voxel reconstruction = 0.9 mm x 0.9 mm x 1 mm. ii) 3DT1-weighted sequence: TR = 7.1 ms, TE = 3.2 ms, flip angle = 9°, 204 contiguous sagittal sections, thickness = 1 mm, FOV = 256 mm x 240 mm, matrix = 256 x 240, voxel reconstruction = 1 mm x 1 mm x 1 mm.

3.7.2 Results

fMRI dual-task vs motor-task at baseline

A direct comparison between the motor-task and the dual-task at baseline in PD patients was performed in order to find out if there is any area specifically related to dual-task interference. During the dual- relative to the motor task, PD patients showed an increased activity of the bilateral anterior supplementary motor area (SMA), left superior and inferior frontal areas and right angular gyrus, and a decreased recruitment of bilateral posterior SMA, bilateral medial orbitofrontal gyri, right superior and inferior parietal

areas, left caudate and right cerebellum lobule VI (supplementary figure 3 and supplementary table 4).

Supplementary table 1 – Comprehensive neuropsychological evaluation in PD-PIGD patients and healthy controls at baseline.

Variable	HC (N=23)	All PD-PIGD (N=25)	DUAL-TASK group (N=12)	DUAL-TASK + AOT-MI group (N=13)	*p* all PD-PIGD *vs* HC	*p* DUAL-TASK group *vs* DUAL-TASK + AOT-MI group
MMSE	29.2 ± 0.9 29 (29 – 30)	28.0 ± 1.9 28 (27 – 30)	28.1 ± 1.6 28 (27.25 – 29.00)	28.0 ± 2.2 28 (26 – 30)	**0.02**	0.81
VERBAL MEMORY						
RAVLT - Immediate recall	49.5 ± 10.8 50.5 (40.75 – 57)	42.0 ± 10.9 44 (35 – 50.5)	46.1 ± 7.1 46.5 (40.75 – 51)	38.2 ± 12.6 38 (25 – 48.5)	**0.04**	0.10
RAVLT - Delayed recall	10.4 ± 2.5 10.5 (8.75 – 13)	8.2 ± 3.5 8 (6 – 10.5)	9.5 ± 3.0 9.5 (8 – 12.25)	6.9 ± 3.5 7 (4.5 – 10)	**0.03**	0.05
RAVLT - Recognition	14.2 ± 0.9 14 (14 – 15)	13.2 ± 2.7 14 (13 – 15)	14.0 ± 1.3 14 (14 – 15)	12.5 ± 3.4 14 (12 – 15)	0.3	0.30
Digit span forward	6.3 ± 1.1 6 (6 – 7)	5.6 ± 1.1 6 (4.5 – 6.5)	5.8 ± 1.1 6 (5 – 6.75)	5.4 ± 1.2 5 (4 – 6.5)	**0.04**	0.47
SPATIAL MEMORY						
Recall of Rey-Osterrieth Complex Figure	-	14.1 ± 5.6 14 (10.25 – 18.25)	14.1 ± 6.9 14.75 (7.63 – 20.75)	14.0 ± 4.5 13.5 (12 – 16)	-	0.85
LANGUAGE						
Token Test	33.7 ± 2.1 34 (33 – 35)	33.2 ± 1.7 34 (32 – 34.75)	33.3 ± 1.7 33.5 (32 – 35)	33.1 ± 1.7 34 (32 – 34)	0.2	0.61
BADA - Names	-	29.6 ± 0.7 30 (29 – 30)	29.7 ± 0.5 30 (29 – 30)	29.5 ± 0.8 30 (29 – 30)	-	0.69
BADA - Verbs	-	27.1 ± 1.3 27 (27 – 28)	26.9 ± 1.7 27.5 (26.25 – 28)	27.3 ± 0.8 27 (27 – 28)	-	0.89
VISUO-SPATIAL ABILITIES						
Copy of Rey-Osterrieth Complex Figure	-	26.9 ± 5.9 28 (24.75 – 31)	25.6 ± 6.8 26.75 (24.63 – 29)	28.2 ± 4.9 29 (24.75 – 32)	-	0.35
Copy of drawings - Freehand	10.4 ± 1.1 10 (9.75 – 11)	10.1 ± 1.5 10 (9.50 – 11.0)	10.3 ± 1.8 10 (9.25 – 12)	10.3 ± 1.8 10 (9.5 – 10.5)	0.6	0.50
Copy of drawings with landmarks	68.2 ± 2.1 69 (67.75 – 70)	64.2 ± 6.4 67 (60.5 – 68.5)	65.5 ± 6.5 68 (63 – 69.75)	62.9 ± 6.3 65 (59 – 68)	**0.01**	0.14
Benton Judgment of Line Orientation Test	-	15.8 ± 3.4 16.5 (12.75 – 18.75)	16.1 ± 2.5 17 (15 – 18)	15.5 ± 4.1 15 (11 – 19)	-	1.00
ACE-R	-	14.2 ± 1.7 15 (13.5 – 15)	13.8 ± 2.1 14.5 (12.5 – 15)	14.5 ± 1.2 15 (13.5 – 15)	-	0.47
EXECUTIVE FUNCTIONS, ATTENTION AND WORKING MEMORY						

Attentive matrices	54.0 ± 4.7 54 (52 – 58)	50.5 ± 6.3 52 (46.5 – 55.5)	49.7 ± 6.5 52 (43 – 54.25)	51.2 ± 6.3 53 (46.5 – 56)	0.06	0.44
Digit span backwards	4.9 ± 1.2 4.5 (4 – 6)	4.2 ± 1.3 4 (3 – 5)	4.3 ± 1.0 4.5 (3.25 – 5)	4.2 ± 1.6 4 (3 – 5)	0.1	0.50
Clock drawing test	-	7.6 ± 3.0 9 (7 – 10)	7.3 ± 3.5 9 (3.25 – 10)	7.8 ± 2.5 8 (7.5 – 9.5)	-	0.77
Trail making test A	29.9 ± 9.7 27.1 (23.48 – 33.80)	47.7 ± 25.5 38 (32.49 – 53.16)	45.7 ± 17.7 46.22 (32.23 – 51.62)	49.6 ± 31.7 36.69 (31.5 – 65.1)	**<0.001**	0.94
Trail making test B	92.9 ± 33.2 85 (72.5 – 105)	127.6 ± 50.4 121.99 (90.25 – 156.68)	125.7 ± 57.2 123.61 (74 – 146.91)	129.6 ± 45.2 120.37 (100 – 186)	**0.01**	0.80
Trail making test B-A	63.0 ± 27.4 56.74 (46.81 – 67.04)	87.2 ± 42.2 79.13 (55.83 – 99.44)	84.3 ± 50.0 74.11 (44.19 – 97.58)	90.1 ± 35.0 80 (71.95 - 104)	**0.04**	0.48
Phonemic fluency	39.4 ± 8.2 38.5 (35.5 – 43.5)	34.5 ± 10.3 35 (29.5 – 38.5)	34.2 ± 7.0 36 (27 – 38.75)	34.9 ± 13.0 33 (29.5 – 42.5)	**0.03**	0.73
Semantic fluency	50.4 ± 9.3 49 (46 – 57.5)	43.6 ± 10.9 44 (38.5 – 49)	43.1 ± 9.8 41.5 (38.25 – 48.25)	44.0 ± 12.2 47 (35.5 – 51.5)	**0.02**	0.54
MCST Categories	4.4 ± 1.1 4.5 (3 – 5)	3.9 ± 1.7 4 (3 – 5)	4.0 ± 1.8 5 (3.25 – 5)	3.9 ± 1.6 4 (3 – 5)	0.58	0.54
MCST Perseverations	3.68 ± 3.2 3 (1 – 5.5)	4.7 ± 4.0 4 (1 – 5.5)	3.5 ± 3.6 3 (1 – 5)	5.8 ± 4.2 5 (3 – 9)	0.42	0.14
MOOD						
BDI	7.5 ± 5.5 5 (4 – 11.5)	8.4 ± 5.8 7 (4 – 10)	10.8 ± 7.4 10 (4 – 18)	6.2 ± 2.6 6 (4.5 – 8.5)	0.61	0.19
HAMA	-	5.2 ± 3.4 4 (3 – 8)	5.7 ± 4.0 4.5 (3 – 10.75)	4.8 ± 2.8 4 (2.5 – 7.5)	-	0.65
Apathy rating scale	8.0 ± 5.6 7 (4 – 12)	7.6 ± 4.6 7 (4 – 13)	8.4 ± 4.7 9 (4.25 – 13)	6.9 ± 4.7 6 (3 – 11.5)	0.91	0.57
QUIP-RS	-	5.2 ± 8.4 0 (0 – 7)	3.7 ± 7.6 0 (0 – 3)	6.5 ± 9.3 3.5 (0 – 8.5)	-	0.24
SHAPS anhedonia	0.3 ± 0.6 0 (0 – 0.75)	0.7 ± 1.0 0 (0 – 1)	0.9 ± 1.3 0 (0 – 2.25)	0.6 ± 0.5 1 (0 – 1)	0.19	0.97
SHAPS 16 items	56.0 ± 4.0 56 (53.25 – 58)	54.6 ± 4.9 53 (50 – 60)	53.6 ± 4.8 52 (49 – 60)	55.6 ± 5.1 54 (51 – 60)	0.17	0.30
SHAPS 14 items	49.3 ± 3.4 49 (48 – 51.75)	48.2 ± 4.6 47 (44 – 52)	47.4 ± 4.8 46 (43 – 52)	49.1 ± 4.5 49 (44 – 52)	0.25	0.37
NPI	-	8.0 ± 7.3 7 (1.5 – 16)	8.3 ± 8.1 5 (1.75 – 18.25)	7.3 ± 7.0 8 (0 – 14)	-	0.91
HDRS	-	4.6 ± 2.5 4 (3 – 5)	5.4 ± 3.3 4.25 (3 – 8.75)	4.1 ± 1.7 4 (3 – 5)	-	0.66

Values are mean ± standard deviation in the first row and median (1st quartile – 3rd quartile) in the second row. p values refer to Mann Whitney test. Statistical significance was accepted for values of $p < 0.05$. ***Abbreviations:*** *ACE-R= Addenbrooke's Cognitive Examination Revised; AOT-MI= action observation and motor-imagery; BADA= Battery for Assessment of Aphasic Disorders; BDI= Beck Depression Inventory; HAMA= Hamilton Anxiety rating Scale; HC= healthy controls; HDRS= Hamilton Rating Scale for Depression; MCST= Modified Card Sorting test; MMSE= Mini Mental State Examination; N= number; NPI= Neuropsychiatric Inventory; PD= Parkinson's disease; PIGD= postural instability and gait disorders phenotype; RAVLT= Rey Auditory Verbal Learning Test; QUIP-RS= Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease–Rating Scale; SHAPS= Snaith-Hamilton Pleasure Scale.*

Supplementary Table 2. fMRI patterns of activation in PD-PIGD patients relative to healthy controls at baseline during the fMRI motor-task.

fMRI Motor-task T0								
PD-PIGD > HC								
kE	***p***	p_{FWE}	**T**	**mni x**	**mni y**	**mni z**	**BA**	**Area**
94	<0.001	0.34	3.98	30	-74	-34	-	Right cerebellum crus 1
83	<0.001	0.26	4.12	0	-80	-30	-	Left cerebellum crus 2
	<0.001	0.55	3.70	8	-78	-32	-	Right cerebellum crus 2
PD-PIGD < HC								
39	<0.001	0.31	4.03	-32	32	2	45	Left inferior frontal gyrus (pars triangularis)
14	<0.001	0.56	3.70	50	38	8	45	Right inferior frontal gyrus (pars triangularis)

X, y, and z coordinates referred to the Montreal Neurological Institute (MNI) space. *p* refers to two-sample t-test with 5000 random permutations; p_{FWE} refers to two-sample t-test with 5000 random permutations, Family-Wise-Error corrected. ***Abbreviations:*** *BA= Brodmann area; HC= healthy controls; kE= cluster extent; mni= Montreal Neurologic Institute; PD= Parkinson's disease; PIGD= postural instability and gait disorders phenotype; T0= baseline.*

Supplementary Table 3. fMRI patterns of activation in PD-PIGD patients relative to healthy controls at baseline during the fMRI dual-task.

fMRI Dual-task T0								
kE	***p***	p_{FWE}	**T**	**mni x**	**mni y**	**mni z**	**BA**	**Area**
PD-PIGD > HC								
231	<0.001	<0.001	5.48	6	26	62	8	Right medial superior frontal gyrus
	<0.001	0.11	4.56	4	50	46	9	
	<0.001	0.16	4.43	-2	56	40	9	Left medial superior frontal gyrus
35	<0.001	0.49	3.85	-4	-2	70	6	Left supplementary motor area
12	<0.001	0.43	3.94	-24	-14	68		
24	<0.001	0.56	3.75	-32	-76	-40	-	Left cerebellum crus 2
14	<0.001	0.57	3.74	20	-10	70	6	Right supplementary motor area
PD-PIGD < HC								
9	<0.001	0.63	3.66	-6	8	-8	-	Left caudate

X, y, and z coordinates referred to the Montreal Neurological Institute (MNI) space. *p* refers to two-sample t-test with 5000 random permutations; p_{FWE} refers to two-sample t-test with 5000 random permutations, Family-Wise-Error corrected. ***Abbreviations:*** *BA= Brodmann area; HC= healthy controls; kE= cluster extent; mni= Montreal Neurologic Institute; PD= Parkinson's disease; PIGD= postural instability and gait disorders phenotype; T0= baseline.*

Supplementary Table 4. fMRI patterns of activation during fMRI motor-task relative to dual-task in PD-PIGD patients at baseline.

fMRI Dual-task > Motor-task								
kE	***p***	p_{FWE}	**T**	**mni x**	**mni y**	**mni z**	**BA**	**Area**
279	<0.001	<0.01	6.43	-4	12	58	6	Left anterior supplementary motor area
				4	16	51	6	Right anterior supplementary motor area
				-3	19	44	8	Left superior frontal gyrus
313	<0.001	0.02	5.92	-40	6	30	8	Left inferior frontal gyrus
				-45	20	28	9	Left inferior frontal gyrus
65	<0.001	0.19	4.78	30	-66	48	39	Right angular gyrus
15	<0.001	0.43	4.26	-24	12	52	8	Left middle frontal gyrus
fMRI Dual-task < Motor-task								
109	<0.001	0.045	5.66	-8	58	-8	10	Left medial orbitofrontal gyrus
327	<0.001	0.049	5.61	58	-28	34	40	Right supramarginal gyrus
62	<0.001	0.34	4.43	18	-54	64	7	Right superior parietal gyrus
25	<0.001	0.34	4.42	30	-46	-30	-	Right cerebellum lobule VI
59	<0.001	0.56	4.04	10	58	-6	10	Right medial orbitofrontal gyrus
6	<0.001	0.60	3.98	-14	24	8	-	Left caudate
27	<0.001	0.61	3.97	-8	-12	62	6	Left posterior supplementary motor area
31	<0.001	0.64	3.91	8	-6	50	6	Right posterior supplementary motor area

X, y, and z coordinates referred to the Montreal Neurological Institute (MNI) space. *p* refers to paired t-test with 5000 random permutations; p_{FWE} refers to paired t-test with 5000 random permutations, Family-Wise-Error corrected. ***Abbreviations:*** *BA= Brodmann area; kE= cluster extent; mni= Montreal Neurologic Institute.*

Supplementary Table 5. fMRI changes after training in DUAL-TASK *vs* DUAL-TASK+AOT-MI PD-PIGD groups during the fMRI motor-task.

fMRI Motor-task W6 vs T0								
kE	***p***	p_{FWE}	**T**	**mni x**	**mni y**	**mni z**	**BA**	**Area**
DUAL-TASK group: W6 < T0								
45	<0.001	0.62	5.10	-24	-60	-46	-	Left cerebellum lobule VIII
	<0.001	0.66	5.00	-14	-50	-44	-	Left cerebellum lobule IX
DUAL-TASK + AOT-MI group: W6 > T0								
22	<0.001	0.51	5.33	8	-44	-6	-	Right cerebellum lobules IV-V
DUAL-TASK + AOT-MI group: W6 < T0								
8	<0.001	0.68	4.93	-10	62	24	10	Left medial superior frontal gyrus
DUAL-TASK AOT-MI group > DUAL-TASK group								
24	<0.001	0.15	4.94	6	-68	-44	-	Right cerebellum lobule VIII
12	<0.001	0.54	4.09	-2	-48	-22	-	Vermis IV-V
DUAL-TASK AOT-MI group < DUAL-TASK group								
16	<0.001	0.69	3.87	-36	24	28	9	Left middle/inferior frontal gyrus
				-36	24	32		

X, y, and z coordinates referred to the Montreal Neurological Institute (MNI) space. *p* refers to paired t-test (W6 *vs* T0 in each group) or GLM model (group-by-time interaction) with 5000 random permutations; p_{FWE} refers to paired t-test or GLM model with 5000 random permutations, Family-Wise-Error corrected. ***Abbreviations:*** *AOT-MI= action observation and motor-imagery;*

BA= Brodmann area; kE= cluster extent; mni= Montreal Neurologic Institute; PD= Parkinson's disease; PIGD= postural instability and gait disorders phenotype; T0= baseline; W6= six weeks (post-treatment).

Supplementary Table 6. fMRI changes after training in DUAL-TASK *vs* DUAL-TASK+AOT-MI PD-PIGD groups during the fMRI dual-task.

fMRI Dual-task W6 vs T0								
kE	***p***	p_{FWE}	**T**	**mni x**	**mni y**	**mni z**	**BA**	**Area**
DUAL-TASK group: W6 > T0								
13	<0.001	0.20	7.62	20	-64	42	7	Right precuneus
13	<0.001	0.58	5.75	48	-24	14	40	Right superior temporal gyrus
13	<0.001	0.85	4.88	22	-48	60	7	Right superior parietal gyrus
9	<0.001	0.78	5.13	18	-42	72	5	Right postcentral gyrus
DUAL-TASK group: W6 < T0								
12	<0.001	0.74	5.25	2	4	64	6	Right supplementary motor area
DUAL-TASK + AOT-MI group: W6 > T0								
23	<0.001	0.57	5.19	-30	-46	-30	-	Left cerebellum lobule VI
DUAL-TASK + AOT-MI group: W6 < T0								
9	<0.001	0.66	4.96	34	34	-10	47	Right inferior frontal gyrus (pars orbitalis)
DUAL-TASK + AOT-MI group < DUAL-TASK group								
18	<0.001	0.61	4.09	26	48	14	10	Right superior/middle frontal gyrus
				26	50	14		

X, y, and z coordinates referred to the Montreal Neurological Institute (MNI) space. *p* refers to paired t-test (W6 *vs* T0 in each group) or GLM model (group-by-time interaction) with 5000 random permutations; p_{FWE} refers to paired t-test or GLM model with 5000 random permutations, Family-Wise-Error corrected. ***Abbreviations:*** *AOT-MI= action observation and motor-imagery; BA= Brodmann area; kE= cluster extent; mni= Montreal Neurologic Institute; PD= Parkinson's disease; PIGD= postural instability and gait disorders phenotype; T0= baseline; W6= six weeks (post-treatment).*

Supplementary table 7 – Correlation between significant changes during fMRI tasks and clinical improvements in the DUAL-TASK and DUAL-TASK+AOT-MI groups.

fMRI Motor-task										
	+/-	**kE**	***p***	p_{FWE}	**T**	**mni x**	**mni y**	**mni z**	**BA**	**Area**
DUAL-TASK+AOT-MI group										
ABC scale T0-W6	-	13	<0.001	0.79	5.07	-44	42	2	46	Left inferior frontal gyrus
MiniBESTest T0-W6	-	32	<0.001	0.55	5.68	-30	48	30	10	Left middle frontal gyrus
Peak turning velocity TUG T0-W6	-	151	<0.001	0.37	7.20	-22	17	46	8	Left superior frontal gyrus
						-25	16	46	8	Left middle frontal gyrus
DUAL-TASK group										
Peak turning velocity TUG T0-W6	-	23	<0.001	0.29	7.04	-8	-54	-50	-	Left cerebellum lobule IX

fMRI Dual-task										
DUAL-TASK+AOT-MI group										
MiniBESTest T0-W6	+	9	<0.001	0.78	4.96	-20	-74	-16	-	Left cerebellum lobule VI
	-	6	<0.001	0.92	4.39	40	28	46	8	Right middle frontal gyrus
AST percent correct trials (incongruent) T0-W6	+	17	<0.001	0.72	6.42	28	-32	-28	-	Right cerebellum lobule IV-V-VI

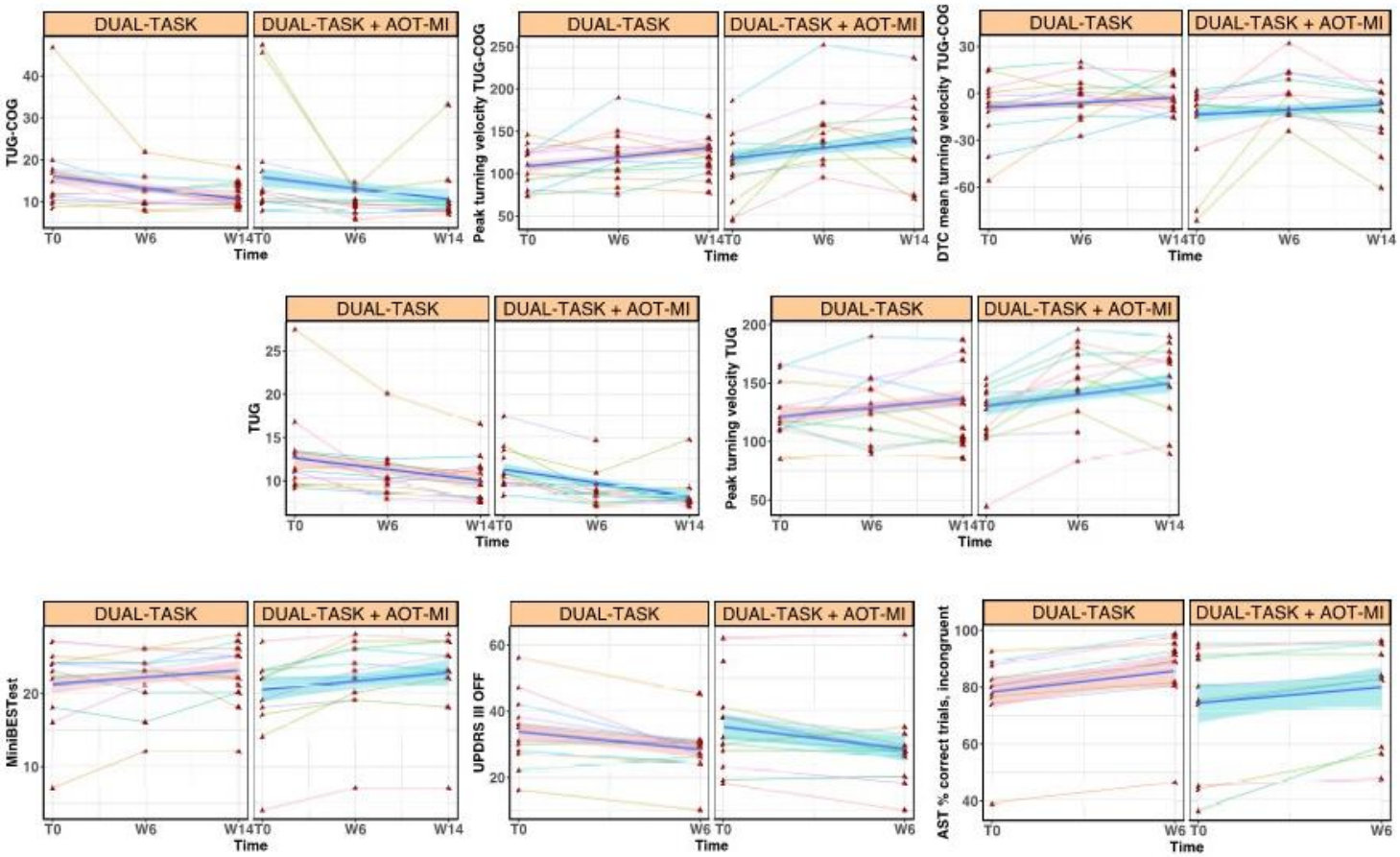
X, y, and z coordinates referred to the Montreal Neurological Institute (MNI) space. Positive correlation (+) means that both the fMRI brain activity and the clinical value decrease or increase; negative correlation (-) means that if clinical value increases, fMRI value decreases or vice-versa. *p* refers to multiple linear regression models with 5000 random permutations; p_{FWE} refers to multiple linear regression models with 5000 random permutations, Family-Wise-Error corrected. ***Abbreviations:*** *+/-= positive/negative correlation; ABC= Activities Balance Confidence scale; AST= attention switching task; BA= Brodmann area; kE= cluster extent; MiniBESTest= Mini Balance Evaluation Systems Test; mni= Montreal Neurologic Institute; r= Spearman correlation coefficient; T0= baseline; W6= six weeks (post-treatment).*

