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IDENTIFICATION OF COGNITIVE SIGNATURES IN PARKINSON'S DISEASE AND IN ITS PRODROMAL STAGES WITH A FOCUS ON RETROGRADE PROCEDURAL MEMORY

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I hereby confirm that the PhD thesis entitled "*Identification of cognitive signatures in Parkinson's disease and in its prodromal stages with a focus on Retrograde Procedural Memory*" has been written independently and without any other sources than cited.

Luxembourg, 2024-03-18 PAULY Laure

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Laure

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List of abbreviations

Abbreviation	Meaning
AD	Alzheimer's Disease
AI	Artificial Intelligence
B-SIT	Brief – Smell Identification Test
BDI	Beck Depression Inventory
CBS	CorticoBasal Syndrome
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CHL	Centre Hospitalier de Luxembourg
CUPRO	CUbe drawing PROcedure
COMT	Catechol-O-MethylTransferase
DAT SPECT	DopAmine Transporter Single-Proton Emission Computed Tomography
DBS	Deep Brain Stimulation
DLB	Dementia with Lewy Body
FAQ	Functional Activities Questionnaire
FOG	Freezing of Gait
GBA	Glucocerebrosidase
iRBD	Idiopathic REM-Sleep Behavior Disorder
IQCODE	Informant Questionnaire on Cognitive Decline in the Elderly
$IS_{1/2}$	Intermediate Score 1 or 2
JLO	Judgment of Line Orientation
LCSB	Luxembourg Centre for Systems Biomedicine
LEDD	Levodopa Equivalent Daily Dose
LIH	Luxembourg Institute of Health
LRRK2	Leucine-Rich Repeat Kinase
MCI	Mild Cognitive Decline
MDS	Movement Disorder Society
MMSE	Mini-Mental Screening Evaluation
MoCA	Montreal Cognitive Assessment
MSA	Multiple System Atrophy
OPDC	Oxford Parkinson's Disease Center
P-PD	Prodromal Parkinson's Disease
PD	Parkinson's Disease
PDD	Parkinson's Disease with Dementia
PDQ-39	Parkinson's Disease Questionnaire – 39 items
PINK1	PTEN INduced putative Kinase 1
PPMI	Parkinson's Progression Markers Initiative
PRC	Parkinson's Research Clinic
pRBD	Probable REM-Sleep Behavior Disorder
PSP	Progressive Supranuclear Palsy

RBD	REM-Sleep Behavior Disorder
REM	Restless-Eye-Movement
rTMS	Repetitive Transcranial Magnetic Stimulation
SAS	Starkstein Apathy Scale
SCD	Subjective Cognitive Decline
SNCA	Alpha-synuclein (α-synuclein) gene mutation
SOP	Standard Operating Procedure
SRTT	Serial Reaction Time Task
tDCS	Transcranial Direct Current Stimulation
TMT	Trail-Making-Task
UPDRS	Unified Parkinson's Disease Rating Scale

List of publications

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Manuscripts presented in the cumulative thesis are marked with a *

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- *L. Pauly, C. Pauly, M.Hansen, V. E. Schröder, A. Rauschenberger, A. K. Leist and R. Krüger on behalf of the NCER-PD Consortium. Retrograde Procedural Memory in Parkinson's Disease: A Cross-Sectional, Case-Control Study. *Journal of Parkinson's Disease* 12,. 1013-1022 (2022); doi: 10.3233/J PD-213081 IOS Press [1].

- *L. Pauly, C. Pauly, M. Hansen, V. E. Schröder, A. Rauschenberger, A. K. Leist and R. Krüger on behalf of the NCER-PD Consortium. Retrograde Procedural Memory is impaired in people with Parkinson's Disease with Freezing of Gait. *Frontiers Aging Neuroscience* 15:1296323 (2024); doi: 10.3389/fnagi.2023.1296323 [2].

- *L. Pauly, A. Rauschenberger, C. Pauly, V. E. Schröder, G. Van Cutsem, A. K. Leist and R. Krüger on behalf of the NCER-PD Consortium. Cognition and other non-motor symptoms in an at-risk cohort for Parkinson's disease defined by REM-Sleep-Behavior-Disorder and Hyposmia. *Journal of Parkinson's Disease* (accepted for publication).

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- A. Hanff, C. Pauly, L. Pauly, V. E. Schroeder, M. Hansen, G. R. Meyers, A. Kaysen, L. Hansen, F. Wauters, R. Krüger. Unmet needs of people with Parkinson's Disease and their caregivers during COVID-19 related confinement: an explorative secondary data analysis. *Front Neurol.* 11,. (2021); doi: 10.3389/FNEUR.2020.615172 [4].

- C. Pauly, F. Ribeiro, V. E. Schröder, L. Pauly, R. Krüger, A. K. Leist & the CON-VINCE Consortium. The moderating role of resilience in the personality-mental health relationship during the COVID-19 pandemic. *Front. Psychiatry* 12,. (2021); doi: 10.3389/FPSYT.2021.745636 [5].

- A. K. Leist, M. Klee, I. Paccoud, L. Pauly, S. Ghosh, J. Fritz, M. O'Sullivan, B. Rommes, P. Wilmes, R. Krüger, CON-VINCE Consortium. CoVaLux Which Demographic and Socio-economic Factors are Associated with Vaccination Willingness and Beliefs Towards Vaccination. *Report* (2021); http://hdl.handle.net/10993/48567 [6].

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- V. E. Schröder, A. Skrozic, D. Erz, A. Kaysen, J. V. Fritz, J. M. Loureiro, D. McIntyre, L. Pauly, J. Kemp, S. K. Schmitz, S. Wagner, M. Reyes, R. Soare, V. Satagopam, C. Vega, P. Gawron, K. Roomp, P. M. Conde, J. Klucken, S. Köhler, T. Hartmann, R. Dodel, A. K. Leist, E. Kalbe, R. Krüger. Programme dementia prevention (pdp): A nationwide programme for personalized prevention in Luxembourg. *Journal of Alzheimer Disease* 97 (2024); doi: 10.3233/JAD-230794 [9].

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Vaillant, J. V. Fritz, M. Ollert, R. Krüger, on behalf of CON-VINCE/ORCHESTRA Consortium. Creation of a pandemic memory by tracing COVID-19 infections and immunity in Luxembourg (CON-VINCE). *BMC Infectious Diseases* 24:179 (2024); doi: 10.1186/s12879-024-09055-z [10].

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Abstract

Today, more than two centuries after Dr. James Parkinson's initial description of Parkinson's disease (PD), our point of view has been broadened beyond the perception of PD as a motor disorder. Nowadays, we recognise a multitude of non-motor symptoms such as cognitive impairment in PD and its substantial impact on quality of life.

Through this doctoral dissertation, we aim to acquire novel insights into the cognition of people with PD and in its prodromal stages, with a focus on retrograde procedural memory. As an initial step, we focused on the development of the CUPRO (CUbe drawing PROcedure), an extended evaluation system of the Cube Copying Task, suggestive for assessing retrograde procedural memory. We applied the CUPRO evaluation system to the Luxembourg Parkinson's Study cohort and compared the performances of people with PD to age- and sex-matched control subjects. We observed significantly lower CUPRO scores in people with PD, suggestive of impaired functioning of retrograde procedural memory in PD. In a second step, we compared cognition, with a focus on the CUPRO performance in people with PD and Freezing of Gait (FOG), a de-automatization disorder of walking, to a counter group of PD without FOG. Besides significantly lower global cognition and mental flexibility, the deficit of retrograde procedural memory was significantly more prominent in PD with FOG compared to the matched counter group. Lastly, we focused on the pre-motor stages of PD, the prodromal PD (P-PD). We investigated non-motor symptoms, focusing on cognition in people at high risk of developing PD, presenting risk factors such as probable Restless-Eye-Movement (REM) Sleep Behaviour Disorder (RBD) and olfactory dysfunction. In this P-PD group, we described early global cognitive, executive, and visuo-constructive function deficits compared to the matched control group. No significant differences have been observed for the retrograde procedural memory at the P-PD stages.

The thesis summarizes the present status of research in the field of PD and offers a thorough exploration of the cognitive changes spanning from the prodromal to the already advanced disease stage. All in all, our primary goal was to gain a deeper understanding of the often invisible symptoms of PD, the cognitive impairment and to, in the future, reduce the burden for people with PD and contribute to the early recognition of PD, helping to get a better prognosis, as soon as disease-modifying treatments are available.

Aims and Objectives

Through this doctoral dissertation, I aim to acquire novel insights into the cognition of people with PD (Chapter I) and in its prodromal stages (Chapter II), with a focus on procedural skills.

In Chapter I, we aimed to develop a cognitive evaluation tool, named CUPRO, for assessing the functioning of retrograde procedural memory, and to apply it to people with PD (Chapter IA) and a subgroup of people with PD and FOG (Chapter IB). As deficits in previously acquired procedural behaviours, such as walking [11] or writing [12], have been described in PD, we hypothesized that the CUPRO performance, suggestive of the functioning of the retrograde procedural memory, the memory of automatized skills, is significantly lower in people with PD compared to their age- and sex-matched control group (Chapter IA). Furthermore, given that FOG in PD is characterized as a de-automatization disorder [11], we hypothesized that the retrograde procedural memory deficit will be more prominent in people with PD and FOG compared to people with PD without FOG (Chapter IB).

In Chapter II, we aimed to investigate non-motor symptoms, focusing on cognition in people at high risk to develop PD, in the prodromal stages of PD. Global cognitive impairment has been added recently to the MDS research criteria for prodromal PD [13]. However, observations on the impairments in the different sub-domains of cognition are still controversial. The main aim of the study is to describe cognition and other non-motor symptoms in the P-PD stages and to define specific prodromal patterns that might support the early recognition of PD. Therefore, we compared the performance of extensive neuropsychological assessment and scores on diverse non-motor symptoms. To our knowledge, retrograde procedural memory has not yet been assessed in the P-PD.

Through this translational, transversal research, applying novel methodological approaches, we aim to make our contributions to the field of cognitive research in PD, to better understand these, often invisible and yet so distressing symptoms of PD and to, in a next step, contribute to the early recognition of the disease.

Synopsis

Parkinson's disease (PD) is currently the fastest-growing neurodegenerative disease worldwide with a substantial impact on the quality of life of people affected and an increasing socioeconomic burden for our ageing societies [14,15]. Besides the typical motor symptoms, the relevance of non-motor symptoms is more and more recognized [16,17]. Many of these non-motor symptoms, such as cognitive deficits, may even precede the motor symptoms related to PD, the prodromal PD (P-PD) stage [13]. A better characterization of cognitive deficits observed in P-PD may help in adapting reeducation therapies and, in combination with other prodromal markers, in recognising the disease at its early stage. In a future step, this will have implications when disease-modifying treatments become available.

The following synopsis is divided into three sections. The first section introduces Parkinsonism, PD and its prodromal stage. The second section concentrates on the cognitive impairment described in PD. The third section highlights the importance of research on cognition, focusing on procedural memory in PD. The fourth and last section summarizes and discusses research findings presented in the following manuscripts.

Parkinson's disease

Parkinsonism

Parkinsonism is an umbrella term used to describe a clinical syndrome, characterized by tremor, rigidity and slowness of movement (bradykinesia). Parkinsonism includes idiopathic PD, the best-known and the most common of this syndrome, and further atypical parkinsonism. Atypical parkinsonism includes Multiple System Atrophy (MSA), Dementia with Lewy Bodies (DLB), Progressive Supranuclear Palsy (PSP) and CorticoBasal Syndrome (CBS). Besides these primary causes represented by neurodegenerative diseases, secondary or symptomatic causes of parkinsonism exist, such as Normal Pressure Hydrocephalus, Vascular Parkinsonism or Drug-Induced Parkinsonism [18]. These different forms of parkinsonism may phenotypically look similar, especially in the early disease stages, by sharing the same cardinal symptoms. However, they may be distinguished based on certain key features, so-called "red flags", appearing along with disease progression, such as vertical supra-nuclear gaze palsy, early postural instability or alien-limb phenomenon, indications for these alternative atypical parkinsonism [18,19]. Neurodegenerative parkinsonian disorders are

defined by the accumulation of specific misfolded proteins and classified based on the most prevalent protein aggregation: PD, DLB and MSA are α-synucleinopathies; PSP and CBS are tauopathies [18].

Parkinson's disease

Epidemiology

First described by Dr. James Parkinson in 1817 [20], PD is currently the fastest-growing neurodegenerative condition affecting over six million people worldwide [14,15]. Incidence and prevalence have risen strongly in the past two decades and the numbers are expected to double by 2040 [21]. The prevalence of PD in Luxembourg, based on data collected between 2007 and 2017, was approximated at 1032 per 100,000 men and 831 per 100,000 women aged 50 years and older [22]. For 2023, this brings us to an estimated prevalence of 2137 people living in Luxembourg who have the PD diagnoses. Every year, between 57 and 100 PD cases are expected to be newly diagnosed in Luxembourg [23,24].

Pathophysiological and clinical features

Although PD is characterized primarily by motor symptoms, such as its cardinal manifestations - tremor, rigidity, and bradykinesia - the global clinical picture is multifaceted and includes many non-motor symptoms such as cognitive decline, depression, olfactory dysfunction (hyposmia) and sleep disorders that may even precede the motor symptoms needed for clinical diagnosis [13,25].

PD is a progressive neurological disease, characterized by a degeneration of dopaminergic neurons and α-synuclein accumulations. The resulting lack of dopamine and accumulation of Lewy Bodies, i.e., are responsible for the previously mentioned disease-typical symptoms. The exact mechanisms underlying this neuronal degeneration in PD remain unclear, potentially defined by a complex interplay of genetic and environmental factors [26,27] possibly characterized by misfolded α-synuclein proteins, neuroinflammation, oxidative stress, autophagy and dysfunction of lysosomes causing deficits in the protein clearance pathways, mitochondrial damage [27–29]. So far, diverse mutations in specific genes, such as SNCA, LRRK2, DJ1, PARK9 and GBA have been identified in people with PD [26]. The different gene mutations affect different pathways increasing the susceptibility to PD, such as abnormal accumulation of α-synuclein protein (e.g., SNCA), impaired mitochondrial homeostasis (e.g., DJ1) impaired lipid metabolism (e.g., GBA) or lysosomal dysfunction (e.g., PARK2) [30]. Environmental and lifestyle factors can be classified into risk- or protective factors. Risk factors

linked to a higher risk of developing PD include having a relative with PD, experiencing tremor, constipation and being a non-smoker [31]. Recent findings identified exposure to pesticides, prior head injury, rural living, beta-blocker use, agricultural occupation and well-water drinking as potential environmental risk factors for PD [26,31]. Protective factors linked to a lower risk of developing PD were smoking, consumption of coffee and alcohol as well as the use of non-steroidal anti-inflammatory drugs and calcium channel blockers [31].

Diagnosis

The clinical diagnosis of PD is based on neurological evaluation, cerebral imaging, and positive response to dopaminergic medication. The neurological evaluation is based on the United Kingdom PD Society Brain Bank Clinical Diagnostic Criteria [32], attributed to the presence of the cardinal motor symptoms, being bradykinesia with tremor and/or rigidity. Cerebral imaging, especially visualization of dopamine transporter by DopAmine Transporter Single-Proton Emission Computed Tomography (DAT SPECT) and testing the response to dopaminergic medication are supportive tools for the PD diagnosis [27].

Recent studies on biological biomarkers made an essential advancement in the knowledge around the biological diagnosis of PD. With the α-synuclein seed amplification assay technique, testing very small amounts of misfolded aggregates of α-synuclein, one can now detect α-synuclein aggregation in blood and in cerebrospinal fluid. These groundbreaking findings allow the identification of people at risk of developing PD and those with non-motor symptoms in the pre-diagnostic PD stage [33,34]. However, definitive diagnosis is only possible by neuropathological examination of postmortem brain tissue, by identifying the presence of Lewy bodies in affected brain regions of a person clinically presenting as PD [26,27,35].

Treatment

Due to the complexity of the disease and the heterogeneity of the underlying causes, there is still no cure or method to stop disease progression. Nonetheless, medications and other treatments are available to address and treat symptoms, such as drug treatments, surgery, or accessory therapies [29]. Levodopa, dopamine precursor amino acid L-DOPA, is the most common PD medication, substituting the lack of dopamine. Other existing PD medication can be based on inhibitors preventing the metabolism of dopamine (e.g.: Catechol-O-MethylTransferase (COMT)) or on dopamine agonists (e.g.: Apomorphine) [36]. Continuous infusion of PD medication such as subcutaneous Apomorphine infusion via an Apomorphine pump or continuous infusion of

intrajejunal DuoDopa, a combination of Levodopa and Carbidopa, gel treatment can reduce fluctuations typically seen in PD patients [37,38]. Given that the dopaminergic medications may not sufficiently control for motor fluctuations, Deep Brain Stimulation (DBS) of the subthalamic nucleus or globus pallidus internus can be a treatment option in some patients, effectively targeting classical symptoms and motor fluctuations, as off-phases or dyskinesia [26,27]. Aside from medication and surgery, multidisciplinary care intervention and non-invasive therapeutic approaches can significantly enhance the quality of life of PD patients [39]. Studies are demonstrating a positive impact of physical activities on quality of life as well as on the positive effect of cognitive rehabilitative training reinforcing cognitive compensation strategies in people with PD [40,41].

Prodromal Parkinson's disease

It is increasingly recognized that clinically diagnosing PD means identifying an already advanced neurodegenerative disorder. PD is typically diagnosed when more than 60% of the dopaminergic cells are degenerated and the first motor symptoms appear [42]. The period between the onset of neuronal degeneration, where symptoms and signs are present, but yet insufficient to define the disease, and the clinical diagnosis is called the prodromal or pre-motor phase and can start up to 20 years before the onset of motor parkinsonism [43,44] (Figure 1). The manifestation of these physical symptoms allows the clinical diagnosis of PD [45].

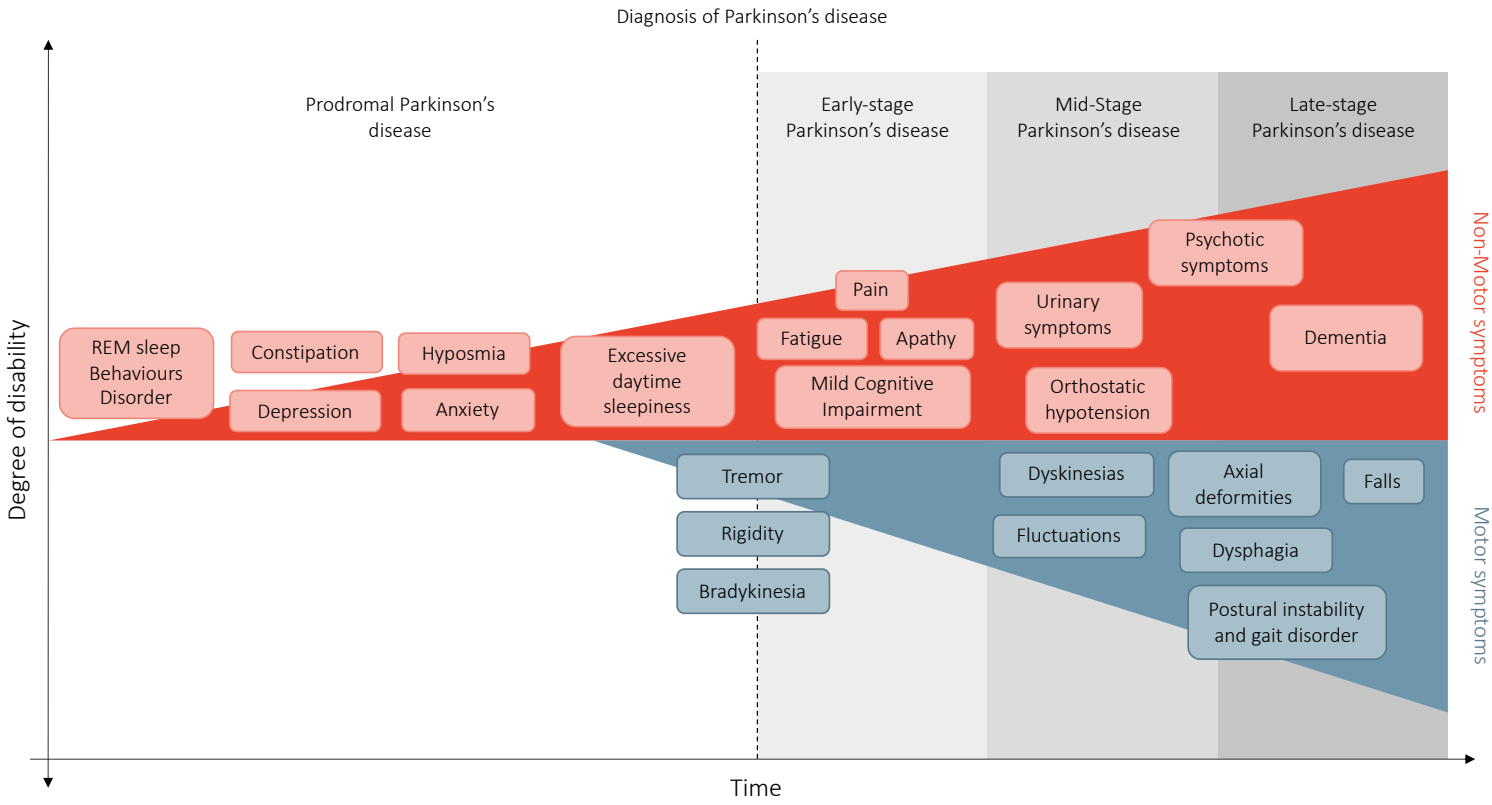
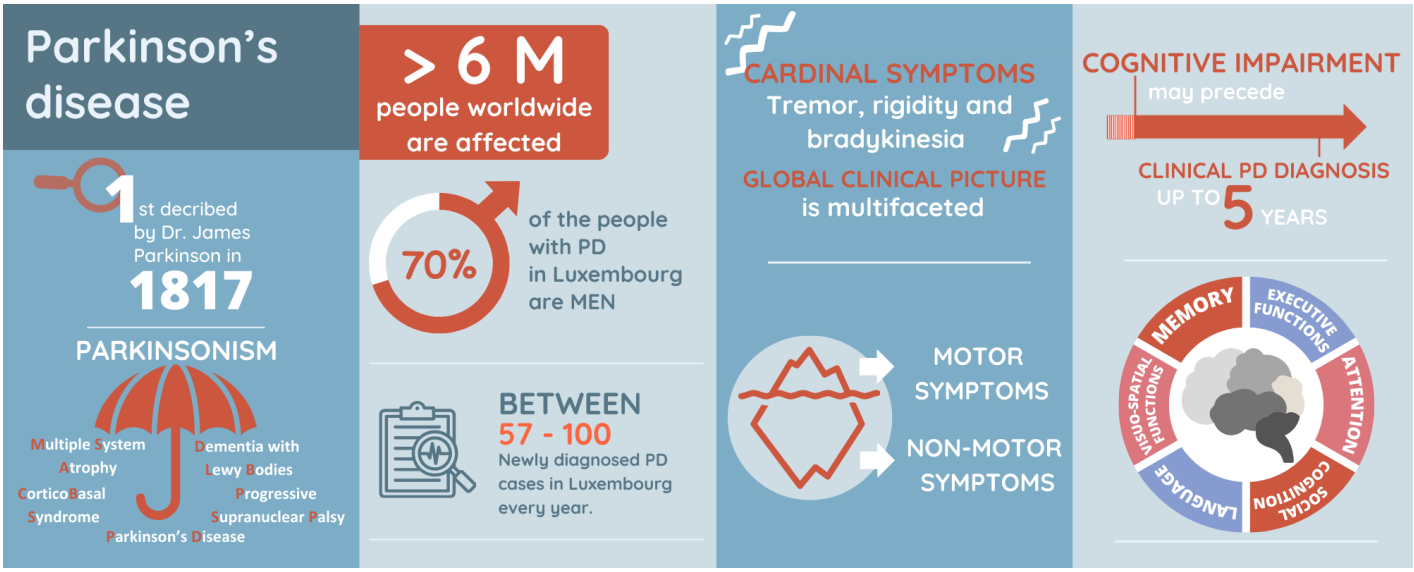


Figure 1. Timeline representing clinical symptoms and course of Parkinson's disease progression. Figure reproduced by Laure PAULY with permission from Springer Nature, Poewe, W. et al. Parkinson disease. Nat. Rev. Dis. Primers 3, 17013 (2017) [36]

The Movement Disorder Society (MDS) established research criteria for this prodromal phase of PD [13,25]. They defined polysomnographically proven Restless-Eye-Movement (REM) Sleep Behavior Disorder (RBD), abnormal dopaminergic PET/SPECT, subthreshold motor parkinsonism symptoms and hyposmia as prodromal markers of PD [25]. At a later stage, global cognitive impairment was

added as an additional prodromal sign of PD [13]. Recent studies indicated that at the time of clinical diagnosis, up to 54% of newly diagnosed patients presented cognitive impairment [46–49] and that it may even precede diagnosis for up to 5 years [43]. Deficits in prodromal PD are most frequently described for global cognition and diverse cognitive sub-domains, mainly executive functions, and less frequently memory [46–48]. However, the available studies are very heterogeneous in their study designs (e.g., recruitment strategies), study populations (e.g., age, education), neuropsychological assessments and the tested cognitive domains, complicating the comparability of results [46–48].

Within this dissertation we aim, among others, to contribute to the understanding of the cognitive profile in prodromal PD. The ultimate objective of research on prodromal PD would be to recognize PD at its early stage, by combining prodromal symptoms, before the onset of motor symptoms in combination with the development of a treatment allowing to stop the disease progression. Therefore, presently defining prodromal markers has not yet a direct clinical advantage but it will have implications when disease-modifying treatments become available.



Infographic 1. Parkinson's disease

Parkinson's disease-associated cognitive impairment

For decades, PD has been primarily described as a motor disorder and the presence of cognitive impairment was questioned for a long time. Nowadays, cognitive impairments are well documented and considered among the most common and disabling non-motor manifestations of PD [16,17].

Spectrum

The appearance, severity and progression speed vary widely among people with PD and range from normal cognition to Subjective Cognitive Decline (PD-SCD), to Mild Cognitive Impairment (PD-MCI) and Dementia (PDD) (Figure 2) [50,51]. Cognitive deficits are already observable in prodromal stages of PD and may precede clinical diagnosis by up to 5 years [43].

