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Brain Connectivity and Plasticity in Neurodegenerative Diseases: From Neuroimaging to Neuromodulation

Habilitation thesis

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Abstract

Neurodegenerative disorders are characterized by progressive and heterogeneous alterations in brain structure, function, and cognition. This habilitation thesis presents a coherent body of research investigating neurodegenerative disorders from three complementary perspectives: structural and microstructural brain changes, dysfunction of large-scale brain networks, and the capacity of the aging and diseased brain for plasticity and therapeutic modulation.

After theoretical introduction, the first section of selected publications focuses on structural neuroimaging biomarkers in Alzheimer's disease, Parkinson's disease, and Lewy body disorders. Using structural MRI and advanced diffusion imaging techniques, the studies characterize disease- and stage-specific patterns of gray matter atrophy and microstructural alterations and examine their relationship with cognitive impairment. Particular attention is devoted to the heterogeneity of neurodegenerative processes and to the identification of early biomarkers, including alterations of the locus coeruleus in prodromal Lewy body disease.

The second part investigates neurodegeneration as a disorder of distributed brain networks. Resting-state and task-based functional MRI studies examine alterations in striato-cortical connectivity, dynamic functional connectivity, and language-related brain systems across Parkinson's disease and Lewy body diseases. These studies support a network-based understanding of neurodegeneration, in which clinical symptoms emerge from dysfunction of distributed and dynamically interacting neural systems.

The third part addresses brain plasticity and its translational potential. Studies using non-invasive brain stimulation demonstrate that targeted modulation can influence cognitive performance and functional brain networks, while also highlighting the importance of structural brain integrity and appropriate experimental control conditions. Complementary research on dance-based interventions shows that behavioral and physical activity can induce measurable changes in brain microstructure and functional organization in older adults. The most recent studies extend this research framework toward clinically scalable biomarkers, particularly digital speech measures reflecting cognitive and language dysfunction across neurodegenerative conditions.

Overall, the presented research demonstrates that cognitive decline in neurodegenerative disorders cannot be understood solely in terms of regional brain damage. Instead, it reflects the interaction between structural degeneration, microstructural alterations, disruption of distributed and dynamic brain networks, and individual differences in neural plasticity.

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List of Abbreviations

AD – Alzheimer's disease

BOLD – Blood Oxygenation Level-Dependent

cTBS – Continuous Theta Burst Stimulation

DAN – Dorsal Attention Network

DLB – Dementia with Lewy Bodies

DMN – Default Mode Network

dMRI – Diffusion Magnetic Resonance Imaging

DKI – Diffusion Kurtosis Imaging

DTI – Diffusion Tensor Imagin

fMRI – Functional Magnetic Resonance Imaging

FPN – Frontoparietal Network

HC – Healthy Controls

iTBS – Intermittent Theta Burst Stimulation

LC – Locus Coeruleus

LTD – Long-Term Depression

LTP – Long-Term Potentiation

MCI – Mild Cognitive Impairment

MRI – Magnetic Resonance Imaging

NIBS – Non-invasive Brain Stimulation

PDD – Parkinson's Disease Dementia

PD – Parkinson's disease

rTMS – Repetitive Transcranial Magnetic Stimulation

rs-FC – Resting-State Functional Connectivity

SMN – Somatomotor Network

SN – Salience Network

TBS – Theta Burst Stimulation

tDCS – Transcranial Direct Current Stimulation

tFUS – Transcranial Focused Ultrasound Stimulation

TIS – Temporal Interference Stimulation

TMS – Transcranial Magnetic Stimulation

VN – Visual Network

1 Introduction

1.1 Preface

My fascination with the human brain, particularly its connectivity and capacity for plasticity, began in 2012 when I started my postdoctoral training in neuroscience under the supervision of Professor Irena Rektorová, MD, PhD. I was fortunate to join her newly established research group at CEITEC Masaryk University during its formative years, as the institute was preparing to open its state-of-the-art research facilities dedicated exclusively to human brain research. Working in such an inspiring and interdisciplinary environment provided a unique opportunity to develop my scientific interests and acquire expertise in advanced neuroimaging and neuromodulation techniques.

My initial research focused primarily on neurodegenerative disorders, particularly Alzheimer's disease (AD). During this period, I gained practical experience with transcranial magnetic stimulation (TMS), which introduced me to the exciting possibility of non-invasively modulating brain connectivity and promoting neuroplasticity. I also became interested in identifying structural neuroimaging markers that could capture the effects of TMS-induced neuroplastic changes.

In parallel, I joined a research project led by Kateřina Skřivanová, PhD, entitled *The Patient's Personality as a Determinant of Success of Cancer Treatment*. This interdisciplinary project, situated at the interface of clinical psychology, oncology, and psychoneuroimmunology, resulted in several of my early scientific publications. However, as its primary focus lies beyond the scope of the present habilitation thesis, the following chapters concentrate exclusively on my research in the field of neurodegenerative diseases.

In 2014, I spent ten weeks as a visiting researcher in Professor Ulf Ziemann's Brain Networks and Plasticity Laboratory at Universitätsklinikum Tübingen, Germany. The following year, I completed a four-month research stay in Professor Hartwig Siebner's group at the Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Denmark. These international research placements significantly broadened my expertise in multimodal applications of non-invasive brain

stimulation (NIBS) and advanced neuroimaging techniques for investigating changes in structural and functional brain connectivity.

During this period, I participated as a research team member in two nationally funded projects. The first, *Effect of Intensive Dance-Exercise Intervention on Cognition and Brain Plasticity in Healthy Seniors and Patients with Mild Cognitive Impairment* (2015–2018), investigated whether dance-based exercise could promote neuroplasticity and improve cognitive function through a targeted behavioral intervention. The second project, *Modulation of Cognition and Brain Connectivity by Non-invasive Brain Stimulation in Patients with Mild Cognitive Impairment due to Alzheimer's Disease* (2016–2019), focused on the therapeutic potential of NIBS for enhancing cognitive performance and modulating brain network organization.

Under the supervision of Professor Irena Rektorová, I also participated in three major international collaborative projects: Pre-clinical Genotype–Phenotype Predictors of Alzheimer's Disease and Other Dementias (APGeM; 2014–2016); Novel Network-Based Approaches for Studying Cognitive Dysfunction in Behavioral Neurology (CoBeN; 2017–2022); and Novel, Individualized Brain Stimulation, Network-Based Approaches to Improve Cognition (NiBS-iCog; 2021–2024). In 2016, I successfully defended my doctoral thesis, The Effect of rTMS on Cognitive Functions in Patients with Neurodegenerative Diseases, and was awarded a PhD in Neuroscience.

Participation in international projects and my international research internships considerably broadened the scope of my research. My interests gradually expanded from TMS to other forms of NIBS, from AD to other neurodegenerative disorders, particularly synucleinopathies, and from general cognition to language dysfunction. Language impairment was one of the central themes of the CoBeN project, in which collaborating centers in the Czech Republic, Hungary, and the United States collected harmonized neuroimaging, neuropsychological, and language data from patients with various neurodegenerative diseases speaking typologically distinct languages. These collaborations substantially influenced my subsequent research direction.

Building on this experience, I successfully obtained two national grants as principal investigator. The first project, *The Effect of Transcranial Direct Current Stimulation on Visual Attention in Mild Cognitive Impairment: A Combined MRI and Non-invasive Brain Stimulation Study* (2018–2022), investigated the neural mechanisms underlying

cognitive enhancement induced by transcranial direct current stimulation (tDCS). The second project, *Language and Lewy Body Diseases: Sentence Comprehension Deficits and Their Modulation by Non-invasive Brain Stimulation* (2023–2026), focuses on language impairments in synucleinopathies and explores the therapeutic potential of home-based neuromodulation.

Most recently, I have been leading one of the three work packages within the Jan Amos Komenský Operational Programme (OP JAK) project *A Lifetime with Language: The Nature and Ontogeny of Linguistic Communication* (LangInLife; 2025-2028), coordinated by Pavel Caha, PhD. Within this multidisciplinary consortium, our research investigates age-related and neurodegeneration-related changes in language processing, with particular emphasis on identifying the neural mechanisms responsible for language decline and potential approaches to modulate communication abilities in patients with neurodegenerative diseases using NIBS.

1.2 Scope and Organization of the Thesis

This habilitation thesis is organized into four main chapters. Following this introductory chapter, Chapter 2 reviews the current understanding of brain connectivity and neuroplasticity in the healthy and aging brain. Chapter 3 summarizes the principal neurodegenerative diseases investigated throughout this work, with particular emphasis on their associated alterations in structural and functional brain networks. Chapter 4 discusses current approaches to modulating brain plasticity, including NIBS and behavioral interventions, and outlines future directions for translational neuroscience.