Supplementary Table 8. Exercises description

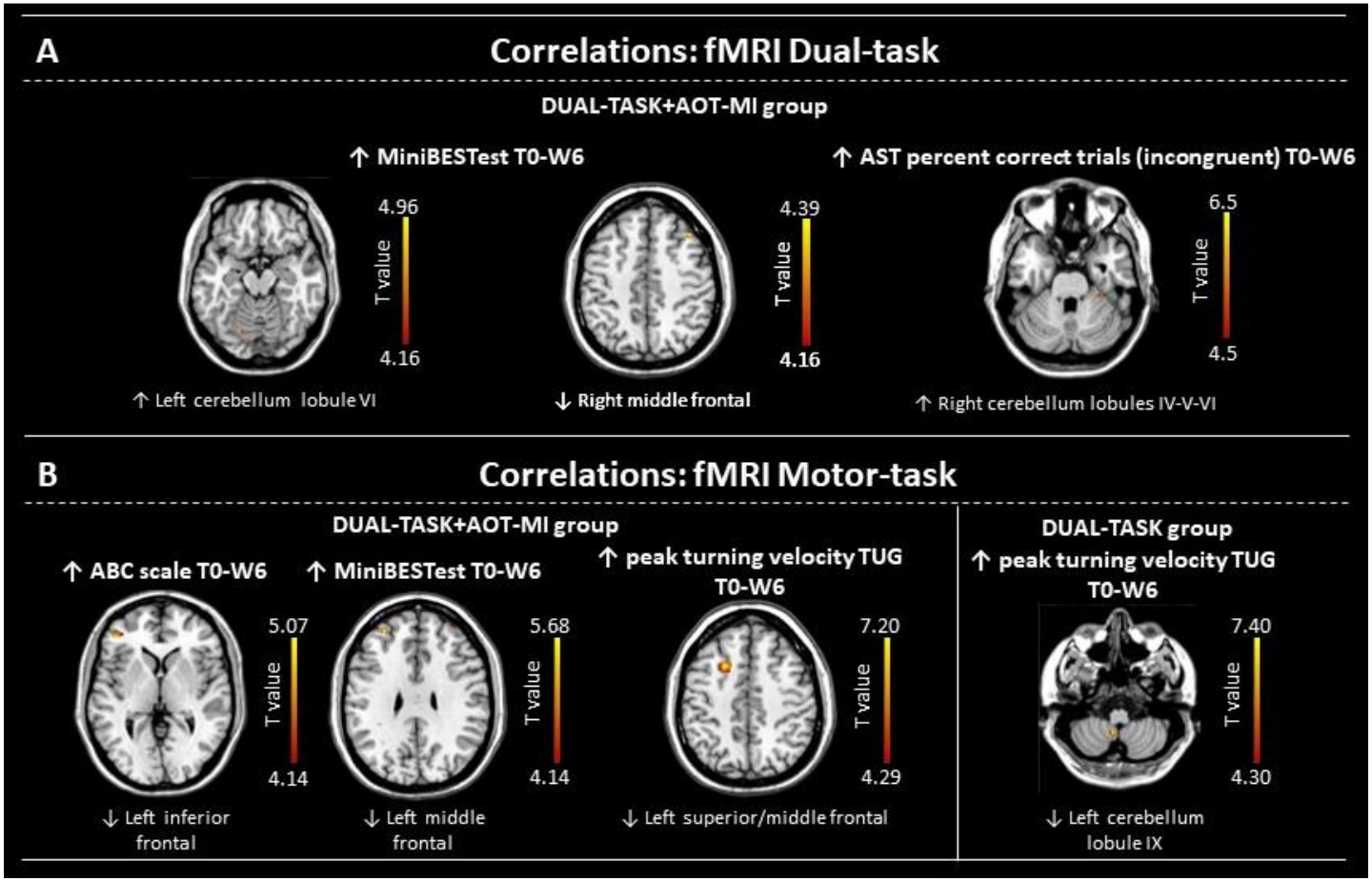
Type of training	Rationale	Exercises description
Balance training	Enhancing anticipatory postural adjustments Balance correcting responses – "feet-in-place"	• Sit-to-stand • Step initiation • Going from bipodalic stance to monopodalic stance • Standing with narrow legs • Standing on a foam pad with eyes open/closed • Standing on a foam with eyes open/closed and narrow legs • Maintaining stance in tandem with and without a foam pad • Maintaining stance with narrow legs on a foam pad, turning the head and the trunk on the left and on the right • Maintaining stance with narrow legs on a foam pad with or without turning head and trunk and with cognitive interference • Semi-monopodalic stance with one leg on the floor and one leg on a step • Semi-monopodalic stance with one leg on the floor and one leg on a foam cushion • Semi-monopodalic stance with one leg on the floor and one leg on a foam cushion with cognitive interference • Semi-monopodalic stance one leg on the floor and one leg on a step, turning the head and the trunk on the left and on the right • Semi-monopodalic stance with one leg on the floor and one leg on a foam cushion, turning the head and the trunk on the left and on the right • Semi-monopodalic stance with one leg on the floor and one leg with the foot on a ball • Maintaining monopodalic stance with and without cognitive interference • Maintaining monopodalic stance while moving the contralateral leg in all directions

	Balance correcting responses – "change-in-support"	• Walk in place • Walk in place alternatively touching a step with the feet • Walk in place alternatively touching a foam cushion with the feet, with and without cognitive interference • Walk in place alternatively touching a ball with the feet, with and without cognitive interference • Walk in place on a foam pad alternatively touching a foam cushion with the feet • Walk in place on a foam throwing and catching a ball • Walking in tandem • Walking in tandem with cognitive or motor interference • Walking in tandem on a foam carpet • Walking in tandem on a foam with cognitive or motor interference • Walking on a foam carpet overcoming obstacles • Walking on a foam carpet overcoming obstacles with cognitive interference
Gait training	Gait initiation and maintenance	• Walking straight • Walking straight and turning 180° • Sit-to-stand and walking straight • Sit-to-stand, walking straight and turning 180° • Tandem walking • Walking backward
	Gait parameters restoring	• Walking straight at maximum speed • Walking speed straight and turning at maximum speed • Walking and turning as few steps as possible (big steps) • Walking straight and turning raising the knees • Walking overcoming obstacles with different heights and at different distances
	Enhancing gait during dual-task conditions	• Walking straight with motor interference • Walking straight with cognitive interference • Walking backward with motor interference • Walking overcoming obstacles with cognitive interference

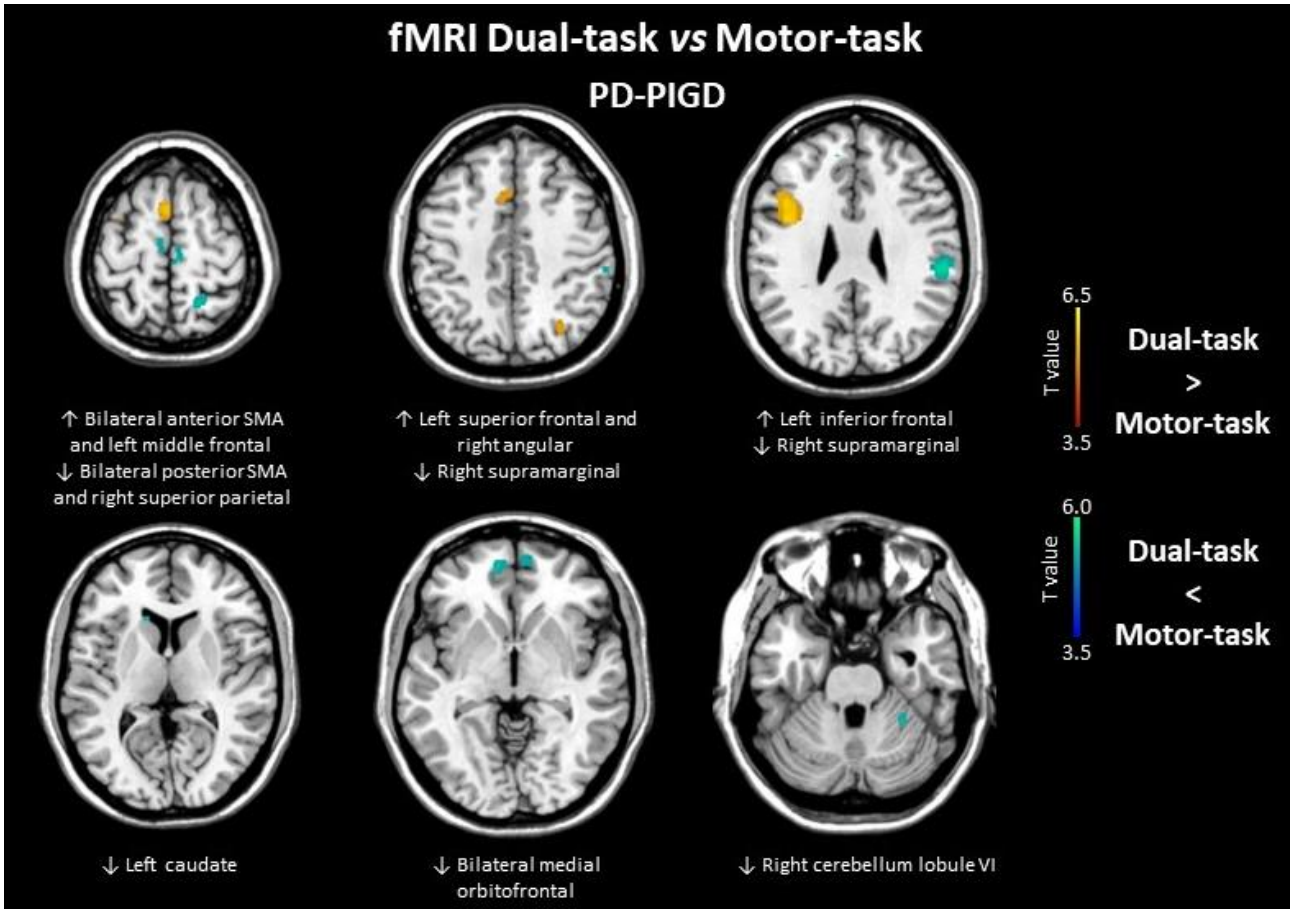
		• Change direction or stop/start walking according to associative cues • Change direction or stop/start tandem walking according to associative cues • Walking, turning and walking through narrow spaces with combined motor and cognitive interference
	Enhancing gait during freezing of gait evoking situations	• Walking through narrow walkway/door and turning as few steps as possible • Walking through narrow walkway/door, stopping, raising one leg and coming back walking through narrow walkway/door again • Walking through narrow walkway/doors overcoming obstacles • 360° turning within a square on the floor



Supplementary Figure 1: Spaghetti plot of clinical variable trajectories over time plotted separately for DUAL-TASK group (left panel) and DUAL-TASK + AOT-MI group (right panel). *Each subject is represented by a red triangle at each timepoint (T0-W6-W14 or T0-W6). Each line represents a person's score across conditions (T0-W6-W14 or T0-W6). Longitudinal changes of clinical variables were assessed in DUAL-TASK and DUAL-TASK+AOT-MI groups using linear mixed-effect models. The two-blue heavy-weighted lines represent the trajectories over time for both PD groups. 95% confidence intervals are indicated by the shaded area (DUAL-TASK group in pink and DUAL-TASK +AOT-MI group in light blue).*



Supplementary Figure 2: Regions where fMRI changes at W6 relative to baseline correlated with clinical changes at W6 in the DUAL-TASK+AOT-MI group. A) *fMRI dual-task;* ***B)*** *fMRI motor-task. Arrows represent increased/decreased (↑↓) brain activity or higher/lower (↑↓) clinical values after training. All findings are shown at p<0.001 uncorrected (5000 permutations) and only clusters greater than 5 voxels are reported. Results are shown on axial sections of the Montreal Neurological Institute standard brain. Color bars denote T values. Color bars denote T values*



Supplementary Figure 3: fMRI differences between dual-task and motor-task at baseline in PD-PIGD patients. *Areas of reduced activity (green-blue) and areas of increased activity (red-yellow) during dual-task relative to the motor-task are reported. All findings are shown at p<0.001 uncorrected (5000 permutations) and only clusters greater than 5 voxels are reported. Results are shown on axial sections of the Montreal Neurological Institute standard brain. Color bars denote T values. Color bars denote T values.*

Chapter 4 – Physiotherapy with dual-tasks improves cognition and resting-state functional connectivity in Parkinson's disease with postural instability and gait disorders

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ABSTRACT

Objectives. To assess whether dual-task gait/balance training with Action Observation Training (AOT) and Motor Imagery (MI) ameliorates cognitive performance and resting-state (RS) brain functional connectivity (FC) in Parkinson's disease (PD) patients with postural instability and gait disorders (PIGD).

Methods. 21 PD-PIGD patients were randomized into 2 groups: i) DUAL-TASK+AOT-MI group performed a 6-week training consisting of AOT-MI combined with practicing observed-imagined gait and balance exercises; ii) DUAL-TASK-group performed the same exercises combined with landscape-videos observation. All patients underwent a computerized cognitive assessment and RS-fMRI scans at baseline and after training. Cognitive and RS-FC changes (and their relationships) over time within and between-groups were assessed.

Results. After training, all PD-PIGD showed improved accuracy in test assessing executive-attentive (mainly dual-task) skills. Within-group analyses showed that: DUAL-TASK+AOT-MI group had increased RS-FC within the Anterior Salience Network (aSAL), and reduced RS-FC within the anterior Default Mode Network (aDMN), right Executive Control Network and Precuneus Network, while the DUAL-TASK group showed increased RS-FC within the Visuospatial Network. GroupxTime interactions showed that, compared to DUAL-TASK group, DUAL-TASK+AOT-MI group showed reduced RS-FC within the aDMN, which correlated with higher accuracy in dual-task executive-attentive tests.

Conclusions. In PD-PIGD patients, both trainings promote cognitive improvement and brain functional reorganization. The DUAL-TASK+AOT-MI training induced specific functional reorganization changes of extra-motor brain networks; these changes are associated with those cognitive domains which are the most challenging for these patients.

4.1 Introduction

PD is a neurodegenerative disorder associated with a loss of dopaminergic neurons, which is clinically defined by the presence of bradykinesia as cardinal motor symptom together with rigidity and rest tremor. In addition to motor aspects, PD patients present with a multitude of non-motor features, such as cognitive deficits, autonomic dysfunction, sleep disorders, mood disorders and smell impairment (Poewe, Seppi et al., 2017). To date, a pharmacological treatment that could prevent PD progression is not yet available, and, although the available drugs are efficient at the symptomatic level, they can lead to several side effects (Bloem, Okun et al., 2021). For this reason, the identification of additional non-pharmacological interventions (including motor and cognitive trainings) is crucial to improve patients' quality of life (Caspersen, Powell et al., 1985).

Gait and posture control are abilities that, once learned, should be automatic, meaning that attentional control is not required to accomplish movements. However, PIGD are common motor features in PD (Wu, Hallett et al., 2015), and patients with this clinical phenotype show difficulties in managing dual-task situations, due to a loss of automaticity (de Souza Fortaleza, Mancini et al., 2017). Cognitive overload (especially in terms of executive and attentive requirements) while dealing with dual-task situations can lead to FoG, which is a very common and disabling symptom in PD (Okuma & Yanagisawa, 2008).

Several studies highlighted the importance and efficacy of motor and cognitive trainings in PD patients, also shedding light on brain plasticity mechanisms (Clark, Bhattacharya et al., 2012, Fisher, Wu et al., 2008, Frazzitta, Maestri et al., 2015, Klaus, Hauser et al., 2009, Maidan, Rosenberg-Katz et al., 2017, Petzinger, Holschneider et al., 2015, Sehm, Taubert et al., 2014). For example, one study demonstrated that motor-cognitive combined training improved attentive skills and walking speed, and reduced the number of falls in PD patients, with all these changes being related to a decreased need of brain activation in frontal regions post-training (Maidan et al., 2017). Another study reported that, over 6 weeks, balance training was associated with specific patterns of structural brain plasticity changes involving the right anterior precuneus, left inferior parietal cortex, left ventral premotor cortex, bilateral anterior cingulate cortex and left middle temporal gyrus, which are all brain regions involved in motor control, coordination and learning (Sehm et al., 2014).

Other emerging mental practice approaches targeted to improving motor learning in PD are AOT and MI (Sarasso, Gemma et al., 2015), which heavily rely on the functioning of the MNS. Recently, AOT has been employed in rehabilitation because it takes advantage of the possibility to exercise motor networks offline; furthermore, it has been demonstrated that it can improve postural control (Patel, 2017), balance (Kim & Lee, 2013), working memory and executive-attentive abilities (Agosta, Gatti et al., 2017, Buccino, 2014). MI regards the ability to imagine a movement without actual performance of that movement, with activation of the same cortical-subcortical network involved in active motor execution (Abbruzzese, Avanzino et al., 2015). A previous study demonstrated that PD patients can modulate movements' amplitude during imitation of observed actions, and that the combination of AOT and MI could boost this effect (Bek, Gowen et al., 2019); and recent findings suggested that the combination of AOT and MI improves balance performance by reducing postural sway, and induces functional brain activation in regions involved in the execution of balance tasks (Taube, Lorch et al., 2014, Taube, Mouthon et al., 2015).

However, MRI findings on the combination of AOT and MI are still scarce and on small patient cohorts. A previous study from our research group showed that four weeks of AOT alone in PD patients reduces FoG and motor disability, improves walking speed, quality of life, and balance. Furthermore, we observed that AOT is associated with increased recruitment of fronto-parietal areas during fMRI tasks, which was related to clinical improvements after training and with the maintenance of the effect over one month follow-up (Agosta et al., 2017). Another recent study in PD patients from our research group demonstrated that dual-task gait/balance training promotes functional reorganisation of brain areas involved in motor control tasks and dual-task, and was associated to an amelioration of executive-attentive functioning skills and long-lasting effects on dual-task mobility and balance (Sarasso, Agosta et al., 2021). However, the relationship between resting-state (RS) functional reorganisation mechanisms and cognitive changes after dual-task gait/balance training with AOT-MI has never been investigated.

Based on previous findings reported in Chapter 3 (Sarasso et al., 2021), the aim of the present study is to demonstrate whether a 6-week physiotherapy training consisting of dual-task gait/balance exercises with AOT and MI ameliorates cognitive performance

(assessed with a computer-based battery) and brain RS functional connectivity (RS-FC) in PD patients with PIGD. Furthermore, our aim is also to investigate the relationship between cognitive and RS-FC changes due to physiotherapy training.

4.2 Materials and Methods

4.2.1 Subjects and study design

Twenty-one idiopathic PD (Hughes, Daniel et al., 1992) cases with PIGD (PD-PIGD) who underwent a 6 weeks dual-task gait/balance training and who had available baseline (T0, before training) and longitudinal (W6, after training) clinical and computer-based cognitive assessments were retrospectively selected. From this group, 17 patients had also available structural and RS-fMRI scans before (T0) and after training (W6). These patients were a subsample of a larger PD-PIGD group from Chapter 3 (Sarasso et al., 2021), where we described the beneficial effect of the dual-task gait/balance training in combination with AOT-MI on movement performances, as well as on motor and dual-task brain functional activity of patients during fMRI.

All patients were recruited at the Neurology Unit, IRCCS Ospedale San Raffaele, Milan, Italy according to the following inclusion criteria: H&Y score ≤ 4 (Hoehn & Yahr, 1967); PIGD phenotype (Stebbins, Goetz et al., 2013); stable dopaminergic medication for at least four weeks and without any changes during the observation period (6 weeks total); no dementia (Litvan, Goldman et al., 2012) and MMSE ≥24 (Folstein, Folstein et al., 1975); no significant head tremor. Eligibility for the dual-task gait/balance training was assessed through neurological, neuropsychological, and motor functional evaluations performed at study entry (T0). The same visits were also performed at the end of training (W6). In general, exclusion criteria were: the presence of medical illnesses or substance abuse that could interfere with cognition; any (other) major systemic, psychiatric, neurological, visual and musculoskeletal disturbances or other causes of walking inability; contraindications to undergo MRI examination; brain damage at routine MRI, including lacunae and extensive cerebrovascular disorders.

A sample of twenty-three age- and sex-matched, right-handed, healthy controls was recruited by word of mouth among non-consanguineous relatives and institute personnel and underwent a neuropsychological assessment at T0 (please refer to Tables 1-2 for socio-demographic and neuropsychological features of healthy controls). Furthermore,

an independent group of thirty-three young healthy controls (age: 24.9 ± 2.8 years; 14 [42%] women; education: 15.4 ± 3.1 years) was also recruited among students at the Vita-Salute San Raffaele University in Milan in order to generate independent components (ICs) networks of interest representing the FC of the human brain at rest (see details below).

All controls were recruited based on the following criteria: no family history of neurodegenerative diseases and normal neurological and cognitive assessment. The relationship between cognitive and RS-FC changes was assessed on the sub-sample of seventeen PD-PIGD patients (please refer to Table 3 for socio-demographic and cognitive performance at the computer-based battery at baseline), who had available both baseline and longitudinal cognitive assessments and RS-FC scans.

As described previously in Chapter 3 and in our previous study (Sarasso et al., 2021), after screening evaluations, patients were equally randomized in two training groups (DUAL-TASK + AOT-MI [N=11] and DUAL-TASK [N=10] groups; see details in the next paragraph) by using minimization method. All neurological, motor and cognitive evaluations, and dual-task gait/balance treatment were performed in ON condition (i.e., under regular dopaminergic medication); neurological assessment was also performed in OFF state. The same blinded assessors performed evaluations at each time-point.

Local ethical standards committee on human experimentation approved the study protocol and all subjects provided written informed consent prior to study participation.

4.2.2 Physiotherapy

As reported in Chapter 3 and elsewhere in further details (Sarasso et al., 2021), the dual-task gait/balance training lasted 6 weeks for both DUAL-TASK + AOT-MI and DUAL-TASK groups. The DUAL-TASK + AOT-MI group performed a gait/balance training consisting of AOT and MI in combination with observed-imagined exercises (specifically, 2 minutes of task observation, 5 minutes of task execution, 2 minutes of task imagination, 5 minutes of task execution). On the other hand, the DUAL-TASK group performed the same number of exercises combined with watching landscape videos instead of observation/imagination.

4.2.3 Neurological and motor evaluation

At T0 and W6, a blinded and experienced neurologist performed the following evaluations: H&Y scale (Goetz, Poewe et al., 2004), MDS-UPDRS-II (Goetz, Tilley et al., 2008), and MDS-UPDRS-III (Goetz et al., 2008).

A blinded, experienced physiotherapist performed the following motor functional evaluations: Pre-assessment Information Form (Paul, Canning et al., 2013); MiniBESTest (King & Horak, 2013); TUG (Morris, Morris et al., 2001); TUG with cognitive (TUG-COG) and manual dual-task (TUG-MAN), consisting respectively of TUG while counting backwards by seven starting from 100 and holding in the right hand a glass full of water (Hofheinz & Schusterschitz, 2010, Lundin-Olsson, Nyberg et al., 1998); 10MWT (Johnston, de Morton et al., 2013); ABC Scale (Powell & Myers, 1995); PDQ-39 (Peto, Jenkinson et al., 1995); NFoG-Q (Shine, Moore et al., 2012). Motor functional evaluations data and results are reported in Chapter 3 and elsewhere (Sarasso et al., 2021).