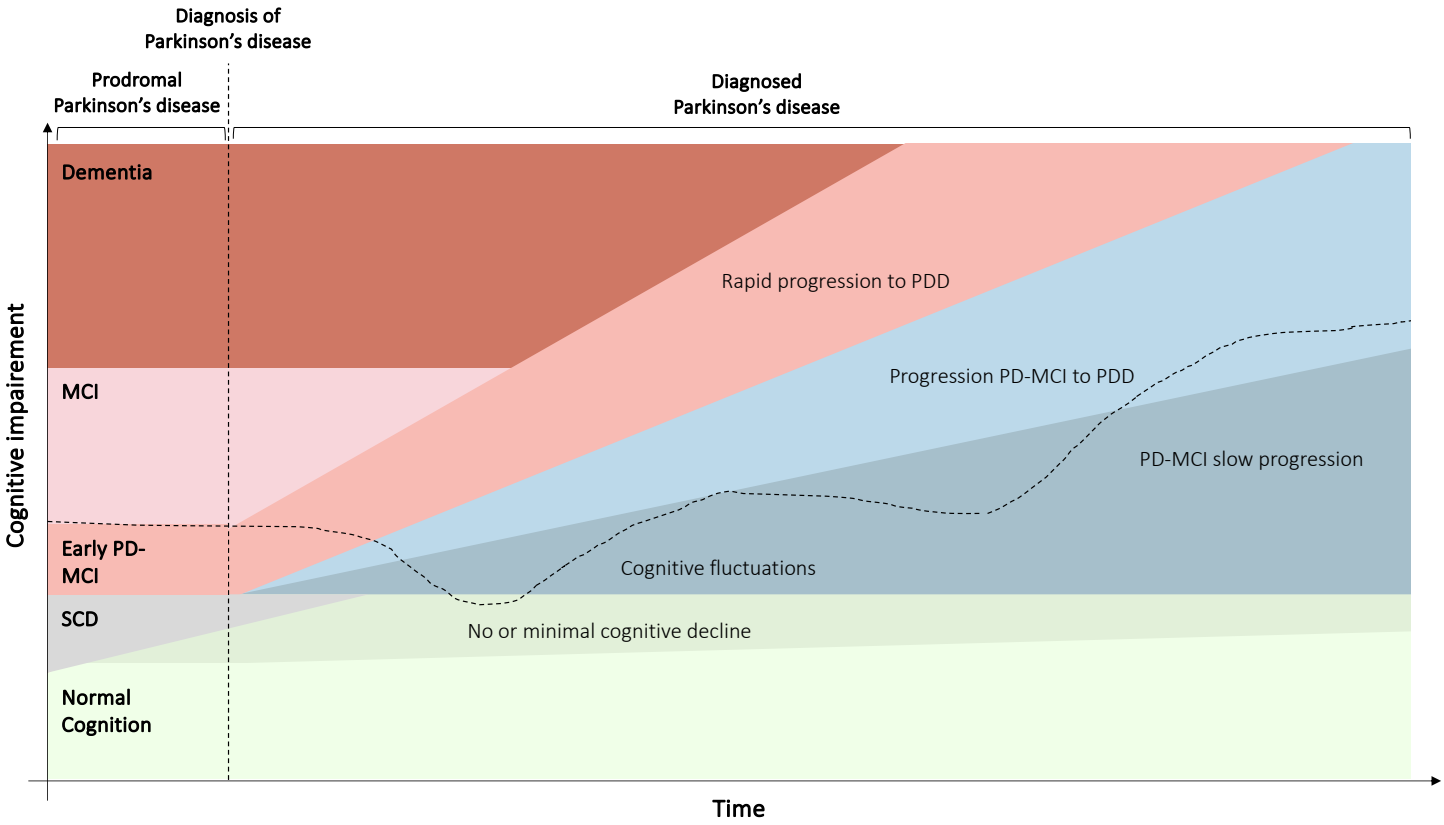


Figure 2. Cognitive spectrum and heterogeneity of progression of cognitive impairment in Parkinson's disease. Figure reproduced by Laure PAULY with permission from Springer Nature, 7:47, Aarsland et al. [50]*, Parkinson's Disease-associated cognitive impairment, Copyright (2021).*

Given that **PD-SCD** has not yet been clearly defined in the PD research field [52], the definition of SCD that has been conceptualized in the field of Alzheimer's research is primarily used for describing SCD in PD. They defined SCD as a self-perceived persistent cognitive decline, relative to a previously

normal cognitive status and unrelated to an acute event, that cannot be validated by neuropsychological testing, describing an unimpaired cognition from an objective point of view [52–54]. PD-MCI is defined as a gradual cognitive impairment, caused primarily by the underlying disease progression, reported either by the patient himself, an informant or the clinician and confirmed by neuropsychological measurements [51]. This cognitive impairment should, however, not be sufficient to significantly interfere with the patient's daily-life functional independence [51]. PDD is defined as a cognitive impairment with deficits in different cognitive domains, being sufficiently severe to affect daily life activities and the patient's functional independence [55,56].

Epidemiology

Despite the numerous studies that have investigated cognition in PD, findings on its prevalence vary severely [50,57]. Previous findings described that, approximately 30 to 40% of people with PD present a PD-SCD and that they were more likely to develop PD-MCI at follow-up [57–59]. Further studies demonstrated that between 15 and 54% of newly diagnosed PD patients experience already PD-MCI [46–49,60,61]. Its frequency increases with age and disease duration [62,63]. Furthermore, people with PD have a 2.5-6 times higher risk of converting to PDD compared to people without PD of similar age [50,64]. The cumulative prevalence of PDD varies between 17%, 46% and 83% at 5, 10 and 20 years after diagnosis, respectively [50].

Longitudinal studies addressing conversion in people with PD between normal cognition, PD-MCI and PDD, described that after 3 years of follow-up, i) in those with PD and no cognitive impairment at baseline, 25% converted to PD-MCI and 2% to PDD; ii) in those with PD-MCI at baseline, 20% converted to PDD while 28% converted back to normal cognition [65]. This observed reversion to normal cognition in PD (Figure 2) might be due to different factors influencing the neuropsychological assessment, such as tiredness, nervosity, stress or medication, highlighting the fact that MCI is known as a transient and fluctuating state [65]. Other researchers state that this observed reversion might be due to small fluctuations around a precise cut-off, and they claim that it is not due to a reversion back to normal cognition [50].

Changes in cognition

The cognitive profile in PD is very heterogeneous, with diverse cognitive functions affected (Figure 3) [51,62,66]. Despite the numerous studies on cognition in PD, we are still confronted with a debate about the precise nature and pattern of the cognitive impairments typically observed in PD [62,66]. Findings describe impairments in cognitive domains such as executive and visuospatial functions, attention and memory [63,67] as well as language dysfunctions [51,62,66]. Deficits can be observed in a single cognitive domain or multiple cognitive domains [68]. Some declare the non-amnestic, single-domain impairment as the most frequent subtype of PD-MCI [62,63]. In contrast to others, that define the amnestic, multi-domain impairment as the most frequent PD-MCI subtype [60].

Figure 3. Graphical representation of cognitive functions known to be impaired in Parkinson's disease. Graphic produced by Laure PAULY based on summary of Brown et al. (2022) [66].

Different hypotheses exist attempting to explain this heterogeneity. Kehagia and colleagues [69] established the "dual syndrome hypothesis" describing two sub-types of cognitive impairment in PD: A frontal-striatal subtype, with predominant executive deficits related to increased dopaminergic loss and a posterior and temporal subtype with predominant visuospatial, memory and language deficits, related to increased cholinergic loss. Others defined possible factors that may explain the heterogeneity of cognitive impairment in PD, such as genetics, premorbid functioning, cognitive reserve, the environment, the applied MCI definition, and the comparison of different neuropsychological assessment tools [70]. Furthermore, the severity and location of the neurodegeneration as well as other concomitant diagnoses may have an impact on cognition in PD [66].

Pathophysiology

The exact underlying mechanisms of cognitive impairment in PD are still not completely understood and are potentially defined by multiple factors. Proposed contributors include typical histopathological characteristics such as the accumulation of α-synuclein in affected brain regions as well as neurotransmitter deficit, functional and structural brain changes that might cause neurodegeneration [68]. Furthermore, synaptic dysfunction, genetics, fatty acid oxidation, inflammation, and oxidative stress, exosomal dysfunction, the gut–brain axis involving the

autonomic and enteric nervous systems may be involved in the explanation of the cognitive decline observed in PD [68]. Furthermore, other age-related diseases can coexist with PD and impact indirectly cognition [50], such as sleep disorders or depression.

Risk factors

Given the complexity and importance of cognitive decline in PD, it is a research priority to identify potential risk factors for cognitive decline in PD. Previous studies identified demographic and clinical risk factors for cognitive decline in this neurodegenerative condition. The following variables, ranked in descending order of importance, were associated with future cognitive impairment: hallucination, older age, severity of motor symptoms, speech impairment, older age at disease onset, bradykinesia severity, higher disease stage, axial impairment, low education level, depression, and male sex [71]. In addition, comorbidities coming along with PD may contribute to cognitive decline. A recent study on the Parkinson's Progression Markers Initiative (PPMI) cohort described that besides mood disorders, some other modifiable comorbidities such as sleep disturbances, overweight were associated with a faster rate of cognitive decline [72]. Furthermore, extensive research has been conducted on the identification of risk factors for cognitive decline in general, which can also be applied to PD. Findings highlight the importance of dementia prevention, defining twelve modifiable risk factors to develop severe cognitive impairment, education, hypertension, diabetes, physical inactivity, obesity, smoking, alcohol consumption, traumatic brain injury, air pollution, hearing loss, depression and social isolation [73,74]. Genetic variation might also have an impact on the observed cognition decline. In a recent review on cognition in genetic forms of PD, cognitive impairment was described as being higher in *Glucocerebrosidase*- (GBA) and *α-synuclein gene mutation*- (SNCA) related PD, lower in Parkin- and *PTEN Induced Putative Kinase 1*- (PINK1) related PD [30].

Treatment

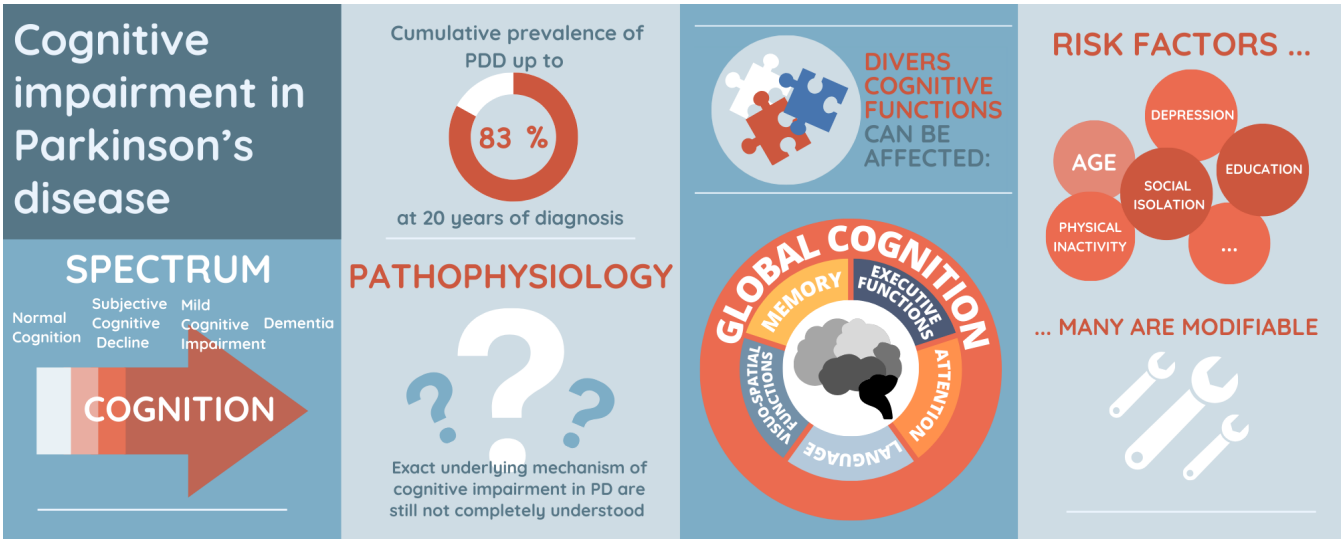
Despite the rising interest, knowledge of treatment options for cognitive difficulties in PD lags far behind our knowledge of treatment options for PD motor symptoms [50]. Since the underlying pathophysiology of cognitive impairment in PD remains unclear, treatment options targeting the cognitive impairment are limited [68]. Pharmacological treatment agent such as Memantine, Rivastigmine and Donezepil have been defined for treating cognitive impairment in PD [75]. Non-pharmacological treatments such as cognitive and physical rehabilitation, nutrition, non-invasive brain stimulation (such as repetitive Transcranial Magnetic Stimulation (rTMS) or transcranial Direct Current Stimulation (tDCS)) and invasive brain stimulation (such as DBS) have shown positive effects

on cognition [68,76–78]. Furthermore, identifying and reducing the previously described modifiable risk factors, e.g., with personalized lifestyle interventions, should be a priority in patients with PD with cognitive complaints.

Impact and burden

Cognitive impairment causes a significant burden for people living with PD as well as economic consequences [16,79,80]. Previous findings demonstrated that non-motor symptoms and their progression, such as cognitive decline, can even have more consequences on quality of life than motor symptoms [81]. Already at an early disease stage, cognitive impairment is an independent factor contributing significantly to poorer quality of life in newly diagnosed PD [79]. In particular, impaired attention had the greatest predictive power for lower quality of life [82]. In addition, cognitive impairment in people with PD is a significant burden for relatives, care partners and caregivers [83]. Thereby the importance to gain better knowledge on the different cognitive impairments in PD at different disease stages. Despite increased interest in cognitive impairment in PD over the past decades, our knowledge is still limited. Continued efforts for a better understanding of this complex symptom in PD are required [50]. Furthermore, severe cognitive impairment impacts possible therapy choices, given that it is an exclusion criterion for DBS [84].

Even though PD was first described in 1817 [20], and the numerous studies that followed, the disease is still not fully understood, and its conceptualization is still ongoing. We need a better characterization of the cognitive impairments observed in PD to be able to develop effective reeducation therapies and to be able to target specific disease processes [85].



Infographic 2. Cognitive impairment in Parkinson's disease

Procedural memory in Parkinson's disease

Procedural memory

General introduction - memory

For decades, memory was defined as a unitary entity, then as a dichotomous distinction. The acceptance of the notion of memory as a complex, involving different systems, only came later.

The first dichotomous theories on memory relevant to current neuroscience started in the 19th century when William James suggested a conceptualization differing between memories and habits [86]. In the following years, memory continued being defined as a dichotomy, differentiating between "memory" and "habit" [87], "explicit" and "implicit memory" [88,89], "knowing how" and "knowing what" [90], and "declarative" and "procedural knowledge" [91,92]. The first neuropsychological, experimental evidence of spatial and conceptual dissociation of declarative versus non-declarative memory came from Brenda Milner. She and her team, pioneers in the field of cognitive neurosciences, made substantial contributions to our understanding of the complexity of the memory systems, among others procedural memory within their famous case study of Henry Molaison (H.M.) [93,94]. This distinction was confirmed by Mishkin and Appenzeller [95] with studies on subhuman primates. Induced brain lesions helped define two designable memory concepts, which underlay different anatomical and functional networks and structures.

During the 1980s and 1990s, the perspective of a dichotomous distinction changed to a multiple memory system point of view. Given that studies continued making observations on a wide variety of learning and memory phenomena, different memory systems, knowledge types or learning strategies, a more complex definition of memory was needed [96,97]. The dichotomous distinctions changed to a "declarative" & "non-declarative" distinction, with the "non-declarative" as an umbrella term referring to multiple memory systems that are not declarative [98], including classical conditioning, non-associative learning, priming and perceptual learning and procedural memory. The "declarative" memory concerns the storage and retrieval of information that an individual can consciously recall – *such as recalling the capital city of a country*; and "non-declarative" memory concerns memories that are unconsciously retrieved – *such as playing an instrument.*

See Figure 4. for the multiple memory system taxonomy based on Squire & Dede's taxonomy for a recent organization of long-term memory systems as well as the brain structures related to each memory form [99].

Definition of procedural memory

General definition

Procedural memory is a non-declarative, implicit long-term memory concept in charge of encoding, storing, and retrieving procedures underlying unconscious motor, cognitive and perceptual skills [100,101], also known as "memory of skills and habits" [102]. Procedural memories are typically acquired through repetition, characterized by an improvement in performance, followed by automatization [100,101]. Automatization is reached when the neural network involved in performing the task can execute it without the need for conscious control or attention. Once acquired, these unconscious memories are crucial for a person's ability to complete automatic activities of daily living, such as walking, typing, and playing instruments. Procedural memory is characterized by its robustness and durability, the capacity to maintain knowledge over a long period, even if it is not regularly consolidated [100]. Clinically relevant examples of procedural memories are writing one's signature, driving a car, tying one's shoes, or playing an instrument [101,103–105].

Retrograde and anterograde procedural memory

As procedural memory is often imprecisely defined, we felt the essential need to define this memory concept in detail before moving along with our project. Judging Crystal's and colleagues' [104] definition, of separating procedural memory into an anterograde and a retrograde component, as the most accurate, we applied it to our project:

The anterograde procedural memory involves the acquisition of new skills, *e.g.: learning to play a new composition on the piano.* Whereas the ability to execute skills acquired in earlier life stages is part of the retrograde procedural memory – *e.g.: playing a piano composition from memory that has been learned in earlier life stages* [104].

An impaired anterograde procedural memory represents problems with the ability to learn new skills, by repetition. An impaired retrograde procedural memory demonstrates a problem in recalling and executing learned procedural skills, that had once reached automatization.

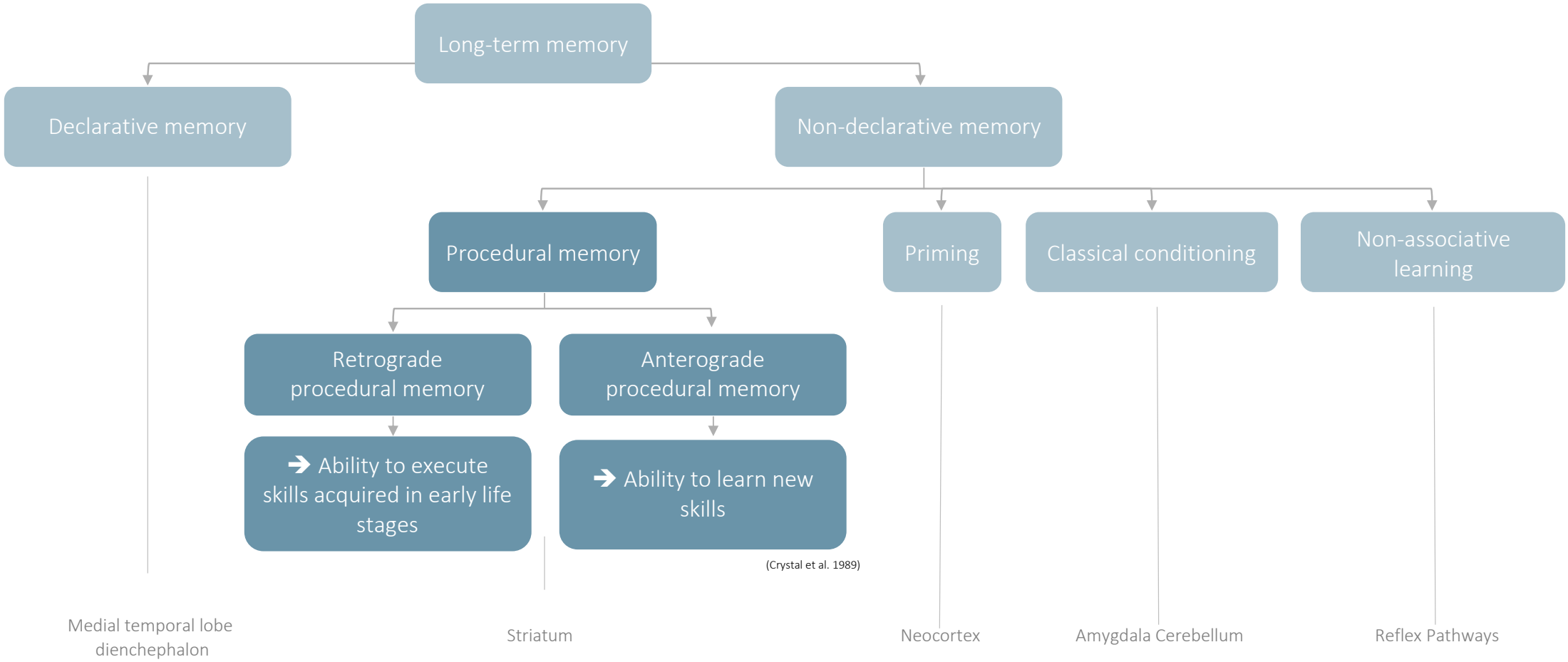


Figure 4. Taxonomy of long-term memory systems with a focus on the division of procedural memory into an anterograde and a retrograde component.

The representation lists brain regions assigned to the different memory systems as defined by Dede & Squire (2015) [99] *Figure reproduced by Laure PAULY based on Dede & Squire (2015), Conscious and unconscious memory systems, Copyright (2015), with permission from Cold Spring Harbor Perspectives in Medicine.*

Neural basis of procedural memory

In the previous decades many studies aimed to better understand the functional anatomy and mechanisms involved in procedural memory. Based on studies on participants with focal lesions [106–108], on people with PD [11,105,107–133] or Huntington's disease [113,114,134] and functional neuroimaging studies [109,135–138], one can summarize that the main brain areas involved in the procedural memory are the basal ganglia, the cerebellum and their associated structures forming the cortico-striatal and cortico-cerebellar systems [100]. Other brain areas like the frontal motor cortex [101,126,139,140] and the limbic system [100] are also involved. Despite the numerous studies that aimed to investigate the mechanisms and areas involved in procedural memory, there are still many divergences. This might be explained by the inconsistent definitions of this memory concept and the varying nature of the applied tasks. Concerning the inconsistent definition of the memory concept, most of the studies focused on the procedural learning phase, the anterograde procedural memory [107,112,114,115,121,129,132,133], only a limited number of studies focused on the long-term retention of a newly learned skill (3-18 month) or the very long-term aspect of this memory concept, the retrograde procedural memory [12,108,126,141].

Some of these findings support the global efficiency hypothesis, which states that during the automatic performance, specific brain areas necessary for performing the skill (e.g.: motor brain regions) are working more efficiently requiring less activation in participants that have developed the skill [127,142]. For example, musicians showed less activation in the motor cortex compared to musically naïve controls when playing an instrument [142]. Other studies concluded that in the early learning stages of over-learned sequential motor tasks (e.g.: knitting) both the cortico-cerebellar and the cortico-striatal networks are activated. However, after the automatization of the skill, the activation of the cerebellum is not needed anymore, and the long-lasting representation of the automatized skill now implicates mainly the basal ganglia and associated cortical regions [140,143]. Some findings on motor learning describe the activation of the cortico-striatal-loop during the early learning phases and the cortico-cerebellum-loop at a later stage [136]. In other motor learning studies, the opposite was found, observing first an activation in the cerebellar-cortical network that transferred later to a striatal-cortical network activation [138]. Regarding the varying nature of the applied tasks, studies defining brain areas involved in motor skills [107,127] are compared to studies relying on cognitive skills [109]. One could hypothesize that the nature of the skill (e.g.: motor or cognitive) and the learning vs. memory aspect are going to implicate different brain networks.

Some models discuss a compensation strategy in PD in case of an impaired procedural memory system. Vandenbossche and colleagues [11] hypothesized that in case of an affected procedural memory system in PD, a shift in neural activation is expected from sub-cortical to cortical brain regions as a compensatory strategy, mainly compensating the procedural memory deficit by applying more executive functions. Vandenbossche applies this model to explain the pathogenesis of FOG in PD. Recent functional brain imaging studies supports this compensatory model by describing increased involvement of attention as a compensatory strategy in PD compared to control subjects after motor learning [144]. In controls, directing attention to automatic movement resulted in increased activation of the dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex and rostral supplementary motor area. The motor cortex received more influence from the cortical motor association regions. Activity of the striatum remain consistent with the automatic stage. In contrast, in people with PD, attention to automatic movement resulted in increased activity of DLPFC, premotor cortex and cerebellum but the connectivity from the putamen to the motor cortex dropped [144]. They observed a shift from the automatic mode back to a controlled pattern within the striatum in PD. They also aimed to explain with their result of dopamine depletion in the sensorimotor striatum the observed deficits of previously stored automatic programs [144].

Assessments of procedural memory

Overview

In adults, procedural memory can be assessed with neuropsychological assessments based on perceptual-motor tasks (e.g., Serial Reaction Time Task, Rotor Pursuit Task, Mirror Tracing) or cognitive pattern-analyzing tasks (e.g. Mirror Reading, Tower of London). Some of the most used assessment tools for evaluating the procedural memory system are presented below.

The **Serial Reaction Time Task** (SRTT) is a choice reaction time task, in which the subject's task is to respond as fast as possible to a visual stimulus at one of several different spatial positions on a keyboard. The study participant should not be aware that the visual stimulus follows a repeating sequence [145]. The **Rotor Pursuit Task** is a device that allows analyzing the visual-motor tracking skills and hand-eye coordination by requiring the participant to follow a moving object with a cursor [112]. In the **Mirror Tracing Task** the participant should trace a star while seeing their hand movements only as a reflection in a mirror [93]. Not receiving the expected visual feedback, the subject needs to overcome the reversed visual field by concentrating. In the **Tower of Hanoi** or

Tower of London the participant needs to find a strategy to transfer a pile of disks from one tower to another while following certain rules [146,147].

These assessment tools measure mainly time and the number of mistakes made. If the participants react quicker after several repetitions and perform without too many mistakes, this represents successful unconscious procedural learning. Furthermore, these assessment tools can be used to measure different cognitive aspects. When only used over a few trials, and the participant gets specific explicit instructions, these assessment tools allow the measurement of specific non-procedural cognitive functions such as planning functions for the Tower of Hanoi. However, when the test is repeated over many learning sessions, and they are unbeknownst about, e.g. in the SRTT a repeating sequence, they may also assess cognitive procedural learning. For example, the SRTT can be used as an explicit task, measuring declarative learning. In this condition, the participant is told that there is a sequence and that they should learn it to improve their performance speed. Used as an implicit task, the participant is never told about a sequence. Under this condition, a participant with intact implicit learning capacitates will learn the sequence unconsciously, and implicitly and will perform the task quicker by repetition.

Procedural memory in PD

Current knowledge and critical appraisal of procedural memory in PD

In people with PD, difficulties in performing everyday automatic activities such as handwriting [148], playing an instrument [101], walking [127], speaking [149] and habitual components of actions such as arm-swinging, have been repeatedly described in the literature [150,151].

Despite the numerous studies that have investigated the cognitive function behind these automatic activities in PD, the conclusions are inconsistent. Studies on procedural memory investigated mainly the acquisition and immediate retrieval of the automation. While most of these studies have described an impairment of the acquisition of a new procedural skill in PD people [105,107,108,110,112–122,124–133,152–155] others did not observe any significant differences [109,156–160] or only mild to moderate or partial impairments [106,116,152,161,162]. Even though, the unique characteristic of procedural memory is its robustness in time, and its longevity, only a very limited number of studies investigated the long-term retention of a learned skill (3-18 months) [12,108,126,141]. In most of these studies, participants learned a new skill (e.g., motor sequence by

SRTT) and they were retested some months after the learning. Most of the studies described impaired long-term retention in people with PD. This observation has also been confirmed by a recent review stating that the retention of (motor) skills is mainly impaired in PD, despite preserved learning [163]. Some studies investigating both, the learning and the long-term retention of the learned skill, described no impairment in the learning stage; however, they observed that the people with PD had difficulties maintaining the newly learned skill over time [141,159,164–166]. People with PD were able to learn a new skill and performed similarly to their control groups. However, the observed improvement disappeared within a few months, whereas the controls retained the learned skills.