The theoretical part is followed by a compilation of the original peer-reviewed publications that constitute the core of this habilitation thesis. To reflect the evolution of my research, these publications are organized into three thematic sections:

- Part I – Structural Neuroimaging Biomarkers
- Part II – Brain Network Dysfunction in Neurodegenerative Disorders
- Part III – Modulation of Brain Plasticity and Translational Neuroscience

1.3 Overall Aim and Specific Objectives

The research presented in this habilitation thesis reflects more than a decade of work dedicated to understanding the neural mechanisms underlying cognitive dysfunction

in neurodegenerative diseases. Although the individual studies address diverse research questions, they are united by a common aim: to advance the understanding of brain connectivity and neuroplasticity in neurodegenerative diseases by integrating multimodal neuroimaging, neuromodulation, and behavioral intervention approaches to identify biomarkers of cognitive dysfunction and explore mechanisms that may support the preservation of cognitive function.

More specifically, this work sought to characterize structural and functional brain network alterations associated with cognitive impairment and evaluate their potential as imaging biomarkers and therapeutic targets for interventions promoting adaptive neuroplasticity.

Objectives of this habilitation thesis were as follows:

1. To characterize alterations in structural and functional brain connectivity associated with cognitive impairment across neurodegenerative diseases using multimodal neuroimaging techniques.

2. To identify neuroimaging biomarkers of neurodegeneration and cognitive decline, and to determine their relationship with cognitive domains including attention, executive functions, memory, visuospatial processing, and language.

3. To investigate the capacity for neuroplasticity in the aging and diseased brain by evaluating the effects of NIBS and behavioral interventions on brain connectivity and cognitive function.

This thesis is based on the hypothesis that disruption of structural and functional brain networks constitutes a common mechanism underlying cognitive impairment across neurodegenerative diseases. Furthermore, multimodal neuroimaging can identify sensitive biomarkers of these network alterations, while neuromodulation and behavioral interventions can induce measurable neuroplastic changes that support cognitive function. These complementary approaches may facilitate earlier diagnosis, improve monitoring of disease progression, and inform the development of individualized therapeutic strategies for patients with neurodegenerative disorders.

2 Brain Networks and Neuroplasticity

2.1 Structural, functional and effective connectivity

The human brain is an extraordinarily complex organ. To illustrate this complexity, although it typically weighs less than 1,500 g, it contains an estimated 61–99 billion neurons, which form approximately 100 trillion synaptic connections. These figures are approximate and vary depending on the methodology used for estimation. Consequently, the exact numbers remain uncertain and cannot be determined with absolute precision[1].

Historically, the brain was viewed as a collection of anatomically segregated regions, each associated with specific cognitive or sensory functions. This concept of functional segregation was largely based on anatomical and lesion studies. The advent of non-invasive neuroimaging, particularly functional magnetic resonance imaging (fMRI), shifted this perspective toward functional integration, emphasizing that brain functions arise from interactions among distributed regions. This paradigm shift led to the emergence of network neuroscience, a field that characterizes the brain as a complex network of interconnected regions, and ultimately to the concept of the human connectome, a comprehensive map of the brain's structural and functional connectivity[2].

Structural connectivity refers to actual organization of complex local and long-range fiber pathways that connects neurons in the brain. Early investigations of brain connectivity relied predominantly on invasive techniques, including histological staining, axonal tracer studies, serial electron microscopy, and fluorescence imaging[3]. These methods have provided detailed insights into the organization of neuronal circuits and remain the gold standard for mapping anatomical connections, particularly in animal models. The development of non-invasive magnetic resonance imaging (MRI) techniques, especially diffusion MRI (dMRI) and tractography, represented a major breakthrough by enabling the in vivo reconstruction of white matter pathways[3].

In white matter, water diffusion is constrained by axonal membranes and myelin sheaths, resulting in preferential diffusion along fibre orientations rather than across them. By measuring diffusion in multiple directions, dMRI enables the reconstruction of white matter architecture. The most widely used technique, diffusion tensor imaging

(DTI), models diffusion as a Gaussian process and provides quantitative measures such as fractional anisotropy and mean diffusivity. More advanced dMRI techniques, such as diffusion kurtosis imaging (DKI), provide a more accurate characterization of tissue microstructure, while methods based on high-angular or multi-shell acquisitions can additionally resolve crossing fibres[4].

Functional connectivity refers to the statistical relationship between the activity of spatially separated brain regions. It is most commonly quantified as the temporal correlation of neurophysiological signals, such as fluctuations in the blood oxygen level-dependent (BOLD) signal measured with fMRI. Regions that exhibit highly correlated activity are considered functionally connected, although such correlations do not necessarily indicate a direct anatomical pathway or causal interaction. In contrast, effective connectivity describes the directed influence that one brain region exerts on another. Rather than measuring statistical associations alone, it aims to infer causal interactions within neural networks using computational models of brain dynamics[5].

2.2 Large-Scale Brain Networks

At the beginning of the 21st century, researchers began exploring the brain's intrinsic activity using fMRI to investigate functional connectivity. Functional connectivity can be studied both during task performance and at rest, the latter being referred to as resting-state functional connectivity (rs-FC). It was discovered that even when the brain is not engaged in any specific task, it continues to exhibit spontaneous, low-frequency fluctuations in the fMRI signal. These fluctuations are organized into functionally connected regions, known as resting-state networks, which reflect the brain's intrinsic functional organization in the absence of goal-directed activity or external stimuli. Numerous resting-state fMRI studies have consistently identified similar network patterns, highlighting the reliability and functional significance of these intrinsic brain networks[6].

Approximately three decades of research on large-scale brain networks have resulted in more than 25,000 publications. Although some inconsistencies remain in the nomenclature of these networks, most brain atlases derived from resting-state fMRI data consistently identify the visual (VN), somatomotor (SMN), dorsal attention (DAN), frontoparietal (FPN), salience (SN), and default mode (DMN) networks. The DMN is

primarily associated with spontaneous, internally directed, and self-referential cognition. In contrast, the FPN, also referred to as the central executive network, supports executive functions, including working memory, cognitive control, and decision-making. The DAN mediates visuospatial attention and the voluntary allocation of attention to external stimuli. Together, the FPN and DAN form the task-positive network, which is typically anti-correlated with the DMN, reflecting the reciprocal engagement of internally and externally oriented cognitive processes. The SN is responsible for detecting behaviorally relevant stimuli and coordinating dynamic switching between the DMN and the FPN. The SMN supports motor planning, execution, and somatosensory processing, whereas the VN is dedicated to visual perception and the processing of visual information. In addition to these major networks, resting-state fMRI studies have identified several other functionally connected systems, including the auditory, language, basal ganglia, and cerebellar networks[7].

Resting-state fMRI data can be analyzed using both model-driven and data-driven approaches. Seed-based correlation analysis assesses functional connectivity between a predefined brain region and the rest of the brain. Independent component analysis identifies spatially independent resting-state networks without prior assumptions, whereas regional homogeneity measures the synchronization of neighboring voxels to assess local connectivity. Graph theory characterizes the topological organization and efficiency of whole-brain networks, while the amplitude of low-frequency fluctuations quantifies the intensity of spontaneous regional brain activity by measuring low-frequency BOLD signal oscillations. Functional connectivity in task-based fMRI can be assessed using psychophysiological interaction analysis, which measures task-dependent changes in connectivity between a predefined seed region and other brain areas. Beta-series correlation estimates trial-specific connectivity by correlating activation across individual trials. More advanced methods, such as dynamic causal modeling and Granger causality analysis, evaluate the direction and causal influence of interactions between brain regions[5,7].

2.3 Brain Plasticity in Aging and Neurodegeneration

Brain plasticity, or neuroplasticity, is the brain's ability to modify its structure and function by forming and reorganizing neural connections. It was once believed that neuroplastic changes occurred only during early development and that this capacity

was largely lost in adulthood. However, extensive research has demonstrated that neuroplasticity persists throughout the lifespan, enabling the brain to learn new skills, form and consolidate memories, and adapt to environmental changes, injury, and neurological disease[8]. A fundamental mechanism underlying neuroplasticity is spike-timing-dependent plasticity, a Hebbian learning process in which synaptic strength is regulated by the precise timing of neuronal activity. When a presynaptic neuron fires shortly before a postsynaptic neuron, the connection between them is strengthened through long-term potentiation (LTP). In contrast, when presynaptic activity follows postsynaptic firing, the synapse is weakened through long-term depression (LTD). These timing-dependent changes occur within a window of milliseconds and provide a mechanism for activity-dependent modification of neural circuits, contributing to learning and memory formation[9].

During healthy aging, several models have been proposed to describe functional changes in brain organization and compensatory mechanisms that support cognitive performance. The Hemispheric Asymmetry Reduction in Older Adults (HAROLD) model, introduced by Cabeza (2002), describes a reduction in hemispheric specialization in older adults, particularly in prefrontal regions, compared with younger individuals[10]. Similarly, the Posterior-Anterior Shift in Aging (PASA) model proposed by Davis et al. (2008) suggests that aging is accompanied by increased recruitment of frontal regions and decreased activity in posterior areas, reflecting a compensatory shift that helps maintain cognitive performance[11]. The Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) further proposes that older adults recruit additional neural resources even during tasks with relatively low demands; however, this compensatory mechanism becomes insufficient when task complexity exceeds available neural capacity[12]. Building upon these concepts, the Scaffolding Theory of Aging and Cognition (STAC) proposes that the aging brain adapts to neural decline by establishing alternative or supportive neural networks to preserve cognitive function[13]. The revised STAC-r model further incorporates lifestyle and environmental influences, suggesting that factors such as physical activity, higher education, cognitive training, and multilingualism enhance neural reserve, whereas conditions including obesity, smoking, diabetes, chronic stress, and depression contribute to its reduction[14].