4.2.4 Standard neuropsychological assessment

At T0, blinded and experienced neuropsychologists, who were trained by the same senior Neuropsychologist, performed a cognitive screening evaluation to both PD patients and the age- and sex-matched healthy controls. For PD-PIGD patients, we tailored our neuropsychological battery according to the specific guidelines for PD-MCI level II category (Litvan et al., 2012). Accordingly, our neuropsychological testing comprehended at least two tests within each cognitive domain. In PD patients we investigated the following domains: global cognition with the MMSE (Folstein et al., 1975); memory with the digit span forward (Orsini, Grossi et al., 1987), the RAVLT (Carlesimo, Caltagirone et al., 1996), and the recall of the Rey-Osterrieth Complex Figure (Caffarra, Vezzadini et al., 2002); executive functions with the Ten-point Clock Drawing Test (Manos, 1999); the Modified Card Sorting Test (Caffarra, Vezzadini et al., 2004); the phonemic and semantic verbal fluency tests (Novelli, Papagno et al., 1986); attention and working memory with the attentive matrices(Spinnler H, 1987), the TMT (Giovagnoli, Del Pesce et al., 1996), and digit span backward (Monaco, Costa et al., 2013); visuospatial abilities with the copy of the Rey-Osterrieth Complex Figure (Carlesimo et al., 1996), the freehand copying of drawings with and without landmarks (Carlesimo et al., 1996), the Benton judgment of line orientation test (Qualls, Bliwise et

al., 2000), and the visuospatial subtests of the ACE-R (Mioshi, Dawson et al., 2006); language with the confrontation naming subtests of the BADA battery (Miceli, Laudanna et al., 1994), and the token test (De Renzi & Vignolo, 1962); mood and behaviour with the BDI (Beck, Ward et al., 1961), the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960), the Hamilton anxiety rating scale (HAMA) (Hamilton, 1959), the Apathy Rating Scale (Starkstein, Merello et al., 2009), the SHAPS (Snaith, Hamilton et al., 2018), and the QUIP-RS (Weintraub, Mamikonyan et al., 2012). The NPI (Cummings, Mega et al., 1994) was administered to the caregivers for having further information on patient behaviour. The group of old healthy controls underwent the same neuropsychological evaluation, except for Rey-Osterrieth Complex Figure, BADA subtests, Benton Judgment of Line Orientation Test, ACE-R visuospatial subtests, Ten-point Clock drawing test, HAMA, QUIP-RS, NPI and HDRS scales.

4.2.5 Cambridge Neuropsychological Test Automated Battery (CANTAB)

To detect cognitive changes related to dual-task gait/balance training, PD-PIGD patients were monitored through an electronic neuropsychological tablet-based assessment, the CANTAB. CANTAB is a computer-based cognitive battery, which includes a range of cognitive tests assessing accuracy and reaction times in several domains. According to the CANTAB Cognitive Test Selector, we selected the most suitable tests suggested for detecting cognitive changes in PD and which are highly sensitive to disease progression. Specifically, we selected the following sub-tests (https://www.cambridgecognition.com/cantab/test-batteries/parkinsons-disease/): Motor Screening Test (MOT), AST, One Touch Stockings of Cambridge (OTS), Spatial Recognition Memory (SRM), and Spatial Working Memory (SWM). Supplementary Figure 1 reports a scheme for each sub-test of the selected battery. The overall assessment lasted about 40 minutes; since the time interval between T0 and post-training visit was 6 weeks, our patients were administered parallel and randomized versions for each sub-test in order to avoid learning effects. A description of each selected CANTAB sub-test is reported in Supplementary -Table 1. For further details relatively to the CANTAB subtests, please refer to: http://www.cambridgecognition.com/cantab/cognitive-tests/.

4.2.6 MRI acquisition

Using a 3.0 Tesla scanner (Ingenia CX, Philips Medical Systems, Best, The Netherlands) MRI scans were obtained between noon and 1 PM during OFF time (i.e., at least 12 hours after their regular evening dopaminergic therapy administration), to mitigate the pharmacological effects on neural activity. RS-fMRI scans were obtained at T0 and the day after the end of training (W6), with a tolerance of 3 days. RS-fMRI was obtained using a T2* weighted echo planar imaging sequence with the following parameters: echo time (TE) = 35 ms, repetition time (TR) = 1572 ms, flip angle = 70°, field of view (FOV) = 240 × 240 mm, matrix = 96 × 94, 48 contiguous axial sections, thickness = 3 mm, acquisition time = 3 min and 57 sec, voxel reconstruction 2.5 x 2.5 x 3 mm. Before starting the RS-fMRI scanning, the technician talked with the participants through their earphones instructing them to remain motionless, to keep their eyes closed, not to fall asleep, and not to think about anything in particular. At the end of the RS-fMRI scanning, the technician talked again with the participants asking whether they remained awake during the sequence. The following structural MRI sequences were acquired at baseline and after training to exclude subjects with eventual structural brain alterations: i) 3DT1-weighted sequence: TR = 7.1 ms, TE = 3.2 ms, flip angle = 9°, 204 contiguous sagittal sections, thickness = 1 mm, FOV = 256 mm x 240 mm, matrix = 256 x 240, voxel reconstruction = 1 mm x 1 mm x 1 mm; ii) 3D T2-weighted sequence: TR = 2500 ms, TE = 330 ms, flip angle = 90°, 192 contiguous sagittal sections, thickness = 1 mm, field of view (FOV) = 256 mm x 256 mm, matrix = 256 x 258, voxel reconstruction = 0.9 mm x 0.9 mm x 1 mm. iii) 3D-FLAIR sequence was acquired only at baseline: TR = 4800 ms, TE = 269 ms, flip angle = 40°, 192 contiguous sagittal sections, thickness = 1.5 mm, FOV = 256 mm x 256 mm, matrix = 256 x 256. Voxel size 1 x 1 x 1.

4.2.7 MRI analysis

MRI preprocessing and analysis was performed at the Neuroimaging Research Unit, IRCCS Scientific Institute San Raffaele, Milan, Italy, by researchers who were blind to patient group allocation.

4.2.8 Resting-state fMRI pre-processing

RS-fMRI data processing of patients and matched healthy controls, and of young controls was carried out using the FMRIB software library (FSLv5.0) as described previously (Canu, Calderaro et al., 2022, Filippi, Canu et al., 2020). The first four volumes of the RS-fMRI data were removed to reach complete magnet signal stabilization. The following FSL-standard preprocessing pipeline was applied: (1) motion correction using MCFLIRT; (2) high-pass temporal filtering (lower frequency: 0.01 Hz); (3) spatial smoothing (Gaussian Kernel of FWHM 6 mm); (4) single-session independent component analysis-based automatic removal of motion artifacts (ICA_AROMA) (Pruim, Mennes et al., 2015) in order to identify those ICs representing motion-related artifacts.

RS-fMRI data set ('clean' from motion-related ICs) were co-registered to the participant's 3D T1-weighted image using affine boundary-based registration as implemented in FLIRT (Greve & Fischl, 2009) and subsequently transformed to the MNI 152 standard space with 4 mm isotropic resolution using non-linear registration through FNIRT (Andersson, Jenkinson et al., 2007). Pre-processed RS-fMRI data for each subject from the young control group were temporally concatenated across participants to create a single 4D data set. This RS-fMRI data set was then decomposed into ICs with a free estimate of the number of components using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) (Beckmann, DeLuca et al., 2005). The resulting young group-IC maps were spatially correlated with a referent atlas of functional ROIs (http://findlab.stanford.edu/functional_ROIs.html), in order to support the visual classification of the most representative functional networks of the brain at rest (i.e., anterior and posterior salience, anterior and posterior DMN, auditory, sensorimotor, primary and associative visual, basal ganglia, precuneus, visuo-spatial, left and right ECN) (Supplementary Figure 2) (Shirer, Ryali et al., 2012). In order to identify the subject-specific temporal dynamics and spatial maps associated with each group IC, a dual regression analysis was applied for all PD-PIGD patients (Filippini, MacIntosh et al., 2009). Finally, spatial maps of all participants were collected into single 4D files for each original IC (network) and were ready for the statistical analyses at T0. To assess RS-FC changes after training in PD-PIGD patients, delta RS-FC maps for each IC (network)

were obtained by subtracting follow-up (W6) subject-specific spatial maps (in MNI standard space) from baseline (T0) maps.

4.2.9 Statistical analysis

4.2.9.1 Demographic, clinical and cognitive data

Sociodemographic and standard neuropsychological data at T0 were compared between PD-PIGD groups of patients and age- and sex-matched healthy controls using the Kruskal-Wallis test. Clinical and CANTAB subtest differences between PD-PIGD groups at T0 were assessed using the Mann-Whitney tests. Longitudinal CANTAB subtest changes after training were assessed within PD-PIGD groups using linear mixed-effects models. Such models were adjusted for the baseline value of each considered variable and for baseline variable-by-time interaction. Furthermore, in order to adjust for longer reaction times (and therefore motor impairment as a confounding variable in our cohort), those CANTAB variables that indicated response latencies (in AST, MOT and SRM) were adjusted for baseline MOT mean response latency values. Extreme outlier values (i.e., data points that fall more than three times below the first quartile or above the third quartile of the interquartile range) were investigated and removed from the analysis. P values were Bonferroni-corrected for multiple comparisons at $P < 0.05$. All statistical analyses were performed using R Statistical software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria).

4.2.9.2 Network-based functional connectivity: Independent component analysis (ICA)

We performed: a) between-group (DUAL-TASK + AOT-MI vs DUAL-TAST groups) RS-FC comparisons within each IC (network) of interest at baseline; b) within-group analysis of each IC (network) changes after 6-week dual-task gait/balance training; c) Group x Time interaction of RS-FC changes after training; d) correlations between RS-FC changes within each IC (network) of interest and significant measures of CANTAB that changed over time. All analyses were carried out using GLMs in FSL (FSLv5.0), including 4D maps (for T0 analyses) or delta RS-FC maps (for longitudinal analyses) for each IC (network) of each group of PD-PIGD patients as dependent variables. Specifically: between-groups comparison at baseline (a) was performed with GLMs including 4D spatial maps of each IC (network) and each PD-PIGD group as dependent

variable; within-group analyses (b) were performed with GLMs including delta RS-FC maps of IC (network) and each PD-PIGD group, separately, as dependent variables; Group x Time interactions of RS-FC changes after training (c) were performed with GLMs including delta RS-FC maps of each IC (network) and each PD-PIGD group as dependent variables. Finally, for correlations (d), GLMs included delta RS-FC maps masked for significant findings obtained in step (c) for each significant IC network and each PD-PIGD group separately as dependent variables, and delta CANTAB scores (baseline - follow-up) which significantly changed over time as covariates of interest, with baseline CANTAB scores of subtests of interest added as nuisance variables.

Nonparametric permutation tests (5000 permutations) were used, and analyses were restricted within the spatial RS-networks of interest using binary masks obtained by thresholding the corresponding Z map images ($Z>2.3$). FWE correction for multiple comparisons was performed, implementing the threshold-free cluster enhancement using a significance threshold of $p<0.05$. Significant findings that did not survive the FWE correction were also observed at an uncorrected $p<0.05$ threshold.

4.3 Results

4.3.1 Socio-demographic and clinical results at baseline

At T0, all PD-PIGD patients were matched with healthy controls for age and sex and were similar for educational levels (Table 1). Furthermore, the DUAL-TASK + AOT-MI and DUAL-TASK groups of patients were similar in terms of sociodemographic and clinical features, both in ON and OFF states (Table 1). In addition, no significant differences in terms of socio-demographic and clinical variables were retrieved between those PD PIGD patients (N=17) with longitudinal RS sequence and CANTAB assessments (Table 3).

4.3.2 Neuropsychological evaluation at baseline

At T0, compared to healthy controls, all PD-PIGD patients performed slightly worse in tests assessing general cognition (MMSE), verbal memory (RAVLT immediate recall), attention (Attentive matrices), set-shifting abilities (TMT), executive functions (semantic fluency), and visuospatial skills. The DUAL-TASK + AOT-MI and DUAL-TASK groups

performed similarly in all cognitive domains and they did not differ also in terms of mood and behavioural measures (Table 2).

4.3.3 CANTAB evaluation at baseline

At T0, no significant differences were observed between DUAL-TASK + AOT-MI and DUAL-TASK groups of patients in any CANTAB sub-tests (Tables 3-4).

4.3.4 Longitudinal cognitive changes (CANTAB assessment)

After training, both DUAL-TASK+AOT-MI and DUAL-TASK patients ameliorated in terms of accuracy in the AST in set-shifting (incongruent) conditions (Table 4 and Figure 1). Group x Time interaction did not show significant differences between the two patient groups.

4.3.5 Network-based functional connectivity: ICA

4.3.5.1 Within-group longitudinal analysis

At T0, we did not observe significant differences between DUAL-TASK + AOT-MI and DUAL-TASK groups in any IC (network) of interest. After the 6-week training, within-groups analysis indicated that DUAL-TASK + AOT-MI patients showed reduced RS-FC of the right frontal pole within the anterior DMN (aDMN), and of the left precuneus within both the right ECN and the Precuneus Network. In addition, this patient group showed increased RS-FC in the left anterior prefrontal cortex and left superior temporal regions within the anterior SAL(aSAL) (Table 5, Figure 2A, upper part). On the other hand, the DUAL-TASK group showed increased RS-FC of the right superior parietal gyrus within the Visuospatial Network (Table 5, Figure 2A, lower part).

4.3.5.2 Between-groups longitudinal analysis

Group x Time interaction analyses showed that, after training, compared to DUAL-TASK group, the DUAL-TASK + AOT-MI group showed more increased RS-FC in the left anterior prefrontal cortex within the aSAL and more reduced RS-FC in the right anterior prefrontal cortex and right frontal pole within the aDMN (Table 5, Figure 2B).

4.3.5.3 Correlation analyses: RS-FC and cognitive changes after training

We observed that, after training, reduced RS-FC of the frontal pole within the aDMN in the DUAL-TASK + AOT-MI group was related to the group better accuracy in AST set-shifting condition (Table 6; Figure 2C).

4.4 Discussion

To our knowledge, this is the first study which aimed to assess whether dual-task gait/balance training combined or not with AOT-MI could determine both cognitive and RS-FC changes in two groups of PD-PIGD patients.

In order to detect cognitive and RS-FC changes specifically associated with the performed training, patient groups were well characterized and comparable among each other in terms of socio-demographic, clinical (i.e., disease duration, disease staging and motor severity), and neuropsychological features. The patients' clinical and cognitive matching was furthermore confirmed by similar RS-FC profiles at study entry. In addition, compared to a sample of age-, sex- and education-matched healthy controls, our patients showed only a slightly worse cognitive performance in terms of verbal memory, visuospatial, attentive and executive functioning.

In our study, we observed that both groups which underwent dual-task gait/balance training ameliorated over 6 weeks in terms of accuracy in an attentive-executive task relying on set-shifting. In this specific test from a computer-based battery (i.e., the CANTAB), an arrow is displayed on either side of the screen (left or right) and can point in either direction (left or right). Participants must select the left or right button on the screen according to "the side on which the arrow appeared" or the "direction in which the arrow was pointing", shifting from one request to the other by paying attention to suppress irrelevant stimuli (e.g., arrow appears on the right, but the correct answer is 'left'). A few studies demonstrated that a dual-task training positively improves some aspects of cognition, such as mental flexibility and processing speed (Fritz, Cheek et al., 2015, Silsupadol, Siu et al., 2006). In our study the positive changes that we observed after a common dual-task training in both groups might be explained by a better functioning of PD patients to focus on the required task, to process parallel information at multiple levels, and to inhibit irrelevant information.

However, even though there was a similar cognitive improvement in the two PD groups after training, our patients presented distinct brain functional reorganization processes. Specifically, we observed that, compared to the other group, DUAL-TASK + AOT-MI patients showed more substantial brain functional changes, with reduced RS-FC in frontal polar regions within the aDMN, and in the visuo-motor associative area of the precuneus within both the ECN and the Precuneus networks. Reduced activity in cerebral frontal areas, specifically in orbitofrontal regions, can be explained as a patient's more efficient and optimal motor control, together with lower reliance on attentive resources (Maidan et al., 2017); in fact, due to the loss of automaticity and of motor control typical of PD patients, the activation of frontal areas is generally increased in dual-task or complex situations for monitoring needs (Wu, Liu et al., 2015). Previous findings demonstrated that the combination of AOT and MI might compensate for decreased automaticity and restore motor function, therefore reducing the need of attentive control performed by frontal regions in more complex conditions (Thumm, Maidan et al., 2018). After training, at the brain functional level, DUAL-TASK + AOT-MI patients rely less on frontal lobe activation compared to the DUAL-TASK group, suggesting a reduction in the attentive overload to accomplish complex requirements. In line with this hypothesis and with previous findings (Sarasso et al., 2021, Thumm et al., 2018), in the DUAL-TASK + AOT-MI group, we further observed that frontal functional reorganization processes were associated to better accuracy in set-shifting, meaning that this type of training might improve attentive-executive functioning.

Furthermore, compared to the DUAL-TASK group, DUAL-TASK + AOT-MI patients showed decreased RS-FC of the precuneus within the ECN and the Precuneus networks. This finding is in line with previous results, which evidenced reduced RS-FC of these circuits in association with motor and cognitive improvements after AOT training in multiple sclerosis patients (Cordani, Valsasina et al., 2021). Specifically, the precuneus belongs to the medial prefrontal-middle parietal neural network (which partially overlaps with the MNS) and has connections with lateral parietal regions and the supplementary motor area; the anterior portions of the precuneus have been linked to mental and visuo-spatial imagery, specifically in setting-up spatial attributes and in the generation of spatial information for imagined movements (Ogiso, Kobayashi et al., 2000). Furthermore, a possible role of the precuneus in internally guided attention and manipulation of mental

images, which occurs also during MI practice, has been observed (Cavanna & Trimble, 2006). We observed a substantial reduced RS-FC of this brain region after AOT-MI training, which has been observed in other studies in healthy subjects when they were required to actually execute (and not imagine) goal-directed actions (Cavanna & Trimble, 2006, Shulman, Fiez et al., 1997).

Finally, compared to the DUAL-TASK group, the DUAL-TASK + AOT-MI patients showed, after training, increased RS-FC of the anterior prefrontal and superior temporal cortices within the aSAL. Previous findings reported that, in highly demanding cognitive situations, an anti-correlated coupling mechanism occurs between the SAL and the DMN; while the first RS-FC network is activated, the latter shows the opposite pattern, with reduced activity. These patterns of activation have been associated in healthy subjects to optimal cognitive performance (Putcha et al., 2016); thus, we suppose that AOT can boost executive functioning skills in our patients by training them focusing on relevant salient stimuli, therefore reducing the attentional control performed by more anterior brain regions.

On the contrary, we observed only a few brain functional reorganizational changes after training in the DUAL-TASK group, specifically in extra-motor areas of the visuospatial network, which are associated to sensorimotor integration and usually hyperactivated in dual-task situations (Wu & Hallett, 2008). Even though DUAL-TASK group patients improved over time in attentive-executive tasks as well as the DUAL-TASK + AOT-MI group, their functional reorganization occurred in a single network only, suggesting the specificity of their improvement for dual-task conditions and, likely, a lower grade of training generalization for other motor and cognitive functions. Although we cannot exclude that, compared to DUAL-TASK + AOT MI, the DUAL-TASK group at baseline had a RS-FC more similar to that of healthy controls, we can speculate that a training with AOT-MI promotes a greater functional reorganization involving different and crucial networks serving several motor control and task performances. In our previous study (Sarasso et al., 2021), we observed improvements in motor performances in both groups, with substantial changes especially in the DUAL-TASK + AOT-MI. However, in the present study we were not able to assess how the combination of dual-task with AOT-MI, more than dual-task alone, has a specific impact on cognition. We can hypothesize that this lack of differences in our groups might be explained by the

training length or the time of observation. Future studies might address whether lengthier trainings and/or longer follow-ups would evidence the different impact of the training type on patient cognitive functions, especially in the DUAL-TASK + AOT-MI group; on the other hand, they can demonstrate whether AOT and MI are more specific to induce motor changes rather than cognitive improvements. Finally, we can speculate that those brain functional reorganizational changes that we observed in the DUAL-TASK + AOT-MI group would subtend possible cognitive changes in this group, but more time of training or of observation are probably needed to retrieve significant variations.

The present study has some limitations: first, the current patient sample is small, mainly when the patient groups were split according to different trainings, thus reducing the statistical power of our analyses. Second, we did not have comparable RS functional MRI sequences for healthy controls; for this reason, we were unable to established whether (and how much) the RS-FC of patient groups was different from controls at the baseline and whether these potential differences reduced after training. Third, we did not test several (other) aspects of the training, such as different total duration or week frequency, which are relevant for a comprehensive definition of the intervention. Finally, longer training periods and/or follow-up observations are needed to verify whether motor learning approaches, such as AOT and MI, have long-lasting effects, which are crucial in these patient cohorts.

In our study, we observed that both DUAL-TASK and DUAL-TASK + AOT-MI promote cognitive improvement and brain functional reorganization processes. Dual-task gait/balance training + AOT-MI could be useful for obtaining specific functional reorganization of brain areas involved in motor control and executive-attentive abilities.

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4.6 Appendix

Table 1. Sociodemographic and clinical variables of PD-PIGD patients and heathy controls at baseline

Socio-demographic variables	HC	All PD-PIGD	*p* all PD-PIGD *vs* HC	DUAL-TASK group	DUAL-TASK+ AOT-MI group	*p* PD DUAL-TASK *vs* PD DUAL-TASK+AOT-MI
N	23	21	-	10	11	-
Age [years]	63.74 ± 8.69 (52.05-80.08)	66.09 ± 8.31 (48.07-82.71)	0.32	63.43 ± 9.95 (48.07-79.44)	68.50 ± 5.95 (60.42-82.71)	0.12
Sex [M/F]	10/13	12/9	0.37	6/4	6/5	0.57
Education [years]	12.14 ± 3.81 (5-18)	11.48 ± 4.76 (5.00-20.00)	0.62	11.20 ± 5.24 (5.00-20.00)	11.72 ± 4.52 (5.00-17.00)	0.83
PD duration [years]	-	8.19 ± 4.09 (2.00-16.00)	-	8.10 ± 3.84 (2.00-13.00)	8.27 ± 4.50 (2.00-16.00)	0.91
LEDD [mg]	-	675.71 ± 414.94 (76.00-1867.00)	-	549.60 ± 232.26 (204.00-901.00)	790.36 ± 515.20 (76.00-1867.00)	0.24
H&Y [ON state]	-	2.36 ± 0.39 (2.00-3.00)	-	2.30 ± 0.35 (2.00-3.00)	2.41 ± 0.43 (2.00-3.00)	0.59
H&Y [OFF state]	-	2.43 ± 0.39 (2.00-3.00)	-	2.40 ±0.39 (2.00-3.00)	2.45 ± 0.41 (2.00-3.00)	0.76
UPDRS-II	-	12.05 ± 5.34 (4.00-24.00)	-	12.90 ± 5.62 (7.00-24.00)	11.27 ± 5.22 (4.00-20.00)	0.48
UPDRS-III [ON state]	-	29.21 ± 8.71 (13.00-51.00)	-	30.20 ± 8.35 (13.00-41.00)	28.32 ± 9.33 (14.00-51.00)	0.36

UPDRS-III [OFF state]	-	36.71 ± 11.22 (16.00-62.00)	-	35.60 ± 11.21 (16.00-56.00)	37.73 ± 11.67 (23.00-62.00)	0.77

Values are mean ± standard deviation in the first row, and minimum-maximum values in the second row. Categorical variables are reported as frequency. *p* values refer to Kruskal-Wallis Test or Fisher's exact test for categorical variables. ***Abbreviations:*** *AOT-MI=action observation and motor-imagery; HC=healthy controls; H&Y=Hoehn and Yahr score; LEDD=levodopa equivalent daily dose; M/F=male/female; mg=milligrams; N=number; PD=Parkinson's Disease; PIGD=postural instability and gait disorders; UPDRS=Unified Parkinson's Disease Rating Scale.*

Table 2. Neuropsychological features of PD-PIGD patients and healthy controls at baseline.