These discrepancies may be explained by diverse confounding factors such as varying definitions of the memory concept itself (anterograde/retrograde procedural memory), the nature of the used tasks (cognitive/motor) [106,157] or the communication and explanations given to the task (explicit/implicit). Furthermore, the influence of drugs and therapies [129,163], the differences in the study population (clinical and demographical characteristics, sample sizes) [129], or even methodological variations (matching methods, application of neuropsychological assessments sensitive to PD typical symptoms) may explain these controversies [140,163].

Complications encountered when investigating procedural memory in PD

Procedural memory is not routinely evaluated by clinicians and researchers [101]. This may be explained by an a priori assumption that this robust cognitive function is resistant to some neurodegenerative disease but probably also due to the lack of easy-to-apply assessments and the not fully understood theoretical background [167].

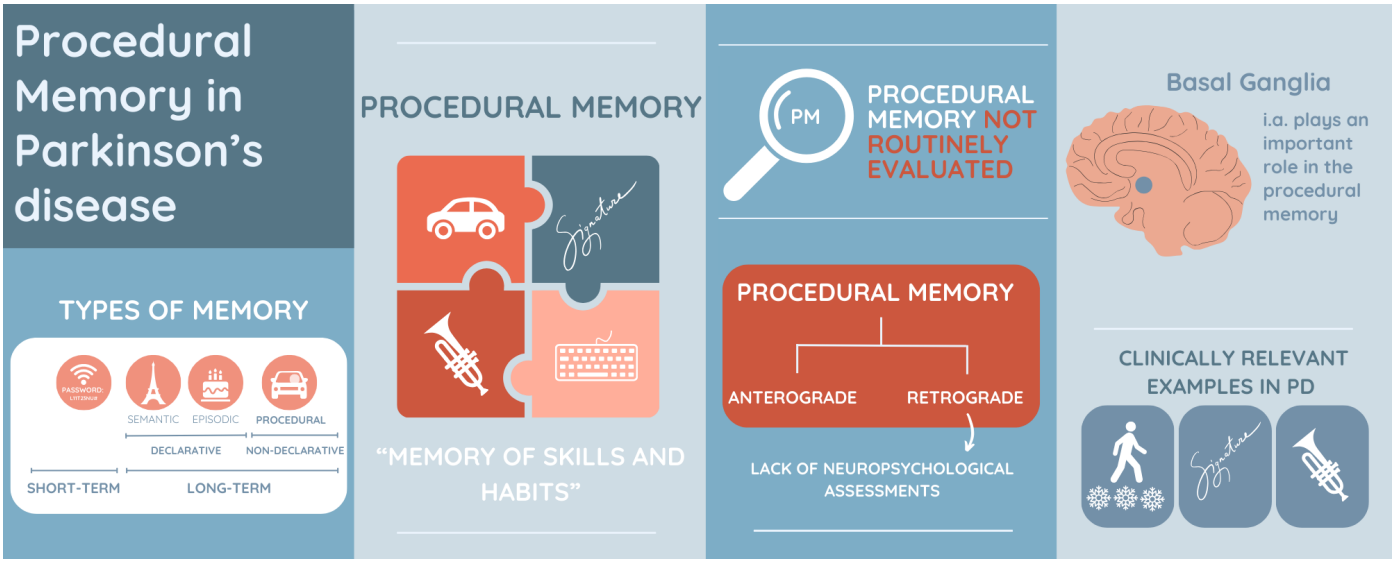
These previously described assessment tools (e.g.: SRTT, Rotor pursuit, Mirror tracing Task) for procedural memory are also frequently used in studies on people with PD. However, two important limitations of these tools explain why they may not be ideal for being applied to people with PD. Firstly, they primarily base their evaluation on the performance speed and secondly, most of the tasks require a lot of motor control. Given that slowness of movement and extrapyramidal symptoms, such as tremor and rigidity, are part of the common PD symptoms, this might cause an important bias. People with PD may be capable of learning a procedural skill but are simply unable to demonstrate this due to a decreased reaction time over trials and motor complications [120,158,160].

Assessment tools that are less influenced by extrapyramidal motor symptoms, focusing more on cognitive tasks, exist but are not frequently applied: In the **Mirror Reading Task** also called the **Inverted Reading Task**, the participant is asked to read mirror-reversed words, which involves learning of a new visual graphic decoding for lexical access [152]. Furthermore, the **Braille Task** is a learning task which involves lexical memory involving tactile and spatial memory [153]. The **Sound and Form Association Task** involves the acquisition of sounds and visual forms [153]. The **Verbal Serial Reaction Time**, a verbal version of the SRTT task where the response, normally performed through a motor response such as pressing a button was replaced by a verbal response [120]. A further alternative assessment is the **Ocular Serial Reaction Time** [133]. However, all these tests are still assessing the possibly biased reaction time. Furthermore, it is important to keep in mind that, as it is the case for most of neuropsychological evaluations, all these evaluation tools implement other cognitive functions, making the analysis for a "pure" procedural evaluation system illusory [168].

These commonly used methods, previously described, measure mainly the learning ability of a procedural skill, the anterograde procedural memory. Representing only one side of the procedural memory concept, it misses the very long-term aspect of the memory, the retrograde procedural memory. Even though memory and learning are very closely related concepts, they are not identical and need to be assessed separately. As mentioned by Cohen [141] *"surprisingly little work has specifically looked at how and whether this learning is maintained in the long-term. Results, which indicate that a new skill information is retained over a testing period, provide no evidence that learning is maintained over a longer period."* Keeping in mind, that exactly this retention and execution of long-term skills has the most important impact on the activities of our daily life.

All in all, assessing procedural memory still encounters difficulties on both levels, theoretical and clinical [167]. On the theoretical level, to our knowledge, there are no easy-to-use and evidence-based protocols available to evaluate specifically this retrograde memory concept [101,167]. On the clinical level, we experience a lack of ecological validity of existing assessment tools for the evaluation of procedural learning. There is no clear linkage with real-world activities between poor performance in the Mirror Tracing Task, SRTT or the Tower of London and possible difficulties in real-world activities and skills [139,167]. To tackle these issues, Doyon and colleagues presented three further options that allow for assessing the automatic performance of a procedural memory [143]: The first option they named is the dual-task paradigm. With the dual-task paradigm, one can

determine whether a secondary task (e.g.: letter-counting task) can be performed with minimal interference on a primary task (e.g.: sequenced motor skill), the procedural task [127]. However, this technique has the important limitation that you cannot be certain that the procedural skill has become completely automatic and that other cognitive functions such as divided attention may play an essential role. The second option they discussed, consists of comparing specific skill performances of participants with over-learned skills (e.g., musicians) to control subjects (e.g. musically naïve controls) [142]. The third option would be to analyze the performance in participants (e.g. skilled knitters) while performing over-learned skills, "automatized condition" (e.g. knitting by applying an over-learned stitching pattern no conscious thinking about the procedure is required) and compare the performance in the same subjects while asking them to do apply the same skill but with a technique that needs to be learned "newly learned condition" (e.g.: knitting by applying a stitching pattern that was unknown to the participant requiring active thinking) [143]. These creative options are however extremely time-consuming and not easily insertable in existing study protocols or applicable clinical setting.



Infographic 3. Procedural Memory in Parkinson's disease

Summary and discussion of the research findings presented in the manuscripts

Chapter I – Retrograde Procedural Memory in PD

In the first part of this dissertation, we aim to take up these previously addressed challenges. By developing the CUPRO [1], the extended evaluation system of the Cube Copying Task, we try to fill the lack of an easy-to-use assessment for the quantification of the functional status of an existing process stored in the procedural memory. The Cube Copying Task, initially evaluating only the final result of the drawing, visuo-constructive functions, on one single point [169], has herewith been extended to a 6-point score. Composed of a first Intermediate Score (IS_1) of three points, evaluating the Cube copying procedure, and the second Intermediate Score (IS_2) of three points, evaluating the final drawing, allows to evaluate both, retrograde procedural memory, and visuo-constructive functions.

With the development of the CUPRO, we tried to consider as many of the previously discussed limitations as possible. The assessment should evaluate a previously automatized unconscious procedure, that is ideally known by most of the assessed population. By copying a Cube, we unconsciously apply a previously acquired procedure. To reduce the burden for study participants and clinicians, this test should be easily incorporated into already existing batteries and should be brief and easy to apply. The CUPRO assessment is easy and quick to administer (<1 minute) and can be evaluated by any trained health professional. It can easily be incorporated into study protocols as the Cube Copying Task is already a commonly used item in standard assessments such as the MoCA [169] and the CERAD [170]. Within these assessments, the CUPRO evaluation can be included without the need to add a new assessment that would increase the burden for study participants and clinicians. Furthermore, ideally, the evaluation should not be influenced by the motor symptoms such as tremor and the mental or motor slowness observed in PD. By concentrating mainly on the drawing procedure and not considering the time needed for the copying, the measurement of procedural memory should not be biased by the typical motor symptoms, such as tremor or slowness. All in all, the CUPRO evaluation system adds valuable information to an already well-established screening tool, the Cube Copying Task, without increasing the burden for study participants.

In **Chapter IA**, we evaluated the Cube copying performances of the PD participants of the Luxembourg Parkinson's Study [24] with the CUPRO evaluation system and compared their performance to an age- and sex-matched control group. We identified that the copying performance was significantly affected in people with PD, suggestive of impaired functioning of retrograde procedural memory in PD. Through evaluating discriminant validity in a subgroup of participants, with several tests representing related constructs, such as motor functions, and visuo-spatial or executive functions, no significant interference has been observed. This may however also be explained by low statistical power and would need to be validated in a larger sample. Lastly, we did not observe a significant correlation between retrograde procedural memory and the years of disease duration in the PD population. This led us to the supra-analysis we performed in Chapter II, hypothesizing that this memory deficit may already be present in the pre-motor stages of PD. Studies for the validation of the CUPRO evaluation system in independent PD cohorts are ongoing.

In **Chapter IB**, we studied cognition focusing on the CUPRO performances in people with PD and FOG. The cognitive contributions to FOG, a common gait disorder in people with PD, have already been described in previous studies [171]. Given that walking is a highly automated skill, it is hypothesized that affected gait, as described in FOG, may be explained by the loss of automaticity [11] supported by procedural memory. Therefore, we compared, additionally to global cognition and mental flexibility, the CUPRO performance between participants with PD and FOG (FOG^+) to age-, sex- and disease duration-matched PD participants without FOG episodes (FOG^-). The main finding of this study was that besides lower global cognition and mental flexibility, the retrograde procedural memory deficit was significantly more prominent in FOG^+ compared to the matched counter group FOG^-.

All in all, our findings in Chapter I (Chapters IA & IB) confirm our hypothesis of lower Cube copying performance, evaluated by the CUPRO evaluation system, in people with PD compared to a matched control group. These observations are suggestive of an affected functioning of retrograde procedural memory in PD. Furthermore, the more prominent deficit of retrograde procedural memory observed in people with PD and FOG compared to non-Freezers may validate the hypothesis that FOG is a de-automatization of walking and, among others, due to the deficit of procedural memory.

Standard Operating Procedure & Training video

To promote consistency, efficiency, quality output and guidance, a written Standard Operating Procedure (SOP) has been established for the CUPRO evaluation system. In addition to a written SOP, we established a self-explanatory training video. The recent SOP and the link for the training video can be found in the Appendices (Appendices 1 & 2). Both include a short introduction defining the task's goal, detailed step-by-step instructions with examples and counterexamples as well as the CUPRO evaluation sheet. Additionally, the training video includes some examples of some Cube drawings. To ensure easy and quick access to the CUPRO SOP, it is available in RedCap [172,173], the used data collection platform, and can be found next to the evaluation items for the CUPRO (see Appendix 1. Figure 3). Given that the clinical teams are constantly growing and are located on two different sites (Parkinson's Research Clinic (PRC) for the assessments of people with PD and Clinical and Epidemiological Investigation Center (CIEC) for the assessments of control subjects), regular joined training were organized, bringing all the members together to ensure consistency in the data collection.

Impact of COVID-19 pandemic on CUPRO project

The last few years have been highly marked by the consequences of the COVID-19 pandemic. Unfortunately, it also had a substantial impact on the research field, halting entire research projects, interrupting participant recruitment, redirecting resources and stopping in-person visits. Even after re-opening, the aftermath of the crisis still impacts research, with delayed participant enrollment and delayed deadlines and timelines [174]. Even after establishing and following strict guidelines for viral infection prevention in the clinics and research centres, many participants, especially vulnerable people, including the elderly, stayed for a long time hesitant to come into the clinic to participate in research. Nevertheless, the situation also introduced innovative and creative solutions to overcome these obstacles. New approaches to continue clinical research have been adopted, such as the replacement of in-person visits with remote visits, by telephone interviews or video calls.

Unfortunately, many of the previously mentioned consequences also impacted this doctoral research project, which only started 3 months before the outbreak of the pandemic. With the temporarily interrupted participant recruitment and follow-up, reduced in-person visits and the higher number of telephone interviews, we encountered a previously unexpected limitation of the CUPRO: Even though it has the important advantage of being language-independent, for its evaluation, the clinicians need visual feedback. This made it impossible to assess the CUPRO during our telephone interviews. In collaboration with the Oxford Parkinson's Disease Center (OPDC), and

after a short inter-rater reliability test, we introduced the CUPRO as a pilot project in their video-call interviews. During the video call, the Cube model of the MoCA test was presented to the participants. They were asked to copy the figure as accurately as possible on a sheet of paper lying in front of them. For the rater to evaluate the Cube drawing procedure, the participants were asked to adapt the position of their camera so that it was directed to the paper sheet, giving the clinician a good view of the drawing procedure. This manoeuvre showed already small complications during the interne testing of the CUPRO over video interview, and this hurdle seemed even more difficult to the people with PD, living with motor symptoms such as poorer fine motor skills and tremor. Furthermore, we also recognized that redirecting the attention of the participant especially to the Cube drawing, by asking them to readjust the camera so that the clinician can observe the drawing procedure, might bias the implicit drawing procedure. The evaluation of the drawing procedure should stay unbeknownst to the subject to ensure that it does not depend on explicit cognitive processes. Based on these two limitations, we came to a joint conclusion, that the CUPRO is not easily accessible via video call and stopped the pilot project and kept the CUPRO evaluation only for the in-person visits. For future projects, one solution would be the application of telehealth administration and the digitalization of the CUPRO, e.g. in the electronic MoCA version, the eMoCA [169].

Overcome limitations of the CUPRO with the CUPRO 2.0 or the eCUPRO

Future research might work on an improved version of CUPRO 2.0. After having collected and discussed received feedback, we suggest reconsidering the scoring system for improvement. In preparation for this updated CUPRO version, CUPRO 2.0, we established a provisory new CUPRO 2.0 evaluation sheet, including some minor updates. These updates aimed to facilitate the evaluation. A juxtaposition of the initial CUPRO version and the CUPRO 2.0 version, with the suggested improvements can be found in Appendix 3. The version CUPRO 2.0 serves as a suggestion for future projects and has not yet been applied. Future research projects should concentrate on expanding and improving the CUPRO evaluation system.

To cope with the previously discussed limitations, it would be substantial to develop an electronic CUPRO version, eCUPRO, allowing the retrospective analysis by a clinician. Furthermore, an automatic evaluation and scoring of the Cube drawing procedure by an Artificial Intelligence (AI), would reduce inter-rater variability and would be an important step for its validation. Integration of electronic measurement into clinical practice may enhance reliability and efficiency of cognitive

assessment [175]. However, it is important to keep in mind that electronic measurements may influence performance and scoring, especially for the drawing assessments, which require physical interaction with the tablet, particularly when administered to an older population that are less familiar with the digital world [176].

Future perspectives for research on procedural memory

As highlighted in the introduction, there is a substantial need for novel assessment tools allowing the evaluation of procedural memory, especially with ecological validity. The development of a multi-approach battery around this memory concept would be of high interest and could cover this need. Therefore, we suggest developing a multi-approach battery, combining different quantitative and qualitative measurements, such as:

i) **Neuropsychological assessments:** for the assessment of anterograde procedural memory, we would suggest the Inverted Reading Task [152] as it is less biased by motor complications and for the retrograde procedural memory the CUPRO evaluation [1].

ii) **Behavioural assessments:** of everyday activities such as shoe tying, knitting, driving, reciting prayers or individualized tasks that need personalized adaptations, e.g.: playing a music instrument for a musician or through an investigation of action slips or habit-driven mistakes [177]. The challenge, however, in this part lies in the standardized evaluation of these activities.

iii) **Self-reported questionnaires or structured interviews:** related to procedural activities in daily living (e.g.: driving, cooking well-known recipes, using the TV remote control).

This multi-measurement approach for a systematic evaluation of the learning of a new skill in combination with the focused evaluation on the procedural memory of previously learned skills would not only fill the previously discussed gap in clinical settings but may also help identify valuable perspectives for future research. Furthermore, it will not only be interesting in PD but also other neurodegenerative disorders, such as Huntington's disease.

Getting a better understanding of this complex memory concept is of great importance given that its integrity plays an essential role in the maintenance of routine daily-life activities and therefore

also their independence. Investigating the relation between the pathophysiology of PD and cognitive functions such as retrograde procedural memory is important, as these insights can lead to new hypotheses on the aetiology of the disease and help us to draw a better picture of cognition in PD.

Chapter II - Cognition and other non-motor symptoms in prodromal PD

In the second part of this dissertation, we focused on the exploration of the non-motor symptoms emphasizing on cognition in people at high risk of developing PD. Diagnosing PD means identifying an already advanced disease, with more than 60% of the dopaminergic neurons already degenerated [32,42]. Therefore, we aimed to describe cognition and other non-motor symptoms in the prodromal PD (P-PD) stages, by taking into consideration recommendations for future perspectives [46,48] and to overcome the limitations of existing studies.

We compared cognitive performance and other non-motor symptoms, such as mood and quality of life, in an at-risk group for PD, defined by probable RBD and hyposmia, with age- and sex-matched control groups. Our findings confirmed a significant deficit of global cognition, executive, and visuo-constructive function in the P-PD group. We carefully interpret, that out of the extensive cognitive assessments, the Trail-Making-Task (TMT) and the Cube Copying Task might be the most sensitive for detecting executive and visuo-constructive changes in PD. Given that we did not find any significant correlation between retrograde procedural memory and disease duration in the previous Chapter, we aimed to explore in a supra-analysis the possibility of an implicit memory deficit in the prodromal stages of PD. However, we did not see any significant difference in retrograde procedural memory in the P-PD compared to the control group. Given that our findings suggest that we work on an early P-PD cohort, this result of intact memory function in early prodromal stages concurs with already existing findings on memory in general [178]. In addition to significant cognitive differences, they had significantly more self-reported motor and non-motor symptoms, such as depression and apathy, and significantly lower quality of life.

All in all, we were able to describe the non-motor symptoms in P-PD and to define a specific non-motor and cognitive profile in our at-risk cohort defined on pRBD and hyposmia. Based on our findings, we suggest considering the addition of executive function, best assessed by the TMT and visuo-constructive deficits, best measured by the Cube Copying Task to the MDS criteria for P-PD.

Having a clear description of the P-PD phenotypes leading to early recognition of PD will be essential as soon as research advances on disease-modifying treatments [26].
The ultimate objective of this research project on P-PD would be to recognize the neurodegeneration causing PD at its early stage. Identification of prodromal stages of PD could be achieved by combining prodromal symptoms before the onset of motor symptoms. Given that we do not yet have a cure that allows us to stop the disease progression of PD, defining prodromal markers has not yet a direct clinical impact but will become beneficial as soon as disease-modifying treatments become available

Future perspectives

According to the MDS criteria for P-PD [25], idiopathic RBD (iRBD) based on polysomnography has a positive likelihood ratio of 130 compared to only 2.3 for the questionnaire-based probable RBD (pRBD). Therefore to follow the gold standard for RBD diagnosis and to enrich this prodromal cohort, our participants undergo video-polysomnography to confirm iRBD. These at-risk participants are followed up yearly to analyze the trajectories of prodromal signs such as cognition, to identify and describe a possible conversion from P-PD to PD. Early recognition of PD may help better prognosis and support the development of neuroprotective therapies.

Given that clinical PD is significantly heterogeneous, the same observation might be the case for P-PD. Our study has the limitation that it focuses on one specific subtype of prodromal PD, defined on hyposmia and pRBD. For future projects, we plan to combine additional prodromal signs, such as constipation, or DAT deficit, and genetical predispositions and to assess if different prodromal subtypes are related to different non-motor, cognitive patterns, of different severities [48]. In addition, we cannot be certain that all the participants at risk of developing PD from our cohort develop PD, given that a proportion of the participants with RBD might develop other neurodegenerative diseases such as DLB or MSA [179]. The understanding of the heterogeneity in P-PD is essential to understanding the diversity of clinical PD and the mechanisms behind this variability. Furthermore, this knowledge might support the targeted development of early neuroprotective therapies specific to prodromal subtypes and the prediction of the progression of pheno-conversion from P-PD to PD.

Materials and Methods

Neuropsychological assessments

The following neuropsychological assessments were applied to evaluate different cognitive domains:

Cognitive functions	Assessments	Chapter
Global cognition	Montreal Cognitive Assessment (MoCA)	IA, IB, II
Memory		
Auditory short-term memory	Digit Span - Forward	II
Auditory working memory	Digit Span - Backward	II
Visuo-spatial short-term memory	Corsi Block Tapping Task - Forward	II
Visuo-spatial working memory	Corsi Block Tapping Task - Backward	II
Episodic verbal long-term memory	CERAD Word List Delayed Recall	II
Learning ability	CERAD Word List Learning	II
Retrograde procedural memory	CUPRO Evaluation System (Intermediate Score 1)	IA, IB, II
Processing speed		
Psychomotor speed, Initiation	Trail Making Test (TMT) - Part A	IB, II
Processing speed	Stroop Test – Word Reading and Color Naming	II
Executive functions		
Mental flexibility, Shifting	Trail Making Test (TMT) - Part B & Delta-TMT*	IB, II
Inhibitory control	Stroop Test - Interference Score	II
Battery for Executive functions	Frontal Assessment Battery (FAB)	IA, II
Mental flexibility	Isaacs Set Test	II
Planning	Rey-Osterrieth Complex Figure – Type	IA
Language		
Language - Denomination	Boston Naming Test	II
Fluency, Word initiation	Semantic Fluency (animals, 2 min)	II
	Phonemic Fluency (letter "F", 1 min)	II
Visuospatial functions		
Visuoconstructive capacities	Qualitative Scoring MMSE Pentagon	IA, II
	Cube Copying Task	IA, IB, II
	CUPRO Evaluation System (Intermediate Score 2)	IA, IB, II
	Rey-Osterrieth Complex Figure – Copy	IA
Visuospatial judgment	Benton Judgment of Line Orientation (JLO)	IA, II

Table 1. **Applied neuropsychological assessments and measured cognitive functions.** N.B.: We allocated cognitive test to cognitive domains. Given that no cognitive assessment evaluates purely one cognitive function, overlap cannot be excluded. * Delta TMT is defined as (TMT-B) – (TMT-A).

Other assessments

The following questionnaires or assessments were applied to evaluate different functions:

Measured function	Assessments	Chapter
Depression	Beck Depression Inventory (BDI-I) Scale	IA, IB, II
Apathy	Starkstein Apathy Scale (SAS)	IA, IB, II
Quality of life	Parkinson's Disease Questionnaire (PDQ-39)	IA, IB, II
Functional Activities	Functional Activities Questionnaire (FAQ)	IB
Relatives-reported cognitive decline	Short IQCODE	IB
Dopaminergic medication	Levodopa Equivalent Daily Dose (LEDD)	IA, IB
Motor and non-motor PD symptoms	MDS-UPDRS I-III	IA, IB, II
Disease stage	Modified Hoehn and Yahr	IA, IB
Olfaction	Burghardt Sniffin'Stick	II
	Brief Smell Identification Test (B-SIT) A	II

Table 2. **Applied questionnaires and assessments and the measured functions.**

Tools

The following tools were used:

Tool	Function	Chapter:
RedCap	Electronic data capture tool	IA, IB, II
Smash	Multi appointment scheduling management tool	IA, IB, II
R & RStudio	Application for statistical analysis	IA, IB, II

Table 3. **Applied tools, programmes and software.**

Results

CHAPTER I - Retrograde Procedural Memory in Parkinson's Disease

CHAPTER IA - Retrograde Procedural Memory in Parkinson's Disease: A Cross-sectional, Case-control Study

Laure Pauly[a,b,c,d], Claire Pauly[c,d], Maxime Hansen[a,d], Valerie E. Schröder[c,d], Armin Rauschenberger[c], Anja K. Leist[e] and Rejko Krüger[a,c,d] on behalf of the NCER-PD Consortium

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Published: 5 April 2022; Journal of Parkinson's Disease

Project description

Pauly et al., 2022 report that by developing and applying a new rating system for the Cube Copying Task, the CUPRO, people with Parkinson's disease showed a significantly lower Cube drawing performance compared to matched control subjects, which is suggestive of impaired functioning of the retrograde procedural memory in Parkinson's disease.

General information

Running title:	Procedural Memory in Parkinson's Disease
Study name:	The Luxembourg Parkinson's Study
Principal Investigator:	Prof. Dr. Rejko Krüger, supervisor of Ph.D. student Laure Pauly
Geographic location:	Parkinson's Research Clinic (PRC) Luxembourg; Sites serviced by the "Flying Team", Mobile team of the PRC; Clinical and Epidemiological Investigation Center (CIEC).
Journal:	Journal of Parkinson's Disease
Type of publication:	Full article – Research report
DOI:	10.3233/JPD-213081 IOS Press

Contributions

LP: Research project: Conception, Organization, Execution; Statistical Analysis: Design, Execution; Manuscript: Writing of the first draft. CP & MH: Research project: Conception, Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique. VS: Research project: Conception, Organization; Statistical Analysis: Review and Critique; Manuscript: Review and Critique. AR: Statistical Analysis: Design, Execution, Review and Critique; Manuscript: Review and Critique. AL & RK: Research project: Conception, Organization, Supervision; Statistical Analysis: Review and Critique; Manuscript: Review and Critique.

Scientific introduction

Procedural memory is a long-term, non-declarative, implicit memory concept in charge of encoding, storing, and retrieving procedures underlying unconscious motor, cognitive and perceptual skills. Clinically relevant examples of procedural memories are driving a car or playing an instrument [101,103].

Given that procedural memory is often very imprecisely outlined, we applied the divided definition of procedural memory into anterograde and retrograde components [104]. The anterograde procedural memory involves the acquisition of new skills, whereas the ability to perform skills achieved in earlier life stages is part of the retrograde procedural memory [104]. The analysis of procedural memory is particularly relevant in PD, due to the central role of the basal ganglia in this memory concept [95,100,143].

Despite numerous studies investigating procedural memory in PD, the conclusions are inconsistent. On one hand, these might be explained using non-PD-adapted neuropsychological assessments, relying primarily on performance speed and motor control, both typically impaired in PD [120,158,160]. On the other hand, it might be explained by the varying definition and nature of the task (anterograde/retrograde; cognitive/motor; implicit/explicit) complicating the comparability [106,157]. Previously described assessments mainly evaluate the learning ability of a new skill, the anterograde procedural memory. Representing only one side of the procedural memory concept, it misses the very long-term aspect of the memory concept, the retrograde procedural memory. Even though this part of memory plays such an essential role in our daily life activities, assessing retrograde procedural memory still encounters difficulties on both levels, theoretical and clinical [167]. On the theoretical level, there are no evidence-based and easy-to-use protocols available to evaluate specifically this memory concept [101]. On the clinical level, we experience a lack of ecological validity of existing assessment tools. There is no clear linkage with real-world activities between a low performance in the Mirror Tracing Task, e.g., and possible difficulties in real-world activities applying procedural memory [139,167].