In neurodegenerative diseases, alterations in brain plasticity may initially serve as an adaptive response to neuronal damage but can gradually become insufficient or

maladaptive as pathological processes progress. During the early stages of disease, the brain can activate compensatory mechanisms, including synaptic strengthening, functional network reorganization, and recruitment of alternative neural pathways, to maintain cognitive and motor functions despite ongoing neuronal loss. However, with advancing neurodegeneration, the capacity for effective plastic adaptation becomes impaired, and disrupted plasticity mechanisms contribute to network dysfunction, reduced functional connectivity, and cognitive decline[15].

3 Neurodegenerative Diseases as Network Disorders

Traditionally, neurodegenerative diseases have been conceptualized as disorders characterized by region-specific neuronal loss. However, advances in non-invasive neuroimaging techniques and analytical approaches have enabled the characterization of large-scale structural and functional brain networks, shifting the perspective toward viewing neurodegenerative diseases as disorders of network organization. Accumulating evidence has demonstrated that neurodegenerative diseases selectively disrupt large-scale structural and functional brain networks with alterations in connectivity detectable before major atrophy and disease-specific proteins (amyloid-β, tau, α-synuclein) may propagate preferentially through connected neural systems[16–19].

3.1 Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia, characterized by the accumulation of amyloid-β plaques and tau tangles in the brain. It causes progressive cognitive and memory decline, with early synaptic dysfunction followed by neuronal degeneration and cell loss[20]. Structural MRI studies have consistently demonstrated progressive brain atrophy in AD, initially affecting the hippocampus and entorhinal cortex before extending to temporal, parietal, and frontal regions[21]. Diffusion MRI further demonstrates impaired white-matter integrity and structural connectivity, with altered network organization detectable even at the stage of mild cognitive impairment (MCI) and associated with amyloid burden and disease severity[22,23].

AD affects multiple brain networks and structures; however, the DMN has emerged as a particularly vulnerable system. Greicius et al.[24] were among the first to demonstrate abnormalities in DMN activity in patients with AD, reporting reduced activity in the posterior cingulate cortex and hippocampus and suggesting disrupted functional connectivity between these regions. Subsequently, Buckner et al.[25] extended these findings by demonstrating a relationship between regions of high default-mode activity, amyloid deposition, and memory impairment and further proposed that highly connected DMN hubs may be particularly susceptible to AD pathology and may augment metabolic cascades associated with neurodegeneration[26].

Specific alterations in rs-FC can be detected already at the stage of MCI, preceding the development of dementia. Early studies reported decreased functional connectivity in MCI patients compared with healthy controls (HC)[27]. However, subsequent studies suggested that an initial maladaptive state of hyperconnectivity may precede the development of hypoconnectivity in affected networks, with connectivity changes following the trajectory of AD neuropathology, which initially affects the medial temporal lobe, followed by posterolateral cortical regions and, in later stages, the frontal cortex[28]. Furthermore, rs-FC disruption progressively increases from mild to moderate and severe stages of AD.[29]. Later systematic review and meta-analysis showed that rs-FC alterations are not restricted to the DMN and also involve other large-scale networks such as the SN and limbic networks[30].

Taken together, evidence from structural and functional neuroimaging suggests that network dysfunction may emerge early in the course of AD, preceding substantial structural damage and clinical symptoms. Recent work using spectral dynamic causal modeling showed that DMN effective-connectivity abnormalities can predict future dementia at the individual level, outperforming structural and conventional functional measures and showing associations with genetic risk for AD and social isolation[31]. These findings highlight the potential of network-level measures, particularly effective connectivity, as sensitive biomarkers for the early detection and characterization of AD-related brain dysfunction.

3.2 Parkinson's disease

Parkinson’s disease (PD) is a chronic neurodegenerative disease characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra and the accumulation of α-synuclein pathology. Although motor symptoms are a hallmark of PD, the disease also involves widespread non-motor and cognitive dysfunction, reflecting disruption of distributed brain networks. Non-motor manifestations such as sleep disturbances, constipation, depression, olfactory impairment, are highly prevalent and may precede the onset of motor symptoms by several years[32].

Structural MRI studies have identified relatively subtle and more widespread regional changes in PD compared with AD, including progressive atrophy involving the caudate, putamen, temporal and hippocampal regions, as well as frontal and parietal areas[33]. White matter microstructural abnormalities affecting major motor and associative tracts

have also been reported in the early stages of PD, potentially indicating that white matter alterations may occur alongside or precede detectable gray matter changes[33,34]. Furthermore, participants with PD have demonstrated reduced structural connectivity involving several brain regions connected by major white matter tracts, including the corpus callosum, fornix, and cingulum, compared with HC[35].

Beyond widespread cortical, subcortical, and white matter alterations, PD is also associated with abnormalities in small brainstem nuclei, particularly the substantia nigra and locus coeruleus (LC). Advanced MRI techniques, including neuromelanin-sensitive and susceptibility-weighted imaging, enable in vivo assessment of these small structures. Neuromelanin-sensitive MRI has demonstrated reduced neuromelanin-related signal and/or volume in these regions, including in patients with de novo PD, highlighting their potential as imaging biomarkers of early neurodegeneration. Furthermore, susceptibility-sensitive MRI techniques can detect abnormalities in nigrosome-1, a dopaminergic subdivision within the substantia nigra pars compacta, providing a potential imaging marker of nigral neurodegeneration and iron-related changes[36].

In parallel with structural alterations, resting-state fMRI studies have also revealed widespread alterations in functional connectivity across multiple large-scale networks in PD, including the DMN, FPN, DAN, SN, SMN and basal ganglia networks. Among these, abnormalities in the DMN are particularly prominent in patients with cognitive impairment, while altered motor and basal ganglia network connectivity is more closely related to motor dysfunction[37–39].

Using dynamic functional connectivity analysis, a recent study revealed disrupted large-scale brain network organization in drug-naïve patients with PD, particularly reduced reconfiguration of the DMN. Longitudinally, network dynamics changed with dopaminergic decline and shifted toward a pattern more similar to that of HC following treatment[40]. Other multisite rs-fMRI findings demonstrated widespread abnormalities in both static and dynamic functional connectivity in PD, with dynamic measures detecting alterations not captured by static analyses, highlighting their potential for characterizing disease progression and treatment response[41].

3.3 Dementia with Lewy bodies

Dementia with Lewy bodies (DLB) is a progressive neurodegenerative disorder characterized by dementia associated with α-synuclein–containing Lewy pathology and clinical features including fluctuating cognition, recurrent visual hallucinations, REM sleep behavior disorder, and parkinsonism. DLB is characterized by cognitive symptoms occurring before or within approximately one year of the onset of parkinsonism, whereas Parkinson's disease dementia (PDD) develops after established PD, typically following a longer period of motor symptoms[42]. Compared with AD, DLB often shows less pronounced hippocampal and medial temporal lobe atrophy and more distributed cortical/subcortical alterations: DLB may show subtle volume reductions in the insula, temporo-occipital cortex, and striatum, although these findings are inconsistent across studies and have limited diagnostic utility for distinguishing different dementia subtypes. Compared with PD, DLB exhibits greater cortical atrophy at comparable levels of dementia severity, suggesting distinct neural substrates underlying cognitive decline in the two syndromes[43].

DTI studies indicate that DLB is associated with widespread white matter microstructural abnormalities and disrupted structural connectivity, despite relatively limited white matter volume loss on conventional MRI. DTI studies suggest that DLB is associated with widespread white matter microstructural abnormalities and disrupted structural connectivity, particularly in parieto-occipital regions and the inferior longitudinal fasciculus, which connects frontal regions with visual areas involved in the ventral visual pathway. Additional alterations have been reported in the uncinate and superior longitudinal fasciculi, inferior fronto-occipital fasciculus, cingulum, and corpus callosum**.** These abnormalities may occur early in DLB and appear to progress more slowly than those observed in AD, suggesting that white matter disruption may represent an early feature of the disease[43,44].

Similarly to PD, advanced MRI techniques, including neuromelanin-sensitive and susceptibility-weighted imaging, can detect abnormalities in DLB. Direct comparisons between PD and DLB suggest that the relationship between loss of neuromelanin-containing nigral neurons and presynaptic dopaminergic degeneration differs between the two disorders, with DLB showing greater presynaptic dopaminergic loss relative to nigral neuronal degeneration[45,46]. Evidence regarding the LC is less conclusive, although its early involvement in Lewy body disease may contribute to the cognitive

and non-motor manifestations of DLB[47]. Recent studies have also demonstrated increased susceptibility abnormalities in DLB compared with HC and cognitively normal PD, whereas comparisons with PDD revealed more widespread susceptibility abnormalities in PDD, including the hippocampus, amygdala, cerebellum, frontal regions, and substantia nigra. These findings suggest that PD-related dementia and DLB may involve distinct patterns of neuronal degeneration and tissue composition, potentially contributing to their different cognitive phenotypes[48].