Variable	HC (N=23)	All PD-PIGD (N=21)	*p* all PD-PIGD *vs* HC	DUAL-TASK group (N=10)	DUAL-TASK + AOT-MI group (N=11)	*p* DUAL-TASK group *vs* DUAL-TASK + AOT-MI group
MMSE	29.23 ± 0.87 (27-30)	28.05 ± 1.98 (24.00-30.00)	**0.04**	28.00 ± 1.63 (24.00-30.00)	28.09 ± 2.34 (24.00-30.00)	0.45
MEMORY						
RAVLT - Immediate recall	49.50 ± 10.76 (29-67)	41.57 ± 11.77 (20.00-57.00)	**0.04**	47.00 ± 7.48 (31.00-57.00)	36.64 ± 13.05 (20.00-57.00)	0.057
RAVLT - Delayed recall	10.41 ± 2.48 (6-14)	8.24 ± 3.75 (0.00-14.00)	0.06	9.70 ± 3.20 (3.00-14.00)	6.91 ± 3.85 (0.00-12.00)	0.08
RAVLT – Recognition	14.18 ± 0.91 (12-15)	13.19 ± 2.86 (4.00-15.00)	0.44	14.20 ± 1.23 (11.00-15.00)	12.27 ± 3.61 (4.00-15.00)	0.12
Digit span forward	6.32 ± 1.13 (4-8)	5.57 ± 1.21 (4.00-7.00)	0.07	5.80 ± 1.13 (4.00-7.00)	5.36 ± 1.29 (4.00-7.00)	0.44
ROCF - Recall	-	14.71 ± 5.83 (4.00-24.00)	-	15.15 ± 7.05 (4.00-24.00)	14.32 ± 4.79 (5.00-22.50)	0.50
LANGUAGE						
Token Test	33.73 ± 2.12 (28.0-36.0)	33.05 ± 1.72 (29.00-35.00)	0.10	33.30 ± 1.78 (29.50-35.00)	32.82 ± 1.72 (29.00-35.00)	0.41
BADA - Names	-	29.52 ± 0.68 (28.00-30.00)	-	29.70 ± 0.48 (29.00-30.00)	29.36 ± 0.80 (28.00-30.00)	0.35
BADA - Verbs	-	27.09 ± 1.37 (22.00-28.00)	-	26.90 ± 1.85 (22.00-28.00)	27.27 ± 0.79 (26.00-28.00)	0.97
VISUO-SPATIAL ABILITIES						
ROCF - Copy	-	27.29 ± 6.12 (8.00-36.00)	-	26.35 ± 7.06 (8.00-34.00)	28.14 ± 5.32 (18.00-36.00)	0.62

Copy of drawings - Freehand	10.41 ± 1.05 (9-12)	10.09 ± 1.58 (6.00-12.00)	0.67	10.40 ± 1.90 (6.00-12.00)	9.82 ± 1.25 (7.00-12.00)	0.22
Copy of drawings - with landmarks	68.23 ± 2.11 (63-70)	64.38 ± 6.89 (47.00-70.00)	**0.03**	65.80 ± 7.11 (47.00-70.00)	63.09 ± 6.74 (48.00-70.00)	0.14
Benton Judgment of Line Orientation Test	-	15.30 ± 3.52 (9.00-20.00)	-	15.89 ± 2.76 (11.00-19.00)	14.82 ± 4.12 (9.00-20.00)	0.59
ACE-R, visuospatial	-	14.19 ± 1.66 (9.00-16.00)	-	13.90 ± 2.02 (9.00-16.00)	14.45 ± 1.29 (12.00-16.00)	0.55
EXECUTIVE FUNCTIONS						
CDT	-	7.67 ± 2.98 (1.00-10.00)	-	7.70 ± 3.40 (1.00-10.00)	7.64 ± 2.73 (2.00-10.00)	0.61
MCST Categories	4.36 ± 1.14 (3-6)	4.05 ± 1.69 (0.00-6.00)	0.83	4.40 ± 1.64 (0.00-6.00)	3.73 ± 1.74 (0.00-6.00)	0.18
Phonemic fluency	39.41 ± 8.16 (18-54)	35.38 ± 10.79 (9.00-58.00)	0.07	35.20 ± 6.23 (26.00-45.00)	35.54 ± 14.06 (9.00-58.00)	0.75
Semantic fluency	50.41 ± 9.30 (26-65)	43.14 ± 11.58 (18.00-62.00)	**0.02**	43.70 ± 10.74 (23.00-62.00)	42.64 ± 12.80 (18.00-61.00)	0.94
ATTENTION AND WORKING MEMORY						
Attentive matrices	54.00 ± 4.73 (40-60)	50.19 ± 6.19 (37.00-57.00)	**0.03**	50.00 ± 6.38 (37.00-57.00)	50.36 ± 6.33 (38.00-57.00)	0.83
TMT-A	29.89 ± 9.72 (16-52)	50.21 ± 26.73 (28.00-136.00)	**<0.01**	46.37 ± 19.11 (28.00-93.00)	53.70 ± 32.74 (28.00-136.00)	0.78
TMT-B	92.86 ± 33.16 (47.47-172.00)	129.80 ± 42.45 (72.00208.00)	**<0.01**	119.43 ± 44.38 (72.00-208.00)	140.17 ± 40.23 (94.00-198.00)	0.35
TMT-B-A	62.98 ± 27.36 (24-139)	88.10 ± 34.82 (44.00-160.00)	**0.01**	78.24 ± 36.68 (44.00-160.00)	97.96 ± 31.82 (59.00-154.00)	0.14

Digit span backward	4.86 ± 1.17 (3-7)	4.29 ± 1.38 (2.00-8.00)	0.13	4.40 ± 0.96 (3.00-6.00)	4.18 ± 1.72 (2.00-8.00)	0.45
MOOD						
BDI	7.50 ± 5.46 (0-20)	9.14 ± 6.02 (1.00-22.00)	0.13	12.00 ± 7.41 (2.00-22.00)	6.54 ± 2.73 (1.00-10.00)	0.09
HAMA	-	5.19 ± 3.64 (0.00-11.00)	-	5.90 ± 4.36 (0.00-11.00)	4.54 ± 2.91 (1.00-10.00)	0.55
ARS	8.00 ± 5.63 (0-19)	8.67 ± 4.29 (1.00-14.00)	0.54	9.80 ± 3.70 (4.00-13.00)	7.64 ± 4.69 (1.00-14.00)	0.43
QUIP-RS	-	6.10 ± 9.02 (0.00-30.00)	-	4.56 ± 8.25 (0.00-19.00)	7.50 ± 9.89 (0.00-30.00)	0.29
SHAPS 16 items	55.95 ± 3.95 (46-62)	51.16 ± 13.35 (0.00-64.00)	0.07	47.40 ± 17.42 (0.00-60.00)	55.33 ± 4.69 (50.00-64.00)	0.19
NPI	-	8.87 ± 7.34 (0.00-19.00)	-	9.80 ± 8.17 (2.00-19.00)	7.33 ± 7.02 (0.00-14.00)	0.65
HDRS	-	4.69 ± 2.75 (1.00-11.00)	-	5.67 ± 3.88 (2.00-11.00)	4.10 ± 1.79 (1.00-7.00)	0.70

Values are mean ± standard deviation in the first row and minimum-maximum values in the second row. *p* values refer to Kruskal-Wallis test. Statistical significance was accepted for values of p <0.05. ***Abbreviations:*** *ACE-R= Addenbrooke's Cognitive Examination Revised; AOT-MI= action observation and motor-imagery; ARS= Apathy rating scale; BADA= Battery for Assessment of Aphasic Disorders; BDI= Beck Depression Inventory; CDT=Clock Drawing Test; HAMA= Hamilton Anxiety rating Scale; HC= healthy controls; HDRS= Hamilton Rating Scale for Depression; MCST= Modified Card Sorting test; MMSE= Mini Mental State Examination; N= number; NPI= Neuropsychiatric Inventory; PD= Parkinson's disease; PIGD= postural instability and gait disorders phenotype; RAVLT= Rey Auditory Verbal Learning Test; ROCF= Rey-Osterrieth Complex Figure; QUIP-RS= Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease–Rating Scale; SHAPS= Snaith-Hamilton Pleasure Scale; TMT= Trail Making Test.*

Table 3. Sociodemographic, clinical and CANTAB variables of those PD-PIGD patients with longitudinal RS sequence and CANTAB assessments at baseline.

	DUAL-TASK group	**DUAL-TASK+ AOT-MI group**	***p* PD DUAL-TASK *vs* PD DUAL-TASK+AOT-MI**
N	9	8	-
Sociodemographic characteristics			
Age [years]	63.13 ± 7.09 (53.39-77.28)	65.71 ± 4.67 58.57-72.10	0.25
Sex [M/F]	7/2	6/2	0.39
Education [years]	11.56 ± 4.85 (6.00-20.00)	13.25 ± 2.71 9.00-17.00	0.28
PD duration [years]	7.67 ± 3.80 (2.00-13.00)	8.50 ± 4.10 3.00-16.00	0.70
Clinical characteristics			
H&Y [ON state]	2.28 ± 0.36 2.00-3.00	2.31 ± 0.37 2.00-3.00	0.83
H&Y [OFF state]	2.33 ± 0.35 2.00-3.00	2.44 ± 0.42 2.00-3.00	0.60
UPDRS-II	10.56 ± 2.51	9.13 ± 4.99	0.41

	7.00-14.00	1.00-17.00	
UPDRS-III [ON state]	27.00 ± 8.44 13.00-39.00	21.94 ± 6.84 14.00-33.00	0.18
UPDRS-III [OFF state]	30.56 ± 8.22 16.00-42.00	28.25 ± 7.79 18.00-38.00	0.66
CANTAB variables			
AST, percent total correct trials [%]	85.31 ± 6.84 75.62-96.25	90.52 ± 6.26 83.75-96.87	0.20
AST, percent total correct trials (simple condition) [%]	89.21 ± 8.45 75.00-100.00	96.25 ± 2.96 92.50-100.00	0.14
AST, percent total correct trials (set-shifting condition) [%]	81.40 ± 6.35 73.75-92.50	84.79 ± 9.66 73.75-95.00	0.56
AST, mean response latency (simple condition) [msec]	799.34 ± 134.71 612.56-982.31	681.84 ± 105.69 554.91-875.08	0.08
AST, mean response latency (set-shifting condition) [msec]	876.34 ± 161.71 697.02-1100.45	773.84 ± 142.40 605.61-967.54	0.14

MOT, mean response latency [msec]	778.10 ± 214.75 552.80-1102.80	715.56 ± 146.53 528.60-951.90	0.56
OTS, first choice [errors]	5.75 ± 2.71 2.00-9.00	4.71 ± 2.56 1.00-9.00	0.64
SRM, percent total correct trials [%]	72.50 ± 13.88 45.00-90.00	71.66 ± 13.29 55.00-95.00	0.65
SRM, mean response latency [msec]	3021.09 ± 955.97 1825.66-4874.46	2558.16 ± 955.26 1569.40-4185.50	0.42
SWM [total errors]	25.25 ± 6.31 16.00-37.00	14.66 ± 10.01 1.00-27.00	0.08
SWM, strategy [accuracy score*]	18.62 ±1.99 16.00-21.00	16.00-3.09 11.00-20.00	0.08

Values are mean ± standard deviation in the first row and minimum-maximum values in the second row. Categorical variables are reported as frequency. *p* values refer to Mann-Whitney Test or Fisher's exact test for categorical variables. ***Abbreviations:*** *AOT-MI=action observation and motor-imagery; AST=Attention Switching Task; CANTAB=Cambridge Neuropsychological Automated Test Battery; H&Y=Hoehn and Yahr score; M/F=male/female; MOT=Motor Screening Task; msec=milliseconds; N=number; OTS=One Touch Stockings of Cambridge; PD=Parkinson's Disease; PIGD=postural instability and gait disorders; SRM=Spatial Recognition Memory; SWM=Spatial Working Memory; UPDRS=Unified Parkinson's Disease Rating Scale. *higher scores indicate poor use of the best strategy, while lower scores indicate good strategy use.*

Table 4. CANTAB performances of PD-PIGD patients at baseline and changes over time due to dual-task training (T0-W6).

CANTAB measures	**DUAL-TASK group (N=10)**	**DUAL-TASK + AOT-MI group (N=11)**	***p* DUAL-TASK group *vs* DUAL-TASK + AOT-MI group**	***p for linear trend DUAL-TASK***	***p for linear trend DUAL-TASK + AOT-MI***	***p for linear trend DUAL-TASK vs DUAL-TASK+ AOT-MI***
AST, percent total correct trials [%]	82.62 ± 13.22 (50.00-96.25)	83.18 ± 14.78 (57.50-96.87)	0.57	**<0.001**	0.16	1.00
AST, percent total correct trials (simple condition) [%]	87.50 ± 12.33 (61.25-100.00)	94.62 ± 5.03 (82.50-100.00)	0.29	0.056	1.00	0.65
AST, percent total correct trials (set-shifting condition) [%]	82.08 ± 6.28 (73.75-92.50)	73.98 ± 22.04 (36.25-95.00)	0.88	**<0.001**	**0.04**	1.00
AST, mean response latency (simple condition) [msec]	820.90 ± 172.12 (612.56-1153.10)	732.42 ± 140.43 (554.91-1065.05)	0.26	1.00	0.07	0.22
AST, mean response latency (set-shifting condition) [msec]	908.81 ± 212.74 (697.02-1340.45)	830.52 ± 187.78 (605.62-1281.94)	0.48	1.00	0.69	0.37
MOT, mean response latency [msec]	789.52 ± 202.47 (552.80-1102.80)	725.31 ± 117.36 (569.90-951.90)	0.60	0.77	0.69	0.94
OTS, first choice [errors]	5.20 ± 3.01 (0.00-9.00)	4.45 ± 3.17 (0.00-11.00)	0.59	0.21	0.23	0.057

SRM, percent total correct trials [%]	72.00 ± 13.16 (45.00-90.00)	66.50 ± 13.34 (50.00-95.00)	0.27	0.24	0.39	0.95
SRM, mean response latency [msec]	2986.64 ± 846.50 (1825.67-4874.47)	2840.62 ± 844.78 (1569.40-4185.50)	0.72	0.93	0.06	0.12
SWM [total errors]	24.60 ± 6.40 (16.00-37.00)	19.70 ± 10.54 (0.00-33.00)	0.40	1.00	0.06	1.00
SWM, strategy [accuracy score*]	18.20 ± 2.30 (14.00-21.00)	17.30 ± 3.71 (11.00-22.00)	0.70	0.68	0.09	1.00

Values are mean ± standard deviation in the first row and minimum-maximum values in the second row. *p* values refer to Mann-Whitney test. Statistical significance was accepted for values of p <0.05. ***Abbreviations:*** *AOT-MI= Action Observation Training-Motor Imagery; AST= Attention Switching Task; CANTAB= Cambridge Neuropsychological Test Automated Battery; MOT= Motor Screening Task; OTS= One Touch Stockings of Cambridge; SRM= Spatial Recognition Memory; SWM= Spatial Working Memory. *Higher scores indicate poor use of the best strategy, while lower scores indicate good strategy use.*

Table 5. Significant RS-FC differences between and within groups over time.

	RSN	Side	Brain regions (BA areas)	MNI coordinates	N of voxels	Intensity (Index)
Within-groups changes						
DUAL-TASK + AOT-MI						
Increased RS-FC	aSAL	L	Anterior prefrontal cortex (BA10)	x -26; y 58; z 12	10	5.78
		L	Superior temporal gyrus (BA22)	x -58; y -26; z 0	5	5.78
Reduced RS-FC	aDMN	R	Frontal pole	x 2; y 70; z 4	1	4.47
		R	Frontal pole	x 18; y 70; z -4	1	4.87
	Right ECN	L	Precuneus (BA7)	x -6; y -74; z 52	7	5.04
	Precuneus Network	L	Precuneus (BA7)	x -2; y -62; z 56	14	4.58
DUAL-TASK						
Increased RS-FC	Visuospatial Network	R	Superior parietal gyrus (BA7)	x 26; y -62; z 56	3	4.53
Group x Time interaction						
DUAL-TASK + AOT-MI > DUAL-TASK	aSAL	L	Anterior prefrontal cortex (BA10)	x -26; y 58; z 12	9	6.24
DUAL-TASK + AOT-MI < DUAL-TASK	aDMN	R	Frontal pole	x 2; y 74; z 4	6	4.05
		R	Frontal pole	x 6; y 70; z -8	3	4.71
		R	Anterior prefrontal cortex (BA10)	x 18; y 70; z -4	1	4.4

Results are shown at $p<0.05$ FWE corrected for multiple comparisons. Only significant results are reported. ***Abbreviations:*** *AOT-MI=action observation and motor-imagery; BA=Brodmann area; aDMN=Anterior Default Mode Network; aSAL=Anterior Salience Network; ECN=Executive Control Network; L=left; MNI=Montreal Neurological Institute; N=number; R=right; RS-FC=resting-state functional connectivity; RSN=resting-state network.*

Table 6. Significant relationships between RS-FC and CANTAB changes after training.

	RSN	CANTAB Subtests	Side	Brain regions (BA areas)	MNI coordinate	N of voxels	Intensity (Index)
DUAL-TASK + AOT-MI							
Reduced RS-FC	aDMN	Better accuracy in AST, total correct trials in set-shifting condition	R	Frontal pole	x 2; y 74; z 8	2	2.98

Coordinates (x, y, z) are in Montreal Neurological Institute (MNI) space. Results are shown at $p<0.05$ FWE corrected for multiple comparisons. Only significant results are reported. ***Abbreviations:*** *aDMN=Anterior Default Mode Network; AST=Attention Switching Task; BA= Brodmann area; CANTAB=Cambridge Neuropsychological Test Automated Battery; MNI=Montreal Neurological Institute; N=number; R=Right; RS-FC=resting-state functional connectivity; RSN=resting-state network.*

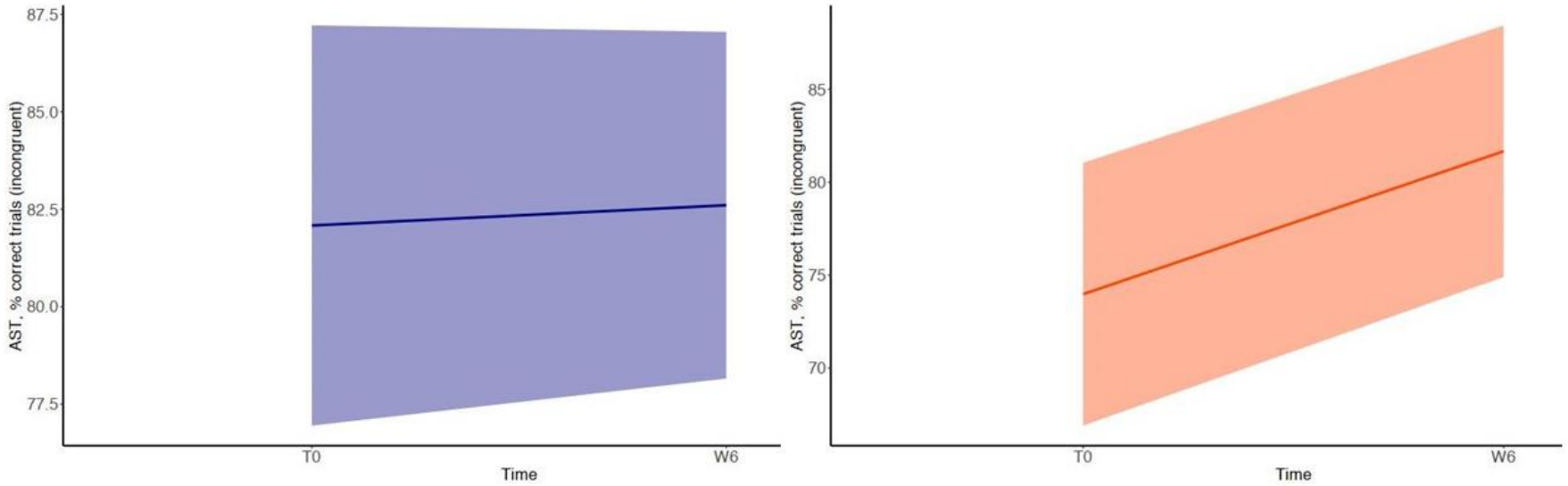


Figure 1. *Cognitive changes assessed with CANTAB battery (T0-W6) in DUAL-TASK patients (left, violet) and DUAL-TASK + AOT-MI patients (right, orange) over the training period. Changes in the AST: percentages of correct trials specifically in set-shifting (incongruent) conditions. Time is reported on the x-axis, while cognitive changes (%) are reported on the y-axis. Only significant results are shown.* ***Abbreviations:*** *AST = Attention Switching Task.*

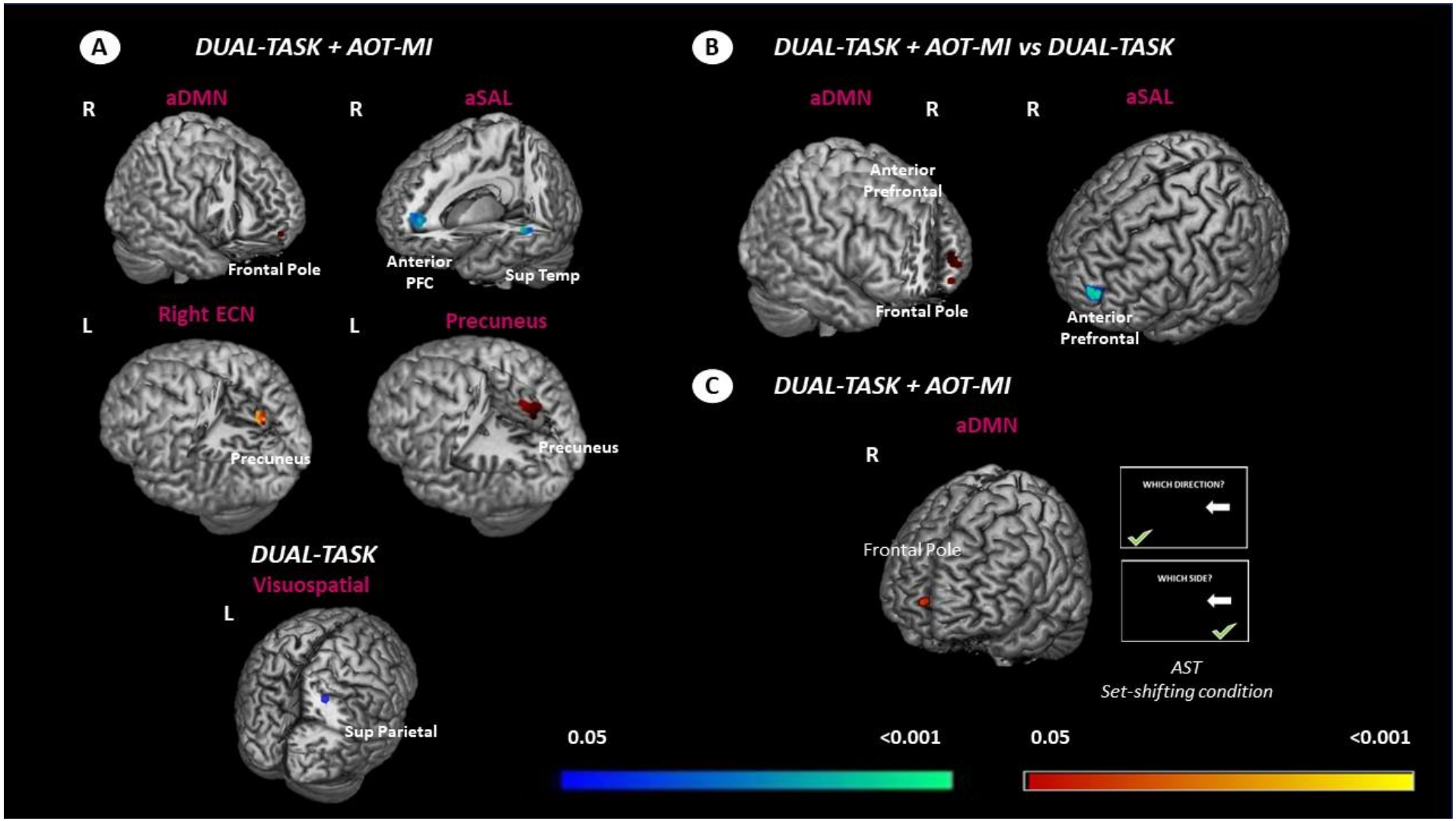


Figure 2. (A) *Within-group RS-FC changes after training in DUAL-TASK+AOT-MI (upper section) and DUAL-TASK (lower section)*