There is a need to develop a quick and easy behavioural assessment tool that provides an evaluation of a previously automatized unconscious procedure. The assessment tool should evaluate an automatic procedure that most of the population knows and has incorporated as procedural memory. It should be simple and easy to administer, not a burden for study

participants or administrators best it should be easily incorporated into already existing study protocols. We aim to fulfil these key points with our CUPRO evaluation system [1].

We observed repeatedly unexpected drawing procedures for copying the Cube, a sub-item of the global cognition assessment tool MoCA, in people with PD. During a pilot study on a group of control individuals, we defined four recurrent standard Cube drawing procedures. Similar observations on standard Cube drawing procedure were also previously made by van Sommers [180,181]. Based on these four typical procedures, the extended Cube scoring system was developed. It evaluates the starting approach of the drawing, the drawing procedure itself, and the accomplishment of the applied drawing procedure. Furthermore, we also extended the scoring system for the final result of the Cube, the classical scoring system of 1 point previously established by Nasreddine and colleagues [182]. The extended evaluation system separately evaluates three-dimensionality, proportions, orientation, and the final result (omission of lines, e.g.). Given that the extended evaluation system of the Cube Copying Task focuses on the evaluation of the Cube drawing procedure, we named it CUPRO, short for CUbe drawing PROcedure. We hypothesized that deficits in applying a previously learned unconscious drawing procedures are suggestive for an affected retrograde procedural memory.

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Retrograde Procedural Memory in Parkinson's Disease: A Cross-Sectional, Case-Control Study

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Abstract

Background: The analysis of the procedural memory is particularly relevant in neurodegenerative disorders like Parkinson's disease, due to the central role of the basal ganglia in procedural memory. It has been shown that anterograde procedural memory, the ability to learn a new skill, is impaired in Parkinson's disease. However, retrograde procedural memory, the long-term retention and execution of skills learned in earlier life stages, has not yet been systematically investigated in Parkinson's disease.

Objective: This study aims to investigate retrograde procedural memory in people with Parkinson's disease. We hypothesized that retrograde procedural memory is impaired in people with Parkinson's disease compared to an age- and gender-matched control group.

Methods: First, we developed the CUPRO evaluation system, an extended evaluation system based on the Cube Copying Task, to distinguish the Cube copying procedure, representing functioning of retrograde procedural memory, and the final result, representing the visuo-constructive abilities. Development of the evaluation system included tests of discriminant validity.

Results: Comparing people with typical Parkinson's disease (n=201) with age- and gender-matched control subjects (n=201), we identified Cube copying performance to be significantly impaired in people with Parkinson's disease (p=0.008). No significant correlation was observed between retrograde procedural memory and disease duration.

Conclusion: We demonstrated lower Cube copying performance in people with Parkinson's disease compared to control subjects, which suggests an impaired functioning of retrograde procedural memory in Parkinson's disease.

Keywords: Parkinson's disease, neurodegenerative disorder, cognitive impairment, memory, habits, neuropsychology

Introduction

Many daily life activities such as driving a car, tying one's shoes, or typing on the computer rely on procedural learning and its automation, the procedural memory. Given that its impaired functioning is linked with significant distress, we must deepen our understanding of this memory concept. This implicit, long-term memory stores information on unconscious cognitive or motor procedures. Procedural memory is characterized by its robustness and its capacity to maintain knowledge over a long period of time, even if it is not regularly consolidated. It is typically acquired through repetition, characterized by an improvement in performance, followed by automatization of the skill [1]. Automatization is reached when the neural network involved in performing the task can execute it without the need for conscious thought [2].

Brenda Milner [3], one of the pioneers in the field of cognitive neurosciences, provided the first solid evidence of spatial and conceptual dissociation of explicit versus implicit memory. She made major contributions to the understanding of the memory systems, among others the procedural memory. Whereas declarative memory appears to be dependent on the medial temporal lobe and the diencephalic structures, the most important brain components involved in the formation and consolidation of non-declarative, procedural memory are the basal ganglia, especially the striatum [4–7].

Procedural memory can be separated into an anterograde and a retrograde component. The anterograde procedural memory involves the acquisition of new skills, whereas the ability to execute skills acquired in earlier life stages is part of retrograde procedural memory [8]. Observations on retrograde procedural memory have been done indirectly in form of case-reports [9] and studies on musical memory or overlearned language (e.g., songs, poems) [8, 10]. However, to our knowledge, validated protocols are missing to evaluate the very long-term retention and retrieval of contents in procedural memory, the retrograde part of the memory concept.

Therefore, we developed a brief and easy to administer assessment tool that allows to evaluate the functioning of retrograde procedural memory. Based on the Cube Copying Task, also called Necker's Cube [11], we established an extended evaluation system that assesses both the copying procedure, representing retrograde procedural memory, and the final result, representing visuo-constructive functions.

The Cube Copying Task, is a short screening tool, widely used in clinical and research settings. It is incorporated in commonly used assessments like the Montreal Cognitive Assessment (MoCA) screening test [12] and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery [13]. The Cube Copying Task is typically applied to evaluate

visuo-constructive cognitive function or constructional praxis, associated with visuo-spatial disorders which are characterized by an impairment in the spatial organization necessary to assemble individual parts to a single entity.

We applied this extended evaluation system of the Cube Copying Task, that we named CUPRO evaluation system (short for Cube drawing PROcedure), on people with typical Parkinson's disease, as this disease is characterized by a loss of dopaminergic innervation in the basal ganglia and as the basal ganglia play a central role in procedural memory [4]. Despite the importance of procedural memory in our daily life activities and the numerous studies that have investigated this topic, there are still many discrepancies. These controversies are mainly due to the varying definitions of the memory concept and to the nature of the used tasks [14]. Until now, assessments primarily evaluated the motor, perceptual and cognitive procedural learning, with tasks such as the pursuit rotor task [15, 16], serial reaction time task [17, 18], and arithmetic alphabet test [19].

Only few studies focused on the suggested long-term retention of new skills (3–18 months) [7,20,21]. As mentioned by Cohen [20] "surprisingly little work has specifically looked at how and whether this learning is maintained in the long-term. Results, which indicate that a new skill information is retained over a testing period, provide no evidence that learning will be retained over a longer period of time".

To our knowledge, this study is the first assessing the very long-term retention and retrieval of contents in procedural memory, that have been learned in earlier life stages in a cohort of deeply phenotyped people with Parkinson's disease [22]. Investigating retrograde procedural memory in Parkinson's disease increases our understanding of the disease's cognitive profile. Gaining insights on impairments in retrograde procedural memory may in the long run even contribute to the treatment of symptoms of Parkinson's disease, since the inability to carry out procedural tasks may have its roots in impaired procedural memory functioning.

The main objectives of our study were, firstly, to develop a tool to assess functioning of retrograde procedural memory by extending the evaluation system of the Cube Copying Task. The development of this CUPRO evaluation system included tests of discriminant validity, given that a wide range of cognitive and neural processing capabilities are required for accurate Cube copying [23]. The second objective was to validate the hypothesis of a deficit of retrograde procedural memory in people with Parkinson's disease compared with control subjects. We hypothesized that people with Parkinson's disease may have more difficulties recalling an acquired copying procedure of the Cube than the control subjects, thereby evaluating two components of the Cube Copying Task, the procedure of copying the Cube and the correctness of the

outcome. To gain further insights into the functioning of retrograde procedural memory, we additionally explored associations between Cube copying performance and disease characteristics.

Material and methods

Participants

All participants were recruited from the Luxembourg Parkinson's Study of the National Centre of Excellence in Research on Parkinson's disease (NCER-PD), a monocentric, observational, longitudinal prospective study with annual follow-ups of people with Parkinson's disease and a control group from Luxembourg and the Greater Region [22]. All participants provided informed consent according to the Declaration of Helsinki. The study was approved by the National Ethics Board (CNER Ref: 201407/13).

In the present study, 402 participants were enrolled, including 201 people with Parkinson's disease and 201 control subjects. Diagnosis of typical Parkinson's disease was based on the United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria [24]. Each subject underwent a detailed neurological examination and provided information on early symptoms, disease history and treatment. Patients were tested while being on their regular medication. Levodopa Equivalent Daily Dose (LEDD) was calculated for each participant according to Tomlinson [25]. The Unified Parkinson's disease Rating Scale MDS-UPDRS-III [26] and the Hoehn and Yahr scale [27] were used to assess motor symptoms and disease stage. Inclusion criteria were age 18 years or older and ability to sign the written informed consent. Excluded were people with Parkinson's disease having undergone brain surgery (i.e., deep brain stimulation) or having been diagnosed with Parkinson's disease with dementia (as defined in [28]), atypical forms of parkinsonism, as well as other neurological diseases. Participants with a history of severe psychiatric disorders (e.g., schizophrenia) or traumatic brain injury were also excluded.

Developing an extended evaluation system of the Cube Copying Task, the CUPRO evaluation system

The Cube Copying Task was initially evaluated with the classical scoring system established by Nasreddine and colleagues [12]. One point was given for a correct final result: Drawing must be three dimensional; the orientation of the drawing must be correct; the final result must be correct (i.e., no line is added/missing, lines are relatively parallel, length of lines is relatively similar). The point was not given if any of these criteria was not met.

Until now, only unsystematic observations in form of case reports [9] or studies on musical memory or overlearned language [8, 10] point to a potential deficit of retrograde procedural memory in Parkinson's disease. Before establishing this study topic, we repeatedly observed that a lot of people with Parkinson's disease applied unexpected procedures for copying the Cube in the MoCA test [12], which is part of the neuropsychological test battery. Drawing geometric forms is taught in primary school [29], so it is reasonable to assume that this skill has been acquired in participants with completed primary education. The Cube Copying Task meets the conditions of assessing retrograde procedural memory: by copying the Cube, a (i) previously learned procedure is (ii) unconsciously applied.

During a pilot study on a group of control subjects (*n*=40), four recurrent procedures were identified as representative patterns and are referred to as "typical" procedures in the following (Fig. 1A–D).

For the procedures A, B, and C, the copying begins with the drawing of one of the six faces of the Cube. Then the copying is differentiated

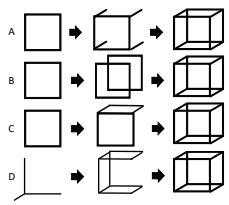
	YES	NO
INTERMEDIATE SCORE 1 - IS_1		
The subject starts with one of the squares / surfaces / with the 3 axes	1	0
 → The subject drew the inside sides → The subject drew a second square (superposition) → The subject drew a second face → The subject drew the 3 axes and continued by drawing any other surface	1	0
The subject fills in the connection lines correctly	1	0
INTERMEDIATE SCORE 1 **IS_1**	/3	
INTERMEDIATE SCORE 2 – IS_2		
The drawing is 3D, the proportions are correct	1	0
The orientation of the drawing is correct (mirror image)	1	0
The final result is correct	1	0
INTERMEDIATE SCORE 2 **IS_2**	/3	
TOTAL SCORE	**/6**	

Fig. 1. Representation of the CUPRO evaluation system, an extended evaluation system for the Cube Copying Task. The first intermediate score (IS1) evaluates the copying procedure, the second intermediate score (IS2) the visuo-constructive functions. A-D)

into three possible procedures: The subject continues by A. drawing the sidelines backwards/forwards; by B. superimposing a second square; by C. drawing any second face of the Cube. For the procedure D, the copying begins with drawing lines similar to the coordinate axes in three-dimensional space (x,y,z). The drawing is completed as soon as all the elements are connected. Similar observations on standard Cube drawing strategies were also made by van Sommers [30, 31].

As a first step, we extended this scoring system to separately assess whether the drawing is three-dimensional (1 point), if the orientation of the drawing is correct (1 point), and if the final result is correct (1 point) (Fig. 1 – Intermediate Score 2 (IS_2)). Subsequently, the Cube Copying Task was further extended to additionally evaluate the copying procedure itself. Based on the four typical procedures observed, the extended scoring system evaluates the starting approach; 1 point is administered if the subject started with one of the squares/surfaces/with the 3 axes. Further, the procedure itself is evaluated on 1 point (A.-D.). The last point is administered if the subject accomplished the copying procedure, by connecting the lines (Fig.1– IntermediateScore1 (IS_1)). The total score of six points of the CUPRO evaluation system is composed of two intermediate scores. The first intermediate score on three points (IS_1) evaluates the copying procedure. The second intermediate score (IS_2) of three points allows us to infer aspects related to visuo-constructive functions.

For the copying of the Cube, a sheet of paper was placed in front of the participant. The participant was asked to copy the drawing as accurately as possible. The drawing procedure was evaluated unbeknownst to the subject to ensure that the copying performance did not depend on explicit memory processes. No time limit was imposed. The tests were administered by a trained neuropsychologist or research nurse and scored according to the procedure described above.

Neuropsychological assessments

The global cognitive function was evaluated with the Montreal Cognitive Assessment [12], part of the basic assessment level (Level A). An optional assessment level (Level B) including a variety of other neuropsychological assessments was also proposed to the participants [22]. This level included inter alia, the Judgment of Line Orientation test used for measuring of visuospatial judgment [32], the Qualitative Scoring MMSE Pentagon test for the visuo-constructive abilities [33], the Complex Rey Figure for the visuo-constructive and planning functions [34] and the Frontal Assessment Battery for the assessment of executive functions [35].

Self-assessment questionnaires

Three different self-rating questionnaires were used: the Beck Depression Inventory (BDI-I) questionnaire [36], the Starkstein Apathy Scale (SAS) [37] and the Parkinson's Disease Questionnaire (PDQ-39) [38] to assess the presence of depression symptomatology, apathy, and quality of life in people with Parkinson's disease, respectively.

Statistics

The two groups were matched by age and gender by Propensity Score Matching (matching tolerance=0.05). Differences in demographic and clinical characteristics as well as the Cube performance differences between the groups were analyzed using the non-parametric Mann-Whitney U test and Pearson's chi-squared test (two-tailed). Correlations were tested with the bivariate Spearman correlation test. The significance threshold was set at $p \leq 0.05$. The *p*-values were assessed for significance using a Bonferroni corrected significance level. All statistical analyses were performed using RStudio version 1.3.1093 (RRID:SCR 000432; R Version 4.0.3 (2020-10-10)).

Results

For statistically significant results, we report the estimated correlation coefficients (Spearman correlation test), the observed percentages (Pearson's chi-squared test), and the mean difference between groups (Mann-Whitney U test).

Confirming successful matching, the groups did not differ significantly in gender (p=0.920), age (p=0.943), years of education (p=0.128), handedness (p=0.139), and MoCA score (p=0.246). As expected, people with Parkinson's disease presented significantly higher scores on the BDI-I (MD=3.37, p<0.001), the SAS (MD=3.79, p<0.001), and the MDS-UPDRS-III (MD=28.21, p<0.001) compared to the control subjects. Concerning number of languages spoken, people with Parkinson's disease spoke significantly fewer languages than the control subjects (MD=–0.75, p<0.001) (Table 1).

Within the PD group, those with impaired retrograde procedural memory were significantly older (MD=4.18, p=0.009), lower educated(MD=–1.08, p=0.023), more likely to be female (54.43% versus 38.52%, p=0.039), and had lower MoCA scores (MD=–1.39, p<0.001) compared with those with unimpaired retrograde procedural memory. No significant differences on motor symptoms, LEDD, and disease duration were observed.

Group differences were found in the total score of the Cube copy in both classical and extended evaluation system of the Cube Copying Task: According to the classical evaluation system, people with Parkinson's disease had a significantly lower average score than the control subjects (p<0.001). With the extended evaluation system (CUPRO), people with Parkinson's disease had significantly lower IS_1 (MD=–0.38, p=0.008) and IS_2 scores (MD=–

0.33, p=0.013) than the control subjects. Investigating the differences in IS_1 scores, we took a closer look at the distribution of the use and non-use of the pre-defined procedures (Table 2).

In people with Parkinson's disease, age and quality of life were negatively correlated with retrograde procedural memory performance (IS_1) (R=–0.228; p=0.001 and R=–0.173; p=0.018). Furthermore, higher MoCA scores and education were associated with a better retrograde procedural memory (R=+0.364, p<0.001 and R=+0.224; p=0.002). We found no significant correlation between IS_1 and disease duration (R=– 0.093; p=0.216), IS_1 and MDS-UPDRS-III score (R=–0.108; p=0.129), IS_1 and LEDD (R=+0.015; p=0.842) and IS_1 and depressive symptoms (R=–0.128; p=0.075) (Table 3).

Additional testing for discriminant validity by investigating associations of Cube copying performance with several related constructs was done with a subgroup of participants for which relevant tests were available (34≤N≤73). Neither visuo-constructive, visuo-spatial, planning nor executive functions significantly interfered with the score representing retrograde procedural memory (Supplementary Material).

Table 1. Demographic and clinical data for people with Parkinson's disease (*n*=201) and control subjects (*n*=201)

Variable	Descriptive statistics						*p*
	PD (*n* = 201)			CS (*n* = 201)			PD vs. CS
	Mean	*SD*	*Range*	*Mean*	*SD*	*Range*	
Gender, M / F	111 / 90		–	109 / 92		–	0.920
Handedness, R / L / A	170 / 14/ 7^{+10na}		–	180/6/10^{+5na}		–	0.139
Age, y	64.84	10.20	22–87	64.71	10.18	30–86	0.943
Education, y	13.60	3.80	4–25	14.25	3.96	4–24	0.128
MOCA total score (/30)	26.58	2.68	21–30	26.97	2.29	21–30	0.246
MDS-UPDRS-III (/132)	32.80	13.40	7–88	4.59	5.10	0–27	<0.001*,**
Hoehn and Yahr	2.06	0.53		0.00	0.00		<0.001*,**
Stage 1 / 1.5 / 2 / 2.5 / 3 / 4 / 5	19 / 13 / 119 / 29 / 18 / 2 / 0 + ^{1na}						
BDI-I (/63)	8.32	6.36	0–34	4.95	4.72	0–27	<0.001*,**
SAS (/42)	13.63	5.49	1–32	9.84	4.75	0–25	<0.001*,**
Languages spoken	2.81	1.10	1–4	3.56	0.78	1–4	<0.001*,**
Disease duration, y	5.37	4.39	0–24	–	–	–	–
LEDD	596.35	391.30	50–2062	–	–	–	–

SD, standard deviation; PD, people with Parkinson's disease; CS, control subjects; M, male; F, female; R, right-handed; L, left-handed; A, ambidextrous; na, not available; n, sample size; MDS-UPDRS, Movement Disorder Society - Unified Parkinson's Disease Rating Scale; BDI, Beck Depression Inventory; SAS, Starkstein Apathy Scale; MoCA, Montreal Cognitive Assessment; LEDD, Levodopa Equivalent Daily Dose. *Significant at the 5% level (2-tailed). **Significant at the Bonferroni-adjusted 5% level (*p*<=0.05/10)

Table 2. Cube Scoring according to the classical evaluation (evaluated with one point) and extended evaluation system of the Cube (evaluated with six points; divided into two intermediate scores: IS_1 (assesses retrograde procedural memory) and IS_2 (assesses the visuo-constructive functions)

		Descriptive statistics				*p*
	Variable	PD ($n=201$)		CS ($n=201$)		PD vs. CS
		Mean	*SD*	*Mean*	*SD*	
Extended evaluation system of the Cube Copying Test	IS_1 (/3)	2.05	1.13	2.43	0.90	0.008*,**
	IS_2 (/3)	2.26	1.10	2.59	0.84	0.013*,**
Classical evaluation score of the Cube Copying test (Nasreddine et al.) [12]	% of participants with correct result	*Mean* 65.67		*Mean* 83.58		<0.001*,**

The Cube Copying total score (classical evaluation system) on one point evaluates the final result of the Cube; one point is administered if the copy is identical to the model. In the extended evaluation system: the first intermediate score (IS_1) evaluates the drawing procedure. The second intermediate score (IS_2) evaluates visuo-constructive functions. SD, standard deviations; PD, participants with Parkinson's disease; CS, control subjects; IS, intermediate score. *Significant at the 5% level (2-tailed). **Significant at the Bonferroni-adjusted 5% level ($p<=0.05/3$).

Table 3. Correlations for the Intermediate Scores 1 in the PD and the CS group

	Spearman Correlations					
	PD ($n = 201$)			CS ($n = 201$)		
	Spearman – Correlation coefficient R		p	Spearman – Correlation coefficient R		p
Disease Duration	–0.093		0.216	–	–	–
MDS-UPDRS-III	–0.108		0.129	–0.225	*	0.010
LEDD	+0.015		0.842	–	–	–
Education	+0.224	*, **	0.002	+0.106		0.135
MoCA total score	+0.364	*a,**	< 0.001	+0.203	*	0.004
Age	–0.228	*a	0.001	–0.006		0.931
BDI-I	–0.128		0.075	–0.060		0.404
SAS	–0.189	*	0.009	–0.092		0.201
Hoehn and Yahr	–0.150	*	0.035	–	–	–
PDQ-39	–0.173	*	0.018	–	–	–

PD, people with Parkinson's disease; CS, Control subjects; UPDRS, Unified Parkinson's Disease Rating Scale; LEDD, Levodopa Equivalent Daily Dose; MoCA, Montreal Cognitive Assessment; BDI, Beck Depression Inventory; SAS, Starkstein Apathy Scale; PDQ-39, Parkinson's Disease Questionnaire – 39 items. *Significant at the 5% level (2-tailed). *[a]Significant at the 1% level (2-tailed). **Significant at the Bonferroni-adjusted 5% level (p<=0.05/16)

Discussion

Summary of findings

By developing and applying a new rating system of the Cube Copying Task, we demonstrated that people with Parkinson's disease showed a lower Cube copying performance compared to control subjects, which suggests an impaired functioning of retrograde procedural memory in Parkinson's disease. The intermediate score, representing the procedure of Cube copying (IS_1), as a surrogate for functioning of cognitive retrograde procedural memory, was significantly reduced in people with Parkinson's disease compared to age- and gender-matched controls (Table 2). The intermediate score could thus discriminate between people with and without Parkinson's disease, reflecting known-group validity. Furthermore, our results support previous studies which assessed retention three to 18 months after learning of a new skill: people with Parkinson's disease were less efficient than control subjects in maintaining skills over time[7,20, 21]. In comparison with the control group, the patient group presented impaired visuo-constructive functions, in line with previous findings on Parkinson's disease [39].

Elevated levels of depression, assessed by BDI-I, were observed between patients and control subjects at baseline. This observation at baseline is not unexpected, as depression is found in approximately 30–40% of people with Parkinson's disease and may even precede motor symptoms [40]. Interestingly however, deficits in retrograde procedural memory in people with Parkinson's disease were not correlated with symptoms of depression. Contrary to what might have been expected, no significant correlation was observed between retrograde procedural memory performance and the disease severity, defined by LEDD, MDS-UPDRS-III score, and disease duration, in Parkinson's disease patients.

The significant correlation, observed between retrograde procedural memory and quality of life in people with Parkinson's disease, highlights the importance of investigating this memory.

Within the Parkinson's disease patients, people with impaired retrograde procedural memory were more likely to be female, older, lower educated, and had lower cognitive performance than those with unimpaired retrograde procedural memory. Women may be more likely to show impairments on retrograde procedural memory due to lower visuo-spatial skills [41]. In research on Parkinson's disease, education has been shown to predict lower risk of cognitive decline in Parkinson's disease [42].

Strengths and limitations

The new extended evaluation system was tested in a comparatively large sample of

people with Parkinson's disease and age- and gender-matched controls and excluded several alternative explanations of impaired functioning of retrograde procedural memory by testing and controlling for a set of confounders. Our evaluation system has a number of strengths, such as specifically assessing recall of previously learned procedures. As it is simple and easy to administer, it can be evaluated by any trained health professional. The time required for the CUPRO evaluation system is short (<1 minute), and once familiar with it, the examiner can grade the Cube copying performance, while simultaneously observing the subject during copying the figure. The Cube Copying Task is widely used in clinical and research settings and is already incorporated in standard assessments, i.e., in the MoCA Screening test. Therefore, the CUPRO evaluation system can be easily integrated without the need to include a new test. It adds valuable information to an already well-established screening tool without increasing the burden for patients. Furthermore, the novel test has potential for wide application, filling the gap of techniques to reliably assess functioning of retrograde procedural memory in clinical settings and giving valuable perspectives for future research. Moreover, for the evaluation of retrograde procedural memory, we focused on the procedure and not on the final result of the Cube drawing. As such, it does not directly involve motor components, contrary to most of the already existing procedural memory tasks [43].

Through evaluating discriminant validity with several tests representing related constructs, we could not find evidence that motor deficits such as tremor and rigidity prevalent in Parkinson's disease as well as deficits in visuo-constructive, visuo-spatial, planning or executive functions interfered with Cube copying performance, further consolidating the value of the new extended evaluation. However, these results thus need to be interpreted with caution, as the absence of significant correlation could also be explained by low statistical power due to the small sub-sample.

A possible bias could be related to socio-cultural components, given that Luxembourg is characterized by a multinational society. However, after verification, no significant difference was observed in the intermediate score 1 for participants from geographical Europe in comparison to participants from other regions.

Indeed, how a Cube is drawn is part of the primary or lower secondary school curriculum [44]. Schooling curricula may have differed across countries; however, anecdotal evidence from neighboring countries, suggests similarities of the timing when Cube drawing is taught at school. Regarding the current Luxembourgish school program, the

drawing of geometric figures is scheduled at latest in the 6th year of schooling [29]. According to the study conducted by Cox [45] six years of education are sufficient for participants to know how to draw a Cube. In this study, most of the participants (98.5%) had a duration of education of ≥6 years, consistent with rates of lower secondary education completion in many developed countries over the last decades. Therefore, we assume that most adults in developed countries will have acquired this faculty before the onset of the pathology. However, it can not be scientifically proven that all participants learned the drawing of geometric forms and the non-conscious acquiring of skills [46, 47] makes it difficult to gain insights into if and how the strategy of Cube drawing has been acquired.

Outlook

Our findings suggest that impaired functioning of retrograde procedural memory could be already detectable in a prodromal, non-motor stage of the disease and perhaps in the future be used as an early marker of Parkinson's disease. Therefore, it would be of great interest to further investigate how this impairment evolves in relation to the disease progression in Parkinson's disease. People with atypical parkinsonism have different and variable neuropsychological profiles. Future studies may compare the performance of retrograde procedural memory between the different forms of parkinsonism. Additionally, future research should validate the CUPRO evaluation system in independent Parkinson's disease cohorts and with attention to possible relationships between impaired Cube drawing performance in low and very low educated participants which we were not able to systematically test in our high-educated sample. Furthermore, future work should also provide a convergent test of the proposed evaluation tool with similar already existing assessments for the procedural memory, such as mirror tracing task and serial reaction time task.