Furthermore, resting-state fMRI studies in DLB have identified abnormalities in functional connectivity within the DMN, FPN, SN, SMN, VN, and basal ganglia networks[44,49,50]. Compared with AD, DLB shows greater disruption of the DMN, SN, and executive networks, while direct comparisons with PDD suggest more pronounced alterations in FPN in DLB and greater involvement of motor networks in PDD[51,52]. Together, these findings suggest that DLB is characterized by disrupted FPN and motor connectivity and occipital disconnection, potentially underlying its characteristic attentional, parkinsonian, visuospatial, and hallucination symptoms and distinguishing its functional network phenotype from those of PD and AD[43].

4 Modulation of Brain Plasticity

4.1 Non-Invasive Brain Stimulation Methods

In the previous chapters, we explained that patients with neurodegenerative diseases experience not only neuronal loss but also impairments in synaptic plasticity and network communication. If pathological network activity can be identified and plasticity mechanisms remain at least partially preserved, NIBS may offer a means of restoring or compensating for dysfunctional network dynamics. NIBS methods could therefore provide an ideal tool for studying and modulating brain function in vivo, with potential therapeutic applications[53].

Transcranial magnetic stimulation (TMS) is a NIBS method that was first described and introduced by Barker et al. in 1985[54]. Since then, TMS has undergone rapid development and has become an increasingly important tool in various areas of neurophysiological and neuroscience research. During TMS, a time-varying magnetic field generated by an electromagnetic coil placed against the scalp induces an electric field in the underlying neural tissue. When the induced electric field is sufficiently strong, it can depolarize neuronal membranes and trigger action potentials. Electromagnetic induction offers several advantages: magnetic fields can pass through the scalp and other soft tissues of the head with relatively little attenuation, while specially designed coils allow the induced electric field to be relatively focused. Figure-of-eight coils are commonly used for this purpose, providing greater focality than circular coils. The magnetic field generated by TMS can reach approximately 2 T, although the strength of the induced electric field and its physiological effects decrease with increasing distance from the coil. The primary effects are therefore strongest in cortical regions close to the scalp, while TMS can also produce indirect effects in other brain regions that are anatomically or functionally connected to the stimulated area[55].

TMS techniques can be broadly classified into single-pulse TMS, paired-pulse TMS, repetitive TMS (rTMS), and theta-burst stimulation (TBS). Single- and paired-pulse protocols are primarily used to assess cortical excitability, whereas rTMS and TBS deliver repeated stimulation and can induce longer-lasting changes in cortical excitability and plasticity. rTMS is commonly divided into low-frequency (≤1 Hz) and high-frequency (≥5 Hz) protocols, while TBS includes continuous (cTBS) and

intermittent (iTBS) protocols. Although high-frequency rTMS and iTBS are generally associated with increased cortical excitability and low-frequency rTMS and cTBS with decreased excitability, these effects are not always consistent and may depend on stimulation parameters and individual characteristics[56].

TMS intensity is typically determined relative to the resting or active motor threshold, and stimulation parameters should follow established safety guidelines[57]. to minimize the risk of adverse effects. Precise targeting can be achieved using frameless stereotactic neuronavigation based on anatomical or functional MRI data[58]. In therapeutic applications, the strongest evidence for rTMS has been demonstrated for specific protocols targeting neuropathic pain, depression, and motor recovery after stroke, for which Level A evidence has been reported[59].

Transcranial direct current stimulation (tDCS) is a noninvasive neuromodulation technique that was introduced in its modern form by Nitsche and Paulus in 2000[60]. tDCS delivers a low-intensity direct electrical current, typically at 1–2 mA, through electrodes placed on the scalp using conductive gel or saline-soaked sponges. The electrical current flows from the positively charged anode toward the negatively charged cathode, generating an electric field in the underlying brain tissue that can modulate neuronal excitability[61]. Traditionally, anodal stimulation has been associated with an increase in cortical excitability, whereas cathodal stimulation has been associated with a reduction in excitability. However, these polarity-dependent effects are not universal and may vary depending on stimulation parameters, electrode montage, current orientation, brain region, and individual characteristics[62].

tDCS produces a relatively weak and sustained modulation of neuronal excitability rather than directly inducing action potentials. By altering the membrane potential of neurons, it can modify the responsiveness of cortical networks and influence subsequent synaptic plasticity, including LTP- and LTD-like mechanisms[62]. The effects of tDCS can extend beyond the stimulation period and may also influence functionally connected brain regions[63]. Compared with TMS, tDCS offers several practical advantages, including relatively portable and inexpensive equipment, minimal acoustic stimulation, and the possibility of delivering stimulation while participants simultaneously perform behavioral or cognitive tasks. These characteristics make tDCS particularly suitable for repeated stimulation and for combining neuromodulation with task-based interventions, including home-based applications[64]. However, unlike

TMS, tDCS provides less focal and temporally precise stimulation, as the applied electric field is distributed over a broader area of the brain.

These two most common NIBS methods have been investigated as a potential approach for modulating cognitive and motor functions in neurodegenerative diseases. In AD, both rTMS and tDCS have shown potential to improve cognitive function, particularly memory. Evidence from the past decade suggests that high-frequency rTMS and anodal tDCS administered for at least 2 weeks may improve cognitive performance and help attenuate cognitive decline[65]. A recent meta-analysis found small but significant improvements in memory following tDCS and rTMS, with tDCS showing greater effects when applied over temporal regions and rTMS when targeting frontal areas[66]. In PD, TMS and tDCS have also demonstrated potential to improve cognitive and motor functions, but recent evidence suggests that the effects of tDCS on cognition and motor symptoms are variable and may depend on stimulation parameters and patient characteristics (more effective in younger PD patients with milder symptoms)[65,67]. In DLB, evidence is considerably more limited, with preliminary studies suggesting possible benefits of TMS for neuropsychiatric symptoms and tDCS for attention; however, the available evidence remains insufficient to draw firm conclusions[68]. Overall, NIBS represents a promising adjunctive approach, but larger, well-controlled trials are needed to establish its clinical efficacy and long-term effects across neurodegenerative diseases.

In addition to TMS and tDCS, several other NIBS techniques have been developed. Transcranial alternating current stimulation applies a sinusoidal alternating current to modulate ongoing neuronal oscillations and can be used to target brain activity at specific frequencies. Transcranial random noise stimulation delivers currents with varying frequencies and amplitudes and may influence cortical excitability and endogenous neural activity[69]. Temporal interference stimulation (TIS) is a more recent approach that applies multiple high-frequency electrical currents with slightly different frequencies, generating a low-frequency amplitude-modulated envelope through their interaction. This approach may enable the modulation of deeper brain structures while limiting stimulation of the overlying cortex, potentially addressing an important limitation of conventional transcranial electrical stimulation[70]. Transcranial focused ultrasound stimulation (tFUS) represents another emerging technique that uses acoustic energy to modulate neuronal activity and offers the potential for noninvasive

targeting of both superficial and deep brain regions[71]. Although these techniques show considerable potential, particularly for targeting neural oscillations or deep brain structures, their mechanisms and therapeutic applications are still being investigated.

4.2 Behavioral Interventions

Beyond NIBS, several other nonpharmacological approaches can influence brain plasticity. These include behavioral interventions such as cognitive training, cognitive rehabilitation, physical exercise, and motor training, which can promote neuroplastic changes through repeated engagement of relevant neural networks.

Cognitive training and cognitive rehabilitation are nonpharmacological approaches that aim to maintain or improve cognitive functioning by repeatedly engaging specific cognitive processes. In AD, cognitive interventions have been associated with modest improvements in global cognition and selected cognitive domains, although the magnitude and persistence of these effects vary across interventions[72]. In PD, cognitive training has been associated with small but significant improvements in overall cognition, particularly in domains such as working memory, executive function, and processing speed[73]. Positive effects have also been reported with computerized cognitive training, including remotely delivered interventions[74]. In DLB, the evidence remains limited, but preliminary findings suggest that cognitive rehabilitation may have beneficial effects on cognition, mood, and quality of life, although larger controlled studies are needed[68].

Physical exercise and motor interventions represent additional nonpharmacological approaches that may promote neuroplasticity and help maintain motor and cognitive function in neurodegenerative diseases. In AD, physical exercise has been associated with modest improvements in cognitive function, with some evidence suggesting greater benefits from aerobic exercise compared with non-aerobic interventions[75]. In PD, aerobic exercise, balance and gait training, and dance-based interventions have demonstrated beneficial effects on both motor and cognitive functions, with some benefits persisting over the longer term[76,77]. Dance may be particularly valuable because it combines physical activity with motor planning, coordination, rhythm, and cognitive engagement, and has been associated with improvements in gait, balance, and aspects of cognition in PD[78,79]. In DLB, evidence remains limited, but preliminary

findings suggest that physical therapy may have beneficial effects on motor and executive functions, although further studies are needed to establish its efficacy[68].