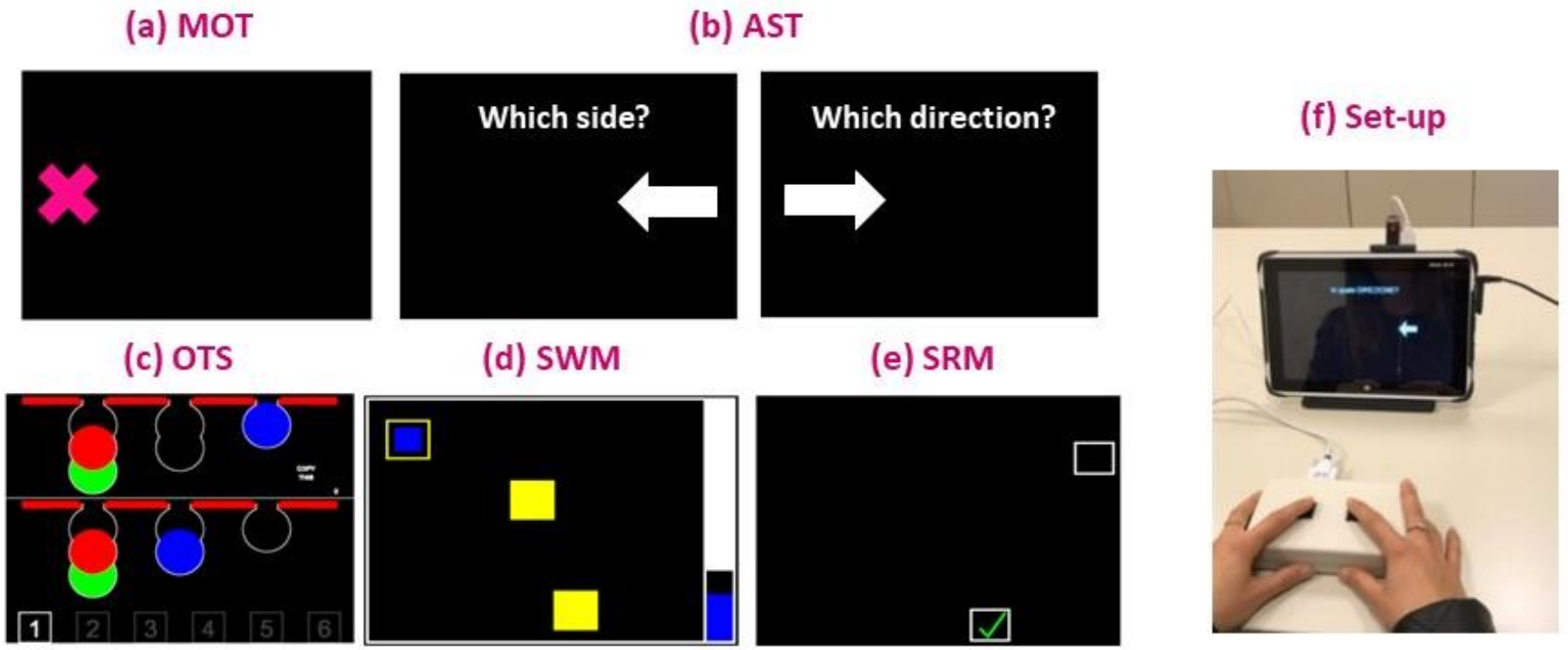
groups; ***(B)*** *Between-groups RS-FC changes after training in the DUAL-TASK + AOT-MI > DUAL-TASK group;* ***(C)*** *Cognitive-fMRI correlations within the anterior Default Mode Network (aDMN) in the DUAL-TASK + AOT-MI group. Results are overlaid on the Montreal Neurological Institute (MNI) standard brain in neurological convention and displayed at p<0.05 Family-wise error corrected for multiple comparisons. Only significant results are reported. Coloured bar represents p values. Abbreviations: AST = Attention Switching Task.*

4.7 Supplementary materials

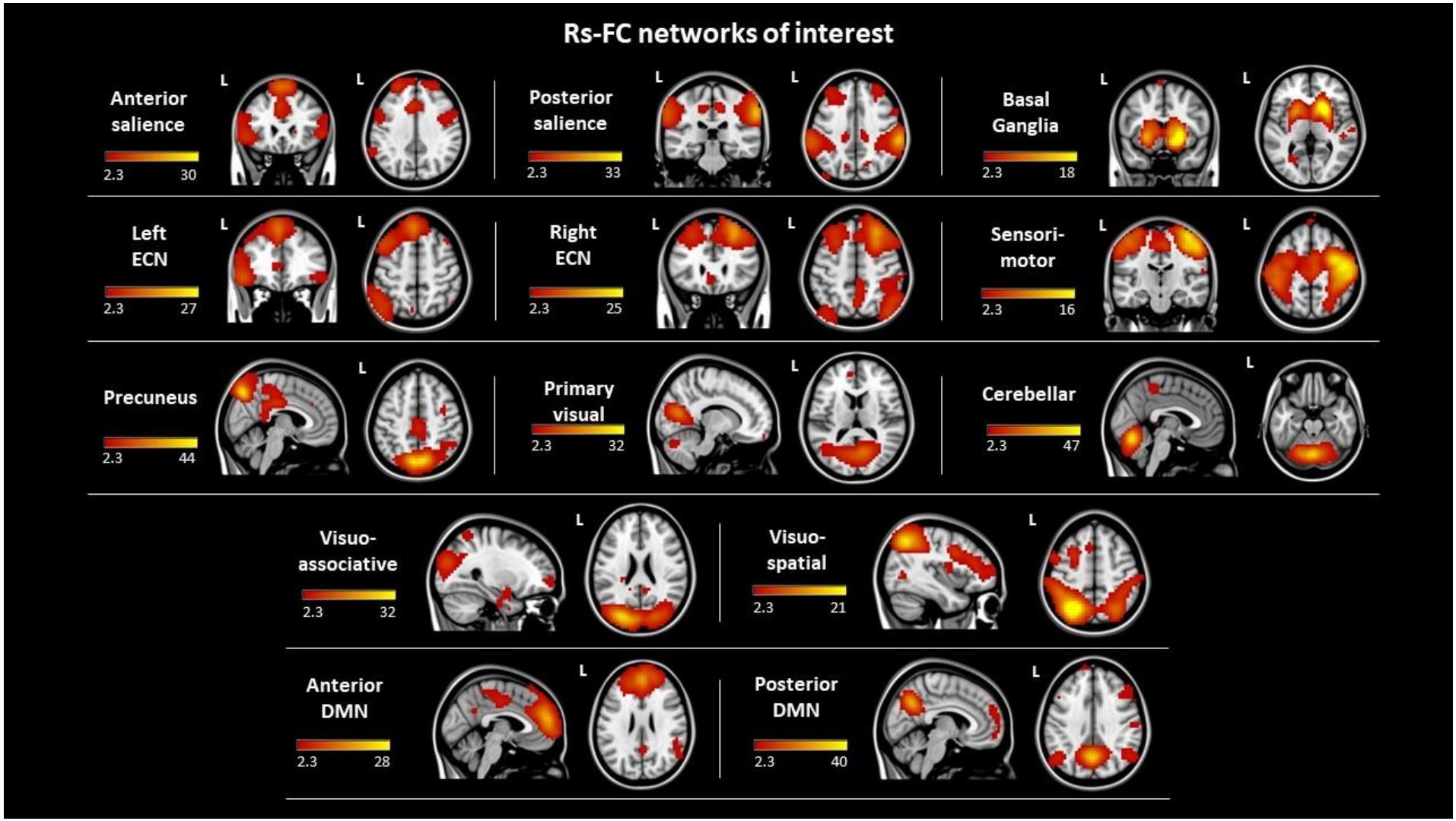
Supplementary Table 1. Summary of the selected CANTAB neuropsychological battery tests.

Cognitive CANTAB sub-tests	Cognitive domain	Outcome measures	Administration Modality
Motor Screening Task (MOT)	Sensorimotor function and comprehension	• Response latency • Accuracy	Coloured crosses are presented on the screen (one at a time). Participants must press the cross as quickly and accurately as possible.
Attention Switching Task (AST)	Multitasking ability	Ability to manage multitasking and interference of incongruent task-irrelevant information in terms of: • Response latency • Accuracy	An arrow is displayed on either side of the screen (left or right) and can point in either direction (left or right). Participants must select the left or right button on the screen according to "the side on which the arrow appeared" or the "direction in which the arrow was pointing". The test is divided in single *vs* multitasking blocks.
One Touch Stockings of Cambridge (OTS)	Planning ability	• Number of problems solved on first choice • Response latency	Two displays containing three coloured balls held in stockings or socks are presented. The experimenter first demonstrates how to move the balls in the lower display to copy the above pattern. Afterwards, the participant must work out how many moves the solutions require to copy the above display.

Spatial Recognition Memory (SRM)	Visuo-spatial recognition memory	• Number of correct trials • Percentage of correct trials • Response latency	Participants are presented with a white square, which appears in a sequence at five different locations on the screen. Afterwards, the participants might choose which square (among pairs) he/she has previously seen.
Spatial Working Memory (SWM)	Working memory and strategy	• Strategy in completing the task • Working memory errors	A number of coloured boxes appear on the screen, and the aim is that by selecting the boxes and using an elimination strategy, the participants should find one yellow "token" in each of a number of boxes. The participants need to use the tokens to fill up an empty column on the right section of the screen.



Supplementary Figure 1. *Schematic representation of CANTAB sub-tests:* ***(a)*** *Motor Screening Test;* ***(b)*** *Attention Switching Task;* ***(c)*** *One Touch Stockings of Cambridge;* ***(d)*** *Spatial Working Memory;* ***(e)*** *Spatial Recognition Memory and* ***(f)*** *example of CANTAB set-up.*



Supplementary Figure 2. *Resting state functional connectivity networks of interest. Results are overlaid on the Montreal Neurological*

Institute (MNI) standard brain and displayed from Z= 2.3 threshold. Abbreviations: rs-FC=resting state functional connectivity; L=Left. Coloured bars represent Z values.

Chapter 5 – Clinical and brain functional MRI effects of a rehabilitative training of upper limb using immersive Virtual Reality in people with Parkinson's disease: an ongoing study

5.1 Introduction

As described in Chapter 1, paragraph 1.2.3, physiotherapy is considered as an useful approach for the motor rehabilitation of patients with PD. Over the last years, increasing attention has been paid to non-pharmacological approaches as complementary treatment in addressing PD symptoms (Connolly & Lang, 2014). Non-pharmacological treatments include, for example, physiotherapy, cognitive rehabilitation, exercise training, treadmill practice and, more recently, technology-based interventions. Specifically, the field of neurorehabilitation aims to maintain motor function and increase patients' independence and safety through continuous and repeated practice, thanks to the possibility to modify brain functional connectivity and activation of motor and cognitive networks, which are usually impaired in PD.

Upper limb deficits significantly limit motor performance during activities of daily living in patients with PD (Muslimovic, Post et al., 2008). Among motor features, bradykinesia has an impact on gait and balance, and it significantly affects upper limb functions in many daily activities, such as handwriting, smartphone/tablet management and appropriate use of cutlery for eating (Radder, Sturkenboom et al., 2017). Considering the early development of upper limb deficits in patients with PD and that antiparkinsonian dopaminergic medication can only partially improve these skills, it is of utmost importance to define non-pharmacological interventions addressing upper limb impairment for these subjects to maintain a satisfying quality of life (Nackaerts, Vervoort et al., 2013). In the last few years, increasing attention has been paid to novel physiotherapy approaches aimed at improving upper limb motor activities in PD patients (Nackaerts, Nieuwboer et al., 2016). A visually cued amplitude training has been shown to improve handwriting skills, suggesting promising possibilities to modify upper limb motor behaviour in individuals with early to mild PD (Nackaerts et al., 2016). Besides the pure motor impairment, recent studies in PD suggested alterations in movement perception and a consequent diminished awareness of the acting self (Kloeters, Hartmann et al., 2017, Sakurada, Knoblich et al., 2018). These cognitive alterations may affect movement production by contributing to the progressive reduction of movement amplitude and speed during a repetitive motor task such as handwriting (Kloeters et al., 2017, Sakurada et al., 2018).

As previously detailed in paragraph 1.2.3, VR is a new technology consisting in a computer-generated scenario where the user's physical presence is projected onto a screen for allowing the user-VR interaction. The peculiar characteristic of immersive VR is the possibility to induce strong sensations of "presence in" and "interaction with" a fictitious environment. In the last years, VR has been successfully used as a rehabilitation tool to promote motor learning in a safe environment with particular focus on balance and locomotion deficits in different neurological disorders (Corbetta, Imeri et al., 2015, Dockx, Bekkers et al., 2016, Mirelman, Maidan et al., 2013). To date, this intervention is still underused for training upper limb functions in patients with PD (Muslimovic et al., 2008), with only one study suggesting the successful effect of VR on PD upper limb motor control (Arias, Robles-Garcia et al., 2012). Finally, neural modifications induced by VR training are still poorly understood. FMRI has been successfully used to understand the mechanisms underlying the efficacy of non-pharmacological treatments in many neurological conditions including PD (Agosta, Gatti et al., 2017, Canu, Sarasso et al., 2018) and it has proved able to detect subtle effects associated with low-intense or brief duration trainings (Agosta et al., 2017, Canu et al., 2018). A quite recent fMRI study investigated brain plasticity changes associated with a VR training aimed at improving walking abilities in PD patients (Maidan, Rosenberg-Katz et al., 2017). This study showed that VR gait training relative to gait training alone was associated with reduced fall rates and decreased functional activity in the prefrontal cortex, likely reflecting increased brain efficiency in trained patients (Maidan et al., 2017). Another work which studied the synergistic effect of VR and Exergaming on motor and cognitive aspects of PD, and on RS-FC compared to standard treatment showed that rehabilitation with VR and Exergaming ameliorates motor aspects (such as balance and gait), general cognition (specifically in attentive-executive functions) and increased brain functional connectivity of the precuneus in PD patients (Hajebrahimi, Velioglu et al., 2022).

Motor behaviour strictly depends on the interaction between three core elements: the brain, the body, and the environment (Nackaerts et al., 2016, Radder et al., 2017). Rehabilitative treatments addressed to modify motor behaviour are usually targeted at promoting a change in the relationship between the body and the brain, while VR offers the unique possibility to act on the environment, thus manipulating the relationship

among all the elements of the brain-body-environment system and providing a more effective change on motor behaviour.

Humans feel in charge and responsible for their own actions in the environment, and this outcome has been defined as "awareness of action" or, more commonly, "sense of agency". James W. Moore in 2016 defined the sense of agency as *"this feeling of being in the driving seat when it comes to our actions"* (Moore, 2016). However, there is evidence that sometimes individuals feel that this experience of "being in charge" is not accurate and corresponding to reality. In fact, our brain appears to actively construct the sense of agency and our experiences of agency can be quite divorced from the facts of agency. Furthermore, sense of agency is extraordinarily flexible and subject to modifications. Several studies reported that the sense of agency is disrupted in several psychiatric and neurological conditions; among neurological disorders, movement disorders seem to be particularly affected since the neural correlates of sense of agency partially overlap with those brain areas involved in motor control of voluntary movements, such as the supplementary and pre-supplementary motor areas (Zapparoli, Seghezzi et al., 2020). Therefore, impairment in these motor networks might end up in disruption of the subjective experience of agency. Furthermore, agency impairments can also be attributed to a side-effect of dopaminergic treatment in the specific case of patients with PD (Moore, Schneider et al., 2010).

In the last twenty years, several neuroimaging studies tried to explore the neural correlates of sense of agency: specifically, the inferior parietal cortex and the angular gyrus seem to be involved in the sense of agency experience (Preston & Newport, 2008), with the parietal cortex being activated also in conditions of mismatch between the expected outcome and the actual action ; moreover, sense of agency generation is attributed to the involvement of frontal and prefrontal cortices (Renes, van Haren et al., 2015). However, recent studies pointed out that, given the complexity of sense of agency phenomenon, it is not accurate to narrow down sense of agency correlates to single brain areas, but rather to brain networks. A quite new hypothesis is that the key core of sense of agency resides in the connectivity between pre-supplementary motor area (which is involved in action generation) and parietal areas deputized to monitoring action consequences (Haggard, 2017).

Behaviour in PD patients is mostly guided by external rather than internal cues, which is the reason why motor deficits occur in the absence of external references, and they ameliorate when external cues are provided. Sense of agency disruption in PD might therefore be explained as a failure of weighting internal and external cues in normal motor experiences (Jahanshahi, 1998), but the number of studies supporting this hypothesis is still scarce, and a comprehensive understanding of this phenomenon in PD is still lacking.

Starting from these assumptions and previous findings from the available scientific literature, we hypothesize that the employment of a physiotherapy training in immersive VR (VR-training), which provides an enhanced visual feedback of spatio-temporal movement features during upper limb motor functions and specific activities (in our case, handwriting and touch screen technology usage), might induce long-term modification of the motor behaviour in people with PD. Furthermore, we want to demonstrate that VR-training would enhance brain functional efficiency (as assessed with fMRI) in both sensorimotor and cognitive associative networks, thus contributing to improve motor performance in PD subjects. In addition, we assume that, by providing precise visually cued feedback and inducing strong sensations of "presence in" and "interaction with" the environment, VR-training might ameliorate bodily self-awareness and sense of agency in people with PD.

Specifically, we propose a study which aims are:

1. to assess the efficacy of an 8-week physiotherapy training on dominant upper limb motor function (i.e., in terms of speed and amplitude of movement) and activity (e.g., handwriting and touch screen technology usage) in PD subjects performing a VR-training relative to a real setting training (RS-training);
2. to assess the effects of VR-training relative to RS-training in PD subjects on brain fMRI activity during hand-tapping tasks in both VR environment (VR-motor task) and real setting (RS-motor task); and to investigate the association between brain functional activity modifications and patient clinical outcomes;
3. to define the effects of VR-training relative to RS-training in PD subjects on training-specific (bodily self-awareness and sense of agency) and PD-related cognitive functions (executive functions, memory and visuospatial abilities).

Enrolment, evaluations and training of participants are still ongoing, therefore only the methodological aspects of the project and study materials will be described in the following sections.

5.2 Materials and methods

5.2.1 Participants

The study is an ongoing monocentric (San Raffaele Hospital – Milan, Italy), single-blinded randomized clinical trial. We plan to include a total of 40 patients with PD and 30 age- and sex-matched healthy controls. Right-handed PD outpatients with idiopathic PD (Hughes, Daniel et al., 1992) will be recruited at the Movement Disorders Unit, Unit of Neurology, IRCCS Ospedale San Raffaele, Milan, Italy and screened by neurologists with expertise on movement disorders. On the other hand, age- and sex-matched right-handed healthy controls will be recruited by word of mouth among nonconsanguineous relatives and institute personnel. To date, we enrolled 16 healthy controls and 14 PD patients.

5.2.2 Study design

PD patients and healthy controls are screened to evaluate their eligibility (see paragraph 5.2.3 for inclusion and exclusion criteria) and undergo the same clinical (except for neurological assessment), cognitive and MRI evaluations. Patients with PD will be randomized into two groups, VR-training and RS-training, via minimization method to balance the groups for the following variables: age, sex, education (≤8 years / 9-13 years / >13 years), presence/absence of MCI, right hand motor impairment (MDS-UPDRS-III items 3, 4 or 3.5 ≤2 / >2). All participants with PD will participate in an 8-week training program twice a week. Participants with PD will undergo clinical and MRI assessments before training (T0), after training (8 weeks – W8) and after a 3-month follow-up (20 weeks – W20). Healthy controls will be assessed only at T0. Assessors blinded to participants' allocation will carry out the evaluations. To date, we enrolled 14 PD patients (7 allocated in the VR-training group and 7 in the RS-group) who underwent all screening evaluations and T0 assessment; among them, 11 patients have completed both W8 and W20 assessments, while 3 patients are currently undergoing either VR- or RS-training.

In the VR-training group only, PD subjects will wear a head-mounted display and their upper limb movements will be captured by a motion tracker, transmitted to a computer, and processed to create an upper limb avatar. The avatar image will be then projected to the patient head-mounted display. Participants will be asked to observe the avatar of their upper limb while performing exercises. Figure 1 shows exercises proposed to patients in the VR-training group.

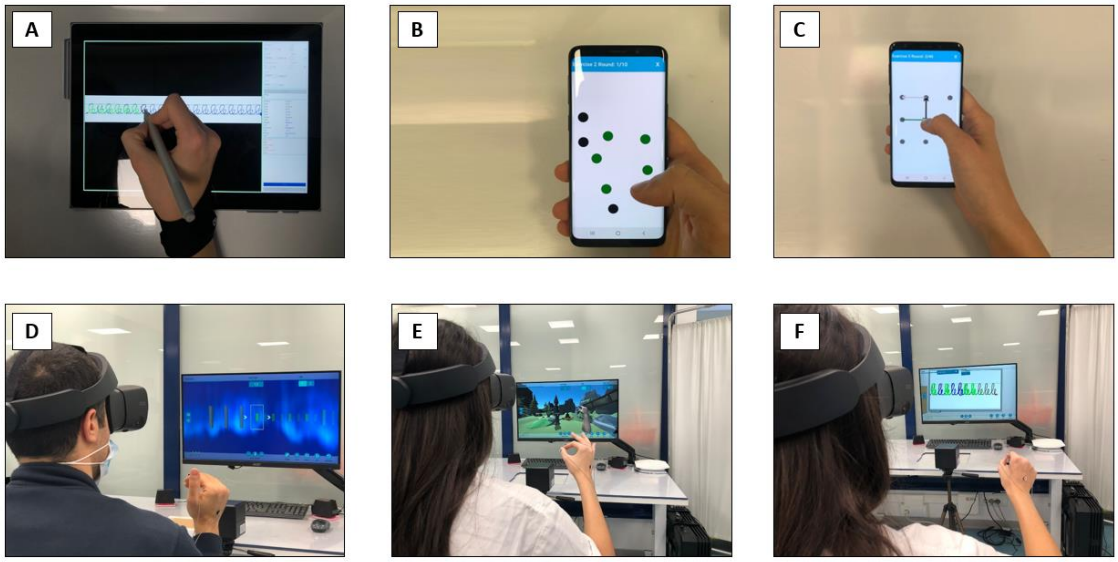


Figure 1. VR-training exercises. *(A) Tablet-based propaedeutic exercises for writing skills; (B-C) Smartphone-based exercises to improve manual dexterity; (D-F) Examples of VR-based exercises to improve movement amplitude and speed.*

Differently from the VR-training group, the RS-training group will perform the same exercises, but without the use of technological devices, such as head-mounted display, smartphone and tablet. All PD patients will undergo fMRI scans on a 3T scanner at T0, W8 and W20 during the performance of two tasks: RS- and VR-motor tasks. During fMRI, all participants will wear an MRI-compatible sensor-based smart glove and will perform a hand-tapping motor task consisting of continuously opening and closing the hand as faster and ampler as possible. Hand motor speed and amplitude parameters will be acquired through the glove and transmitted via optical fibers to a computer. In the VR-motor condition only, participants will also wear MRI-compatible smart glasses. In this condition, motor parameters will be processed by the computer to create an avatar of the participant hand. Avatar images will be then projected onto the display of the participant glasses. Participant will be asked to observe the avatar of their proper hand while performing hand-tapping. The amplitude and speed parameters of the avatar movements

will be reduced to create a perceptive mismatch between the real participant movement and the observed avatar movement. The perceptive mismatch will stimulate the participants to perform faster and ampler movements. At T0, W8 and W20, all PD participants will undergo cognitive assessments including: i) Rubber Hand Illusion (RHI paradigm) to assess bodily self-awareness and sense of agency (please refer to paragraph 5.2.4.4), and ii) CANTAB computer-based sessions (please refer to paragraph 5.2.4.5) to investigate PD-related cognitive functions (executive functions, memory and visuospatial abilities). Healthy controls will undergo the same assessment only at T0. Study design is summarized and depicted in Figure 2.

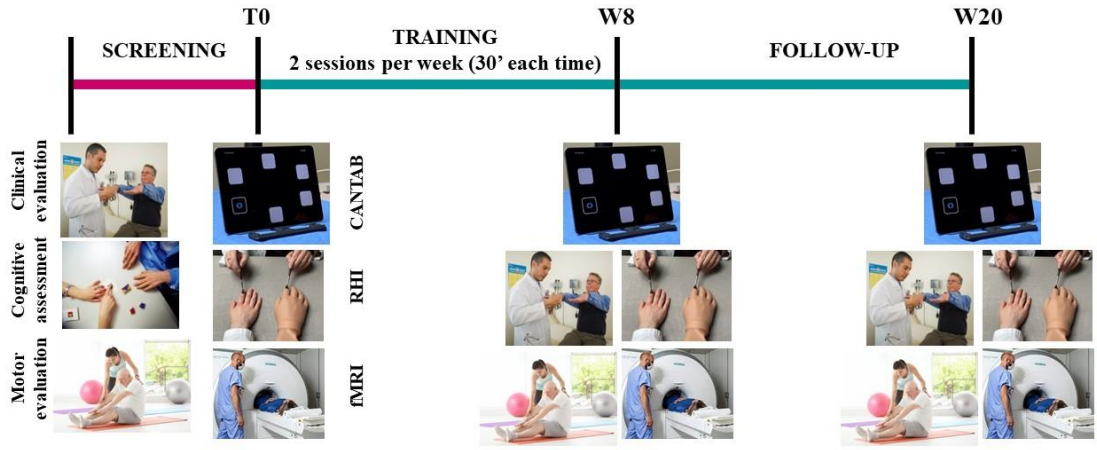


Figure 2. Study design. *Abbreviations: CANTAB=Cambridge Neuropsychological Test Automated Battery; fMRI=functional magnetic resonance imaging; RHI=Rubber Hand Illusion.*

5.2.3 Inclusion and exclusion criteria

PD patients will be enrolled according to the following inclusion criteria:

- H&Y ≤3 while on medication (ON state);
- age ≤ 85 years;
- stable dopaminergic medication for at least 4 weeks and without any changes during the observation period;
- right-side involvement according to H&Y and MDS-UPDRS III;
- handwriting difficulty defined by a score greater than or equal to 1 on item II.7 of the MDS-UPDRS;

- oral and written informed consent to study participation.