Conclusion

It is of great importance to get a deeper knowledge of the functioning of retrograde procedural memory, as the integrity of this part of the memory is crucial for a person's ability to conduct routine activities of daily living, which ultimately serve to maintain independence. This study established a new tool to assess functioning of retrograde procedural memory and showed deficits in retrograde procedural memory in people with Parkinson's disease compared with control subjects. The CUPRO evaluation system will not only fill the gap of techniques for reliably assessing functioning of retrograde procedural memory in clinical settings but may also help to identify valuable perspectives for future research.

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Conflict of interest

The authors have no conflict of interest to report.

Data availability statement

All supporting material, data and software are available here: https://doi.org/10.17881/7bwb-aj16.

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Supplementary Material

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Table 1. Correlations for the Intermediate Scores 1 and possible interfering factors

		N	Spearman – Correlation coefficient R	p
Visuo-constructive abilities	**Qualitative Scoring MMSE Pentagon Test**	54	- 0.103	p = 0.462
Visuo-spatial abilities	**Judgment of Line Orientation Benton**	56	+ 0.058	p = 0.249
Executive functions	**Frontal Assessment Battery**	73	+ 0.102	p = 0.392
Visuo-constructive abilities	**Rey-Osterrieth Complex Figure - Copy**	34	+ 0.169	p = 0.338
Planning functions	**Rey-Osterrieth Complex Figure - Type**	34	- 0.381	p = 0.831

Figure 1. Example of a Cube drawing without a typical procedure. This participant would get a correct final result by copying the Cube line by line, without using a pre-defined procedure.

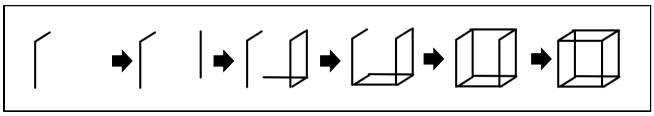
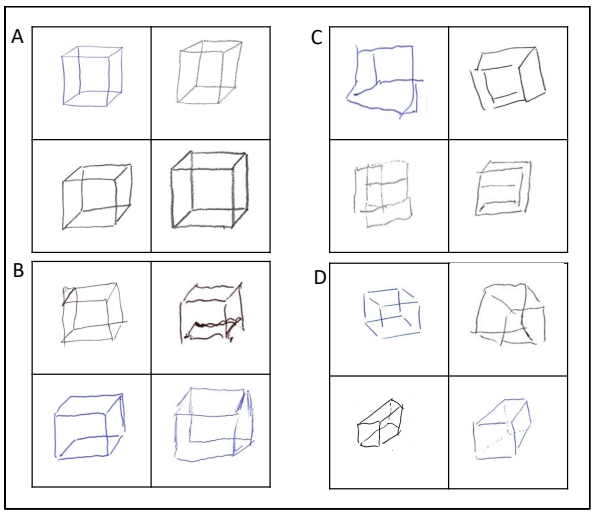


Figure 2. Examples of Cube drawings and their evaluation scores. (A) $IS_1 < 3$ and $IS_2 = 3$ or $IS_1 = 3$ and $IS_2 = 3$; (B) $IS_1 < 3$ and $IS_2 < 3$; (C) $IS_1 = 0$ and $IS_2 = 0$; (D) $IS_1 = 3$ and $IS_2 < 3$.



Further qualitative analyses revealed four possible scenarios for the final results of the Cube copying:

A. A correct final result of the Cube drawing ($IS_2 = 3$), that was obtained by the application of a copying procedure ($IS_1 = 3$). Even when not using a typical copying procedure ($IS_1 < 3$) it was possible to get a correct final result ($IS_2 = 3$), by simply copying the Cube line by line, continuously comparing their drawing with the figure without using a typical procedure (Fig.2 & 3A).

B. A slightly incorrect final result of the Cube drawing ($IS_2 < 3$), often characterized by the addition and/or omission of an element during the copying of the Cube, in those who copied the Cube line by line without a clear procedure ($IS_1 < 3$) (Fig.3B).

C. An incorrect final result with almost no resemblance to the Cube ($IS_2 = 0$) did not fulfill the above-described criteria and was therefore not correct ($IS_1 = 0$) (Fig.3C).

D. A procedure was applied ($IS_1 = 3$) but the final result is wrong ($IS_2 < 3$). This observation is reflected by Cubes that are wrongly oriented, wrong proportioned, or in mirror-representation.

CHAPTER IB - Retrograde Procedural Memory is impaired in people with Parkinson's Disease with Freezing of Gait

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Project description

Pauly et al., report that by comparing cognitive performance in people with PD and Freezing of Gait (FOG) and people with PD without FOG, matched on age, sex and disease duration, a significantly lower global cognition, executive function, and retrograde procedural memory functioning.

General information

Running title:	Procedural Memory in Freezing of Gait
Study name:	The Luxembourg Parkinson's Study
Principal Investigator:	Prof. Dr. Rejko Krüger, supervisor of Ph.D. student Laure Pauly
Geographic location:	Parkinson's Research Clinic (PRC) Luxembourg; Sites serviced by the "Flying Team", Mobile team of the PRC; Clinical and Epidemiological Investigation Center (CIEC).
Journal:	Frontiers in Aging Neuroscience
Type of publication:	Brief research report article
DOI:	10.3389/fnagi.2023.1296323

Contributions

LP: Research project: Conception, Organization, Execution; Statistical Analysis: Design, Execution; Manuscript: Writing of the first draft. CP & MH: Research project: Conception, Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique. VS: Research project: Conception, Organization; Statistical Analysis: Review and Critique; Manuscript: Review and Critique. AR: Statistical Analysis: Design, Execution, Review and Critique; Manuscript: Review and Critique. AKL & RK: Research project: Conception, Organization, Supervision; Statistical Analysis: Review and Critique; Manuscript: Review and Critique.

Scientific introduction

Freezing of gait (FOG) is an abnormal gait pattern, defined as "brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk" [183]. People living with PD and FOG describe it as a temporary incapacity in moving their feet, that seem to be "glued" to the floor. Especially during the initiating gait or during turning, FOG episodes may appear. Furthermore, the presence of an obstacle in the walking path, narrow spaces, or crowds, being stressed or rushed can trigger FOG episodes. Up to 65% of people with PD are affected by FOG [184], reducing their quality of life and causing a substantial burden for people with PD and caregivers [185].

Previous studies highlighted the importance of cognitive functions in gait, describing most notably deficits in executive and visuospatial functions in people with PD and FOG [171,186–189]. Even though FOG in PD is described as a de-automatization deficit and the numerous observations that in PD, movements become less automatic [11,183,190,191], only limited studies investigated the relation between FOG and already acquired procedural memory. Deficits in already learned procedural skills, such as handwriting [12,148], as well as deficits in learning a new procedural skill, have been described [132]. However, to our knowledge, aside from the handwriting studies [12,148], retrograde procedural memory, has not been systematically assessed in people with PD and FOG. Therefore, we applied our recently developed CUPRO tool to evaluate this memory concept and compare performance in people with typical PD with FOG, to PDs without FOG. We hypothesized that the retrograde procedural memory deficit will be more prominent in people with PD and FOG compared to people with PD without FOG.

Despite the knowledge that FOG is one of the main causes of falls and reduced quality of life in people with PD [185], treatment options, especially non-invasive therapeutic approaches, are limited. Insights on specific cognitive impairment patterns, such as on retrograde procedural memory, in people with PD and FOG, may lead to a better understanding of the etiology of FOG. This may support the targeted development of cognitive rehabilitation training, aiming to maintain or even reinforce their cognitive functions, indirectly improving their quality of life.

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Retrograde Procedural Memory is impaired in people with Parkinson's Disease with Freezing of Gait

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Abstract

Background: Freezing of gait (FOG), is associated with impairment of different cognitive functions. Previous studies hypothesized that FOG may be due to a loss of automaticity.

Research question: To explore whether FOG is associated with impairment in cognitive functions, focusing on retrograde procedural memory, the memory responsible for the automatic, implicit stored procedures that have been acquired in earlier life stages.

Methods: In this cross-sectional, case–control study, 288 people with typical Parkinson's disease (PD) from the Luxembourg Parkinson's Study were assigned to Freezers (FOG+) and non-Freezers (FOG−) based on the MDS-UPDRS 2.13 (selfreported FOG episodes) and 3.11 (FOG evaluated by clinicians during gait assessment). Both groups were matched on age, sex and disease duration. Global cognition (MoCA), retrograde procedural memory and visuo-constructive abilities (CUPRO), psychomotor speed and mental flexibility (TMT) were assessed. Furthermore, we repeated our analyses by additionally controlling for depression (BDI-I).

Results: Besides lower global cognition (MoCA; $p=0.007$) and mental flexibility (TMT-B and Delta-TMT; $p<0.001$), FOG+ showed a lower performance in retrograde procedural memory (CUPRO-IS1; $p<0.001$) compared to FOG−. After controlling additionally for depression, our main outcome variable CUPRO-IS1 remained significantly lower in FOG+ ($p=0.010$).

Conclusion: Our findings demonstrated that besides lower global cognition and mental flexibility scores, FOG+ showed lower performance in retrograde procedural memory compared to matched FOG-control patients, even when accounting for factors such as age, sex, disease duration or depression.

Significance: In the context of limited treatment options, especially for noninvasive therapeutic approaches, these insights on procedural memory and FOG may lead to new hypotheses on FOG etiology and consequently the development of new treatment options.

Keywords: Freezing of Gait, Parkinson's disease, Cognitive impairment, Procedural memory, Gait impairment

Introduction

Freezing of gait (FOG) is an abnormal gait pattern, defined by brief, temporary episodes of difficulty or even inability to move the feet despite the intention to walk (Nutt et al., 2011). FOG is common in people with Parkinson's disease (PD), affecting approximately 38% in early disease stages and up to 65% in advanced disease stages (Zhang et al., 2021). By reducing quality of life and independence, FOG poses a substantial burden for patients and caregivers (PerezLloret et al., 2014). Besides a proven link with disease progression (Macht et al., 2007), additional symptoms like impaired cognition are observed in People with PD (PwPD) with FOG (FOG^+) (Peterson et al., 2016).

Early findings indicated that gait is controlled by the central pattern generators in the spinal cord and brain stem. Even though these brain areas are highly implicated in locomotion, recent evidence from behavioral and imaging studies demonstrated the implication of higher-level cortical structures in gait, highlighting the importance of cognitive function in the process (Peterson et al., 2016). Nevertheless, studies related to this topic are still controversial. Some studies suggested impaired cognition, most notably in executive and visuospatial functions in FOG^+ (Amboni et al., 2007; Cohen et al., 2014; Jha et al., 2015; Peterson et al., 2016; Gan et al., 2023). Others provided no evidence for differences in cognition (Morris et al., 2020; Taximaimaiti and Wang, 2021). These divergences could be due to the heterogeneity in their study populations (e.g., age, education), covariates (e.g., disease severity and medication), varying definitions of FOG, different applied neuropsychological assessments or to the fact that cognitive functions more directly associated with FOG have not been tested yet.

Despite that FOG in PD has been characterized as a de-automatization deficit (Hallett, 2008; Nutt et al., 2011; Heremans et al., 2013; Vandenbossche et al., 2013b), little is known about the relation between FOG and procedural memory. In PD, movements become less automatic, mainly due to the loss of dopaminergic input to the striatum, a brain area that plays an essential role in procedural skills, such as walking (Lehéricy et al., 2005; Doyon et al., 2009). Therefore, these skills require more attentional control in PD, relying on a shift in neural activation from sub-cortical, implicit and automatic behaviour to cortical brain areas, explicit and goal-directed behavior, as a compensation strategy (Vandenbossche et al., 2013b; Wu et al., 2015).

As procedural memory is often imprecisely defined, and we have judged Crystal's and colleagues' definition, which divides procedural memory into anterograde and retrograde components, as the most accurate, we are applying this terminology in our study (Crystal et al., 1989). Anterograde procedural memory involves the acquisition of new skills, while the ability to execute skills acquired in earlier life stages is part of retrograde procedural memory. An affected anterograde procedural memory demonstrates difficulties with the ability to

learn new skills, by repetition. An affected retrograde procedural memory demonstrates difficulties in recalling and executing learned procedural skills that had once reached automatization. Despite numerous studies on procedural memory in PD, the conclusions are inconsistent. Studies on procedural memory in PD investigated mainly the anterograde procedural memory. While most of these studies have described an impairment of the learning of new procedural skills (Frith et al., 1986; Saint-Cyr et al., 1988; Heindel et al., 1989; Ferraro et al., 1993; Jackson et al., 1995; Roncacci et al., 1996; Westwater et al., 1998; Krebs et al., 2001; Sarazin et al., 2002; Muslimović et al., 2007; Vandenbossche et al., 2013a; Vakil et al., 2021) others did not observe any significant differences (Agostino, 1996; Seidler et al., 2007; Beauchamp et al., 2008; Pendt et al., 2011; Panouillères et al., 2016) or only mild to moderate or partial impairments (Harrington et al., 1990; Pascual-Leone et al., 1993; Allain et al., 1995; ThomasAntérion et al., 1996; Sommer et al., 1999). Even though, the unique characteristic of procedural memory is its robustness in time, and its longevity, only a limited number of studies investigated retrograde procedural memory (Mochizuki-Kawai et al., 2004; Cohen and Pourcher, 2007; Heremans et al., 2016). Assessing procedural memory still encounters difficulties on both levels, theoretical and clinical (Van der Linden and Seron, 2014). This might be explained by the not fully understood theoretical background and the lack of easy-to-apply assessments. Specific impairments in procedural skills, like handwriting (Heremans et al., 2016), and in the acquiring new procedural skills (Vandenbossche et al., 2013a) in FOG^+ compared to a control group without FOG (FOG^-), have been documented. However, to our knowledge, apart from the previously mentioned handwriting studies, retrograde procedural memory has not been systematically assessed in FOG^+. We recently provided the extended evaluation system of the Cube Copying Task, the CUPRO (short for CUbe drawing PROcedure) to assess this memory concept and demonstrated impairments in PwPD compared to matched control subjects (Pauly et al., 2022). We hypothesized that the Cube Copying Task meets the conditions of assessing retrograde procedural memory: by copying the Cube, a (i) previously acquireded procedure is (ii) unconciously applied. Through assessing discriminant validity with several tests representing related constructs, no evidence was found indicating that general motor symptoms prevalent in PD as well as deficits in visuocognition, planning or executive functions might interfere with the Cube coping performance (Pauly et al., 2022). Aiming to investigate the relationship between cognition and FOG, with a focus on retrograde procedural memory, we hypothesized that this memory deficit, already observed in PD (Pauly et al., 2022), may be more prominent among FOG^+ compared to matched FOG^-. In addition to procedural memory, we assessed global

cognition, visuoconstructive function, psychomotor speed and mental flexibility.

Despite that FOG is one of the main causes of falls and reduced quality of life, knowledge of treatment options, especially for non-invasive therapeutic approaches, is limited (Perez-Lloret et al., 2014; Walton et al., 2015). Previous studies demonstrated improved FOG symptoms in PwPD after cognitive rehabilitative training that may lead to neuroplastic changes by reinforcing cognitive strategies (Walton et al., 2018). Therefore, insights on specific cognitive impairment patterns, such as on procedural memory in PwPD and FOG may lead to a better understanding of the etiology of FOG. Consequently, a better characterization of the cognitive impairments observed in PD may support the targeted development of cognitive rehabilitation training and reeducation therapies, aiming to maintain, or even reinforce cognitive function and indirectly improve the quality of life of PwPD.

Materials and Methods

Participants

Participants were recruited from the Luxembourg Parkinson's Study of the National Centre of Excellence in Research on PD (NCERPD) (Hipp et al., 2018) and provided informed consent according to the Declaration of Helsinki. Inclusion criteria were the age of 18 years or older and the ability to sign the consent. We excluded participants with Deep Brain Stimulation, with a Montreal Cognitive Assessment (MoCA) score below 21 or having been diagnosed with PD with dementia (Dubois et al., 2007), atypical forms of parkinsonism, other neurological or severe psychiatric disorders. In the present study, 288 participants with typical PD were selected and two groups were defined differing in FOG status (FOG^+ = 144; FOG^- = 144) propensity score matched on age, sex and disease duration. The diagnosis was based on the UK PD Society Brain Bank Clinical Diagnostic Criteria (Hughes et al., 1992). Each subject underwent a neurological examination and provided information on early symptoms, disease history and current treatment. Patients were tested while being on their regular medication. Levodopa Equivalent Daily Dose (LEDD) was calculated for each participant (Tomlinson et al., 2010). The Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Goetz et al., 2007) and the modified Hoehn and Yahr scale (Goetz et al., 2004) were used to assess motor symptoms and disease stages.

FOG evaluation

Current and past FOG symptoms were explored using information (i) on self-reported FOG episodes (MDS-UPDRS part II sub-item 2.13), and (ii) on FOG symptoms reported by the specialized neurologist during gait assessment (MDS-UPDRS part III sub-item 3.11). Participants were categorized into two groups; the FOG^+ group with participants reporting or presenting FOG episodes (MDS-UPDRS 2.13 or MDS-UPDRS 3.11 score range 1–4) in at least

one of their visits at the research clinic and the FOG-group without FOG symptoms (MDS-UPDRS 2.13 = 0 and MDS-UPDRS 3.11 = 0). A detailed flowchart can be found in the Supplementary Figure S1.

Neuropsychological assessment

With the CUPRO evaluation system, we assessed our main outcome variable, the Cube copying procedure (Intermediate Score 1 – IS1), representing retrograde procedural memory and the final result of the Cube (Intermediate Score 2 – IS2), representing visuoconstructive functions (Pauly et al., 2022). The CUPRO is an extended evaluation score for the Cube Copying Task, that was initially evaluated, with the classical scoring system established by Nasreddine et al. (2005). Following the classical scoring system, one point was administered for a correct final result, assessing visuo-constructive functions: the drawing must be three-dimensional, the orientation of the drawing must be correct, the final result must be correct and the point was not given if any of these criteria were not met. We extended upon this scoring system to separately assess its three-dimensionality (1 point), the accuracy of its orientation (1 point), and the correctness of the final result (1 point) [Intermediate Score 2 (IS_2)]. Subsequently, the Cube Copying Task was further extended to additionally evaluate the copying procedure itself. Based on the four, previously defined typical procedures, the extended scoring system evaluates the starting approach; 1 point is given if the subject started with one of the squares, surfaces, or the three axes. Further, the procedure itself is evaluated on 1 point (A.-D.). The final point is administered if the subject completes the copying procedure, by connecting the lines [Intermediate Score1 (IS_1)]. To summarize, the total score of six points of the CUPRO evaluation system is composed of two intermediate scores. The first intermediate score of three points (IS_1) evaluates the copying procedure. The second intermediate score (IS_2) of three points allows us to infer aspects related to visuoconstructive functions. A detailed description of the development of the CUPRO can be found elsewhere (Pauly et al., 2022).

In addition to retrograde procedural memory, global cognition was evaluated with the MoCA (Nasreddine et al., 2005). Psychomotor speed and mental flexibility were measured with the Trail-MakingTest (TMT) part A and part B, respectively (Godefroy, 2008). Delta TMT is defined as (TMT-B) – (TMT-A).

Questionnaires

The Beck Depression Inventory (BDI-I) (Beck et al., 1961), Starkstein Apathy Scale (SAS) (Starkstein et al., 1992), PD Questionnaire (PDQ-39) (Peto et al., 1995), and the MDS-UPDRS (I&II) (Goetz et al., 2007), were used to assess the presence of depression symptomatology, apathy, quality of life, and non-motor and motor aspects of experiences of daily living, respectively. Functional Activity

Questionnaire (FAQ) (Pfeffer et al., 1982) and the Short Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) (Jorm, 1994) were used to measure functional activity and cognitive decline reported by relatives.

For all the questionnaires, we investigate the summed item scores, except for the PDQ-39 we investigate the summary index, derived by the sum of all 39 items' responses as a percentage score (Jenkinson et al., 1997).

Genetical testing

Targeted Glucocerebrosidase (GBA) screening was performed by real-time single-molecule sequencing developed by Pacific BioScience (PacBio). More details about the GBA screening have been reported elsewhere (Pachchek et al., 2023). In the present study, we define the carriers of a known pathogenic mutation in the GBA gene as GBA^{+} and the non-carriers as GBA^{-}.

Statistics

The two groups were matched by age, sex and disease duration by propensity score matching (matching tolerance = 0.05). Differences in demographic and clinical characteristics as well as cognitive performance were analyzed using the non-parametric Mann–Whitney U test and Pearson's chi-squared test (two-tailed). Correlations were tested with the bivariate Spearman correlation test. The significance threshold was set at $p \leq 0.05$. Where appropriate, we used the Bonferroni correction for multiple testing to prevent alpha error inflation. The same statistical analyses were repeated for the two groups matched additionally for depression (Supplementary material). All statistical analyses were performed using R version 4.2.0 GUI 1.78 and RStudio version 2023.03.1 + 446.

Results

Testing for sociodemographic differences between the groups confirmed successful matching, as the groups did not differ significantly in sex [$\chi2(1, N = 288) = 0.06$, $p = 0.802$], age ($u = 10,379$, $p = 0.988$), or disease duration ($u = 11,740$, $p = 0.677$). After multiple testing corrections, FOG+ presented significantly higher scores for MDS-UPDRS-I ($u = 6116.5$, $p < 0.001$), II ($u = 4955.5$, $p < 0.001$) and III ($u = 7853.5$, $p < 0.001$), BDI-I ($u = 6,418$, $p < 0.001$), SAS ($u = 6,627$, $p < 0.001$), FAQ ($u = 5,483$, $p < 0.001$) and PDQ-39 ($u = 4,820$, $p < 0.001$). FOG+ presented nominally significantly higher scores for the short IQCODE ($u = 6564.5$, $p = 0.039$). FOG+ present shorter education duration and higher LEDD than FOG−, but these differences are insignificant ($u = 11,740$, $p = 0.051$; $u = 7,304$, $p = 0.059$ respectively). Similarly, the number of languages is not significantly different. Given that heterozygous GBA gene mutation carriers (severe, mild and low-risk pathogenic mutations) represent increased susceptibility for PD, gait impairment and cognitive dysfunction (Wang et al., 2014), we tested group differences. No significant difference was observed regarding the number of GBA carriers between the groups [$\chi2(1, N = 259) = 0.89$, $p = 0.583$]. Descriptive statistics on the demographic and clinical data can be found in Table 1.

Our outcome variable of interest, CUPRO-IS1, was significantly lower in the FOG+ compared to FOG− _($u = 12,651$, $p < 0.001$). FOG+ presented significantly lower MoCA total scores ($u = 12264.5$, $p = 0.007$), as well as significantly higher TMT-A and TMT-B time scores ($u = 8047.5$, $p = 0.021$; $u = 7,089$, $p < 0.001$ respectively) and Delta TMT ($u = 7,135$, $p < 0.001$) compared to FOG−. No significant differences were observed for the CUPRO-IS2. Differences in neuropsychological measures between the two groups can be found in Table 2. No significant correlation was observed between the CUPRO scores and the FOG severity (MDS-UPDRS 2.13 /3.11). Results on the Spearman Correlations for the CUPRO scores in the FOG+ group can be found in Table 3.

Given that depression can have an important impact on cognition, we repeated our analyses by additionally controlling for depression. After controlling additionally for this variable, our main outcome variable CUPRO-IS1 ($u = 8,211$, $p = 0.010$), as well as the variables for the TMT (TMTa: $u = 5123.5$, $p = 0.006$; TMTb: $u = 5099.5$, $p = 0.005$; Delta TMT: $u = 5,418$, $p = 0.030$) remained significantly lower in FOG+.

Findings on the additional analyses can be found in Supplementary Tables S1, S2.

Table 1. Demographic and clinical data for FOG+ (N = 144) and FOG- (N = 144)

Variable	Descriptive statistics										P-Values	Significance
	FOG+					FOG-					FOG+ vs. FOG-	
N Total	144					144						
N GBA+ / GBA-	19/119[+6NA]					13/108[+23NA]					p = 0.583	
Sex, M / F	95/49					98/46					p = 0.802	
	Mean	*SD*	*Median*	*IQR*	*N*	*Mean*	*SD*	*Median*	*IQR*	*N*		
Age, in years	66.94	10.11	68.48	14.83	144	67.51	9.21	68.55	12.70	144	p = 0.988	
Disease duration, in years	4.67	4.03	4.00	4.00	144	4.50	3.99	3.50	4.00	144	p = 0.677	
Education, in years	12.99	3.98	12.00	4.25	144	13.87	3.80	13.00	6.00	144	p = 0.051	
Languages spoken	2.90	1.08	3.00	2.00	140	2.81	1.10	3.00	2.00	140	p = 0.527	
MDS-UPDRS I (/52)	11.55	6.38	11.00	8.00	141	7.71	4.91	7.00	6.00	138	p < 0.001	**
MDS-UPDRS II (/52)	12.99	7.30	12.00	9.00	141	7.25	4.82	6.00	5.50	139	p < 0.001	**
MDS-UPDRS III (/132)	37.80	12.98	36.00	16.75	142	32.24	12.64	32.00	17.25	144	p < 0.001	**
Modified Hoehn and Yahr	2.27	0.55	2.00	0.50	143	1.98	0.42	2.00	0.00	144	p < 0.001	**
Stage 1 / 1.5 / 2 / 2.5 / 3 / 4 / 5	2/4/88/26/16/7/0[+1NA]					11/12/99/15/7/0/0						
LEDD	621.7	417.0	540.00	490.00	129	514.4	311.7	420.00	378.6	131	p = 0.059	
BDI-I (/63)	10.68	8.46	9.00	9.00	140	6.48	5.47	5.00	6.50	139	p < 0.001	**
SAS (/42)	15.05	5.84	15.00	7.00	131	12.33	5.59	12.00	7.00	137	p < 0.001	**
FAQ (/30)	3.78	6.01	1.00	5.00	124	1.12	2.29	0	1.00	125	p < 0.001	**
PDQ-39 (%)	28.19	17.83	25.96	22.44	130	15.12	11.49	13.46	14.10	138	p < 0.001	**
Short IQCODE (/5)	3.14	0.51	3.09	0.33	120	3.05	0.38	3.00	0.19	128	p = 0.039	*

Demographic and clinical data for FOG+ and FOG–. Both groups were matched for sex, age and disease duration. SD, standard deviation; IQR, Inter Quartile Range; FOG+, freezers; FOG–, non-freezers; M, male; F, female; R, right-handed; L, left-handed; A, ambidextrous; NA, not available; N, sample size; GBA, glucocerebrosidase gene mutation; MDS-UPDRS, Movement Disorder Society - Unified Parkinson's Disease Rating Scale; LEDD, Levodopa Equivalent Daily Dose; BDI, Beck Depression Inventory; SAS, Starkstein Apathy Scale; FAQ, Functional Activity Questionnaire; PDQ-39, Parkinson's Disease Questionnaire 39-item; IQCODE, short Informant Questionnaire on Cognitive Decline in the Elderly. * Significant at the unadjusted 5% level (value of $p \leq 0.05$) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (value of $p \leq 0.05/16$) (two-tailed).

Table 2. Differences in neuropsychological measures between FOG+ (N = 144) and FOG- (N = 144) matched on age, sex and disease duration.