4.3 Future Therapeutic Perspectives

Future therapeutic strategies are likely to increasingly focus on personalized and network-guided approaches that combine neuromodulation with behavioral or pharmacological interventions. Rather than relying on standardized stimulation protocols, individual neuroimaging, electrophysiological biomarkers, and electric-field modelling may be used to optimize the stimulation target, intensity, timing, and duration for each patient. There is also a growing shift from targeting individual cortical regions toward modulating dysfunctional brain networks and combining different NIBS techniques. Combining NIBS with cognitive or motor training may further enhance neuroplasticity by stimulating neural networks while they are actively engaged in relevant tasks. Emerging approaches such as TIS and tFUS may provide access to deeper brain structures that are difficult to target with conventional TMS or tDCS. Closed-loop stimulation, in which real-time measures of brain activity are used to guide the timing or location of stimulation, may further improve the precision and effectiveness of neuromodulation. Home-based applications could facilitate more frequent and accessible treatment while reducing the burden of repeated visits to clinical settings. Finally, the development of sensitive biomarkers may enable earlier identification of neurodegenerative disease, potentially allowing therapeutic interventions to be initiated during the preclinical or early stages, when they may have the greatest potential to modify disease progression. Overall, future therapeutic strategies are likely to move toward individualized, biomarker-guided, network-based, and multimodal interventions rather than one-size-fits-all stimulation protocols[65,80].

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Collection of Selected Publications with Commentary

List of Selected Publications

Part I. Structural Neuroimaging Biomarkers

1. Distinct Pattern of Gray Matter Atrophy in Mild Alzheimer's Disease Impacts on Cognitive Outcomes of Noninvasive Brain Stimulation (Journal of Alzheimer’s Disease, 2015)
2. Patterns of Grey Matter Atrophy at Different Stages of Parkinson's and Alzheimer's Diseases and Relation to Cognition (Brain Topography, 2019)
3. Diffusion Tensor and Restriction Spectrum Imaging Reflect Different Aspects of Neurodegeneration in Parkinson's Disease (Plos One, 2019)
4. Patterns of Diffusion Kurtosis Changes in Parkinson's Disease Subtypes (Parkinsonism and Related Disorders, 2020)
5. Early Changes in the Locus Coeruleus in Mild Cognitive Impairment with Lewy Bodies (Movement Disorders, 2025)

Part II. Brain Network Dysfunction in Neurodegenerative Disorders

6. Striato-cortical Connections in Parkinson's and Alzheimer's Diseases: Relation to Cognition (Movement Disorders, 2017)
7. Striato-cortical Functional Connectivity Changes in Mild Cognitive Impairment with Lewy Bodies (Parkinsonism & Related Disorders, 2024)
8. Dynamic Functional Connectivity is Related to Cognitive Performance of Prodromal Lewy Body Dementia (Journal of Neural Transmission, 2026)
9. Language Impairment in Parkinson's Disease: fMRI Study of Sentence Reading Comprehension (Frontiers of Aging Neuroscience, 2023)
10. Sentence Comprehension in Lewy Body Diseases: A Functional Magnetic Resonance Imaging Study (Brain Communications, 2025)
11. Language Network Disruption in Patients with Lewy Body Diseases (Journal of Neural Transmission, 2026)

Part III. Modulation of Brain Plasticity and Translational Neuroscience

12. Non-invasive Brain Stimulation of the Right Inferior Frontal Gyrus May Improve Attention in Early Alzheimer's Disease (Journal of the Neurological Sciences, 2014)
13. Cognitive Effects of Repetitive Transcranial Magnetic Stimulation in Patients with Neurodegenerative Diseases – Clinician's Perspective (Journal of the Neurological Sciences, 2014)
14. Theta Burst Stimulation Enhances Connectivity of the Dorsal Attention Network in Young Healthy Subjects (Neural Plasticity, 2018)
15. Theta-Burst Transcranial Magnetic Stimulation Induced Cognitive Task-Related Decrease in Activity of the Default Mode Network (Brain Stimulation, 2020)
16. Is the Vertex a Good Control Stimulation Site? Theta Burst Stimulation in Healthy Controls (Journal of Neural Transmission, 2022)
17. Cognitive Effects of Dance-Movement Intervention in a Mixed Group of Seniors Are Not Dependent on Hippocampal Atrophy (Journal of Neural Transmission, 2019)
18. Multishell Diffusion MRI Reflects Improved Physical Fitness Induced by Dance Intervention (Neural Plasticity, 2020)
19. Dance Intervention Impact on Brain Plasticity: A Randomized 6-Month fMRI Study in Non-expert Older Adults (Frontiers of Aging Neuroscience, 2021)
20. Digital Speech Biomarkers for Assessing Cognitive Decline Across Neurodegenerative Conditions (Computers in Biology and Medicine, 2025)

Part I. Structural Neuroimaging Biomarkers

1. Distinct Pattern of Gray Matter Atrophy in Mild Alzheimer's Disease Impacts on Cognitive Outcomes of Noninvasive Brain Stimulation

Published in Journal of Alzheimer’s Disease. 2015;48(1):251-60. doi: 10.3233/JAD-150067.

Commentary.

This study represents an early step in my research linking structural brain pathology with individual differences in therapeutic response. Mild AD is characterized by considerable heterogeneity in the distribution and severity of cortical atrophy, which may influence the effects of NIBS. The study was performed to investigate whether the anatomical pattern of gray matter loss could explain variability in cognitive outcomes following stimulation. The findings suggested that structural neurodegeneration is an important determinant of cognitive response to stimulation, supporting the concept that brain morphology should be considered when designing individualized interventions. Notably, patients with more pronounced gray matter atrophy appeared to benefit less from brain stimulation, whereas those whose pattern of gray matter volume was closer to that of HC showed greater cognitive improvement. This work extended my interest in cognitive effects of brain stimulation by introducing structural biomarkers as potential predictors of treatment response. It also provided an important conceptual foundation for subsequent work on PD, Lewy body disorders, and brain network plasticity. My personal contribution should emphasize my role in the data collection, interpretation of structural findings, and integration of imaging results with cognitive and stimulation outcomes.

Journal of Alzheimer's Disease 48 (2015) 251–260
DOI 10.3233/JAD-150067
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Distinct Pattern of Gray Matter Atrophy in Mild Alzheimer's Disease Impacts on Cognitive Outcomes of Noninvasive Brain Stimulation

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Abstract.
Background: Repetitive transcranial magnetic stimulation (rTMS) is a promising tool to study and modulate brain plasticity.
Objective: Our aim was to investigate the effects of rTMS on cognitive functions in patients with mild cognitive impairment and Alzheimer's disease (MCI/AD) and assess the effect of gray matter (GM) atrophy on stimulation outcomes.
Methods: Twenty MCI/AD patients participated in the proof-of-concept controlled study. Each patient received three sessions of 10 Hz rTMS of the right inferior frontal gyrus (IFG), the right superior temporal gyrus (STG), and the vertex (VTX, a control stimulation site) in a randomized order. Cognitive functions were tested prior to and immediately after each session. The GM volumetric data of patients were: 1) compared to healthy controls (HC) using source-based morphometry; 2) correlated with rTMS-induced cognitive improvement.
Results: The effect of the stimulated site on the difference in cognitive scores was statistically significant for the Word part of the Stroop test (ST-W, $p = 0.012$, linear mixed models). As compared to the VTX stimulation, patients significantly improved after both IFG and STG stimulation in this cognitive measure. MCI/AD patients had significant GM atrophy in characteristic brain regions as compared to HC ($p = 0.029$, Bonferroni corrected). The amount of atrophy correlated with the change in ST-W scores after rTMS of the STG.
Conclusion: rTMS enhanced cognitive functions in MCI/AD patients. We demonstrated for the first time that distinct pattern of GM atrophy in MCI/AD diminishes the cognitive effects induced by rTMS of the temporal neocortex.

Keywords: Alzheimer's disease, brain atrophy, cognitive functions, noninvasive brain stimulation; source-based morphometry

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common type of dementia in older people. Clinical hallmarks of AD include multiple cognitive deficits (e.g., in

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memory, attention, executive and visuospatial functions, and language), impaired activities of daily living, behavioral and psychiatric disturbances, and specific structural atrophic changes of the brain [1]. Mild cognitive impairment (MCI) due to AD was proposed to recognize as-yet non-demented individuals with some concerns about cognitive decline, objective impairment in one or more cognitive domains, and preserved independent functioning [2]. Due to the limited effectiveness of the current pharmacological treatment for AD patients, which is symptomatic and based on acetylcholinesterase inhibitors and NMDA receptor antagonists (for MCI, no symptomatic treatment exists), non-pharmacological strategies have received more attention.