Exclusion criteria for both patients and healthy controls are:

- MMSE lower than 24 (for patients with PD) or 28 (for healthy controls);
- visual impairments that could interfere with the immersive virtual environment;
- upper limb deficits impeding handwriting;
- history of (other) systemic, neurologic, psychiatric diseases, head injury and cerebrovascular alterations visible at an MRI scan;
- family history of neurodegenerative disorders;
- history of alcohol and/or psychotropic drug abuse;
- contraindications to undergo MRI;
- denied oral and written informed consent to study participation.

5.2.4 Assessments

Patients with PD and healthy controls will both undergo motor functional, neuropsychological and MRI assessments, while PD will undergo also neurological examination.

For PD, all clinical evaluations will be performed in the ON condition (under regular dopaminergic medication), while the MRI scans will be obtained during OFF medication state (at least 12 h after their regular evening dopaminergic therapy administration) to mitigate the effects of medication on neural activity. The same day as MRI acquisition, neurological assessment will be repeated during OFF time.

5.2.4.1 Motor functional assessment

Motor functional assessments consist of a battery of tests and questionnaires performed by an experienced physiotherapist to evaluate handwriting, finger/hand bradykinesia and manual/finger dexterity. Only data from manual/finger dexterity assessments will be included in the analyses of this study.

The following tests will be performed on a Samsung Galaxy S9 smartphone:

- vertical tapping test;

- horizontal tapping test;
- swipe and slide test (upward, downward, leftward, rightward);
- swipe-slide pattern task.

The software to perform these tests has been developed by expert engineers to be compatible with the Khymeia Virtual Reality Rehabilitation System for Windows and a dedicated smartphone application has been developed for the Android phone.

Lastly, the following test and questionnaires will be administered:

- Purdue Pegboard Test (PPT);
- Manual Ability Measurement (MAM)-36;
- Parkinson disease questionnaire (PDQ)-39;

The tests will be performed in a quiet room with the participants sitting at a desk on a height-adjustable armchair. The smartphone is encased in a cover that will prevent unintentional touches of the navigation bar. Before every test, the evaluator will provide an explanation with the help of images and videos shown on the computer display.

The following tests for the evaluation of handwriting function are completed using a Surface Pro 7 tablet and a stylus:

- SOS test;
- repetitive cursive loop test;
- closed loop test;
- funnel test.

The following tests are performed using Polhemus sensors:

- hand tapping test;
- finger tapping test.

The software to perform these tests was developed by expert engineers to be compatible with the Khymeia Virtual Reality Rehabilitation System for Windows. Lastly, SOS test is repeated on paper. The tests are performed in a quiet room with the participants sitting at a desk on a height-adjustable armchair. The tablet is embedded in the desk (to provide support for the forearm and wrist). Before every test, the evaluator provides an explanation with the help of images and videos shown on the computer display.

5.2.4.2 Neurological assessment

An experienced neurologist will perform neurological evaluations at each time point. The following scales and questionnaires will be administered:

- H&Y (during ON and OFF time) (Hoehn & Yahr, 1967);
- MDS-UPDRS-II (Goetz, Tilley et al., 2008);
- MDS-UPDRS-III (during ON and OFF time) (Goetz et al., 2008).

Scores of the items 3.4 and 3.5 of the MDS-UPDRS-III will be used in the randomization with minimization process, in particular a cut-off of 2 will be set to divide patients according to hand motor symptoms severity. These items assess respectively finger- and hand-tapping with the following score:

- 0: no problems;
- 1: any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) the amplitude decrements near the end of the 10 taps;
- 2: any of the following: a) 3 to 5 interruptions during tapping; b) mild slowing; c) the amplitude decrements midway in the 10-tap sequence;
- 3: any of the following: a) more than 5 interruptions during tapping or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the first tap;
- 4: patient cannot or can only barely perform the task because of slowing, interruptions, or decrements.

5.2.4.3 Neuropsychological evaluation

All PD patients and healthy controls will be screened at T0 for inclusion in the present study with a comprehensive neuropsychological assessment. According to MDS Task Force guidelines (please refer to Chapter 1, paragraph 1.1.6) (Litvan, Goldman et al., 2012), we decided to administer a Level II comprehensive assessment considering all cognitive domains of interest and choosing at least two neuropsychological tests for each of the following domains: memory, visuospatial skills, attention and executive functioning, and language.

A blinded and experienced neuropsychologist will perform the following assessments: MMSE (Folstein, Folstein et al., 1975) and Montreal Cognitive Assessment Test (MoCA)

(Santangelo, Siciliano et al., 2015) for global cognition; Modified Card Sorting Test (Caffarra, Vezzadini et al., 2004), phonemic verbal fluency test (Alberici, Geroldi et al., 2007) and Ten-point Clock Drawing Test (Manos, 1999) for executive functions; TMT (Giovagnoli, Del Pesce et al., 1996) and digit span backward (Monaco, Costa et al., 2013) for attention and working memory; RAVLT immediate and delayed recall (Carlesimo, Caltagirone et al., 1996) and recognition and recall of the Rey-Osterrieth Complex Figure (Caffarra, Vezzadini et al., 2002) for memory; copy of the Rey-Osterrieth Complex Figure (Caffarra et al., 2002) and freehand copying of drawings without landmarks (Carlesimo et al., 1996) for visuospatial skills; single-word naming and comprehension (CaGi test) (Catricala, Della Rosa et al., 2013) for language assessment; handwriting abilities will be assessed with the Aachener Aphasie Test (writing sub-test) (Luzzatti, 1994) .

Furthermore, the presence/absence of depression and/or anxiety will be ruled out with the BDI (Beck, Ward et al., 1961) and the HAMA (Hamilton, 1959); the presence of apathy will be investigated with the Apathy Rating Scale (Starkstein, Mayberg et al., 1992) and the Italian validation of the Dimensional Apathy Scale (Santangelo, Raimo et al., 2017). In addition, the NPI (Cummings, Mega et al., 1994) will be used to perform a behavioural assessment of the caregiver.

5.2.4.4 The assessment of sense of agency and embodiment: the Rubber Hand Illusion paradigm

As reported in the Introduction (paragraph 5.1), movement disorders and PD in particular offer a good example for studying impairment in sense of agency and embodiment. Sense of agency relies on the integration between sensory signals coming from different modalities, namely multisensory integration (Stein & Stanford, 2008). Feeling of ownership is the result of the correspondence of spatial, temporal, visual and proprioceptive inputs from a body limb, and this phenomenon might be demonstrated with the "Rubber Hand Illusion" (RHI) experimental paradigm, developed by Botvinick and Cohen in 1998 (Botvinick & Cohen, 1998). This illusion can be induced by placing a rubber hand (RH) in full view on a table in front of a participant, and then by touching synchronously both the rubber and the real participant's hands, the latter being hidden from sight. After this repetitive visuo-tactile stimulation of both hands, participants

usually report to feel the touch where they see it on the RH, rather than where it is applied on their real hand; furthermore, participants report to perceive the RH as their own. On the contrary, asynchronous stimulation, either temporal, or spatial, or both, of the two hands might suppress this illusion (Shimada, Fukuda et al., 2009).

This illusion is commonly investigated with implicit and explicit measures which quantify distinct aspects of the participant's experience of the RHI (Walsh, Guilmette et al., 2015): the implicit measure corresponds to the "proprioceptive drift", which can be defined as a shift in the perceived position of the real hand towards the RH. Proprioceptive drift is measured by asking the participant to estimate the position of the real hand both before and after stimulation: the drift is the result of the difference between these two measures (baseline position – post-stroking position). On the other hand, explicit measure of ownership corresponds to collecting the subjective feeling of body ownership, which is usually obtained by asking the participant to self-report his/her impressions *via* a questionnaire. However, previous studies pointed out a relative variability on both RHI measures based on specific dispositional and personality traits (Kallai, Hegedus et al., 2015).

To date, a scarce number of studies has been published on RHI phenomenon and PD patients (Ding, Palmer et al., 2017, Ding, Palmer et al., 2018, Waldmann, Volkmann et al., 2020). These studies showed that PD patients usually experience a greater proprioceptive drift in both synchronous and asynchronous conditions compared to healthy controls, therefore showing higher proprioceptive bias, which becomes even greater at more advanced disease stages; furthermore, PD patients usually score higher than controls in those RHI questionnaire items relative to the asynchronous condition (which means that they perceived the illusion even in the control condition), while no differences are observed in the synchronous condition compared to controls (Ding et al., 2017, Ding et al., 2018). In our study, we expect to retrieve similar results in line with the previous literature, and we assume that, after the 8-week physiotherapy training, PD patients will ameliorate (and therefore reduce) their proprioceptive bias towards the RH, thus resembling the performance of healthy subjects. Additionally, we also expect to observe a further improvement in VR-training patients compared to RS-training patients. To our knowledge, no previous studies have been published on using fMRI to assess RHI in patients with PD. However, we expect that, at W8, both our patient groups (VR-training

and RS-training) will show brain functional reorganization processes, with the VR-training group in particular revealing brain functional changes in cognitive-associative networks (e.g., areas associated with sense of agency/awareness, attentive-executive and visuospatial networks). Furthermore, according to previous findings, we expect that increased efficiency of cognitive-associative areas can further facilitate the efficiency of the sensorimotor network, and a correlation between brain plasticity and clinical changes in the VR-training group is predictable (Agosta et al., 2017).

In our study, all PD patients and healthy controls will be administered the RHI at T0, and only PD patients will repeat RHI at W8 and W20 (please refer to Figure 2). Patients will perform the RHI in ON state. The RHI set-up that was built for the present study consists of two wooden boards for hands (one for left hand stimulation, the other for the right hand), two prosthetic hands, a numbered ruler, a metallic marker, a black cloth and two paintbrushes (our RHI set-up is displayed in Figure 3).

Figure 3. RHI set-up. *Wooden board for left hand stimulation, left prosthetic hand, two paintbrushes, numbered ruler and the metallic marker are depicted.*

Before stimulation, participants are initially asked to remove all objects or jewellery from both hands, fingers, and wrists to avoid any proprioceptive interference with the experiment. For example, regarding left hand stimulation (which is depicted in Figure 4), participants are asked to insert their left hand into the left compartment on the wooden board, which function is to conceal the real hand of the participant from sight, and the other hand on the wooden board surface. The RH and the real hand are positioned in similar anatomical positions, and their indexes are positioned at a distance of 20 cm. The experimenter then covers both the real hand and the RH with a black cloth, in a way that the participant can only see the RH. Participants are initially asked to stare at the RH for two minutes, and after that, two paintbrushes are employed to stroke either synchronously

or asynchronously (spatially and temporally) both the RH and the real hand (the latter being hidden from sight). During stroking sessions, each lasting 90 seconds, participants are instructed to stare at the RH. The brushstrokes are small and applied on the dorsal surface of the index fingers, at a frequency of approximately 2 Hz. Immediately before and after each stroking session, participants are asked to verbally refer the perceived position of their own real index finger on a ruler (the participant's side is not numbered) positioned in front of them: the experimenter slides a metallic object (depicted in Figure 4) on the numbered ruler, and the participant is instructed to say "STOP" when the metallic object reaches the felt position of the real finger. Figure 4 shows RHI administration from the experimenter's perspective.

Figure 4. RHI administration: view from the experimenter's perspective. *The experimenter is stroking both the real (hidden in the left compartment) and the RH (on the board surface) with two paintbrushes for 90 seconds. After that, the experimenter is going to slide the metallic marker on the numbered ruler and ask the participant to say "STOP" when the marker has reached the perceived position of the participant's index.*

Furthermore, we will also measure the 'proprioceptive drift', which is the difference between the perceived position of the index finger before and after stimulation (baseline position – post-stroking position). Regarding the left hand, in our case (based on the orientation of the numbered ruler on the wooden board – please refer to Figure 4), a negative value for proprioceptive drift indicates that the perceived localization of the real left finger is closer to the RH after stroking (therefore indicating the presence of the RHI), while positive values indicate absence of the RHI phenomenon. For the right hand,

positive values for proprioceptive drift indicate the presence of the RHI, while negative values indicate the opposite situation. To account for potential manual lateralization, each hand will be tested in both conditions (synchronous and asynchronous), resulting in four experimental conditions, which are performed in randomized order (Left hand – synchronous; Left hand – asynchronous; Right hand – synchronous; Right hand – asynchronous). Synchronous and asynchronous conditions are repeated twice for each hand. The Italian version of our scoring sheet used for registering both baseline assessment and post-stroking assessment in the four experimental conditions is reported in the Appendix (Additional Material 1).

After each stroking condition, participants will be asked to judge their sense of embodiment with the RH through a 6-item *ad hoc* questionnaire derived from the one created by Botvinik and Cohen (Botvinick & Cohen, 1998). The presentation order of each item of the questionnaire will be randomized for all participants and all experimental conditions. The first three items of the questionnaire are taken as a global subjective measure of the RHI (Illusion score), while the remaining three items are considered as control affirmations (and therefore referring to a Control score). Participants will be asked to rate their agreement on a 10-point scale (0= "I totally disagree"; 10= "I totally agree"). An example of the scoring sheet for the RHI questionnaire is reported in the Appendix (Additional Material 2).

As reported previously in the present paragraph, to take into account possible individual effects of personality and dispositional traits relatively to the RHI, our participants will be administered the Italian adaptation of the 10-item short version of Big Five Inventory scale (Rammstedt, 2016) (the original one has 44 items) (John, 1991). This questionnaire is a self-report instrument to assess the five main constructs of personality, namely extraversion, agreeableness, conscientiousness, neuroticism, and openness. Figure 5 reports the English version of the questionnaire, while the Italian translation (Guido, 2015) is reported in the Appendix (Additional Material 3).

Instruction: How well do the following statements describe your personality?

I see myself as someone who …	Disagree strongly	Disagree a little	Neither agree nor disagree	Agree a little	Agree strongly
… is reserved	(1)	(2)	(3)	(4)	(5)
… is generally trusting	(1)	(2)	(3)	(4)	(5)
… tends to be lazy	(1)	(2)	(3)	(4)	(5)
… is relaxed, handles stress well	(1)	(2)	(3)	(4)	(5)
… has few artistic interests	(1)	(2)	(3)	(4)	(5)
… is outgoing, sociable	(1)	(2)	(3)	(4)	(5)
… tends to find fault with others	(1)	(2)	(3)	(4)	(5)
… does a thorough job	(1)	(2)	(3)	(4)	(5)
… gets nervous easily	(1)	(2)	(3)	(4)	(5)
… has an active imagination	(1)	(2)	(3)	(4)	(5)

Figure 5. English version of the 10-item short version of Big Five Inventory. *From Rammstedt and John, Journal of Research in Personality 2007.*

In addition, as previously reported, variability in the RHI response could arise from individuals' sensory suggestibility, which is dependent on the presence of ambiguity or uncertainty that might highly influence sensory perception. In the context of the RHI, synchronous stroking of both the real hand and the RH creates perceptual ambiguity, and this phenomenon seems influenced by individual's sensory suggestibility (more susceptible individuals would be more susceptible to experiencing the RHI, while less susceptible participants would experience the RHI poorly) (Marotta, Tinazzi et al., 2016). To this regard, we choose to administer the Sensory Suggestibility Scale (SSS) (Gheorghiu, 1995) to all our participants at the end of each experimental session. The SSS has been used in several studies as a measure of indirect suggestibility: the participant, when tested, does not perceive that his/her suggestibility is going to be tested. This scale is composed by ten experimental and four control exercises where tactile, visual, auditory or taste sensations in the participants through *ad-hoc* stimulation and verbal suggestions are induced by the experimenter. In the experimental exercises, the suggested sensations are not physiologically plausible: highly sensory suggestible participants will usually rate with high scores their subjective experience, meaning that the suggested sensation has been vividly perceived. Conversely, low sensory suggestible participants are more likely to give their subjective experience lower scores. Our adaptation of the SSS scoresheet and relative instructions are reported in the Appendix (Additional Material 4). In our study, we expect to find a correspondence between highly

suggestible participants (as assessed with the SSS) and participants more prone to perceive the RHI.

5.2.4.5 Monitoring of cognitive changes due to rehabilitation: the CANTAB assessment

In the Study design (paragraph 5.2.2 and Figure 2), we specified that both PD patients and healthy controls will perform CANTAB Research Suite computer-based assessment at T0. Furthermore, PD patients will perform CANTAB also at W8 and W20. As previously reported in Chapter 4, CANTAB is a computerized neuropsychological battery, which includes a range of cognitive tests assessing several aspects of various cognitive domains. It has been demonstrated that CANTAB tests are sensitive to detect cognitive impairment (Olde Dubbelink, Stoffers et al., 2013) and predict the conversion to dementia (McKinlay, Grace et al., 2009) in patients with PD. According to CANTAB Cognitive Test Selector, we selected those cognitive tests which were most suitable to detect cognitive changes in patients with PD (please refer to (https://www.cambridgecognition.com/cantab/test-batteries/parkinsons-disease/). Specifically, we selected the following tests and versions (estimated durations of each test are reported in parentheses):

1. Motor Screening Task (MOT): Voice (2 min)
2. Pattern Recognition Memory (PRM): Recommended Standard immediate (5 min)
3. Multi-tasking Test (MTT): Standard (8 min)
4. Paired Associates Learning (PAL) Recommended Standard (8 min)
5. Spatial Working Memory (SWM) Recommended Standard (4 min)
6. Pattern Recognition Memory (PRM): Recommended Standard, delayed 20 minutes

The overall assessment will last about 30-40 minutes; since the time interval between visits is of 8 and 20 weeks from T0, our PD patients will be administered parallel and randomized versions for each sub-test to avoid learning effects that might impact our findings. In our study, some of the selected CANTAB sub-tests have been already presented in Chapter 4 (please refer to paragraph 4.2.5). The only different tests are PRM and PAL. AST is named as MTT in the present study, since a newer version of CANTAB Research Suite has been released after the completion of the previous project (please refer

to Chapter 4). The following table summarises the main features of the new additional tests:

Table 1. Summary of the additional CANTAB neuropsychological battery tests.

Cognitive CANTAB sub-tests	Cognitive domain	Outcome measures	Administration Modality
Pattern Recognition Memory (PRM	Visual pattern recognition memory	• Number of correct trials • Percentage of correct trials • Response latency	Visual patterns are presented, one at a time, in the center of the screen. These patterns are designed so that they cannot easily be given verbal labels. In the recognition phase, the participant is required to choose between a pattern they have already seen and a novel pattern. In this phase, the test patterns are presented in the reverse order to the original order of presentation. This is then repeated, with new patterns. The second recognition phase is administered after a delay period, typically 20 minutes later.
Paired Associates Learning (PAL)	Visual memory and new learning	• Number of errors • Number of trials to correctly locate the pattern(s) • Memory score • Stages completed	Boxes are displayed on the screen and are "opened" in a randomized order. One or more of them will contain a pattern. The patterns are then displayed in the middle of the screen, one at a time and the participant must select the box in which the pattern was originally located. If the participant makes an error, the boxes are opened in sequence again to remind the participant of the locations of the patterns.

An example of CANTAB sub-tests administration is reported in Figure 6.

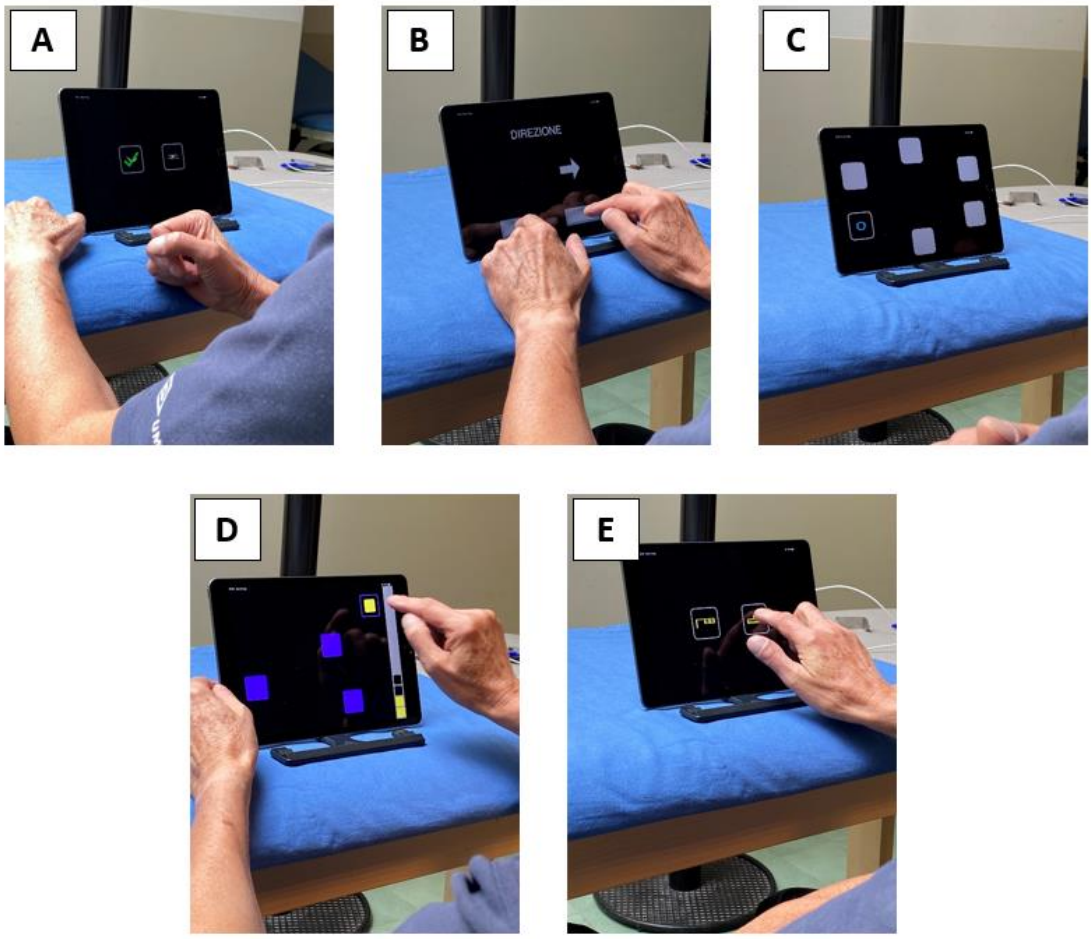


Figure 6. CANTAB Research Suite administration (example) on an I-Pad. *A preliminary motor test is performed to assess motor speed (MOT). After that, five tests are delivered: (A) PRM, immediate recall; (B) MTT; (C) PAL; (D) SWM; (E) PRM, delayed recall (after approximately 30 minutes from immediate recall). Abbreviations: MOT=Motor Screening Task; MTT=Multi-tasking Test; PAL=Paired Associates Learning; PRM=Pattern Recognition Memory; SWM=Spatial Working Memory.*

We expect that, after training, both patient groups will ameliorate their cognitive performances, especially in terms of attention and executive functions, which are the most affected in PD patients. However, we aim to demonstrate whether patients in the VR-training group will perform better than RS-training patients.

5.2.5 MRI

MRI scans will be acquired using a 3.0 Tesla MRI (*Ingenia CX, Philips Medical Systems, Best, The Netherlands*) at San Raffaele Hospital. Both healthy controls and PD will undergo MRI at T0, while PD will be assessed again at W8 and W20 (please refer to Figure 2). Subjects will be placed supine on the bed of MRI. To standardize the

positioning, the head will be placed on the sagittal plane with the canto-meatal line (imaginary line that unites eyes' canto with the external auditory meatus) perpendicular to the horizontal plane of the bed; and on the coronal plane, the laser symmetrically divides the head on the midline. Then the head will be locked, and the upper coil cover will be placed. Structural and functional (RS- and task-based) sequences will be obtained.