	Variable	FOG+ (N = 144)				FOG- (N = 144)				N FOG+ /FOG-	P-Values	Significance
		Mean	*SD*	*Median*	*IQR*	*Mean*	*SD*	*Median*	*IQR*			
CUPRO Evaluation System	IS_1 (/3)	1.81	1.17	2.00	2.00	2.28	1.03	3.00	1.00	144/144	p < 0.001	*
	IS_2 (/3)	2.14	1.01	3.00	2.00	2.38	1.16	3.00	1.00	144/144	p = 0.084	
	CUPRO total score (/6)	3.94	2.10	4.00	4.00	4.66	1.78	6.00	2.00	144/144	p = 0.004	*
Global Cognition	MOCA total score (/30)	25.85	2.68	26.00	4.00	26.71	2.36	27.00	3.25	144/144	p = 0.007	*
Psychomotor speed / Mental flexibility	TMT-A (sec)	56.64	32.28	47.50	29.25	49.25	23.56	45.00	22.00	140/137	p = 0.021	*
	TMT-B (sec)	149.4	80.82	125.00	106.20	115.1	62.99	97.00	51.00	140/137	p < 0.001	*
	Delta TMT (sec)	92.79	71.66	74.50	94.25	65.80	49.60	48.00	46.00	140/137	p < 0.001	*

Neuropsychological assessment. The extended evaluation system: the first intermediate score (IS1) (our outcome variable of interest) evaluates the drawing procedure. The second intermediate score (IS2) evaluates visuo-constructive functions. N, sample size; SD, standard deviations; FOG+, freezers, FOG–, non-freezers; CUPRO, Cube drawing procedure, extended evaluation system of the Cube Copying Task; IS, intermediate score; TMT, Trail-Making-Test; Delta TMT: (TMT-B)–(TMT-A); MoCA, Montreal Cognitive Assessment. *_ _Significant at the 5% level (*p*-value ≤ _0.05) (two-tailed).

Table 3. Spearman Correlations for the CUPRO scores in the FOG^{+} group.

	MDS-UPDRS II.13		MDS-UPDRS III.11		MDS-UPDRS II.13 + III.11	
	Coefficient R	P-Values	Coefficient R	P-Values	Coefficient R	P-Values
IS_1	0.04	0.7	-0.03	0.7	0.01	0.9
IS_2	0.06	0.4	-0.04	0.6	0.03	0.7
IS_1+ IS_2	0.07	0.4	-0.04	0.6	0.03	0.8
Delta TMT	0.08	0.4	-0.06	0.5	0.02	0.8
MoCA total	-0.04	0.6	0.07	0.4	-0.01	0.9

Spearman correlations. IS1: Intermediate Score 1; IS2: intermediate Score 2; MDS-UPDRS: Movement Disorder Society - Unified Parkinson's Disease Rating Scale; Delta TMT: (TMT-B)–(TMT-A); MoCA: Montreal Cognitive Assessment,

Discussion

The present study aimed to investigate if Freezing of gait (FOG) in Parkinson's disease (PD) is associated with impairment in retrograde procedural memory. For this purpose, we used the CUPRO assessment tool (Pauly et al., 2022) and compared performance in FOG+ and FOG−, matched on age, sex and disease duration. The present study demonstrates significantly lower scores representing the performance in retrograde procedural memory in FOG+, even when matched for age, sex and disease duration to the control group. Similar observations on procedural learning have been described in FOG+ (Vandenbossche et al., 2013a).

Furthermore, we tested for differences in other cognitive domains. Previous studies have suggested cognitive deficits in FOG+ (Vandenbossche et al., 2013a; Cohen et al., 2014; Jha et al., 2015; Heremans et al., 2016), but only a few found no differences in cognition (Morris et al., 2020). These discrepancies can be explained by the varying definitions of FOG and non-controlled covariates. Our results support previous studies which demonstrated impaired psychomotor speed, procedural skills (Vandenbossche et al., 2013a; Heremans et al., 2016) and executive functions (Amboni et al., 2007; Peterson et al., 2016), such as mental flexibility in FOG+ compared to FOG−. We did not see any significant differences for visuo-constructive functions. Nevertheless, we need to keep in mind that a small test battery was used. To validate the findings, future research should apply a larger neuropsychological test battery.

Our findings of impaired procedural memory and mental flexibility, part of the executive functions, support the Vandenbossche model (Vandenbossche et al., 2013b). The model hypothesizes that those two functions, regulated by the frontostriatal circuitry, are crucial for understanding the pathogenesis of FOG. In case of disturbances of automaticity/procedural memory, one would expect a shift in neural activation from sub-cortical to cortical brain areas as a compensation strategy. In case of additional impairment of executive functions, this could lead to a FOG episode (Vandenbossche et al., 2013b). Recent brain imaging studies support this finding by describing increased involvement of attention as a compensatory strategy in PD compared to control subjects after motor learning (Wu et al., 2015). Functional neuroimaging studies suggested that FOG in PD is caused by abnormal interactions between frontoparietal cortical and subcortical structures, such as the striatum (Shine et al., 2013). This is in line with our observation of impaired retrograde procedural memory in FOG+, as the basal ganglia, especially the dorsolateral striatum, play an essential role in procedural memory (Mishkin and Appenzeller, 1987).

Measured by the absence of significant correlations, neither, global cognition, mental flexibility, nor retrograde procedural memory, were affected more severely by the worsening of the FOG symptoms. This previously mentioned shift might therefore not be gradual, defined by a temporal gradient but more by a spontaneous shift.

Furthermore, our findings that FOG+ show significantly more non-motor and motor symptoms, lower quality of life and higher disease stages compared to their matched control group, are in line with previous findings (Perez-Lloret et al., 2014). Given that depression can have an important impact on cognition, we repeated our analyses by additionally controlling for depression. The results for the main outcome variable, retrograde procedural memory, remained significantly lower in FOG+. This is in line with our observations made in a previous study comparing retrograde procedural memory in PwPD and control subjects, where we did not find significant associations between depression symptomatology and retrograde procedural memory (Pauly et al., 2022).

We took into consideration recently published recommendations for studies on cognition and FOG in PD (Monaghan et al., 2023); First, we ensured that the FOG cohorts were well characterized for clinical demographics including age, sex, education and, what is often neglected, for disease duration. Second, we reported medication status, by calculating the Levodopa Equivalent Daily Dose (LEDD) for each participant, so that the impact of dopamine medication can be interpreted. In the current study, we did not see any significant difference in LEDD between both groups. Third, apart from the novel CUPRO evaluation system, we used validated neuropsychological assessment tools and questionnaires to facilitate future comparisons across studies. The present study has the advantage that we included people with current and initial FOG symptoms. Given that dopaminergic medication can have a positive impact on gait abnormalities (Giladi, 2008), a medically well-adjusted patient may have his FOG masked.

Although the differences in dopaminergic medication were not statistically significant, they may still influence our outcome variables, as dopaminergic treatments can potentially shape the neural connectivity of cognitive networks in PD (Aracil-Bolaños et al., 2021). Since no significant correlation between retrograde procedural memory and LEDD have been observed in our previous study (Pauly et al., 2022), we do not anticipate a substantial impact on retrograde procedural memory. Furthermore, despite the fact that the years of education did not significantly differ between both groups, we cannot rule out the possibility that it might have an impact on our outcome variable, considering that previous findings have shown that the number of years of education completed is positively

correlated with their cognitive functions (Lövdén et al., 2020).

Even though FOG is one of the main causes of falls and reduced quality of life, knowledge of treatment options, especially for non-invasive therapeutic approaches, is limited. Therefore, getting a deeper understanding of the relation between the pathophysiology of FOG and cognitive functions such as retrograde procedural memory is important, as these insights can lead to new hypotheses on the etiology of FOG. Previous findings demonstrated that cognitive rehabilitative training improves FOG symptoms in PwPD, leading to neuroplastic changes by reinforcing cognitive strategies (Walton et al., 2018). Research developing cognitive rehabilitation training reinforcing cognitive compensation strategies in people with FOG may have the potential to improve the quality of life of FOG patients.

The present study aimed to investigate if Freezing of Gait (FOG) in Parkinson's disease (PD) is associated with impairment in retrograde procedural memory. By comparing retrograde procedural memory performance in FOG+ and FOG−, measured by the CUPRO assessment, we observed significantly lower CUPRO-IS1 scores, suggestive of impaired retrograde procedural memory, in FOG+, even when accounting for possible confounding factors such as age, sex, disease duration or depression.

Although FOG is a significant contributor to falls and a decline in the quality of life, our knowledge of treatment options, particularly non-invasive therapeutic methods, is still limited. Therefore, gaining insights into specific patterns of cognitive impairment, such as procedural memory in PwPD and FOG, and its suggested relationships with other cognitive domains in other studies, may improve our understanding of FOG's causes. Consequently, a more thorough understanding of the cognitive deficits observed in PD may facilitate the targeted development of cognitive rehabilitation training and reeducation therapies. These efforts aim to preserve or even enhance cognitive function, ultimately leading to an improvement in the quality of life for individuals with PD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

All participants taking part in the Luxembourg Parkinson's Study agreed and signed a written informed consent. The study was conducted in accordance with the local legislation and institutional requirements and has obtained a positive opinion from the National Research Ethics Committee (CNER Ref: 201407/13).

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The dataset used for this manuscript is available upon request to the Data and Sample Access Committee of Luxembourg's Parkinson's Study (via email: request.ncer-pd@uni.lu).

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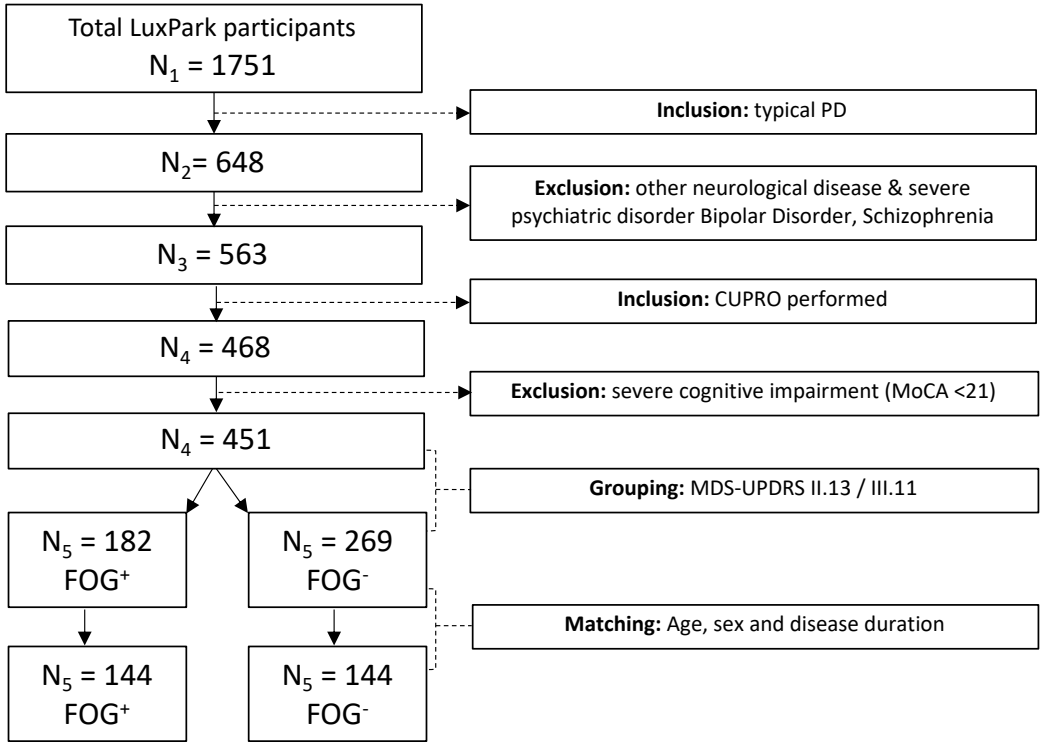
Supplementary Material

Figure 1 Supp. Flowchart



Flowchart. FOG+: Freezers; FOG-: non-Freezers; N: sample size; PD: Parkinson's Disease; CUPRO: CUbe drawing PROcedure, extended evaluation system of the Cube Copying Task; MoCA: Montreal Cognitive Assessment; MDS-UPDRS: Movement Disorder Society - Unified Parkinson's Disease Rating Scale; MoCA: Montreal Cognitive Assessment

Table 1 Supp. Demographic and clinical data for FOG^{+} (N = 118) and FOG^{-} (N = 118) matched on age, sex, disease duration and depression.

Variable	Descriptive statistics										P-Values	Significance
	FOG^{+}					FOG^{-}					FOG+ vs. FOG-	
N Total	118					118						
N GBA+ / GBA-	18 / 98^{+2NA}					9 / 94^{+15NA}					p = 0.188	
Sex, M / F	86 / 32					83 / 35					p = 0.773	
	Mean	*SD*	*Median*	*IQR*	*N*	*Mean*	*SD*	*Median*	*IQR*	*N*		
Age, in years	67.35	10.35	69.12	12.82	118	66.75	9.94	67.53	13.49	118	p = 0.271	
Disease duration, in years	4.66	3.98	4.00	4.75	118	4.94	4.15	4.00	5.00	118	p = 0.493	
Education, in years	13.33	3.71	13.00	4.00	118	14.43	3.79	14.00	5.00	118	p = 0.017	*
Languages spoken	2.98	1.04	3.00	2.00	118	2.76	1.15	3.00	2.00	118	p = 0.527	
MDS-UPDRS I (/52)	10.03	5.98	9.00	7.00	118	8.45	5.12	8.00	6.00	117	p = 0.067	
MDS-UPDRS II (/52)	12.24	7.03	11.00	9.00	118	7.80	5.21	7.00	7.00	117	p < 0.001	**
MDS-UPDRS III (/132)	37.72	12.94	36.00	16.25	116	32.25	12.80	32.00	19.00	118	p = 0.002	**
Modified Hoehn and Yahr	2.26	0.52	2.00	0.50	117	1.96	0.45	2.00	0.00	118	p < 0.001	**
Stage 1 / 1.5 / 2 / 2.5 / 3 / 4 / 5	2/2/72/26/10/5^{+1NA}					12/10/78/12/6/0						
LEDD	631.4	420.8	536.50	487.50	106	531.9	331.6	450.0	381.1	103	p = 0.129	
BDI-I (/63)	8.09	5.86	7.50	6.75	118	7.92	5.61	7.00	6.00	118	p = 0.905	
SAS (/42)	13.83	5.18	14.00	8.00	109	12.82	5.11	12.50	6.00	114	p = 0.134	
FAQ (/30)	3.10	5.32	1.00	4.00	102	1.33	2.35	0.00	2.00	104	p = 0.020	*
PDQ-39 (%)	24.92	17.08	21.79	22.12	107	17.63	11.91	16.03	14.74	113	p = 0.002	**
Short IQCODE (/5)	3.06	0.46	3.00	0.25	100	3.08	0.34	3.00	0.19	106	p = 0.554	

Demographic and clinical data for FOG+ and FOG-. Both groups were matched for sex, age, disease duration and depression. SD: Standard Deviation; IQR: InterQuartile Range FOG+: Freezers; FOG-: non-Freezers; M: Male; F: Female; R: Right-handed; L: Left-handed; A: Ambidextrous; NA: not available; N: sample size; GBA: glucocerebrosidase gene mutation; MDS-UPDRS: Movement Disorder Society - Unified Parkinson's Disease Rating Scale; LEDD: Levodopa Equivalent Daily Dose; BDI: Beck Depression Inventory; SAS: Starkstein Apathy Scale; FAQ: Functional Activity

Questionnaire; PDQ-39: Parkinson's disease questionnaire 39-item; IQCODE: Short Informant Questionnaire on Cognitive Decline in the Elderly. * Significant at the unadjusted 5% level (p-value ≤ 0.05) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (p-value ≤ 0.05/16) (two-tailed).

Table 2 Suppl. Differences in neuropsychological measures between FOG^+ (N = 118) and FOG^- (N = 118) matched on age, sex, disease duration and depression.

	Variable	FOG+				FOG-				N FOG+ /FOG-	P-Values	Significance
		Mean	*SD*	*Median*	*IQR*	*Mean*	*SD*	*Median*	*IQR*			
CUPRO Evaluation System	IS_1 (/3)	1.85	1.16	2.00	2.00	2.22	1.08	3.00	1.75	118/118	p = 0.010	*
	IS_2 (/3)	2.30	1.10	3.00	1.00	2.43	0.94	3.00	1.00	118/118	p = 0.583	
	CUPRO total score (/6)	4.14	2.00	4.00	3.00	4.65	1.78	6.00	2.00	118/118	p = 0.043	*
Global Cognition	MOCA total score (/30)	26.30	2.48	27.00	3.00	26.68	2.28	27.00	3.75	118/118	p = 0.305	
Psychomotor speed / Mental flexibility	TMT-A (sec)	59.05	40.30	48.00	15.00	46.75	18.64	43.00	25.00	115/113	p = 0.006	*
	TMT-B (sec)	136.7	75.02	110.00	97.50	109.10	56.73	95.00	46.00	115/113	p = 0.005	*
	Delta TMT (sec)	77.63	71.52	62.00	73.50	62.37	46.20	46.00	42.00	115/113	p = 0.030	*

Neuropsychological assessment. The extended evaluation system: the first intermediate score (IS_1) (our outcome variable of interest) evaluates the drawing procedure. The second intermediate score (IS_2) evaluates visuo-constructive functions. SD: Standard Deviations; FOG^+: Freezers; FOG^-: non-Freezers; TMT: Trail-Making-Test; Delta TMT: (TMT-B)–(TMT-A); MoCA: Montreal Cognitive Assessment; IS: Intermediate Score. * Significant at the 5% level (p-value ≤ 0.05) (two-tailed).

Additional data

Supplementary analyses: Impact of dopaminergic medication on retrograde procedural memory

Given that recent studies suggested that dopaminergic treatments may shape the neural connectivity of cognitive networks in PD [192], we did some further investigations to study the possible impact of dopaminergic medication on retrograde procedural memory.

In Chapter I, the described findings were collected in people with PD who were ON their regular medication. For every participant with PD, the Levodopa Equivalent Daily Dose (LEDD) was calculated. Considering, that we did not observe any significant correlation between the CUPRO-IS_1 and LEDD, we assumed no direct influence of dopaminergic medication on procedural memory. We hypothesized that there would be no significant differences observed in the CUPRO performances when testing participants with PD in ON compared to their OFF performance. For the validation of this statement, the CUPRO evaluation system was added as a side-project, in an international collaborative neuroimaging project comparing performances in people with PD ON vs. OFF their dopaminergic medication. When investigating preliminary analyses on the current sample of 22 participants with PD, we did, indeed see no significant difference in the Cube Copying performance, when tested in ON or in OFF. We carefully conclude on these results and on the absence of a significant correlation in Chapter I, that the Cube drawing performance, suggestive of retrograde procedural memory, is not influenced by the dopaminergic medication in people with PD. This conclusion concurs with findings indicating that learning a new procedural skill may be independent of dopaminergic medication [49].

CHAPTER II - Cognition and other non-motor symptoms in an at-risk cohort for Parkinson's disease defined by REM-Sleep-Behavior-Disorder and Hyposmia

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Project description

Pauly et al., report, by comparing cognitive performance in people at high risk of developing PD and in sex- and age-matched controls, a significantly lower global cognition, executive function, visuo-constructive functions in the at-risk group. In addition, significantly more difficulties in motor and other non-motor symptoms of experiences of daily living, as well as significantly higher scores for depression and apathy and significantly lower scores for quality of life have been observed in the P-PD group.

General information

Running title: Cognition and non-motor symptoms in prodromal Parkinson's Disease

Study name:	The Luxembourg Sleep Study & The Luxembourg Parkinson's Study
Principal Investigator:	Prof. Dr. Rejko Krüger, supervisor of Ph.D. student Laure Pauly
Geographic location:	Parkinson's Research Clinic (PRC) Luxembourg; Sites serviced by the "Flying Team", Mobile team of the PRC; Clinical and Epidemiological Investigation Center (CIEC).
Journal:	Journal of Parkinson's disease (accepted for publication)
Type of publication:	Full Article – Research Report
DOI:	NA

Contributions

LP: Research project: Conception, Organization, Execution; Statistical Analysis: Design, Execution; Manuscript: Writing of the first draft. AR: Statistical Analysis: Design, Execution, Review and Critique; Manuscript: Review and Critique. CP & GVC: Research project: Conception, Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique. VS: Research project: Conception, Organization; Statistical Analysis: Review and Critique; Manuscript: Review and Critique. AKL & RK: Research project: Conception, Organization, Supervision; Statistical Analysis: Review and Critique; Manuscript: Review and Critique.

Scientific introduction

Diagnostic criteria for Parkinson's disease (PD) are based on the presence of motor symptoms, such as bradykinesia, tremor or rigidity. At the time of diagnosis, more than 60% of the dopaminergic neurons are already degenerated [32,42]. The neurodegeneration begins however already decades before the appearance of motor symptoms, defining the prodromal stage of PD. In this prodromal phase, which can start up to 20 years before the onset of motor parkinsonism [43], non-motor symptoms can appear [13,25]. REM sleep behaviour disorder (RBD) and other non-motor symptoms such as hyposmia, constipation and depression were proposed by the Movement Disorder Society (MDS) as research criteria for prodromal Parkinson's disease (P-PD). Global cognitive deficit was only later recently in 2019 [13].

Cognitive impairment is already present in up to 54% of newly diagnosed PD [6–9]. Recent findings indicate that these cognitive deficits may precede clinical PD diagnosis by up to 5 years [43]. Due to the novelty of the concept and the great effort needed to study participants at high risk for PD, knowledge is limited on the nature of these prodromal cognitive changes and results are still controversial. Global cognition and diverse cognitive sub-domains, mainly executive functions, less frequently visuospatial functions, memory and language, have been shown impaired in P-PD [193–196]. The controversy of the findings might be due to the heterogeneity of the existing studies, in their study designs (e.g. recruitment strategies), study populations (e.g. age, education), neuropsychological assessments and the tested cognitive domains, complicating the comparability of results [46–48]. Therefore, following previously published recommendations [46,48] results on cognition in P-PD need validation i) in a deep-phenotyped population, ii) combining strong predefined prodromal markers, iii) on normative-controlled cognitive data, iv) based on a broad variety of commonly used cognitive assessments to evaluate both global cognition and domain-specific cognition, v) with at least two tests per cognitive domain. Furthermore, in the supra-analysis, we try to broaden our findings by adding the CUPRO (Cube drawing PROcedure) evaluation system.

The main aim of the study is to describe cognition and other non-motor symptoms in prodromal PD, defined by pRBD and hyposmia, compared to a matched counter group. Defining additional specific prodromal patterns will support the early recognition of PD.

Copy of the submitted manuscript

Cognition and other non-motor symptoms in an at-risk cohort for Parkinson's disease defined by REM-Sleep-Behavior-Disorder and Hyposmia

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Abstract

Background: REM-sleep Behavior Disorder (RBD) and other non-motor symptoms such as hyposmia were proposed by the Movement Disorder Society as research criteria for prodromal Parkinson's Disease (P-PD). Global cognitive deficit was later added.

Objectives: To compare non-motor symptoms, focusing on cognition, between a P-PD group and a matched control group.

Methods: In this cross-sectional, case-control study, in a first set of analyses, we performed extensive cognitive testing on people with (n=76) and a control group without (n=195) probable RBD and hyposmia. Furthermore, we assessed motor and non-motor symptoms related to PD. After propensity score matching, we compared 62 P-PD with 62 age- and sex-matched controls. In addition, we performed regression analyses on the total sample (n=271). In a second set of analyses, we used, a.o., the CUPRO to evaluate retrograde procedural memory and visuo-constructive functions.

Results: People with P-PD showed significantly poorer performances in global cognition, visuo-constructive and executive functions, mainly in mental flexibility ($p<0.001$; $p=0.004$; $p=0.003$), despite similar educational levels ($p=0.415$). We observed significantly more motor and non-motor symptoms ($p<0.001$; $p=0.004$), higher scores for depression ($p=0.004$) and apathy ($p<0.001$) as well as lower quality of life ($p<0.001$) in P-PD.

Conclusion: Our findings confirm that global cognitive, executive, and visuo-constructive deficits define P-PD. In addition, depression, apathy, and lower quality of life were more prevalent in P-PD. If replicated in other samples, executive and visuo-constructive deficits should be considered in non-motor P-PD. Determining specific patterns will support early recognition of PD, secondary prevention of complications and the development of neuroprotective treatments.

Keywords: Parkinson Disease, Cognition, Executive Function, Population at Risk, Quality of Life

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder with increasing prevalence. It is mostly diagnosed when more than 60% of the dopaminergic neurons are degenerated and first motor manifestations, such as tremor, rigidity and slowness of movement, appear [1,2]. The period between the onset of neuronal degeneration, where symptoms and signs are present, but yet insufficient to define the disease, and the clinical diagnosis is called the prodromal or pre-motor phase and can start up to 20 years before the onset of motor parkinsonism [3,4]. Given that diagnosing PD means identifying an already advanced neurodegeneration, it is essential to focus on its early detection, by defining patterns of cognitive and other non-motor symptoms.

Research criteria for prodromal PD (P-PD) were proposed by the Movement Disorder Society (MDS) [5,6]. Their findings suggest that polysomnographically proven REM-Sleep Behavior Disorder (RBD), abnormal dopaminergic brain imaging (PET/SPECT), subthreshold motor parkinsonism and olfactory dysfunction are the prodromal markers with the highest likelihood to predict α-synucleinopathies, such as PD. Global cognitive deficit was only later added as a criterion for prodromal PD [6].

Cognitive impairment already defines the early stages of PD. In the Luxembourgish PD cohort, approximately 45% of newly diagnosed typical PD patients (disease duration ≤ 1 year) presented cognitive impairment (MoCA < 26). These findings are in line with previous observations of 24 to 54% of cognitive impairment in newly diagnosed PD [6–9]. These deficits may precede clinical PD diagnosis by up to 5 years [3]. Longitudinal studies comparing converters to non-converters describe a prevalence of 42% of cognitive impairment at baseline [7]. Knowledge on the nature of these prodromal cognitive changes is still limited, probably due to the novelty of the concept. Recent studies on cognitive deficits in prodromal PD described that global cognition and diverse cognitive sub-domains, mainly executive functions, less frequently visuospatial functions, memory and language, may be prodromal cognitive features of PD [8–11]. The available studies are very heterogeneous in their study designs (e.g., recruitment strategies), study populations (e.g., age, education), neuropsychological assessments and the tested cognitive domains, complicating the comparability of results [12–14]. Therefore, following previously published recommendations [5,6,12,13], results on cognition in prodromal PD need validation i) in a deep-phenotyped population, ii) combining predefined prodromal markers, iii) on normative-controlled cognitive data, iv) based on a broad variety of commonly used cognitive assessment tools to evaluate both global cognition and domain-specific

cognition, v) with at least two tests per cognitive domain.

In the present cross-sectional, case-control study, we performed extensive cognitive testing in an at-risk group for developing PD, defined by probable RBD and hyposmia and compared them with Besides testing different cognitive functions, we investigated additional features such as non-motor (e.g., psychological factors and quality of life) and motor symptoms.

The main aim of this study was to describe non-motor symptoms in P-PD and to define its specific profile focusing on cognition. In the future, participants will be followed-up yearly to capture possible phenoconversion from P-PD to PD, allowing us to determine specific patterns supporting the definition of further possible prodromal markers. Early recognition of PD could not only allow better prognosis but also help the development of neuroprotective therapies.