Different noninvasive brain stimulation (NIBS) techniques, including transcranial magnetic stimulation (TMS), can be used to study and influence brain plasticity in humans *in vivo*. Repetitive TMS (rTMS) is able to induce changes that outlast the duration of the stimulation and may cause plasticity modifications locally [3] as well as in distant connected areas [4]. A recent study showed that a multiple rTMS session protocol increased functional connectivity among cortical-hippocampal network regions and improved memory performance in healthy adults; the effects persisted for up to 24 h after the stimulation [5]. These results represent a major rationale for the use of rTMS in AD research.

rTMS is a noninvasive, painless, and safe method that is based on the application of a train of magnetic pulses to a specific target location of the brain. The stimulation induces electric currents in underlying neuronal tissue. The effects of stimulation decrease rapidly with the distance from the coil [6]. In general, cortical excitability is reduced when low-frequency rTMS ($\leq$1 Hz) is applied over the motor cortex and increased when high-frequency rTMS (>1 Hz) is applied. Compared to quite extensive research in motor cortices, less is known about non-motor networks that can be accessed by rTMS using frameless stereotaxy for the coil navigation. The offline approach (performing a task prior to and after rTMS) can be used to study possible after-effects of the stimulation with the potential for therapeutic use [7]. Certain behavioral after-effects of rTMS applied mostly over the left dorsolateral prefrontal cortex have been shown in subjects with MCI or AD, but the results have thus far been inconsistent and more studies are needed; for deeper insight, see a recent review [8].

In the current proof-of-concept study, our main goal was to identify the possible effects of rTMS on cognitive functions in patients with MCI due to AD and mild AD (MCI/AD). Our main assumption was that NIBS may interact with compensatory brain mechanisms in order to enhance adaptive changes or diminish maladaptive changes that occur in the brains of these patients. We also addressed the possible effects of brain atrophy, which has been overlooked in most studies so far. MCI/AD subjects are hypothesized to benefit more from rTMS than patients in severe stages [9].

The choice of the targeted areas and behavioral tasks to measure the possible effects of the stimulation was largely based on results of our previous functional magnetic resonance imaging (fMRI) and rTMS studies [10–15]. We decided to target two cortical areas of interest: 1) the right inferior frontal gyrus (IFG) with the aim of modulating attention and cognitive speed; and 2) the right superior temporal gyrus (STG) in order to manipulate visual processing, attention, visual memory encoding, and recognition. In our preliminary data analysis of 10 MCI/AD patients, we observed a trend toward improvement of attention (as assessed by the Trail Making test) after one session of rTMS applied over the right IFG [16]. While the improvement was significant after the IFG stimulation, we were not able to directly compare the effects between active and control stimulations due to the small sample size. In this original research paper, the final results of the complete data set are presented.

METHODS

Participants

Twenty right-handed patients (11 women, 9 men; age: 73 $\pm$ 7 years; disease duration: 3 $\pm$ 2 years; 13 with 9–12 years of education, 7 with more than 12 years of education) participated in the proof-of-concept controlled study. All patients were longitudinally followed in the Center for Cognitive Impairment and Dementia, First Department of Neurology, Masaryk University and St Anne's Hospital, Brno, Czech Republic. Twelve subjects had a mild form of AD [1] and 8 subjects had MCI due to AD [2] at the time of stimulation. According to the most recent neurological or neuropsychological examination (the end of 2014 or the beginning of 2015), five out of eight MCI patients progressed into clear AD (mild and moderate dementia stage), two MCI patients progressed with respect to their cognitive performance decline since the time of stimulation but still remained in MCI category and one MCI can be characterized as non-progressor during the follow-up.

The exclusion criteria of this study were: a cardio pacemaker or any MRI-incompatible metal in the body, epilepsy, any diagnosed psychiatric disorder, alcohol/drug abuse, and lack of cooperation. Prior to the study commencement, each subject underwent an MRI examination and complex neuropsychological testing evaluating global cognitive functions, memory, attention, psychomotor speed, working memory, executive functions, visuospatial functions, language, activities of daily living, and depression; see Table 1 for the neuropsychological results of the whole group of patients. Each participant signed an informed consent form and the study was approved by the local ethics committee.

For the gray matter volume (GMV) analysis, T1-weighted images of 3 MCI/AD patients were not included into further analysis due to major MRI artifacts. The sample of 17 healthy controls (HC; 7 men, age: 69 ± 7 years) were matched according to gender and age to the remaining 17 MCI/AD patients (7 men, age: 73 ± 7 years). HC were scanned using the same 1.5T Siemens Symphony MR machine and all of them underwent a neuropsychological screening evaluation. For the comparison of HC and MCI/AD scores, see Table 2.

rTMS protocol, frameless stereotactic navigation of the coil, study design

Preceding TMS application, high-resolution anatomical T1-weighted images were acquired for each subject using the 1.5T Siemens Symphony MR machine and MPRAGE sequence with 160 sagittal slices, matrix size 256×256 resampled to

Table 1
Neuropsychological characteristics of MCI/AD patients ($n = 20$)

		Mean	SD	Med	Min	Max
Global cognitive functions	ACE-R (all subjects, $n = 20$)	70.8	9.5	73.5	47	82
	ACE-R (AD subjects, $n = 12$)	67.0	10.1	72	47	80
	ACE-R (MCI subjects, $n = 8$)	76.4	4.8	77	67	82
	MMSE (all subjects)	24.0	3.4	24	18	28
	MMSE (AD subjects, $n = 12$)	21.8	2.5	23	18	24
	MMSE (MCI subjects, $n = 8$)	27.3	1.0	27.5	25	28
Memory	RCFT Immed. Recall	5.2	5.3	4	0	21.5
	RCFT Delayed Recall	4.6	5.2	4	0	20.5
	RCFT Recog. Task	15	5.5	16	0	22
	WMS-III World list I	15.1	6.3	14.5	7	31
	WMS-III Logical Memory I	17	9.2	17	2	33
	WMS-III World list II	0.6	1.8	0	0	8
	WMS-III Recognition	16	3.8	16	11	24
	WMS-III Logical Memory II	5.2	6.9	1.5	0	22
Attention, psychomotor speed, working memory	WAIS-III Digit span	13.4	4.9	12	6	24
	WAIS-III Symbol search	27.1	16.4	25.5	1	57
	ST-W	80.9	17.4	80	38	111
	ST-C	61.4	17.8	63	27	98
	TMT-A	107.8	106.7	78.5	32	436
Executive functions	ST-CW	36.6	12.1	35	8	58
	VFT sem.	17.7	9.4	16	6	51
	VFT lex.	28.2	10.4	28	10	46
	TMT-B	303	291	210	89	1320
	Clock Test	3.3	1.4	3	1	5
Visuospatial functions	JLO	22	6.3	23.5	5	30
	RCFT Copy	26.4	9.5	30	2	36
Language impairment	MAST Total index	96.7	3.7	98	87	100
	MAST Expres. index	48	2.5	49	42	50
	MAST Recep. index	48.7	2.5	50	42	50
ADL	FAQ	6.6	6.5	4	0	20
Depression	MADRS	6.3	4.7	6	0	20

ACE-R, Addenbrooke's Cognitive Examination Revised; MMSE, Mini-Mental State Examination; RCFT, Rey-Osterrieth Complex Figure Test (Copy, Immediate Recall, Delayed Recall, Recognition Task); WMS-III, Wechsler Memory Scale III (Word List I, Logical Memory I, Word List II, Recognition Task, Logical Memory II); WAIS-III, Wechsler Adult Intelligence Scale III (Digit span, Symbol Search); ST, Stroop Color and Word Test (W-Word, C- Color, CW – Color-Word score); TMT-A/B, Trail Making Test, Part A/Part B; VFT, Verbal Fluency Test (sem., semantic; lex., lexical); JLO, Judgment of Line Orientation Test; MAST, Mississippi Aphasia Screening Test (Expressive, Receptive, and Total index); ADL, Activities of Daily Living; FAQ, Functional Activities Questionnaire; MADRS, Montgomery-Asberg Depression Rating Scale; SD, standard deviation; Med, median; Min, minimum; Max, maximum.

Table 2
Neuropsychological Assessment of MCI/AD patients and HC

	MMSE (M ± SD)	ACE-R (M ± SD)	Attention (M ± SD)	Memory (M ± SD)	Executive functions (M ± SD)	Language (M ± SD)	Visuospatial functions (M ± SD)
MCI/AD (n = 17)	24.1 ± 3.7	70.1 ± 10.2	14.6 ± 3.0	10.2 ± 4.1	9.1 ± 2.1	23.4 ± 2.5	12.7 ± 3.1
HC (n = 17)	29.1 ± 0.9	92.4 ± 3.7	17.6 ± 0.6	22.4 ± 2.3	11.2 ± 2.0	25.6 ± 0.5	15.6 ± 0.8
Maximum possible score for the given (sub-)test	30	100	18	26	14	26	16

MMSE, Mini-Mental State Examination; ACE-R, Addenbrooke's Cognitive Examination Revised and its subscores (Attention, Memory, Executive functions, Language, Visuospatial functions); n, number of subjects; M, mean; SD, standard deviation.