5.2.5.1 Structural MRI

The parameters of structural MRI sequences are the following:

- 3D T1-weighted sequence: Repetition Time (TR) = 7.1 ms, Echo Time (TE) = 3.2 ms, flip angle = 9°, 204 contiguous sagittal sections, thickness = 1 mm, Field of View (FOV) = 256 mm x 240 mm, matrix = 256 x 240. Voxel size 1 x 1 x 1;
- 3D T2-weighted sequence: TR = 2500 ms, TE = 330 ms, flip angle = 90°, 192 contiguous sagittal sections, thickness = 1 mm, FOV = 256 mm x 256 mm, matrix = 256 x 258. Voxel size 0.9 x 0.9 x 1;
- 3D Flair sequence: TR = 4800 ms, TE = 269 ms, flip angle = 40°, 192 contiguous sagittal sections, thickness = 1.5 mm, FOV = 256 mm x 256 mm, matrix = 256 x 256. Voxel size 1 x 1 x 1.

3D T1, T2 and flair sequences are used to exclude subjects with structural brain alterations or excessive vascular lesions.

5.2.5.2 Task-based functional MRI

The parameters of fMRI sequences are the following:

- Echo Planar Imaging for "hand-tapping task": TR = 1572 ms; TE = 35 ms; flip angle = 70°; 48 contiguous axial sections; thickness = 3 mm; FOV = 240 mm x 240 mm; matrix = 96 x 94. Voxel size 2.5 x 2.5 x 3.

Participants will wear an MRI-compatible headset which projects commands and images during the task. Participants will perform a total of three fMRI tasks:

1. Hand-tapping task
2. Hand-tapping task with auditory cue
3. Sense of agency task (congruent, mismatched, and scaled conditions)

In the "hand-tapping task", a block design (ABAB) will be used, with periods of cerebral activation (A) alternated with rest periods (B). Activation A corresponds to the execution of the tasks, while rest condition corresponds to the interruption of the task

(stop). Participants are asked to hand tap alternatively as fast and as ample as possible with the right and left hand with eyes open.

FMRI cycle for these tasks is as follow:

- 4 TR of rest;
- 1 TR of “right” command;
- 6 TR of right hand-tapping;
- 1 TR of “stop” command;
- 4 TR of rest;
- 1 TR of “left” command;
- 6 TR of left hand-tapping;
- 1 TR of “stop” command.

Right- and left-hand-tapping is repeated six times for a total duration of 3 minutes and 46 seconds. Hand-tapping task is performed with participants wearing fMRI compatible optic-fiber gloves that register hands movement. Sandbags are used to position participants’ arms and to prevent unwanted movements. Participants familiarize with the experimental condition outside of the scanner. Only data from the right hand are included in the study.

For the “hand-tapping task with auditory cue”, the same block design and procedure are used. Participants are asked to hand tap alternatively as ample as possible with the right and left hand with eyes open following an auditory cue delivered at a frequency of 2 Hz. fMRI cycle is the same as the hand-tapping task.

The “sense of agency task” uses a block design (ABCABC). Periods of cerebral activation (A) are alternated with judgement (B) and rest (C) periods. Block A corresponds to the execution of right hand tapping while observing the projection of a 3D avatar of a hand. The virtual hand could move in three different manners:

1. exactly mimicking the movements of the participants’ hand (“congruent condition”);
2. in an opposite way, namely the avatar hand opened when the participant closes his hand and vice versa (“mismatched condition”);
3. with scaled down amplitude, either in opening or in closing (“scaled condition”).

During block B participants answer “yes” or “no” to the question “was the projected hand moving as your own hand?” The eye-tracking system implemented in the headset is

used to collect the answers. Finally, during block C the subject rests and observes a static hand.

The fMRI cycle for the sense of agency task is as follow:

- 1 TR of "open and close your right hand" command;
- 4 TR of right hand tapping (with one of the three possible hand projections);
- 2 TR of judgement;
- 2 TR of "observe the hand on the screen and do not move your hand" command;
- 2 TR of rest.

The three conditions are randomized *a priori* and repeated five times each for a total duration of 6 minutes and 52 seconds.

The tasks are performed with participants wearing fMRI compatible optic-fiber gloves that are used to register hands movement. Sandbags are used to position participants' arms similarly to the 3D avatar and to prevent unwanted movements. Given the complexity of the tasks, participants familiarize with the experimental conditions before scanning.

FMRI data are processed using the SPM12 software (www.fil.ion.ucl.ac.uk/spm, Wellcome Trust Center for Neuroimaging, London). The acquired images will undergo a pre-processing procedure, comprising realignment, slice timing correction, coregistration, normalization and smoothing to make the experimental data suitable for statistical analysis. The realignment procedure consists in realigning a time series of scans with respect to the first or the average of the scans, in order to correct any translational and rotational movement of the head of the subject. In this way, the correspondence between a given voxel and the relative reference in the cerebral volume is preserved. All subjects included in the study show a maximal head movement lower than 3 mm in each direction.

Slice timing correction corrects differences in image acquisition time between slices to make the data on each slice correspondent to the same point in time.

Coregistration refers to the alignment and overlay of fMRI data from a single subject with that subject's own anatomic imaging study that has a higher resolution. This improves the possibility to match up with the anatomical details of the template used for group analysis.

The normalization procedure consists in applying a 12-parameter transformation to refer the acquired functional images of each subject to a common space. The procedure

is necessary for group analyses since different brains vary in shape and size. Therefore, the procedure offers the double advantage of eliminating inter-individual variability and enabling to define the coordinates of cerebral activation with stereotactic certainty. The spatial reference was defined with the MNI template, developed at the Neurological Institute of Montreal, Quebec, Canada.

Finally, with the smoothing operation, the normalized images undergo the application of a 10 mm 3D Gaussian filter in the three dimensions to improve the signal-to-noise ratio of the images and reduce inter-individual variability. Conceptually, the procedure consists in replacing the intensity of each voxel with a weighted average of the intensities of the adjacent voxels.

The signal variations of the BOLD effect associated with the execution of all fMRI tasks (considering head movement parameters as confounds) are evaluated voxel by voxel using the GLM and the Gaussian field theory. Specific effects are tested applying appropriate linear contrasts. Significant hemodynamic changes for each contrast are evaluated using SnPM, a toolbox for SPM.

5.3 Future steps

As stated before in the current Chapter, the present study is still ongoing, therefore only methodological aspects were described and commented. Our next steps will be to continue and finalize patients' and healthy controls' screening and enrolment in order to increase statistical power of our analyses. In addition, we aim to observe the following outcomes:

- after training, both patient groups will improve in upper limb motor function and writing/technology usage abilities; specifically, we expect that VR-training group will show significantly higher improvements in upper limb motor function and writing/technology usage abilities than RS-training group;
- after training, we hypothesize that both patient groups will show improved brain functional efficiency in sensorimotor areas, while only the VR-training group will show brain functional changes in cognitive-associative networks (i.e., brain areas associated with sense of agency/awareness, attentive-executive and visuospatial networks). We expect that an increased efficiency of cognitive-associative brain networks can further facilitate the efficiency of the sensorimotor network; thus, we

expect also a significant correlation between brain plasticity and clinical changes in the VR-training group;

- after training, both patient groups will improve their cognitive performance. However, we expect that these changes will be greater in the VR-training group, particularly for the training-specific cognitive domains (bodily self-awareness and sense of agency).

We do not exclude that some risks and possible problems might occur. Here follows a lists of limitations of our study with possible mitigation actions:

- Drop-out of our participants: we plan to increase our sample size by 10%;
- Therapeutic interventions may modify functional activity over time: MRI analyses will be adjusted for pharmacological treatment at baseline and after training;
- Longitudinal MRI studies require monitoring of MRI data stability over time: the same MRI scanner will be used for the entire duration of the study;
- Repeating neuropsychological tests after a short time period is at risk of learning effects: we adopt subtest randomization procedures and parallel versions for each test;
- Patient motor deficits may affect cognitive performance, mainly the reaction times: CANTAB includes a motor screening test (MOT) that allows to account for motor deficits.

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5.5 Appendix

Additional Material 1

Rubber Hand Illusion – Scoring Sheet (Italian version)

Cognome.....
Nome.....

Data di nascita ..... Data di somministrazione .....

MANO SINISTRA

Condizione	Baseline Assessment (mm)	Giudizio post-stroking (mm)	Proprioceptive drift (mm)
Sincrono			
Asincrono			
Sincrono			
Asincrono			

MANO DESTRA

Condizione	Baseline Assessment (mm)	Giudizio post-stroking (mm)	Proprioceptive drift (mm)
Sincrono			
Asincrono			
Sincrono			
Asincrono			

Additional Material 2

Rubber Hand Illusion Questionnaire (Italian version)

Mano:

- ○ Sinistra
- ○ Destra

Condizione sperimentale specifica:

- ○ Sincrona 1
- ○ Asincrona 1

Per favore, metti una crocetta sopra il numero corrispondente al grado di accordo o disaccordo con le seguenti affermazioni.

1. Mi sembrava di sentire il tocco del pennello nella posizione dove vedevo toccare la mano finta.

2. Mi sembrava come se il tocco che sentivo sulla mia mano fosse causato dal pennello che toccava la mano finta.

3. Sentivo la mano finta come se fosse la mia.

4. Sentivo come se la mia mano stesse fluttuando lentamente verso l'alto.

5. Sembrava ci fosse la possibilità che avessi più di una mano ed un braccio sinistri.

6. Sembrava come se il tocco che sentivo avesse origine da un punto collocato tra la mia mano e la mano finta.

Additional Material 3

Questionario BF-10

Buongiorno,
di seguito trova elencate delle caratteristiche che possono riguardarLa o meno. Per esempio, è d'accordo di essere una persona a cui piace passare del tempo con gli altri?
Per favore scelga una risposta sotto ogni affermazione che indichi quanto Lei è d'accordo o in disaccordo con quell'affermazione.

1. In disaccordo fortemente
2. Un po' in disaccordo
3. Né d'accordo né in disaccordo
4. Un po' d'accordo
5. D'accordo fortemente

***Campo Obbligatorio**

IO MI VEDO COME UNA PERSONA CHE...

1. **Io mi vedo come una persona che è riservata***
    1. In disaccordo fortemente
    2. Un po' in disaccordo
    3. Né d'accordo, né in disaccordo
    4. Un po' d'accordo
    5. D'accordo fortemente

2. **Io mi vedo come una persona che di solito** (generalmente) **si fida***
    1. In disaccordo fortemente
    2. Un po' in disaccordo
    3. Né in accordo, né in disaccordo
    4. Un po' d'accordo
    5. D'accordo fortemente

3. **Io mi vedo come una persona che tende ad essere pigra***
    1. In disaccordo fortemente
    2. Un po' in disaccordo
    3. Né in accordo, né in disaccordo
    4. Un po' d'accordo
    5. D'accordo fortemente

4. **Io mi vedo come una persona che è rilassata, gestisce** (sopporta) **bene lo stress***
    1. In disaccordo fortemente
    2. Un po' in disaccordo

3. Né in accordo, né in disaccordo
4. Un po' d'accordo
5. D'accordo fortemente

5. **Io mi vedo come una persona che ha pochi interessi artistici***
   1. In disaccordo fortemente
   2. Un po' in disaccordo
   3. Né in accordo, né in disaccordo
   4. Un po' d'accordo
   5. D'accordo fortemente

6. **Io mi vedo come una persona che è estroversa** (spigliata)**, socievole***
   1. In disaccordo fortemente
   2. Un po' in disaccordo
   3. Né in accordo, né in disaccordo
   4. Un po' d'accordo
   5. D'accordo fortemente

7. **Io mi vedo come una persona che tende a trovare da ridire sugli** (i difetti negli) **altri***
   1. In disaccordo fortemente
   2. Un po' in disaccordo
   3. Né in accordo, né in disaccordo
   4. Un po' d'accordo
   5. D'accordo fortemente

8. **Io mi vedo come una persona che è un lavoratore affidabile** (Coscienziosa sul lavoro)*
   1. In disaccordo fortemente
   2. Un po' in disaccordo
   3. Né in disaccordo, né in accordo
   4. Un po' d'accordo
   5. D'accordo fortemente

9. **Io mi vedo come una persona che si innervosisce** (agita) **facilmente***
   1. In disaccordo fortemente
   2. Un po' in disaccordo
   3. Né in disaccordo, né in accordo
   4. Un po' d'accordo
   5. D'accordo fortemente

10. **Io mi vedo come una persona che ha un'immaginazione attiva** (fervida immaginazione)*
    1. In disaccordo fortemente

2. Un po' in disaccordo
3. Né in disaccordo, né in accordo
4. Un po' d'accordo
5. D'accordo fortemente

Elemento aggiuntivo opzionale – Gradevolezza (punteggio reale):

11. Io mi vedo come una persona che è gentile e premurosa con quasi tutte le persone

1. In disaccordo fortemente
2. Un po' in disaccordo
3. Né in disaccordo, né in accordo
4. Un po' d'accordo
5. D'accordo fortemente

Grazie per aver compilato tutte queste affermazioni!

Additional Material 4

SENSORY SUGGESTIBILITY SCALE
(Gheorghiu, Keller, Kreisel, Kroeger)

INTRODUZIONE (per lo sperimentatore)
L'intero test consiste di 12 prove, delle quali 10 sono prove vere (+) e 2 sono false (* - prova 1, prova 6a). Le prove false sono costruite in modo tale da causare una reazione in tutti i soggetti, e non saranno considerati nella valutazione finale.

INTRODUZIONE (per il soggetto)
Le sensazioni che sperimenteremo nelle prove che seguiranno sono a volte legate all'immaginazione. Può accadere che alcune volte tu non sia sicuro di aver veramente percepito una sensazione o di averne avuto solo l'immaginazione. Ma non ti devi preoccupare per questo, perché le nostre percezioni sono sempre associate all'immaginazione, ma non sempre siamo in grado di rilevarle.
Le sensazioni che possono scaturire dai test che ti saranno somministrati possono essere di intensità differenti per differenti persone. Per valutare se saranno percepite e quanto saranno forti queste sensazioni, ti darò un foglio di risposta dove ti sarà chiesto di valutare quanto è stata intensa ogni sensazione dopo ciascun esercizio. I punteggi sono 0 = nessuna sensazione, 1 = qualche sensazione, 2 = sensazione media, 3 = sensazione forte, 4 = sensazione molto forte (consegnare foglio di risposta al soggetto).
Prima di iniziare ogni prova, la nominerò e te la spiegherò e ti dirò su quale sensazione dovrai concentrarti. Qualche prova consiste di due parti e tu dovrai concentrarti su due differenti emozioni, ma te lo farò notare prima di cominciare. Deciderai, se

porti gli occhiali, se é meglio toglierli oppure no. Vedrai che tutto ciò sarà facile.
Alla fine, ti chiederò di chiudere gli occhi, durante le prove, per una migliore concentrazione sulle sensazioni attese.
Adesso, se non hai da fare ulteriori domande, potremmo iniziare.

ISTRUZIONI

1. SENSAZIONE DI FRUSCIO – SUONO DEL MARE (*)
Nella prova seguente cercherai di avvertire un fruscio simile a quando tieni una conchiglia sul tuo orecchio.

Adesso cominceremo la prova.
Per favore chiudi gli occhi e copri le orecchie con le mani per proteggerle dai rumori esterni. Quando focalizzi attenzione sui suoni che provengono dall'interno, puoi avvertire un fruscio simile al suono del mare. Concentrati completamente sul fruscìo.

(10 secondi)

Per favore esprimi la tua sensazione sul foglio-risposta.

2. SENSAZIONE DI CALORE IN UNA META' DEL VISO (+)
Nella prova seguente sperimenteremo la sensazione di calore che si può avvertire se si orienta una torcia su una metà del viso. Le torce, infatti, non trasmettono solo luce, ma anche piccole quantità di calore, che possono essere avvertite da parti molto sensibili del corpo.

Cominciamo ora la prova.

Per favore prendi in mano la torcia e accendila. Chiudi gli occhi e avvicinala ad una tua guancia a circa 3 centimetri. Per favore concentrati completamente sulla sensazione di calore in quella parte del tuo viso.

(10 secondi)

Per favore esprimi la tua sensazione sul foglio risposta.

3. VALUTAZIONE DI SAPORE DOLCE O SALATO (+)

Questa prova consiste di due parti.

È noto che i sapori, come dolce, salato o aspro, sono percepiti in parti differenti della lingua. Può accadere che i differenti sapori possono essere provocati dalla stimolazione per contatto di determinate parti della lingua. In questa prova, prima produrremo il sapore salato, poi quello dolce. Prima di cominciare devi sciacquare la bocca con un po' di acqua minerale per eliminare ogni altro sapore che può essere presente nella nostra bocca.

Adesso ti do un bicchiere di acqua minerale, per favore, sciacquati la bocca e poi deglutisci l'acqua. L'acqua che rimane sarà usata nella seconda parte della prova.

Cominciamo ora la prova.

a) Sapore salato:

Per favore, chiudi gli occhi e sfrega la parte esterna della lingua con i denti per un istante. Poi concentrati per sentire se avverti su un sapore salato.

(10 secondi).

Per favore, sciacquati la bocca con un po' di acqua minerale ancora una volta.

b) *Sapore dolce:*

Ora chiudi gli occhi e sfrega la punta della lingua con i denti anteriori per un istante. Quindi concentrati completamente per avvertire un sapore dolce.

(10 secondi).

Per favore, annota le tue sensazioni sul foglio-risposta.

4. PERCEZIONE CONTINUA DEL BATTITO CARDIACO (+)

Come sai, il battito cardiaco è percepibile in tutto il corpo. Nella prova seguente vogliamo rilevare il battito con la punta delle dita.

Ora cominciamo la prova.

Sfrega la punta delle dita (indice e medio) della tua mano destra per sensibilizzare i recettori posti sotto la pelle. Per avere una idea delle pulsazioni cardiache, prendiamo le pulsazioni ponendo la punta dell'indice e del medio della mano destra sulla tempia destra.

Ora, senti le pulsazioni sulla tua tempia destra e chiudi gli occhi. Concentrati completamente sulle pulsazioni per vedere se e quanto forte tu possa sentire le pulsazioni sulla punta delle tue dita.

(10 secondi).

Adesso togli le dita e concentrati per sentire se e quanto avverti le pulsazioni sulla punta delle tue dita.

Per favore annota la tua sensazione sul foglio-risposta.

5. PERCEZIONE ACUSTICA DEL BATTITO CARDIACO (+)

In questo esercizio cercheremo di sentire acusticamente le pulsazioni che percepiamo sulle nostre tempie. Vuole essere un esempio di come uno stimolo tattile viene percepito anche acusticamente.

Ora cominciamo la prova.

Cerca le pulsazioni con le dita della mano sinistra (medio e anulare) sulla tempia destra. Se le hai trovate chiudi gli occhi. Cerca per favore di percepire le pulsazioni nel modo più chiaro possibile e contale.

Per favore, adesso togli le dita della mano sinistra dalla tempia destra e usale per chiudere il tuo orecchio destro per escludere suoni esterni che potrebbero interferire con la tua percezione acustica. Ora concentrati per sentire le pulsazioni.

(10 secondi).

Per favore annota la tua sensazione sul foglio-risposta.

6. LA SENSAZIONE DI CALORE (*) E DI FORMICOLIO SULLA MANO (+)

Questa prova si compone di due parti.

Nella prima parte, proverai ad avvertire una sensazione di calore sulla tua mano destra. La sensazione di calore è spesso accompagnata da un formicolio; nella seconda parte della prova, vedremo se avverti una sensazione un formicolio nella tua mano.

Adesso cominciamo la prova.

a) Sensazione di calore:

Strofina le mani perché si scaldino un po'. Chiudi gli occhi e concentrati per vedere se puoi avvertire una sensazione di calore nella tua mano destra

(10 secondi).

b) Sensazione di formicolio

Per favore, adesso concentrati, con gli occhi ancora chiusi, e prova a vedere se oltre ad una sensazione di calore puoi avvertire un formicolio nella mano destra.

(10 secondi).

Per favore, annota la tua sensazione sul foglio risposta.

7. SENSAZIONE DI FREDDO E INTORPIDIMENTO NELLA MANO (+)

Nella prova seguente ti viene chiesto ancora una volta di percepire due sensazioni, una sensazione di freddo e una di intorpidimento nella tua mano. Cercheremo di scoprire se una sensazione di freddo o di intorpidimento può essere trasmessa dall' avambraccio alla mano.

Ora iniziamo l'esercizio.

Per favore scopri il tuo braccio destro e poggialo ad angolo difronte a te. Prendi con l'altra mano questa fredda lamina di metallo e poggiala sulla parte interna del tuo avambraccio, al di sopra del gomito. Chiudi gli occhi. Mentre questa lamina sta toccando il tuo braccio, conta per favore da 1 a 3, lentamente. Ora togli la lamina fredda. Concentrati sulla sensazione di freddezza in questa zona del braccio e

sulla sensazione di freddo e di intorpidimento che si è irradiato nella tua mano destra.

(10 secondi).

Annota per favore le tue sensazioni sul foglio-risposta.

8. RAFFIGURAZIONE DEI CONTORNI DELLE DITA E SENSAZIONE DI BLU (+)

La prossima prova consiste di due parti.
Nella prima parte ti viene chiesto di raffigurare il contorno delle tue dita, con gli occhi chiusi.
Per la seconda parte è importante sapere che con una leggera pressione sulle palpebre si può percepire una sensazione di colore. Cercherai quindi di percepire una sensazione di blu causata da una leggera pressione delle dita sulle tue palpebre.

Ora iniziamo la prova.

a) Raffigurazione del contorno delle dita
   Allunga il tuo braccio destro all'altezza dei tuoi occhi e osserva attentamente il contorno delle tue dita.
   Ora copri con la mano sinistra il tuo occhio sinistro e dirigi lentamente la mano destra verso l'occhio destro. Mentre stai facendo ciò, per favore continua a fissare la punta delle dita. Quando con la mano sei vicino all'occhio, per favore chiudi gli occhi. Ora concentrati se puoi raffigurarti il profilo delle dita.

   (10 secondi)

b) Sensazione di colore:
   Tieni per favore gli occhi chiusi e premi con la punta delle dita della mano destra sull'occhio

destro per qualche istante. Concentrati se puoi percepire una tinta blu.

(10 secondi).

Annota per favore le tue sensazioni sul foglio-risposta.

FOGLIO-RISPOSTA DELLA SENSORY SUGGESTIBILITY SCALE

Cognome.....Nome.....
....

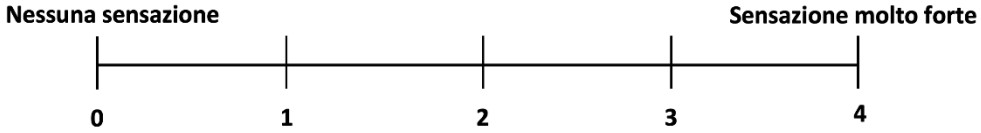
Data di nascita ..... Data .....

Per favore, metti una crocetta sopra il numero corrispondente al grado di sensazione da te percepito.

I numeri segnati corrispondono a:
0 = nessuna sensazione;
1 = qualche sensazione;
2 = sensazione media;
3 = sensazione forte;
4 = sensazione molto forte.

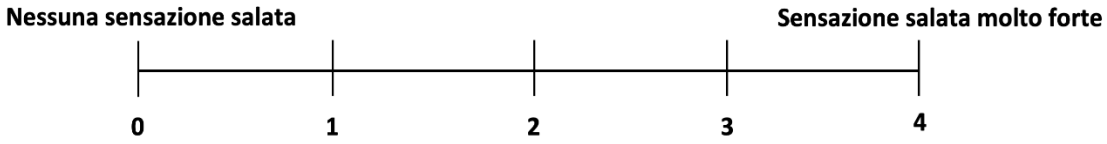
1. Sensazione di fruscio

2. Sensazione di calore in una metà del viso

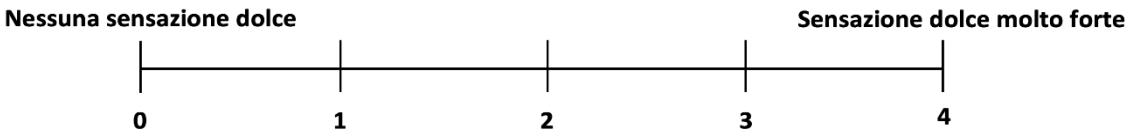


3. Valutazione di sapore dolce o salato

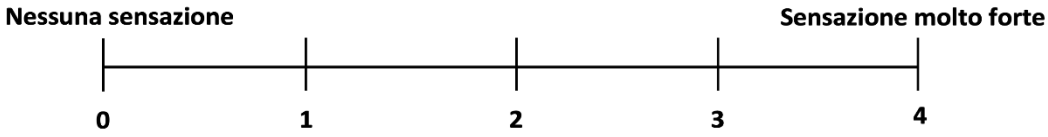
A.