Material and Methods

Participants

All participants were recruited from the Luxembourg Parkinson Study of the National Centre of Excellence in Research on Parkinson's disease (NCER-PD). NCER-PD is a monocentric, observational, longitudinal prospective study including a PD, an enriched P-PD as well as a control cohort from Luxembourg and the Greater Region [15], an area of cross-border cooperation between Luxembourg, Germany, Belgium and France. All participants provided informed consent according to the Declaration of Helsinki. The study is approved by the National Ethics Board (CNER Ref: 202001/03 and 201407/13). The detailed study design, recruitment and screening steps have been described elsewhere [15,16].

The classification of probable RBD was based on the RBD Screening Questionnaire (RBDSQ score ≥ 7 [17]). The Brief Smell Identification Test (A) (B-SIT) [18] or Sniffin'Stick Identification Test [19] (B-SIT score < 8 [18] or Sniffin'Stick score ≤ 12 [20]) were used to assess olfaction. Each subject underwent a detailed neurological examination by a physician trained in movement disorders and provided information on probable symptoms and disease history. The Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS) [21] was used to assess motor and non-motor symptoms. Inclusion criteria were age 18 years or older and ability to sign the written informed consent. People with PD or other known neurological diseases as well as participants with a history of severe psychiatric disorders were excluded (Figure 1).

Approach

We defined two sets of analyses: The first set of analyses (Set 1) (Flowchart, Figure 1 (A) & (B) – Supplementary Material), capitalized on the extensive neuropsychological assessment.

We adjusted for the effects of other variables (and tested the effect of the variable of interest) with (i) propensity score matching (followed by testing whether the outcome differs between the two groups) and (ii) multiple regression (followed by testing the effect of the group on the outcome).

For the second set of analyses (Set 2) (Flowchart, Figure 1 (C) – Supplementary Material), we compared cognitive performances measured by the CUPRO evaluation system [22]. The size of the P-PD and matched control sample differ slightly between sets, since CUPRO was more recently added to the neuropsychological assessment.

Neuropsychological assessments

For the first set of analyses, study participants underwent detailed neuropsychological assessments, selected previously based on recommendation by Goldman and colleagues [23] (Table 1). Cognitive measures were combined to evaluate global cognition and the following five cognitive domains: memory, processing speed, executive functions, language, and visuospatial functions.

For the second set of analyses, we applied the CUPRO, (short for CUbe drawing PROcedure) evaluation system to assess the cube copying procedure (Intermediate Score 1 – CUPRO-IS1), representing retrograde procedural memory and the final result of the cube (Intermediate Score 2- CUPRO-IS2), representing visuo-constructive functions [22]. Furthermore, Montreal Cognitive Assessment (MoCA) [24] and Trail-Making-Test [25] were also assessed in this set of analyses.

Mild and severe cognitive impairment were defined as impaired global cognition based on MoCA < 26 and < 21, respectively [24,26]. Participants with MoCA ≥ 26 were classified as cognitively normal.

Self-assessment questionnaires

The Beck Depression Inventory-I (BDI-I) [27], the Starkstein Apathy Scale (SAS) [28] and the Parkinson's Disease Questionnaire (PDQ-39) [29] were applied to assess symptoms of depression and apathy, and quality of life, respectively. Participants reported non-motor and motor aspects of experiences of daily living in the MDS-UPDRS Part I and II.

Statistics

Two different statistical methods were used to adjust for the effects of potential confounders, namely propensity score matching and multiple regression.

In a first step we chose to test differences between samples, both groups were matched by age and sex (propensity score matching; matching tolerance = 0.05). As many outcomes are not normally distributed, differences in demographic and clinical characteristics as well as cognitive performance between the groups were analyzed using the Mann-Whitney U test (two-tailed) for numerical variables (which might be non-normally distributed) and

Pearson's chi-squared test (two-tailed) for binary variables (Figure 1 (A) & (C) – Supplementary Material), (Table 2 & 3) and (Table 1 & 2 – Supplementary Material). We corrected for multiple testing using the Bonferroni correction ($p \leq 0.05/n$, n = number of comparisons) (**).

To validate the findings in a larger sample and to further assess the relationship between the groups and demographic, clinical and cognitive factors, controlled for sex, age and education depending on P-PD status, we applied, in a second step, multiple linear and logistic regressions (Figure 1 (B) – Supplementary Material) (Table 4 & 5). The significance threshold was set up at p-value ≤ 0.05. We corrected for multiple testing ($p \leq 0.05/n$, n = number of comparisons) (**).

To evaluate the assumptions of the linear and logistic regressions, we confirmed in a first step that the samples are independent; No participant was included twice or more and they have not been measured under two or more conditions. However, we cannot exclude that we might have included participants that share a family link. In a second step, we assessed the variance inflation factor (VIF), measuring of how much the variance of the estimated regression coefficients increases due to multicollinearity. We could not detect any VIF greater than 2 and excluded therefore multicollinearity. To verify the linearity assumption, we examined scatter plots of the residuals against the predictors. As we did not observe any relationship between the residuals and the predictors, we have no evidence of any non-linear effects. To verify the linearity assumption for the logistic regressions we plotted the partial residuals against predictors and observed a linear relationship between each predictor variable and the log-odds of the response variable

All statistical analyses were performed using R version 4.2.0 GUI 1.78 and RStudio version 2023.03.1+446.

Results

For Set 1, in total, 271 participants fulfilled the inclusion criteria, 76 participants with probable RBD and hyposmia and 195 control subjects without RBD and without hyposmia (Figure 1 (A) & (B) – Supplementary Material).

A. Propensity score matching

After matching for age and sex, we compared 62 P-PD participants with 62 control subjects (Figure 1 (A) – Supplementary Material) (Table 2 & 3). Confirming successful matching, the groups did not differ significantly in sex ($p = 1.000$) or age ($p = 0.793$). Furthermore, they did not differ on years of education ($p = 0.415$). After multiple testing correction, the P-PD group presented significantly higher scores in SAS ($p < 0.001$), BDI-I ($p = 0.004$), MDS-UPDRS I & II ($p < 0.001$, $p = 0.004$, respectively) and a significantly lower score

for PDQ-39 ($p < 0.001$) compared to the matched control subjects.

Significant group differences were found in cognition (Table 3). The P-PD group presented significantly lower scores in MoCA ($p < 0.001$) and Delta-TMT scores ($p = 0.003$) compared to the control group. We observe a tendency for deficits in the Cube Copying Task in the P-PD, however the difference is not significant after correction for multiple testing.

When investigating the distribution of the total MoCA score, we observed that 53/62 (85%) and only 37/62 (60%) participants presented normal cognition (based on MoCA ≥ 26 [24]) in the control group, respectively the P-PD group; 7/62 (11%) and 23/62 (37%) participants presented Mild Cognitive Impairment (based on 21 > and MoCA < 26) in the control group [24], respectively the P-PD group. Furthermore, 2/62 (3%) and 2/62 (3%) participants presented severe cognitive impairment (based on MoCA < 21 [26]) in the control group, respectively the P-PD group (Figure 1).

B. Regressions

After adjusting for age, sex and education as well as multiple testing correction, the P-PD group was associated with significantly different scores on MoCA, TMT-B, Delta-TMT, Cube Copying Task, BDI, SAS, PDQ-39 as well as on the MDS-UPDRS I and II. Furthermore, nominal significant different scores were observed for the Stroop Interference Score, FAB and Isaacs Set test (Table 4 & 5).

Both analytical strategies (Figure 1 (A) & (B)) – Supplementary Material) yielded consistent results: Both sets of findings indicate impaired global cognition, executive and visuo-constructive functions in the P-PD group compared to the matched control group. With the matching analyses (Figure 1– Supplementary Material), significantly lower performances in executive functions were only observed in one cognitive test (Trail-Making-Test, TMT); the difference in visuo-constructive abilities was only nominally significant. However, in the regression analyses (Figure 1 (B) – Supplementary Material), taking the total sample into consideration, we observed that several cognitive assessments measuring executive functions were nominally significant impaired in the P-PD group compared to the matched control group (Stroop Interference Score; Isaacs Set Test; Frontal Assessment Battery, FAB), which were however not significant after Bonferroni correction ($p = 0.011$, $p = 0.009$, $p = 0.005$, respectively). Differences in visuo-constructive abilities were significant in the larger sample ($p < 0.001$). Furthermore, in both analyses, scores for depression (BDI-I), apathy (SAS), motor and non-motor symptoms (MDS-UPDRS I and II) were significantly higher and the score for quality of life (PDQ-39) was significantly lower in P-PD.

Results of the Set 2 are presented in the Supplementary Material (Table 1 & 2 – Supplementary Material).

Table 1. Neuropsychological assessments and measured cognitive functions

Cognitive functions	Assessments	
Global cognition	Montreal Cognitive Assessment (MoCA)	[24]
Memory		
Auditory short-term memory	Digit Span - Forward	[43]
Auditory working memory	Digit Span - Backward	[43]
Visuo-spatial short-term memory	Corsi Block Tapping Task - Forward	[44]
Visuo-spatial working memory	Corsi Block Tapping Task - Backward	[44]
Episodic verbal long-term memory	CERAD Word List Delayed Recall	[45]
Learning ability	CERAD Word List Learning	[45]
Processing speed		
Psychomotor speed, Inititation	Trail Making Test (TMT) - Part A	[25]
Processing speed	Stroop Test - Word Reading	[46]
Executive functions		
Mental flexibility, Shifting	Trail Making Test (TMT) - Part B & Delta-TMT*	[25]
Inhibitory control	Stroop Test - Interference Score	[25,46]
Dysexecutive syndrome	Frontal Assessment Battery (FAB)	[47]
Mental flexibility	Isaacs Set Test	[48]
Language		
Language - Denomination	Boston Naming Test – short form	[44]
Fluency, Word initiation	Semantic Fluency (animals, 2 min)	[25]
	Phonemic Fluency (letter "F", 1 min)	[24]
Visuospatial functions		
Visuoconstructive capacities	Qualitative Scoring MMSE Pentagon	[49]
	Cube Copying Task	[24]
Visuospatial judgment	Benton Judgment of Line Orientation (JLO)	[50]

Neuropsychological assessments and measured cognitive functions. N.B.: We allocated cognitive test to cognitive domains. Given that no cognitive assessment evaluates purely one cognitive function, overlap cannot be excluded. *Delta-TMT is defined as (TMT-B) – (TMT-A).

Table 2. Demographical and clinical information for prodromal PD (P-PD) and control group

Variable	Descriptive statistics						P-Values	
	Prodromal PD n = 62			Control n = 62			Prodromal PD vs. Control	
	Mean	*SD*	*n*	*Mean*	*SD*	*n*		
Sex, M / F	37/25		62	38/24		62	p = 1.000	
Age, in years	63.52	5.96	62	63.70	8.04	62	p = 0.793	
RBDSQ (/13)	9.07	1.83	62	2.00	1.79	62	p < 0.001	
Sniffin'Stick (/16)	10.11	3.19	53	14.18	1.06	62	p < 0.001	
BSIT-A (/12)	6.48	1.94	61	NA	NA	0	NA	
Education, in years	13.00	4.25	60	13.76	3.66	62	p = 0.415	
MDS-UPDRS I (/32)	8.22	5.96	46	4.65	4.49	62	p < 0.001	**
MDS-UPDRS II (/32)	2.68	4.01	57	1.10	1.77	62	p = 0.004	**
MDS-UPDRS III (/132)	4.71	6.57	59	4.19	5.06	59	p = 0.872	
BDI-I (/63)	8.69	6.89	54	5.34	5.13	62	p = 0.004	**
SAS (/42)	13.56	5.32	55	9.93	5.09	61	p < 0.001	**
PDQ-39 (%)	13.32	12.63	53	5.87	6.15	61	p < 0.001	**

Demographical and clinical information for prodromal PD (P-PD) and control group. Both groups were defined on RBDSQ, Sniffin'Stick and BSIT-A and matched for sex and age. SD: Standard Deviation; M: Male; F: Female; n = sample size; MDS-UPDRS: Movement Disorder Society - Unified Parkinson's Disease Rating Scale; RBDSQ: REM Sleep Behavior Disorder (RBD) Screening Questionnaire; BSIT: Brief Smell Identification Test; BDI-I: Beck Depression Inventory; SAS: Starkstein Apathy Scale; PDQ-39: Parkinson's disease questionnaire 39-item. * Significant at the unadjusted 5% level (p-value ≤ 0.05) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (p-value ≤ 0.05/7) (two-tailed).

Table 3. Results of neuropsychological assessments for prodromal PD (P-PD) compared to the control group

Variable	Descriptive statistics						Significance	
	Prodromal PD n = 62			Control n = 62			Prodromal PD vs. Control	
	Mean	*SD*	*N*	*Mean*	*SD*	*N*		
Montreal Cognitive Assessment (MoCA) (/30)	26.05	2.47	62	27.39	2.43	62	p < 0.001	**
Trail-Making-Test Part A (TMT-A) (sec)	45.13	37.52	61	44.73	36.94	62	p = 1.000	
Trail-Making- Test Part B (TMT-B) (sec)	111.7	61.77	61	89.66	38.50	62	p = 0.030	*
Delta-TMT (TMT-B) – (TMT-A)	66.52	49.74	61	44.94	23.30	62	p = 0.003	**
Digit Span Test Forwards (/16)	8.61	1.73	62	8.47	1.66	62	p = 0.668	
Digit Span Test Backwards (/14)	5.90	1.63	62	6.16	1.87	62	p = 0.651	
Corsi Block-Tapping Test Forward (/16)	8.21	1.50	62	8.00	1.34	61	p = 0.450	
Corsi Block-Tapping Test Backward (/14)	7.74	1.59	62	7.59	1.94	61	p = 0.628	
Kaplan Stroop Interference Score (sec)	64.90	35.97	59	52.35	22.99	62	p = 0.146	
Semantic Fluency Test (N Letter F, 1 min)	10.37	4.43	60	11.45	4.50	62	p = 0.307	
Phonemic Fluency Test (N Animals, 1 min)	29.07	9.62	61	30.41	7.10	61	p = 0.258	
Isaacs Set Test (N)	32.47	7.58	58	34.98	6.09	58	p = 0.058	
Interlocking Pentagons Test (incorrect/correct)	2/60		62	4/58		62	p = 0.676	
Cube Copying Task (incorrect/correct)	24/38		62	9/53		62	p = 0.004	*
Benton's Judgment of Line Orientation Test (/30)	24.11	4.88	61	24.45	4.35	62	p = 0.877	
Frontal Assessment Battery (FAB) (/18)	15.48	2.17	52	16.21	1.53	62	p = 0.091	
CERAD Word list (Learning) (/30)	22.60	3.93	62	23.05	3.29	62	p = 0.722	
CERAD Word list (Delayed Recall) (/10)	7.10	2.28	60	7.59	1.77	61	p = 0.327	

Results of neuropsychological assessments for prodromal PD (P-PD) compared to the control group. SD: Standard Deviation; CERAD: Consortium to Establish Registry for Alzheimer Disease. * Significant at the unadjusted 5% level (p-value ≤ 0.05) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (p-value ≤ 0.05/18) (two-tailed).

Table 4. Regression analyzing the relationship between the groups and the demographical and clinical factors, controlled for age, sex and education

Dependent variables		Independent variables			
		Age	Sex	Education	Prodromal PD
MDS-UPDRS I	Estimate	0.042	1.457	-0.019	3.491
	p-value	p = 0.159	p = 0.018 *	p = 0.811	p < 0.001 **
MDS-UPDRS II	Estimate	0.021	-0.087	-0.082	1.462
	p-value	p = 0.199	p = 0.781	p = 0.043 *	p < 0.001 **
MDS-UPDRS III	Estimate	0.118	-0.736	-0.138	0.807
	p-value	p < 0.001 **	p = 0.222	p = 0.074	p = 0.227
BDI	Estimate	0.018	2.050	-0.066	2.931
	p-value	p = 0.601	p = 0.004 *	p = 0.462	p < 0.001 **
SAS	Estimate	0.032	-0.017	-0.316	2.957
	p-value	p = 0.307	p = 0.979	p < 0.001 **	p < 0.001 **
PDQ-39	Estimate	-0.020	1.085	-0.260	6.043
	p-value	p = 0.718	p = 0.318	p = 0.059	p < 0.001 **

Linear regression analyzing the relationship between the groups (N prodromal PD = 76; N control group = 195) and the demographical and clinical factors, controlled for age, sex and education. * Significant at the unadjusted 5% level (p-value ≤ 0.05) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (p-value ≤ 0.05/23) (two-tailed).

Table 5. Regression analyzing the relationship between the groups and the cognitive factors, controlled for age, sex and education

Dependent variables		Independent variables			
		Age	Sex	Education	Prodromal PD
MoCA	Estimate	-0.033	0.463	0.191	-1.166
	p-value	p = 0.015 *	p = 0.089	p < 0.001 **	p < 0.001 **
TMT-A	Estimate	0.642	6.490	-0.480	1.401
	p-value	p = 0.002 **	p = 0.107	p = 0.351	p = 0.751
TMT-B	Estimate	1.016	-3.057	-3.323	23.380
	p-value	p < 0.001 **	p = 0.541	p < 0.001 **	p < 0.001 **
(TMT-B) – (TMT-A)	Estimate	0.374	-9.547	-2.843	21.978
	p-value	p = 0.091	p = 0.032 *	p < 0.001 **	p < 0.001 **
Digit Span Test Forward	Estimate	-0.019	-0.382	0.006	-0.112
	p-value	p = 0.096	p = 0.093	p = 0.836	p = 0.655
Digit Span Test Backwards	Estimate	-0.025	-0.070	0.066	-0.113
	p-value	p =0.027 *	p = 0.754	p = 0.020 *	p = 0.646
Corsi Block Tapping Forward	Estimate	-0.033	-0.047	0.040	0.311
	p-value	p < 0.001 **	p = 0.799	p = 0.086	p = 0.124
Corsi Block Tapping Backward	Estimate	-0.049	-0.445	0.115	0.464
	p-value	p < 0.001 **	p = 0.057	p < 0.001 **	p = 0.071 .
Stroop Interference Score	Estimate	0.842	3.289	-0.577	9.170
	p-value	p < 0.001 ***	p = 0.307	p = 0.162	p = 0.011 *
Semantic Fluency	Estimate	-0.152	1.171	0.431	-0.851
	p-value	p = 0.005 *	p = 0.273	p = 0.002 **	p = 0.470
Phonemic Fluency	Estimate	-0.037	0.809	0.235	-0.369
	p-value	p = 0.165	p = 0.131	p < 0.001 **	p = 0.533
Isaacs Set Test	Estimate	-0.139	1.313	0.404	-2.354
	p-value	p < 0.001 **	p = 0.107	p < 0.001 **	p = 0.009 *
Interlocking Pentagons	Estimate	-0.050	-0.872	0.212	0.951
	Odds Ratio	0.952	0.418	1.236	2.587
	p-value	p = 0.190	p = 0.226	p = 0.019 *	p = 0.272
Cube Copying Task	Estimate	-0.008	-0.403	0.198	-1.235
	Odds Ratio	0.992	0.668	1.219	0.291
	p-value	p = 0.706	p = 0.255	p < 0.001 **	p < 0.001 **
Benton JLOT	Estimate	-0.075	-3.619	0.310	-0.688
	p-value	p = 0.003 *	p < 0.001 **	p < 0.001 **	p = 0.212
FAB	Estimate	-0.032	0.212	0.130	-0.688
	p-value	p = 0.002 **	p = 0.318	p < 0.001 **	p = 0.005 *
CERAD Word List learning	Estimate	-0.088	2.108	0.172	-0.021
	p-value	p < 0.001 **	p < 0.001 **	p = 0.002 **	p = 0.965
CERAD Word List Delayed Recall	Estimate	-0.040	0.982	0.095	-0.083
	p-value	p < 0.001 **	p < 0.001 **	p = 0.001 **	p = 0.739

Regression analyzing the relationship between the groups (N prodromal PD = 76; N control group = 195) and the cognitive factors, controlled for age, sex and education. Multiple logistic regression for "Interlocking Pentagons" and "Cube Copying Task" otherwise multiple linear regression.* Significant at the unadjusted 5% level (p-value ≤ 0.05) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (p-value ≤ 0.05/23) (two-tailed).

Discussion

The aim of the present study was to investigate non-motor symptoms focusing on the cognitive profile in a prodromal PD (P-PD) cohort with self-assessments and extensive cognitive testing. We compared presence and level of non-motor symptoms, focusing on cognition, between P-PD and age- and sex-matched control subjects. The present study demonstrates that cognitive performance was impaired in the enriched at-risk group for developing PD compared to the control group. More precisely, participants with P-PD present significantly lower scores in global cognition, executive and visuo-constructive functions (in tasks with higher complexity) compared to the matched control group. In addition, we observed significantly more difficulties in motor and other non-motor symptoms of experiences of daily living, as well as significantly higher scores for depression and apathy and significantly lower scores for quality of life in the P-PD group.

The observation of global cognitive and executive deficits are consistent with the conclusion of recently published studies stating that these cognitive impairments may be prodromal features of PD [8-10,30]. With the screening tool for global cognition (MoCA) employed here, we observed that 40% of the at-risk group presented cognitive impairment, in contrast to only 15% in the matched control group. These results are consistent with findings of 42% with Mild Cognitive Impairment (MCI) and global cognitive deficit at baseline in a longitudinal study comparing converters to non-converters [7] and close to the prevalence of 45% of MCI in newly diagnosed PD patients in our PD cohort [15]. A longitudinal study on RBD P-PD participants found that global cognitive deficits appeared approximately 5 years before phenoconversion to PD compared to age- and sex-matched control subjects [3].

We found tendencies for impaired executive functions in P-PD across all assessments previously defined to evaluate these functions. After correction for multiple testing, significant differences remained for one sub-domain of executive functions: mental flexibility, as measured by the Trail-Making-Test (TMT). Therefore, we carefully interpret that, out of a range of neuropsychological assessments of executive functioning, the TMT might be the most sensitive for detecting executive changes in P-PD. Our findings of significantly impaired mental flexibility and a trend towards impairment in other sub-domains of executive functions are in line with observations in longitudinal and cross-sectional studies in prodromal PD cohorts [8,9,11,30,31]. Furthermore, they match with the cognitive profile of an executive deficit in newly diagnosed PD patients [32] and its association to the frontal lobe and modulation by dopaminergic input [33]. Our observations of early pre-diagnostic impairment of executive

functions lend support to the "Dual Syndrome Hypothesis" [34], describing the possibility of two sub-types of cognitive impairment in PD; the "frontal-striatal subtype", defined by predominant executive deficits related to increased dopaminergic loss starting early in the disease progression; and the "posterior and temporal subtype" with predominant visuospatial, memory and language deficits, related to increased cholinergic loss [34]. In the present study, we did not find significant differences in processing speed, language, learning and memory. Only a few studies tested learning and memory [10-13,30]. Memory impairments have been observed in patients who converted to PD within 2 years but not in the earlier prodromal stages [35]. Based on the results in Set 2, by applying the CUPRO evaluation system [22], we confirmed that the significant difficulties observed in the Cube Copying Task (initial scoring on 1 point [24]) are due to visuo-constructive deficits and not to a deficit of retrograde procedural memory. To our knowledge, the current study is the first to systematically evaluate retrograde procedural memory in P-PD. Given that, in our previous work on retrograde procedural memory in already diagnosed PD, we did not see any significant correlation between this memory concept and disease duration [22], we assumed that retrograde procedural memory might be already impaired in the prodromal stages of PD. Furthermore, we did not find significant differences in processing speed, language, learning and memory. Only a few studies tested learning and memory in earlier P-PD stages, with inconsistent results [10-13]. Memory impairments have been observed in patients who converted to PD within 2 years but not in the earlier prodromal stages [35].

The combination of the absence of memory and language impairment, the visuo-constructive impairment only in the more complex assessments and the previously discussed point of a more "frontal-striatal subtype" suggest that the sample may still be in the early stage of P-PD. Previous findings demonstrated that motor variables have been found to be highly predictive of the phenoconversion to parkinsonism [36]. The fact that we did not see any significant differences for the measured motor assessments and that the UPDRS score is estimated to become only abnormal at 4.5 years before diagnosis [37] are further arguments highlighting the possibility that the cohort is in the early stage of P-PD. This might be explained by our wide study design defining the prodromal cohort based on a population-wide participant recruitment in which we invited the entire Luxembourgish population between 55 and 75 years to participate in an online survey about their sleep quality and possible sleeping difficulties. One additional fundamental strength of the current study is the thorough recruitment steps and deep phenotyping of the participants, involving a complex study design, including self-reported, web-based

questionnaires, telephone interviews, face-to-face assessments, and longitudinal follow-up assessments. Moreover, by combining two validated prodromal markers, RBD and hyposmia, we work on a population that is at high risk to develop PD. Furthermore, as recommended [12], we assured that the groups were well described and matched for possible confounding factors such as age and sex. Domain-specific cognitive deficits were investigated each through several assessments, allowing us to cross-validate our results. While global cognition and executive functions have been frequently evaluated, learning, memory, visuo-spatial cognition, and language abilities are less frequently assessed [12,13]. Although we administered an extensive range of neuropsychological assessments, we acknowledge that no cognitive assessment evaluates purely one cognitive function and impairments in one domain may be reflected in impaired performances on tests assessing other domains. Lastly, we cannot fully exclude the possibility of cognition in P-PD being affected by sleep problems and depressive mood. Sleep abnormalities and mood disorders such as depression are validated signs for P-PD [5,6]. We repeated our regressions by additionally controlling for apathy and depression and the adjustment for apathy and depression does not change our conclusions on the effects of the disease status on the outcomes (significant vs. insignificant). Given that the study participants are characterized with sleep abnormalities and knowing that sleep quality plays a crucial role in the well-functioning of cognition, cognitive performance may be affected by these confounders [38].

Our study has the limitation that it focuses on an at-risk cohort based on pRBD and not on a polysomnographically proven idiopathic RBD (iRBD). According to the MDS criteria for P-PD, iRBD based on polysomnography has a positive likelihood ratio of 130 compared to only 2.8 for the questionnaire-based pRBD [6]. Therefore, to follow the gold standard for RBD diagnosis and to enrich the prodromal cohort, participants with pRBD are currently undergoing video-polysomnography to confirm the diagnosis of RBD. Furthermore, given that our P-PD cohort is defined by pRBD and hyposmia, we need to highlight that our observations focus mainly on one specific subtype of P-PD, as we do not control for all potential prodromal markers and that it cannot be generalized on all the P-PD subtypes. Especially because iRBD in PD has been associated with higher burden of non-motor symptoms, such as impaired cognitive functions [36,39]. In order to address the heterogeneity in P-PD, we are currently working on the investigation of a population based on additional prodromal signs, e.g.: by combining alternative prodromal signs, such as constipation and genetic predispositions [40]. Exploring different markers may yield valuable insights, particularly if they are

associated with distinct cognitive patterns and varying degrees of severity [13]. Another limitation lies in the fact that not all participants with RBD might develop PD, as RBD is also a risk factor for other synucleinopathies, such as DLB or MSA [41]. The understanding of the heterogeneity in P-PD is essential to understanding the diversity of clinical PD and the mechanisms behind this variability.