512 × 512, slice thickness = 1.17 mm, TR = 1700 ms, TE = 3.96 ms, FOV = 246 mm, FA = 15°. Brainsight TMS neuronavigation software was used with the patients' own T1 scans to position the TMS coils and maintain their exact location on the target area throughout the duration of the stimulation. TMS was applied using a Magstim Super Rapid Stimulator (Magstim Company, Whitland, UK) with a focal 70 mm figure of eight air-cooled coil.

A crossover design was employed. Each patient received three stimulation sessions in a random order: over the right IFG, over the right STG, and over the vertex (VTX, a control stimulation site). The sessions were separated from each other by at least a one-day interval between them. The stimulation parameters for all three stimulated regions were 90% individual resting motor threshold, 10 Hz, 45 trains of 50 pulses with an interstimulus interval of 25 s, resulting in 2,250 stimuli in one session. This particular TMS protocol parameters were chosen based on the results of the review summarizing the parameters most likely to result in the significant cognitive improvement in patients with neurodegenerative diseases [8] and our previous studies [12, 13] and experience.

For the right IFG and the right STG, the MNI coordinates (R_IFG: 48 21 3 [BA 45] and R_STG: 54 –15 0 [BA 21, 22]) were adapted from our previous studies [10, 14]. The T1 images were spatially normalized to the MNI coordinate system by SPM8 toolbox (Functional Imaging Laboratory, the Wellcome Department of Imaging Neuroscience, Institute of Neurology at University College London, UK) running under R2014a (The MathWorks, Inc.) and the resulting transformation parameters were used to project the MNI coordinates back to the native T1 image coordinate system. The location of VTX was identified as the point midway between the inion and the nasion, equidistant from the left and right intertragal notches. This point was also imported into Brainsight TMS neuronavigation software for online control of the precise location during the whole stimulation procedure.

Neuropsychological methods

The effect of the stimulation was measured using three tests: Trail Making Test (TMT), Stroop test (ST), and Complex Visual Scene Encoding Task (CVSET). They were administered prior to and immediately after each stimulation in a random order. Tests were practiced once prior to the study commencement in order to reduce the practice effects, which are most prominent between the first and the second sessions [17]. We controlled for the possible practice effect in our statistical analysis.

TMT consists of two parts (part A and part B). Both parts of the test evaluate sustained attention, psychomotor speed, and efficacy of cognitive processing. TMT-B is additionally focused on cognitive flexibility, set-switching, and executive functioning, and it poses a greater cognitive demand for the tested subjects [18]. ST consists of three subtests (ST-C, ST-W, ST-CW). ST-C and ST-W are mainly measures of speed of cognitive processing. ST-CW measures cognitive flexibility, divided attention, set shifting, and inhibition of habitual responses which is also a component of executive functions [19]. CVSET evaluates attentional visual processing, encoding, and recognition [20]. We have successfully used this task in our previous fMRI studies [10, 11, 15].

Statistical methods

For data analyses, we applied a paired t-test to compare the pre- and post-stimulation results in cognitive tests for each stimulated region. Linear mixed models were used for the evaluation of the effect of the stimulated site on the difference in cognitive scores. The rationale for the usage of linear mixed models instead of the commonly used analysis of variance (ANOVA) was that the three regions were stimulated in each individual and the cognitive data acquired from relevant pre- and post- stimulation time points were thus not independent. For all the analyses, $\alpha = 0.05$ was used as a level of statistical significance. Statistical analysis

was performed in STATISTICA 12 (StatSoft, Inc.) and SPSS 22 (IBM, Inc.) software.

Source based morphometry (SBM) was performed to explore the effect of brain atrophy on rTMS after-effects [21]. We used SPM8 toolbox running under Matlab R2014a to pre-process the high-resolution anatomical T1-weighted images. We segmented the images into gray and white matter, spatially registered the segments to the MNI coordinate system using DARTEL Tools, Jacobian-modulated in order to obtain GMV images, and smoothed with a spatial filter with a Gaussian kernel (FWHM = 8 mm). The spatial resolution of the resulting GMV images was $1.5 \times 1.5 \times 1.5$ mm.

SBM uses spatial Independent Component Analysis (ICA) to locate naturally grouping spatially independent sources of local GMV variability with common co-variation among subjects. Initially the GMV images from all subjects were concatenated and reshaped to form a single 2D matrix with the number of voxels and the number of subjects as matrix dimensions. To reduce the data dimensionality Principal Components Analysis (PCA) was performed with the Minimum Description Length (MDL) algorithm in order to estimate the optimal number of components [22]. These reduced data were then subjected to spatial ICA using the Infomax algorithm. Each component had two features: a) a vector of subjects' loadings and b) a spatial map. For each component, the vector of subjects' loadings, after filtering out the effects of age, gender, and total intracranial volume, was subjected to inter-group comparison using the nonparametric Mann-Whitney U test. To reveal the potential relationship between the extent of atrophy and the improvement after stimulation, the patient subgroup loadings of significant components were correlated to the difference in scores using the Spearman Rank-Order Correlation and also Spearman partial correlation to control for an effect of cognitive reserve (education level) and cognitive status (Mini-Mental State Examination (MMSE) score). Regions with strong inter-group differences were visualized by thresholding the spatial maps of significant components with $Z > 2.5$ and extent threshold of 100 voxels.

The parallel analysis was performed in order to evaluate the effect of local GMV (l-GMV) on stimulation outcomes. l-GMV was estimated as a sum of GMV image voxel values within specific regions of interest (ROIs; i.e., a sphere of 5-mm radius centered at each ROI coordinate for the right IFG, right STG, and vertex). The subjects' l-GMV were adjusted for the age, gender, and total intracranial volume in the same way as in the SBM analysis. We performed Mann-Whitney U test to assess differences in l-GMV between groups (MCI/AD and healthy controls). We used Spearman Rank-Order Correlation as well as Spearman partial correlation (education level, MMSE score) to calculate the correlation between l-GMV and cognitive rTMS outcomes.

RESULTS

The rTMS protocol used in this study was safe and well-tolerated. We noticed only a few transient side effects: three patients complained about painful scalp sensations during the IFG stimulation. All twenty patients completed the whole protocol and underwent all three sessions of the stimulation. Regarding the cognitive testing, four patients with AD were never able to complete the TMT-B due to the higher cognitive demands as compared to the TMT-A. Two additional patients were unable to complete the TMT-B once.

Cognitive outcomes

Statistically significant improvements were seen when the pre- and post-stimulation behavior data were compared using a paired *t*-test. Specifically, we found that the IFG stimulation induced improvement in the ST-W ($p = 0.016$), the TMT-A ($p = 0.012$), and the TMT-B ($p = 0.007$). After stimulation of the STG and of the VTX, our patients improved in the ST-CW ($p = 0.014$ and $p = 0.011$, respectively). The STG stimulation induced some improvement also in the ST-W but this result missed the threshold of significance ($p = 0.051$). The power analysis revealed that estimated power for ST-W change is 0.71 after IFG stimulation and 0.51 after STG stimulation.

The differences of the pre- and post- stimulation behavior variables were normally distributed, so the use of linear mixed models was possible. The results of the analysis are summarized in Table 3. A statistically significant effect of the stimulated site on the difference in the cognitive tests scores was present only for the ST-W ($p = 0.012$). Table 4 shows that the difference in the ST-W values before and after stimulation are 4.8 times higher with the IFG stimulation than with the VTX stimulation ($p = 0.004$) and 3.6 times higher with the STG stimulation than with the VTX stimulation ($p = 0.029$). We also checked for the effects of a trial (possible learning effect for the relevant cognitive tests), age, and global cognitive performance (as measured by Addenbrooke's Cognitive Examination Revised, ACE-R) on the patients' pre- versus

Table 3
The effect of the stimulated site on the difference in the values of the cognitive scores assessed by linear mixed models

Cognitive scales	F	*p*-value
ST-W	5.00	**0.012***
ST-C	0.36	0.697
ST-CW	0.59	0.559
TMT-A	1.86	0.170
TMT-B	2.72	0.083
CVSET	0.07	0.930

ST, Stroop Color and Word Test (W-Word, C- Color, CW – Color-Word score); TMT-A/B, Trail Making Test, Part A/Part B; CVSET, Complex Visual Scene Encoding Task; statistically significant result is marked by *.

Table 4
Comparison of the difference in values before and after stimulation for ST-W in stimulated areas assessed by linear mixed models

Site/reference site	Risk ratio (95% CI)	*p*-value
IFG/VTX	4.8 (1.6 – 7.9)	**0.004***
STG/VTX	3.6 (0.4 – 6.7)	**0.029***
STG/IFG	–1.2 (–4.4 – 2.0)	0.447

IFG, inferior frontal gyrus; STG, superior temporal gyrus; VTX, vertex; CI, confidence interval; statistically significant result is marked by *.

post-stimulation behavior change. None of those variables had any significant effect on the results.