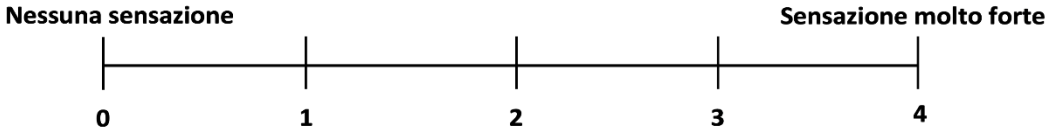
B.



4. Percezione continua del battito cardiaco

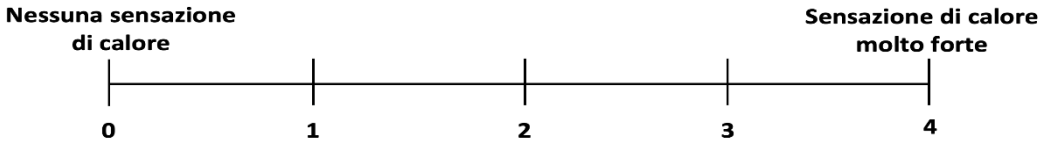


5. Percezione acustica del battito cardiaco

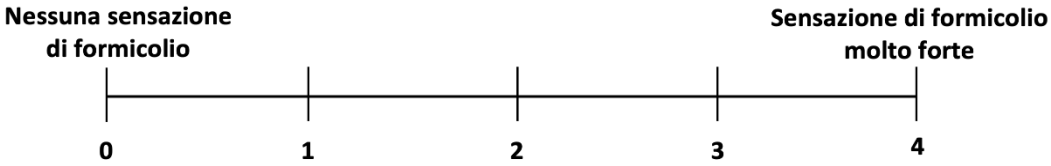


6. Sensazione di calore e di formicolio sulla mano

A.

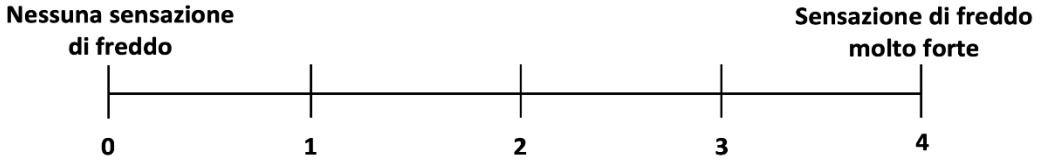


B.

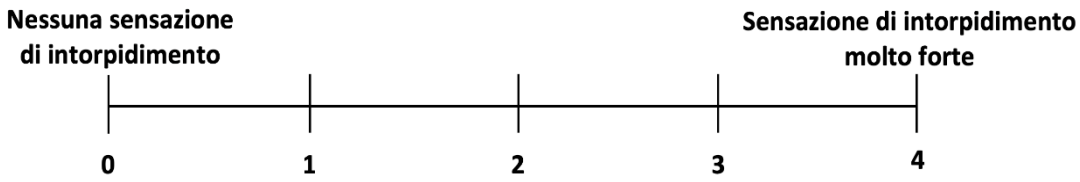


7. Sensazione di freddo e di intorpidimento nella mano

A.

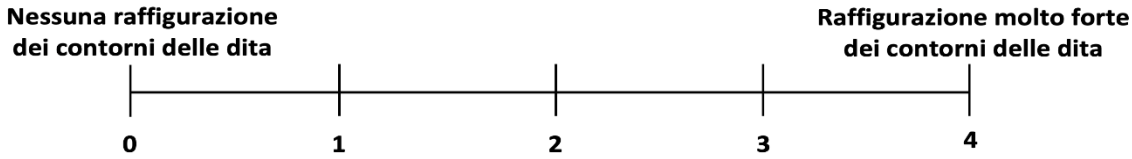


B.

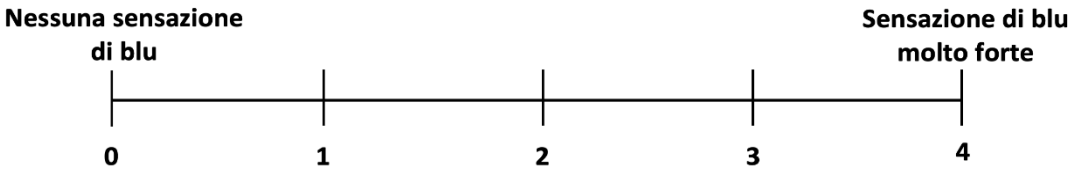


8. Raffigurazione dei contorni delle dita e sensazione di blu

A.



B.



FINE

Chapter 6 – General Discussion

6.1 Discussion

In this thesis, we firstly highlighted the importance of employing advanced brain structural and functional MRI techniques to study clinical evolution and disease progression in PD patients from the early stages of the disease. Specifically, we focused on the assessment of differences in terms of disease progression and worsening between idiopathic cases and patients bearing GBA mutation.

Secondly, the lack of effective pharmacological antiparkinsonian treatments to manage both motor and non-motor symptoms lead to the need of developing non-pharmacological interventions; in this work, we focused on the employment of physiotherapy with dual-task gait/balance training in combination with AOT-MI and VR and possible neurorehabilitation approaches.

In **Chapter 2,** we investigated the longitudinal disease course of GBA-positive PD patients from the very early disease stage compared to non-carriers and normal aging focusing on clinical, cognitive and MRI assessments over a 5-year follow-up (Leocadi, Canu et al., 2022). Although both GBA-positive and GBA-negative patients were accurately matched for all socio-demographic, cognitive, motor and non-motor features, we observed a different disease evolution in the two cohorts, with GBA-positive cases showing a rather worse progression. Compared to non-carriers, we observed that, after a 5-year follow-up period, GBA-positive patients showed a worsening in terms of disease severity and motor symptoms, which is in line with previous findings (Pal, Robertson et al., 2016). In addition, they displayed a relatively worse cognitive profile, specifically in visuospatial skills, which is coherent with cognitive impairment evolution observed in idiopathic PD over the disease course; our findings are in line with previous results, which displayed a rather worse clinical and cognitive profile evolution of genetic cases compared to the non-carriers (Mata, Leverenz et al., 2016, Winder-Rhodes, Evans et al., 2013). Even though no significant differences were observed in the two cohorts at study entry in terms of clinical/cognitive aspects and subcortical GM volumes, we retrieved distinct brain cortical thickness profiles even at the first visit: in fact, GBA-positive patients showed a widespread pattern of cortical damage mainly involving temporo-parietal-occipital cortices, which is consistent with pathological findings on PD evolution of spreading of pathology (Braak, Bohl et al., 2006). Furthermore, after 5 years of observation, GBA-positive individuals accumulated more cortical damage in posterior

regions, which further spread also to frontal and orbitofrontal cortices. Interestingly, we observed that GBA-negative patients reached the same pattern of cortical thinning of GBA-positive individuals at baseline only after five years, therefore highlighting a similar topographic trajectory of brain damage but a faster and more aggressive disease course of the latter cohort. Another interesting finding of our work is the different timeline between cortical and subcortical damage evolutions in the two groups: in fact, both patient cohorts seemed to display the same pattern of subcortical GM atrophy over 5 years, with GBA-positive patients showing only additional atrophy in the right caudate over time, thus resembling disease evolution of idiopathic PD. Our study confirmed the importance of using cortical thickness as a possible *in vivo* biomarker to detect GBA-positive cases in their early disease stages compared to idiopathic PD, with the aim to predict their clinical and motor evolution, which might be crucial also for possible clinical trials.

In **Chapters 3 and 4,** we highlighted the importance of combining non-pharmacological treatments to antiparkinsonian medications for PD motor symptoms management, specifically in those patients manifesting with PD-PIGD, who become less responsive to dopaminergic treatment and show a more rapid disease progression compared to other PD phenotypes over time (Piramide, Agosta et al., 2020). Specifically, we studied the combined effect of dual-task gait/balance training with two motor and cognitive mental practice techniques, namely AOT and MI, which exploit the MNS activity and improve motor recovery by facilitating motor and cognitive pathways (Buccino, 2014, Rizzolatti, Fogassi et al., 2001) on clinical, motor, cognitive and brain functional reorganization activity. In both studies, the two patient groups (DUAL-TASK + AOT-MI and DUAL-TASK) were relatively small but well characterized and well matched in terms of socio-demographic, clinical, motor and cognitive aspects. In **Chapter 3,** we explored whether dual-task gait/balance training with AOT-MI might improve motor learning and reduce attentional needs on control movement, especially in dual-task conditions, which are highly demanding for PD patients. After dual-task gait/balance training, we observed that both groups improved (with even long-lasting effects after 14 weeks) in terms of mobility, gait speed and balance in dual-task situations, which is in line with previous findings (Strouwen, Molenaar et al., 2017, Strouwen, Molenaar et al., 2015). Among the most challenging situations for PD patients, DUAL-TASK + AOT-MI patients furthermore ameliorated in their turning velocity during dual-

task situations, and they improved their balance and their confidence; in line with previous studies (Agosta, Gatti et al., 2017, Pelosin, Bove et al., 2013), these improvements lead to better quality of life and reduction of FoG episodes. In terms of brain functional reorganization, we observed changes in both groups: the DUAL-TASK + AOT-MI group experienced, during fMRI motor- and dual-tasks, reduced recruitment of frontal areas compared to the other group, which we interpreted as a more efficient way to control movement and a lower reliance on executive-attentive resources (Maidan, Rosenberg-Katz et al., 2017). Our interpretation was further validated by the association of these functional rearrangements with improvements in balance, balance confidence and turning velocity. In this group, we also retrieved a correlation between increased activity of the cerebellum with better balance and improved performance in cognitive tests assessing set-shifting abilities (as assessed with CANTAB sub-test), thus suggesting a possible involvement of this brain area in both motor and cognitive abilities. On the other hand, the DUAL-TASK group showed increased recruitment of temporal and parietal areas after training, which are brain areas known to be hyper-activated during dual-task situations (Strouwen et al., 2015, Wu, Hallett et al., 2015). We demonstrated that adding AOT-MI techniques to dual-task gait/balance training could boost functional reorganization processes in brain areas engaged in executive-attentive skills and motor control, therefore inducing long-lasting effects on dual-task mobility and balance in PD patients with PIGD.

Starting from these results and from the same cohort of patients, in **Chapter 4** we discussed whether dual-task gait/balance training with AOT-MI might also improve cognitive performance using a computerized neuropsychological battery (especially for the evaluation of attentive and executive functioning skills) and RS-FC processes in our cohorts. At baseline, DUAL-TASK + AOT-MI and DUAL-TASK were similar among each other in terms of clinical, sociodemographic, cognitive, and RS-FC activity. After 6 weeks of dual-task training, both patient groups ameliorated their cognitive performance in terms of accuracy in attentive-executive tasks, specifically in set-shifting conditions, which are the most challenging for PD patients. These findings are in line with previous evidence of mental flexibility and processing speed improvements after dual-task training (Fritz, Cheek et al., 2015, Silsupadol, Siu et al., 2006), which can be explained by a better functioning of inhibiting irrelevant information to complete a given task. Even though in

this study a distinct cognitive profile was not detected in the two groups after training, the two groups showed distinct RS-FC reorganization processes. Similar to our previous findings (Chapter 3) (Sarasso, Agosta et al., 2021), DUAL-TASK + AOT-MI patients showed reduced activity in frontal areas (in the aDMN) after training, and additionally in visuo-motor associative areas within the ECN and Precuneus networks. As already mentioned in Chapter 1 (in paragraph 1.3.2.1) and in previous studies, we further observed increased activity in the aSAL after 6 weeks. A possible anti-correlated coupling mechanism between the DMN and the SAL in cognitive tasks has been extensively reported in previous studies on PD cohorts (Dosenbach, Visscher et al., 2006, Greicius, Krasnow et al., 2003). In fact, in situations where highly-demanding cognitive efforts are required, these brain networks show the opposite RS-FC pattern: the SAL is generally activated, while the DMN shows reduced activity in turn, which has been associated in healthy controls to optimal cognitive performance (Putcha, Ross et al., 2016). We retrieved this anti-correlated coupling mechanism in our DUAL-TASK + AOT-MI patients when compared to DUAL-TASK patients after training, which suggests that these RS-FC reorganization processes are therefore indicative of cognitive improvement consequent to dual-task gait/balance training in DUAL-TASK + AOT-MI patients. Furthermore, our correlation analyses indicated that suppressed recruitment of frontal areas (aDMN) was related to better accuracy in the AST test of the CANTAB (specifically, in set-shifting conditions), which is highly challenging for these patients. In accordance with the study presented in Chapter 3, at the brain functional level we demonstrated that dual-task gait/balance training with AOT-MI can reduce cognitive overload and attentional control in our PD PIGD patients during dual-task situations, which are highly demanding and frequent in the situations of daily life.

In **Chapter 5,** we proposed a new possible rehabilitation approach which aims to ameliorate upper limb motor function and therefore reduce bradykinesia occurrence in PD patients through the use of new technologies, such as VR. Even though VR settings can be manipulated unlimitedly, with the potential to boost multisensory motor-cognitive integration and to adapt VR protocols to patients' individual needs and specific profile of motor/cognitive deficits (Canning, Allen et al., 2020), little evidence already exists on the benefits of employing VR-training over RS-training in PD populations. As reported in paragraph 5.1, upper limb bradykinesia in PD has a negative impact on daily living

activities such as writing and technology usage. The use of touch screen technology for instance requires a set of motor skills, like fine-tuning of repetitive motor sequences, which are typically difficult for these patients. Thus, rehabilitation strategies focusing on improving bradykinesia during handwriting and technology-based motor skills are urgently needed to improve quality of life in PD. The VR setting implemented in this project will improve bradykinesia through the modulation of movement perception (augmented feedback). VR will also reproduce a motivating setting of training, which we hope can improve subjects' engagement. The use of fMRI will furthermore allow to identify surrogate markers to monitor training efficacy. With the increasing importance of using technologies in modern society, this study proposes a rehabilitative practice that could increasingly become a strategic tool, essential for future generations. To our knowledge, no previous studies tried to determine also the modulations in terms of sense of agency and upper limb embodiment after VR-training in PD patients. Previous studies pointed out that behaviour in these cohorts of patients is mostly guided by external rather than internal cues (Jahanshahi, 1998). We hope that our VR-training, by providing enhanced visual feedback during upper limb movements, would enhance brain functional efficiency in sensorimotor and cognitive associative areas, thus improving motor aspects of PD.

6.2 Conclusions

This thesis has provided important insights regarding the distinct disease evolution trajectories between idiopathic PD and GBA-positive PD patients. In summary, we observed that, starting from similar disease staging and disease severity, GBA-positive individuals have a more rapid disease worsening over time, in clinical, cognitive, motor, and brain structural aspects. Our findings provide further evidence on the need to promptly target these early-stage and genetic cases, in view of possible clinical trials interventions.

In addition, we also evaluated the importance of combining non-pharmacological interventions to antiparkinsonian treatments in PD patients at early disease stages. We explored the motor and clinical implications of dual-task gait/balance training in PD-PIGD patients, evaluating substantial changes in these patients also in term of brain functional reorganizational changes. In addition, these types of training determined

cognitive improvements in attention and executive functioning in our patients, which were also supported by RS-FC findings. Lastly, we proposed a new physiotherapy treatment which employs VR technology for the rehabilitation of the upper limb. The field of neurorehabilitation and specifically the implementation of protocols using VR is still in its infancy, therefore our study could contribute to increasing the knowledge on this field.

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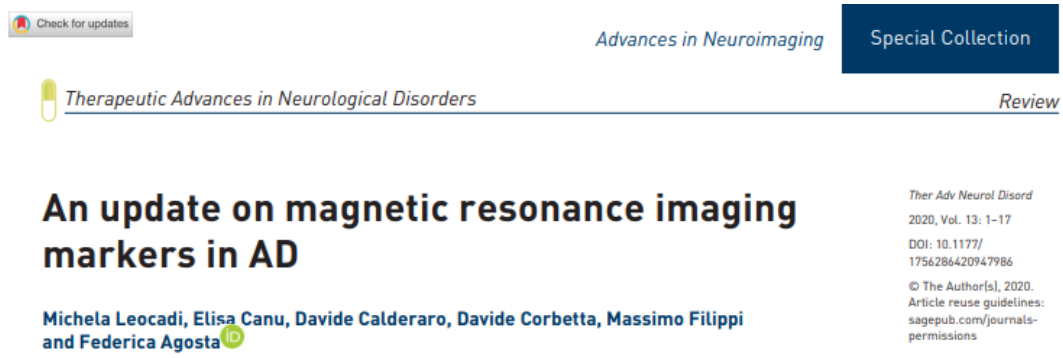
Chapter 7 - Additional publications

1) **Leocadi, M.,** Canu, E., Calderaro, D., Corbetta, D., Filippi, M., Agosta, F., 2020. An update on magnetic resonance imaging markers in AD. Therapeutic advances in neurological disorders, 13, p.1756286420947986.



ABSTRACT

The purpose of the present review is to provide an update of the available recent scientific literature on the use of magnetic resonance imaging (MRI) in Alzheimer's disease (AD). MRI is playing an increasingly important role in the characterization of the AD signatures, which can be useful in both the diagnostic process and monitoring of disease progression. Furthermore, this technique is unique in assessing brain structure and function and provides a deep understanding of in vivo evolution of cerebral pathology. In the reviewing process, we established a priori criteria and we thoroughly searched the very recent scientific literature (January 2018–March 2020) for relevant articles on this topic. In summary, we selected 73 articles out of 1654 publications retrieved from PubMed. Based on this selection, this review summarizes the recent application of MRI in clinical trials, defining the predementia stages of AD, the clinical utility of MRI, proposal of novel biomarkers and brain regions of interest, and assessing the relationship between MRI and cognitive features, risk and protective factors of AD. Finally, the value of a multiparametric approach in clinical and preclinical stages of AD is discussed.

2) Canu, E., Bessi, V., Calderaro, D., Simoni, D., Castelnovo, V., **Leocadi, M.,** Padiglioni, S., Mazzeo, S., Cividini, C., Nacmias, B. Sorbi, S., Filippi, M., Agosta, F., 2020. Early functional MRI changes in a prodromal semantic variant of primary progressive aphasia: a longitudinal case report. Journal of Neurology, 267(10), pp.3100-3104.

Journal of Neurology (2020) 267:3100–3104
https://doi.org/10.1007/s00415-020-10053-9

SHORT COMMENTARY

Early functional MRI changes in a prodromal semantic variant of primary progressive aphasia: a longitudinal case report

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ABSTRACT

Objective: To assess longitudinal patterns of brain functional MRI (fMRI) activity in a case of prodromal semantic variant of a primary progressive aphasia (svPPA).

Methods: Clinical, cognitive and neuroimaging data (T1-weighted and task-based fMRI during silent naming [SN] and object knowledge [OK]) were obtained at baseline, month 8 and month 16 from a 49-year-old lady presenting with anomias and evolving to overt svPPA in 8 months.

Results: At baseline, the patient showed isolated anomias and mild left anterior temporal pole atrophy. During SN–fMRI, she showed bilateral temporal and left inferior frontal gyri (iFG) activations. During OK–fMRI, we observed normal performance and the recruitment of bilateral posterior hippocampi, iFG and left middle orbitofrontal gyrus (mOFG). At month 8, the patient received a diagnosis of svPPA and showed isolated right iFG activity during SN–fMRI, and a borderline performance during OK–fMRI together with a disappearance of mOFG recruitment. At the last visit (after 7-month language therapy), the patient showed a stabilization of naming disturbances, and, compared to previous visits, an increased left iFG recruitment during SN–fMRI. During OK–fMRI, she performed abnormally and did not show the activity of mOFG and iFG. Across all visits, brain atrophy remained stable.

Conclusions: This case report showed longitudinal fMRI patterns during semantic-related tasks from prodromal to overt svPPA. Frontal brain recruitment may represent a compensatory mechanism in patients with early svPPA, which is likely to be reinforced by language-therapy. Brain fMRI is more sensitive compared with structural MRI to detect progressive brain changes associated with disease and treatment.

3) Agosta, F., Canu, E., **Leocadi, M.,** Castelnovo, V., Magno, M.A., Calderaro, D., Filippi, M, 2021. Principles of behavioural and cognitive neurology. In: Encyclopedia of Behavioural Neuroscience, 2e, George Koob, Sergio Della Sala (eds). Elsevier Science.

4) Taheri Gorji, H., **Leocadi, M.,** Grassi, F. and Galati, G., 2021. The art gallery maze: A novel tool to assess human navigational abilities. Cognitive Processing, 22(3), pp.501-514.

Cognitive Processing (2021) 22:501–514
https://doi.org/10.1007/s10339-021-01022-9

RESEARCH ARTICLE

Check for updates

The art gallery maze: a novel tool to assess human navigational abilities

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ABSTRACT

Humans differ widely in their ability to navigate effectively through the environment and in spatial memory skills. Navigation in the environment requires the analysis of many spatial cues, the construction of internal representations, and the use of various strategies. We present a novel tool to assess individual differences in human navigation, consisting of a virtual radial-arm maze presented as an art gallery to explore whether different sets of instructions (intentional or incidental) affect subjects' navigation performance. We furthermore tested the effect of the instructions on exploration strategies during both place learning and recall. We evaluated way-finding ability in 42 subjects, and individual differences in navigation were assessed through the analysis of navigational paths, which permitted the isolation and definition of a few strategies adopted by the incidental and

intentional instructions groups. Our results showed that the intentional instruction group performed better than the other group: these subjects correctly paired each central statue and the two paintings in the adjacent arms, and they made less working and reference memory errors. Our analysis of path lengths showed that the intentional instruction group spent more time in the maze (thus being slower), specifically in the central hall, and covered more distance; the time spent in the main hall was, therefore, indicative of the quality of the following performance. Studying how environmental representations and the relative navigational strategies vary among "intentional" and "incidental" groups provides a new window into the acknowledgment of possible strategies to help subjects construct more efficient approaches in human navigation.

5) **Leocadi M.,** Canu E., Cividini C., Russo T., Cecchetti G., Celico C., Cardamone R., Barcella V., Magnani G., Agosta F., Filippi M. Brain structural abnormalities and cognitive changes in a patient with 17q21.31 micro-duplication and early-onset dementia: a case report. J Neurol 2022 Online ahead of print.

Journal of Neurology
https://doi.org/10.1007/s00415-022-11423-1

SHORT COMMENTARY

Check for updates

Brain structural abnormalities and cognitive changes in a patient with 17q21.31 microduplication and early onset dementia: a case report

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ABSTRACT

Objectives: We described brain structural damage and cognitive profile evolution of an adult patient with 17q21.31 microduplication, a rare condition associated with psychomotor delay, behavioural disturbances, and poor social interaction.

Methods: A.B., 57 years old, male, displayed obsessive and repetitive behaviours, irritability, scarce hygiene, and memory loss at disease onset. He had strong familiarity for adult-onset behavioural alterations (his father and sister) and neuropsychiatric

conditions (his son). Blood and cerebrospinal fluid (CSF) samples revealed 17q21.31 microduplication, shared also by his son and sister, and raised CSF tau, respectively. He was hospitalized one year after disease onset and underwent an MRI scan and a neuropsychological assessment, the latter being repeated 7 months later. To quantitatively investigate patient's gray matter (GM) volume, 16 age- and education-matched male controls were selected, and voxel-based morphometry analysis was performed.

Results: During hospitalization, his behavioural profile was characterized by anosognosia, impulsivity, apathy, and aggressiveness. Cognitive testing revealed main attentive-executive disturbances, and difficulties in understanding non-literal language. Compared to controls, A.B. had greater GM atrophy mainly in the right hemisphere, involving amygdala, hippocampus, inferior/superior temporal gyri, and temporal pole. He received a diagnosis of early onset dementia. After 7 months, he developed empathy loss, perseverative behaviour, changes in eating habits, and worsening in executive-attentive abilities.

Conclusions: In A.B., 17q21.31 microduplication caused a neurodegenerative condition with prevalent right temporal damage, raised CSF tau level, behavioural disturbances, memory impairment, attentive-executive and abstract language dysfunctions, and fast disease progression, thus reflecting the complex interaction between such genetic substrate and clinical phenotypes.