The trends described in the present study highlight the importance of investigating cognitive performances and other non-motor symptoms in populations at risk of developing Parkinson's disease. However, as these findings are based on the cross-sectional analyses it needs validation on longitudinal observations. Therefore, we aim to confirm the reported findings through longitudinal follow-up, currently foreseen. Furthermore, for future projects, it would be interesting to also include subjective cognitive decline (SCD) in prodromal PD, as little knowledge on prevalence and progression of SCD exists in P-PD [13], and to compare different risk factor profiles involved in P-PD.

In conclusion, our findings confirm that global cognitive, executive, and visuo-constructive deficits are present in individuals at risk for PD based on probable RBD and hyposmia. In addition, people with P-PD had significantly more self-reported motor and other non-motor symptoms, such as depression and apathy, and a lower quality of life. The validation of these results, on normative-controlled, extensive cognitive and clinical data in a deep-phenotyped population is essential, as knowledge on the nature of these cognitive changes is still limited due to the novelty of the concept of cognitive deficits in P-PD [14]. Combining non-motor prodromal signs, including global cognition, executive and visuo-constructive functions, depression, apathy and quality of life may improve the description of the P-PD phenotype and allow a clearer identification of the at-risk population for PD. Based on our findings and if replicated in other samples, we suggest considering the addition of executive and visuo-constructive deficits as a non-motor sign in prodromal PD. A clear definition of the P-PD phenotype will have an important impact when disease-modifying treatments will become available [42].

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Conflict of interest

RK serves as Editorial Board Member of the European Journal of Clinical Investigation, the Journal of Parkinsonism and Related Disorders and the Journal of Neural Transmission. RK is an Editorial Board Member of this journal, but was not involved in the peer-review process nor had access to any information regarding its peer-review. RK has received research grants from the Fond National de la Recherche (FNR) as Coordinator of the National Centre for Excellence in Research on Parkinson's Disease (NCER-PD), Coordination of the Study on COvid-10 National survey for assessing VIral spread by Non-affected CarriErs (CON-VINCE). RK received as well as speaker's honoraria and/or travel grants from Abbvie, Desitin, Zambon and Medtronic and he participated as PI or site-PI for industry sponsored clinical trials without receiving honoraria.

AKL served on advisory boards and as speaker for Roche. The other authors have no conflict of interest to report.

Data availability statement

The dataset used for this manuscript is available upon request to the Data and Sample Access Committee of the Luxembourg Parkinson's Study (via email: request.ncer-pd@uni.lu).

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Supplementary Material

Figure 1 Supp. Study Flowchart

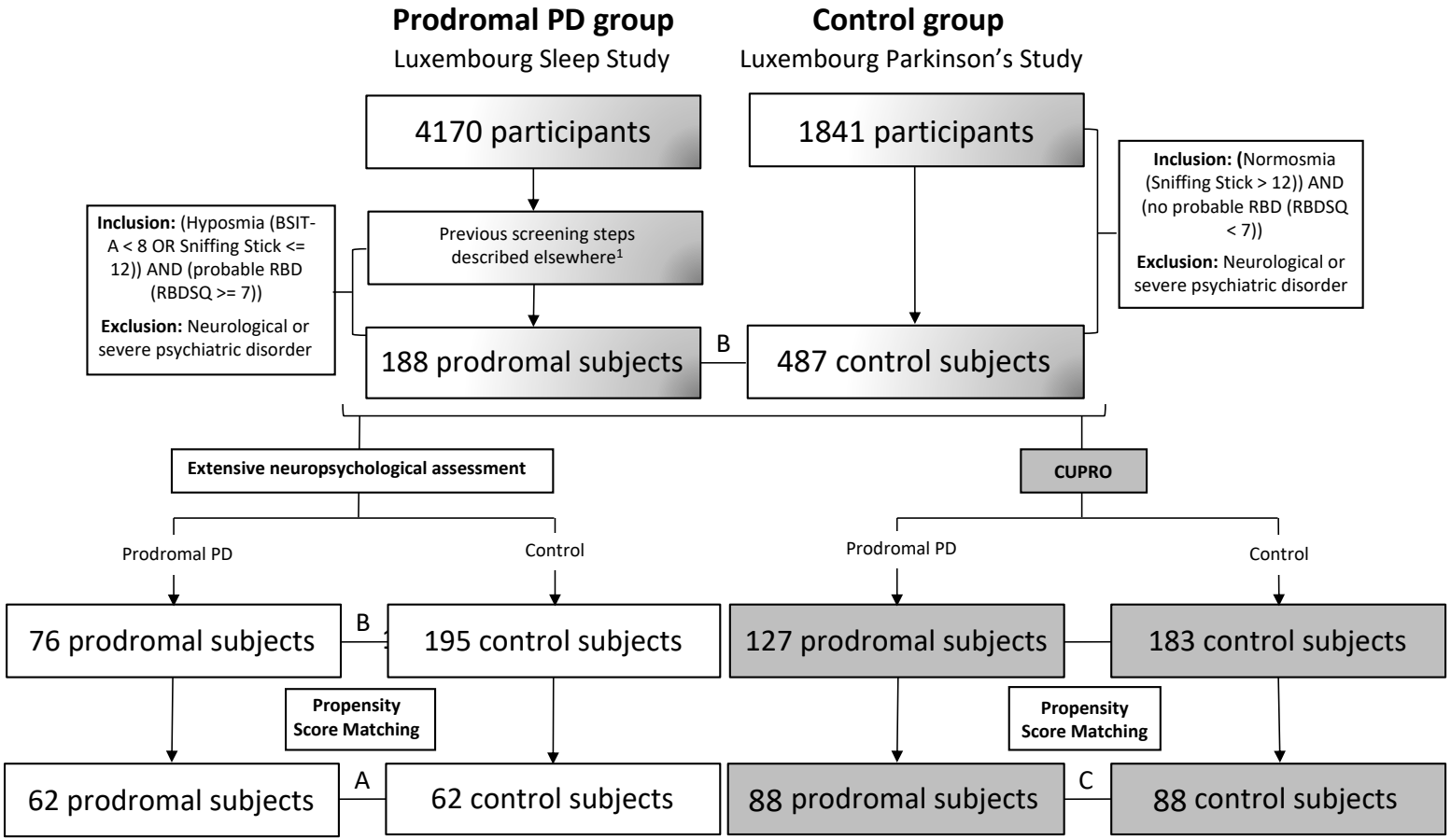

Figure representing inclusion/exclusion criteria, the propensity score (PS) matching (A) and regression (B) steps for both groups, prodromal PD and control group. The main analyses on the extensive cognitive assessment are represented in green, the supra-analyses on a reduced cognitive assessment including the novel assessment the CUPRO is represented in orange. [1] McIntyre et al. 2023 (in preparation)

In the second set of the analyses (see Flowchart, Figure 1 – Supplementary Material) we compared cognitive performances measured by our novel assessment, the CUPRO evaluation system [1]. With the CUPRO evaluation system, we assessed our main outcome variables for this set, the Cube copying procedure (Intermediate Score 1 - IS1), representing retrograde procedural memory and the final result of the Cube (IS2), representing visuo-constructive functions [1]. Given that the CUPRO assessment tool has only been recently developed and integrated in the Luxembourg Parkinson's Study, not all the control participants that have participated in the extensive cognitive testing session (supplementary visit at the research clinic) have a CUPRO evaluation from their cube drawing. Therefore, we decided to observe this variable in an additional analyses, so that by filtering for this assessment, we do not impact the power of our main analyses on the broad cognitive assessment in P-PD.

Table 1 Supp. Demographical and clinical information for prodromal PD (P-PD) and control group

Variable	Descriptive statistics						P-Values	
	Prodromal PD n = 88			Control n = 88			Prodromal PD vs. Control	
	Mean	*SD*	*n*	*Mean*	*SD*	*n*		
Sex, M / F	47/41		88	47/41		88	p = 1.000	
Age, in years	64.98	5.76	88	64.59	5.86	88	p = 0.648	
RBDSQ (/13)	9.03	1.62	88	2.47	1.72	88	p < 0.001	
Sniffin'Stick (/16)	9.91	3.18	78	13.94	1.03	88	p < 0.001	
BSIT-A (/12)	6.64	2.04	88	NA	NA	0	NA	
Education, in years	13.11	4.72	80	14.53	4.07	86	p = 0.058	
MDS-UPDRS I (/32)	8.37	6.21	35	4.81	3.89	87	p = 0.002	**
MDS-UPDRS II (/32)	3.27	4.92	45	0.82	1.28	88	p < 0.001	**
MDS-UPDRS III (/132)	5.96	8.55	49	4.35	4.10	82	p = 0.833	
BDI-I (/63)	9.24	8.08	46	5.18	4.74	88	p = 0.001	**
SAS (/42)	13.29	5.59	45	9.51	4.26	88	p < 0.001	**
PDQ-39 (%)	12.61	13.50	42	6.43	5.78	88	p = 0.017	*

Both groups were defined on RBDSQ, Sniffin'Stick and BSIT-A and matched for sex and age. SD: Standard Deviation; M: Male; F: Female; n = sample size; MDS-UPDRS: Movement Disorder Society - Unified Parkinson's Disease Rating Scale; RBDSQ: REM Sleep Behavior Disorder (RBD) Screening Questionnaire; BSIT: Brief Smell Identification Test; BDI-I: Beck Depression Inventory; SAS: Starkstein Apathy Scale; PDQ-39: Parkinson's disease questionnaire 39-item. * Significant at the unadjusted 5% level (p-value ≤ 0.05) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (p-value ≤ 0.05/7) (two-tailed).

After matching for age and sex, we compared 88 P-PD participants with 88 control subjects (Figure 1 (C) - Supplementary Material). Confirming successful matching, the groups did not differ significantly in sex (p = 1.000), age (p = 0.648). They did not differ significantly in years of education (p = 0.202). After multiple testing correction, the P-PD group presented significantly higher SAS (p < 0.001), BDI-I (p = 0.001), MDS-UPDRS I & II (p = 0.002, p < 0.001, respectively) and nominally significant lower score for PDQ-39 (p = 0.017) compared to the matched control subjects.

Table 2 Supp. Results of neuropsychological assessments for prodromal PD (P-PD) compared to the control group

Variable	Descriptive statistics						Significance	
	Prodromal PD n = 88			Control n = 88			Prodromal PD vs. Control	
	Mean	*SD*	*N*	*Mean*	*SD*	*N*		
CUPRO								
Intermediate Score 1 (IS1) (/3)	2.22	1.07	88	2.40	0.94	88	p = 0.273	
Intermediate Score 2 (IS2) (/3)	2.06	1.14	88	2.63	0.78	88	p < 0.001	**
CUPRO Total score (/6)	4.27	1.94	88	5.02	1.49	88	p = 0.010	*
Montreal Cognitive Assessment (MoCA) (/30)	25.22	3.41	88	27.09	2.55	88	p < 0.001	**
Trail-Making-Test								
Part A (TMT-A) (sec)	43.98	18.73	48	38.42	16.37	88	p = 0.071	
Part B (TMT-B) (sec)	116.4	57.99	48	82.83	28.05	88	p < 0.001	**
Delta-TMT (TMT-B) – (TMT-A)	72.46	51.24	48	44.41	29.43	88	p < 0.001	**

SD: Standard Deviation; CERAD: Consortium to Establish Registry for Alzheimer Disease. * Significant at the unadjusted 5% level (p-value ≤ 0.05) (two-tailed); ** Significant at the Bonferroni-adjusted 5% level (p-value ≤ 0.05/7) (two-tailed).

Significant group differences were found in cognition. The P-PD group presented significantly lower scores in CUPRO Intermediate Score 2 (IS2) ($p < 0.001$), MoCA ($p < 0.001$), TMT-B and Delta-TMT scores ($p < 0.001$) compared to the control group.

In the present study, we found significant differences for the Cube copying task (initial scoring on 1 point [2]) but not for the Interlocking Pentagon copying task. To be able to interpret if this observed difference in the Cube copying task is due to an impaired visuo-constructive functioning or due to retrograde procedural memory deficit, we performed additional analyses in Set 2 (Figure 1 (C) – Supplementary Material). We applied the CUPRO evaluation system allowing the separate assessment of the Cube drawing procedure (CUPRO-IS1), suggestive of retrograde procedural memory, and of the final result of the Cube (CUPRO-IS2), suggestive of visuo-constructive functions. No significant differences

were observed for retrograde procedural memory (CUPRO-IS1), while visuo-constructive functions were affected in the P-PD group (CUPRO-IS2). This is consistent with previous findings, stating that the Cube copying assessment is more sensitive than the Interlocking Pentagon assessment, most likely related to the Cubes' greater complexity [3]. No significant difference had been observed for visuo-spatial judgment. Until now, visuo-cognitive abilities have only been investigated sparsely [4,5] and findings are still controversial [6-9].

References – Supplementary Material

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Conclusion and Perspectives

Chapter I - Retrograde Procedural Memory in Parkinson's Disease

In the first part of my thesis, we focused on the retrograde procedural memory. We developed an evaluation tool for the functioning of this memory and applied it to people with PD (Chapter IA) and people with PD and Freezing of Gait (FOG) (Chapter IB). The underlying hypotheses for these two studies were that people with PD show an affected retrograde procedural memory compared to matched control groups and that the deficit is more prominent in people with PD who experience FOG episodes compared to non-Freezers.

Regarding Chapter IA of the dissertation, we would like to highlight three main findings; Firstly by comparing Cube copying performances evaluated with the CUPRO system in people with PD with age- and sex-matched control subjects, we identified that the performance was significantly affected in people with PD, suggestive for an impaired functioning of retrograde procedural memory in PD. Secondly, through evaluating discriminant validity in a subgroup of participants, with diverse neuropsychological and clinical assessments evaluating related constructs, we could not find any significant interference between motor deficits, as well as visuo-cognitive or executive functions. This absence of significance could however also be explained by low statistical power due to the small sub-sample size. Lastly, no significant correlation was observed between retrograde procedural memory and disease duration. This led us to the supra-analysis we performed in Chapter II, hypothesizing that this memory deficit may already be present in the pre-motor stages of PD. In Chapter IB we compared, additionally to global cognition and mental flexibility, the CUPRO performance between participants with PD and FOG, a de-automatization of walking, to age-, sex- and disease duration-matched PD participants without FOG episodes. The main finding of this study was that besides lower global cognition and mental flexibility, the impaired functioning of retrograde procedural memory was significantly more notable in people with PD and FOG compared to people with PD without FOG.

All in all, our findings in Chapter I confirm our hypothesis of lower Cube copying performance in people with PD compared to matched control subjects, which suggests an affected functioning of retrograde procedural memory in PD, that seems to be more prominent in people with PD and FOG.

Studies for the validation of the CUPRO evaluation system in independent PD cohorts are ongoing. Future research might work on an improved version of CUPRO 2.0. based on the suggestions, previously presented. The digitalization of the CUPRO, the eCUPRO, or even the automatic evaluation and scoring through AI and the reduction of the intra-rater variability would be an important step for its validation. Further ideas for a multi-approach battery around the evaluation of procedural learning and memory have been discussed and could be considered as a future research project.

Chapter II - Cognition and other non-motor symptoms in prodromal PD

The second part of my thesis focused on the exploration of the cognitive profile of people at high risk of developing PD. After highlighting the current challenges that diagnosing PD means identifying an already advanced disease, we discussed the importance of research in non-motor, cognitive profiles in prodromal PD. We conclude with the advancement on our investigation of the non-motor, focusing on the cognitive profile in prodromal PD (P-PD). We investigated the cognitive performance and other non-motor in an at-risk group for PD, defined by probable RBD and hyposmia, with an age- and sex-matched control group. The main findings of this study are the confirmation of early global cognitive, executive, and visuo-constructive function deficits in a group at high risk of developing PD. No significant difference was observed for retrograde procedural memory in the P-PD group. Furthermore, significantly more self-reported motor and non-motor symptoms, such as depression and apathy, and lower quality of life have been described in the P-PD group compared to the matched control group.

We discussed this project's perspectives, on the ongoing addition of the gold standard RBD diagnostic of the participants with probable RBD. Furthermore, yearly follow-up is already ongoing allowing us to investigate the trajectories of prodromal signs such as cognition and to describe conversions from P-PD to PD. For future projects, we consider combining additional prodromal features, such as constipation, or DAT deficit, α-synuclein seeding or genetical predispositions and investigating if different combination of prodromal signs is related to different cognitive patterns and of different severities and different prodromal subtypes [48,179].

All in all, we were able to describe the non-motor symptoms in P-PD and to define a specific non-motor and cognitive profile in our at-risk cohort. As no disease-modifying treatment exists yet,

prodromal markers have presently not yet a direct clinical implication. However, having a clear description of the P-PD phenotypes leading to early recognition of PD will help better prognosis and will be essential for future causative therapeutic interventions to become available [26].

Through this translational, transversal research, applying innovative methodological approaches, we expect to advance knowledge in the field of cognitive research in PD, to support the establishment of knowledge around the cognitive profile of PD and to, in a next step, maybe reduce the burden for people with PD.

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Appendices

Appendix 1: Standard Operating Procedure

SOP – Standard Operating Procedure

Extended Evaluation of the Cube Copying Task
CUPRO Evaluation System

Questions and comments are welcome: laure.pauly@lih.lu

I. Introduction

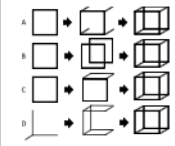
The Cube Copying Test was initially evaluated with the classical scoring system established by Nasreddine and colleagues [1].

We extended this scoring system to separately assess whether the drawing is three-dimensional (1 point), if the orientation of the drawing is correct (1 point), and if the final result is correct (1 point) (Fig.1 - Intermediate Score 2 (IS_2)). Subsequently, the Cube Copying Test was further extended to additionally evaluate the copying procedure itself. Based on the four typical procedures observed, the extended scoring system evaluates the starting approach; 1 point is administered if the subject started with one of the squares/surfaces/with the 3 axes. Further, the procedure itself is evaluated on 1 point (A.-D.). The last point is administered if the subject accomplished the copying procedure, by connecting the lines (Fig.1 - Intermediate Score 1 (IS_1)).

The total score of six points of the extended Cube Copying evaluation system is composed of two intermediate scores:

1) The **first intermediate score** on three points (IS_1) evaluates the **copying procedure**.
2) The **second intermediate score** (IS_2) of three points allows us to infer **aspects related to visuo-constructive functions**.

For the copying of the cube, a sheet of paper was placed in front of the participant. The participant was asked to copy the drawing as accurately as possible. The examinators did not disclose the intention to observe the cube copying procedure, to ensure that the copying performance did not depend on explicit memory processes. No time limit was imposed.

	YES	NO
INTERMEDIATE SCORE 1 - IS_1		
The subject starts with one of the squares / surfaces / with the 3 axes	1	0
 The subject drew the inside sides The subject drew a second square (superposition) The subject drew a second face The subject drew the 3 axes and continued by drawing any other surface	1	0
The subject fills in the connection lines correctly	1	0
INTERMEDIATE SCORE 1 **IS_1**	/3	
INTERMEDIATE SCORE 2 – IS_2		
The drawing is 3D, the proportions are correct	1	0
The orientation of the drawing is correct (mirror image)	1	0
The final result is correct	1	0
INTERMEDIATE SCORE 2 **IS_2**	/3	
TOTAL SCORE	/6	

Evaluation of the drawing procedure (I.A.)

Evaluation of the final result (I.B.)

Figure 1 - Representation of the extended evaluation system for the Cube Copying Test. The first intermediate score (IS_1) evaluates the copying procedure, the second intermediate score (IS_2) the visuo-constructive functions. (A-D) Representation of the four copying procedures.

!! In RedCap we have two evaluations for the cube:

- The Extended Evaluation System of the Cube Copying Task on 6 points (Figure 1)
- The initial MoCA evaluation on 1 point (Figure 2)

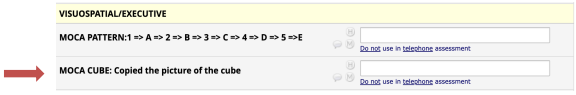


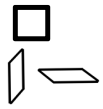
Figure 2. RedCap-Screenshot: The initial cube evaluation from the MoCA [1]

II. Extended Evaluation System

II.A. Intermediate score 1: Evaluation of the drawing procedure

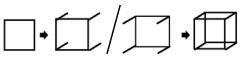
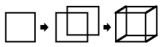
a) The subject starts with one of the squares/surfaces/with the 3 axes

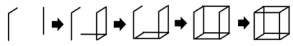
4 answer options:

1. The subject first completed one of the squares → 
   ➔ 1 point is administered
2. The subject first completed one of the surfaces →
   ➔ 1 point is administered
3. The subject first completed the 3 axes (x,y,z) → 
   ➔ 1 point is administered
4. The subject did not start with a square/surface neither with the 3 axes.
   ➔ 0 point

b) The subject drew one of the following 4 options

4 correct answer options:

1. The subject drew the inside sides → e.g.: 
   ➔ 1 point is administered
2. The subject drew a second square → e.g.: 
   ➔ 1 point is administered
3. The subject drew a second face → e.g.:  OR 
   ➔ 1 point is administered
4. The subject drew the 3 axes and continued by drawing any other surface 
   ➔ 1 point is administered

If none of these procedures have been used → e.g.: 
➔ 0 point

c) *The subject fills in the connection lines correctly* *(= does the subject finish the drawing procedure?)*

➔ 2 Answer possibility = 1 or 0

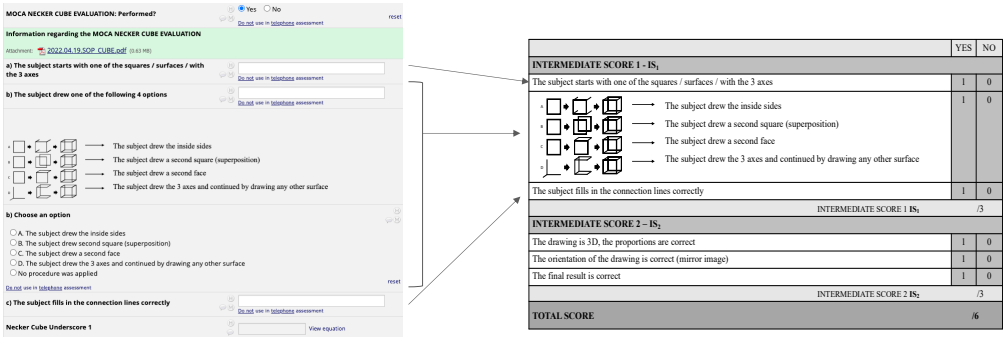


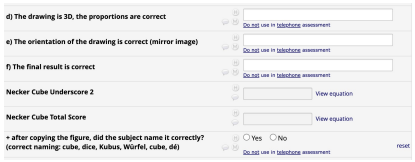
Figure 3 – Mapping of the different items of the intermediate score 1 on RedCap and on the Evaluation Sheet

II.B. Intermediate score 2: Evaluation of the final result

a) *The drawing is 3D, the proportions are correct*
➔ 2 Answer possibility = 1 or 0
Counterexamples:

b) *The orientation of the drawing is correct*
➔ 2 Answer possibility = 1 or 0
Counterexamples:

c) *The final result is correct*
➔ 2 Answer possibility = 1 or 0
Counterexamples:

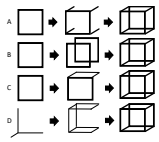


	YES	NO
INTERMEDIATE SCORE 1 - IS_1		
The subject starts with one of the squares / surfaces / with the 3 axes	1	0
0 → The subject drew the inside sides 0 → The subject drew a second square (superposition) 0 → The subject drew a second face 0 → The subject drew the 3 axes and continued by drawing any other surface	1	0
The subject fills in the connection lines correctly	1	0
INTERMEDIATE SCORE 1 IS_1 /3		
INTERMEDIATE SCORE 2 – IS_2		
The drawing is 3D, the proportions are correct	1	0
The orientation of the drawing is correct (mirror image)	1	0
The final result is correct	1	0
INTERMEDIATE SCORE 2 IS_2 /3		
TOTAL SCORE /6		

Figure 4 – Mapping of the different items of the intermediate score 2 on RedCap and on the Evaluation Sheet

III. Evaluation sheet

ND_____ Visit_____

	YES	NO
INTERMEDIATE SCORE 1 - IS_1		
The subject starts with one of the squares / surfaces / with the 3 axes	1	0
 The subject drew the inside sides The subject drew a second square (superposition) The subject drew a second face The subject drew the 3 axes and continued by drawing any other surface	1	0
The subject fills in the connection lines correctly	1	0
INTERMEDIATE SCORE 1 **IS_1**	/3	
INTERMEDIATE SCORE 2 – IS_2		
The drawing is 3D, the proportions are correct	1	0
The orientation of the drawing is correct (mirror image)	1	0
The final result is correct	1	0
INTERMEDIATE SCORE 2 **IS_2**	/3	
TOTAL SCORE	**/6**	

Procedure reproduction:

Naming: _____

Accepted answers: Cube, Die, Dice, Cubus, Würfel, Dé

Appendix 2: Training video

(Paper version)

The training video can be found under this QR Code:

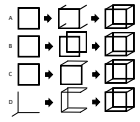
Figure 5. QR Code linked to the CUPRO training video

(Electronic version)

The training video can be found under this link: https://youtu.be/lnCHSVYJxNs

Appendix 3: CUPRO 2.0

A. Inital CUPRO evaluation

	YES	NO
INTERMEDIATE SCORE 1 - IS_1		
The subject starts with one of the squares / surfaces / with the 3 axes	1	0
 The subject drew the inside sides The subject drew a second square (superposition) The subject drew a second face The subject drew the 3 axes and continued by drawing any other surface	1	0
The subject fills in the connection lines correctly	1	0
INTERMEDIATE SCORE 1 IS_1	/3	
INTERMEDIATE SCORE 2 – IS_2		
The drawing is 3D, the proportions are correct	1	0
The orientation of the drawing is correct (mirror image)	1	0
The final result is correct	1	0
INTERMEDIATE SCORE 2 IS_2	/3	
TOTAL SCORE	/6	

B. Suggestions for CUPRO 2.0

	YES	NO	
INTERMEDIATE SCORE 1 - IS_1			
The subject starts with one of the squares / surfaces / with the 3 axes	1	0	
A. The subject drew the inside sides B. The subject drew a second square (superposition) C. The subject drew a second face D. The subject drew the 3 axes and continued by drawing any other surface 2 e.g.* 1	1	0	Evaluation of the drawing procedure
The subject finishes the drawing procedure 3	1	0	3
INTERMEDIATE SCORE 1 IS_1	/3		
INTERMEDIATE SCORE 2 – IS_2			
The drawing is 3D, the proportions are correct	1	0	Evaluation of the final result
The orientation of the drawing is correct (≠ mirror image) 4	1	0	
The final result is correct	1	0	
INTERMEDIATE SCORE 2 IS_2	/3		
TOTAL SCORE	/6		

Figure 6. *Juxtaposition of (A) the initial CUPRO version and (B) the adapted CUPRO 2.0 version. Numbers represent the suggested modifications: 1) Graphic and text switched position. Change of contrast for the parts of the graphic that are not directly evaluated by this item. 2) Addition of "e.g." to highlight that these representations are only examples and "*" to signal that other examples can be found in the SOP. 3) Adjustment to clarify what is already stated in the SOP. 4) Addition of a "≠" sign to define that it is a counter example.*