Impact of gray matter atrophy on cognitive outcomes

We were particularly interested in assessing the global GMV differences between MCI/AD patients and cognitively normal age- and gender-matched HC and in investigating whether the atrophy pattern in our MCI/AD patients correlates with their stimulation outcomes. According to the MDL criterion, the ICA revealed 5 independent components, of which there was one with a significant between-group difference (Mann-Whitney U test; $p = 0.029$, Bonferroni corrected, see also Fig. 1).

MCI/AD patients showed GMV reduction bilaterally in the inferior temporal and fusiform gyri, putamen, and cerebellum as compared to HC (see Fig. 2 and Table 5). We also observed a significant correlation between the component loadings and improvement in ST-W after stimulation over the right STG (Spearman, $R = 0.58$, $p = 0.015$). To control for an effect of cognitive reserve (education level) and cognitive status (MMSE score) we also calculated Spearman partial correlation ($R = 0.68$, $p = 0.005$). MCI/AD patients with more pronounced global GMV atrophy had less benefit from the STG stimulation than patients with minor global GMV changes.

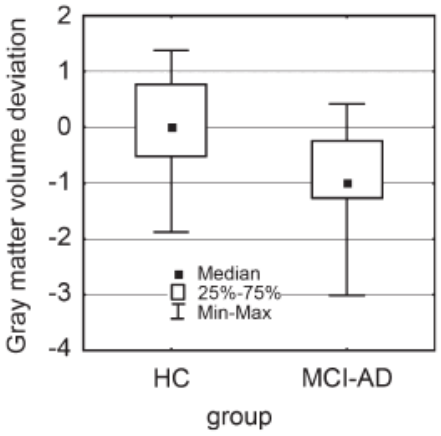

Fig. 1. GMV deviation (in Z score) difference in the significant component between HC and MCI/AD.

The individual baseline values of ST-W seem not to play any role in reported results. We double-checked and specifically looked at the baseline values of ST-W and we found no statistically significant difference between AD and MCI patients. Moreover the baseline values did not correlate with the pattern of GMV atrophy. In fact, when we looked at the correlation between improvement of ST-W after STG-rTMS and the GMV deviation, the MCI and AD patients overlapped (see Fig. 3).

As regards the l-GMV results, there was no statistically significant difference in l-GMV between MCI/AD and HC and no significant correlations (using Spearman Rank-Order Correlation or Spearman partial correlation) between relevant l-GMV and cognitive rTMS outcomes.

DISCUSSION

In this study, we present the results of brain plasticity modulation induced by rTMS applied over the right IFG and the right STG regions in an MCI/AD patient population and demonstrated by significant cognitive improvement in attention and psychomotor speed. It is a proof-of-concept controlled study (using the vertex as the control stimulation site) of an adequately-sized sample of very well characterized MCI/AD patients. Unlike in previous studies, we took into account potential confounders of rTMS after-effects such as subject age and global cognitive

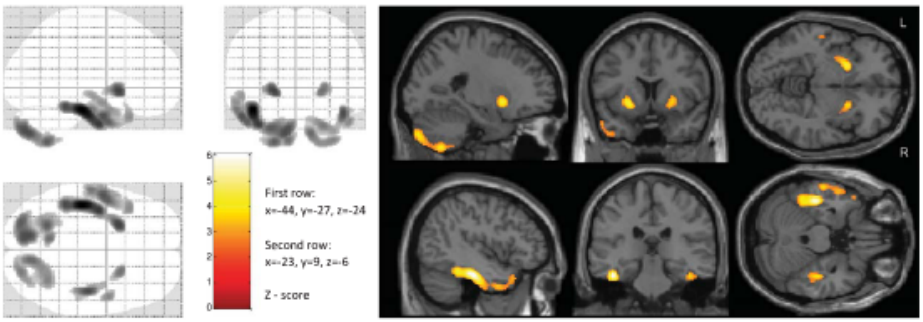

Fig. 2. The areas in which patients with MCI/AD have significantly less GMV as compared to HC, threshold set at Z > 2.5, extent threshold 100 voxels.

Table 5
Structures with GMV reduction in MCI/AD as compared to HC

No. of clusters	Structure	Side	No. of voxels	Z value in maximum	MNI coordinates (mm)		
					X	Y	Z
7	Inferior temporal gyrus, Fusiform gyrus	Left	3911	3.11	−44	−27	−24
	Putamen	Left	532	2.71	−23	9	−6
	Fusiform gyrus	Right	671	2.66	44	−19	−29
	Cerebellum	Left	2890	2.65	−12	−78	−48
	Cerebellum	Right	1982	2.50	12	−75	−48
	Putamen	Right	319	2.46	23	12	−6
	Inferior temporal gyrus	Right	196	2.34	50	0	−36

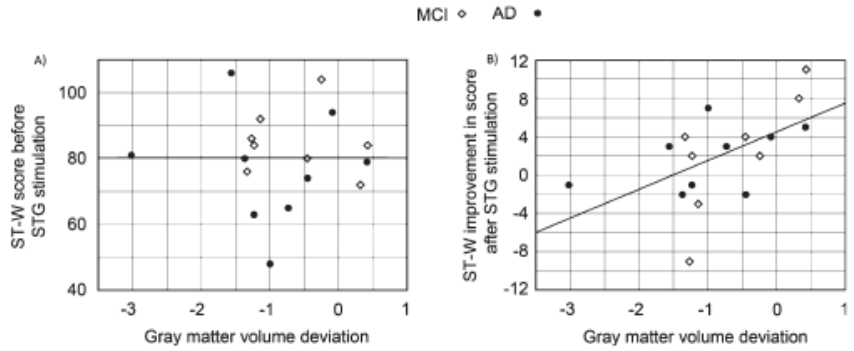

Fig. 3. The correlations between GMV deviation in the significant component and A) ST-W baseline before STG stimulation; B) the improvement in ST-W score after STG stimulation with marked group membership (MCI or AD).

impairment. We also controlled for learning effects of repeated cognitive testing. To our knowledge, this is the first study to explore the impact of GM atrophy on rTMS-induced cognitive outcomes in the MCI/AD patient population.

We demonstrated that stimulation of the right IFG and of the right STG led to improved performance on the task evaluating particularly attention and psychomotor speed in our MCI/AD patients. According to the results of previous studies, the right IFG, as a part of

the ventral attention network, governs stimulus-driven detection, reflexive reorientation, and response inhibition [23–25]. In NIBS study of healthy humans, the dorsal part of the right IFG was critical for visual detection of changes and the ventral portion of the right IFG was engaged in updating the corresponding action plan [26]. Weissman, et al. [27] used fMRI to investigate the relationship between brain activity and response time in healthy subjects. They showed that attentional lapses begin with reduced prestimulus activity in the anterior cingulate and right middle and IFG—areas involved in controlling attention. These lapses were also linked to increased default-mode network activity. The right IFG coordinate in their study (R_IFG: 49, 25, 8) was very similar to our coordinate of stimulation (R_IFG: 48, 21, 3), which revealed decreased connectivity with the posterior cingulate/precuneus (i.e., the major node of the default mode network) in patients with Parkinson's disease with dementia as compared to healthy age-matched controls [10]. rTMS over the right IFG increased the cognitive processing speed in non-demented patients with Parkinson's disease, as shown by improvement in all subtests of the ST [12] or in Parkinson's disease patients with implanted DBS electrodes by the shortening of the cognitive event-related potential (ERP of P3) latency [28]. The right IFG showed reduced activation in AD patients as compared to HC during attentional tasks as assessed by fMRI [29]. It was also found that activation within the right IFG correlated with the TMT scores in MCI subjects [30]. We have already shown in our pilot study that modulating the IFG excitability with rTMS may lead to clinically relevant improvement in attentional task performance in early AD patients [16].

The right STG region is also involved in the ventral attention network, thus engaged in reorienting attention toward behaviorally salient stimuli [25]. This region showed greater activation in response to target stimuli than to non-target stimuli in a visual oddball fMRI task performed in healthy subjects [14]. In AD patients as compared to HC, connectivity changes were found between the posterior cingulate and the STG when switching from baseline to a visual task condition [15]. Kim, et al. [31] demonstrated changes in current source density of the theta band in the STG of AD subjects as compared to HC which was associated with impaired cognitive performance. In MCI subjects, greater activation of the right STG was found as compared to HC during a memory task performance. Moreover, the magnitude of activation was associated with the cognitive performance, suggesting a compensatory function of this region [30]. Taken together, we showed that the stimulation of the IFG and the STG may interfere with underlying pathophysiological mechanisms of AD and/or the compensatory functions of these regions in MCI/AD subjects and lead to improved attention and enhanced cognitive speed.

The rationale for choosing VTX as a control stimulation site rather than choosing a sham stimulation by tilting the coil from the scalp or using commercially available sham coil is that patients are not able to distinguish the nature of this stimulation (real/control) due to the same local somatosensory skin sensation and the same auditory artifacts in each session.

Our MCI/AD patients showed reductions in GMV bilaterally in the inferior temporal and fusiform gyri, putamen, and cerebellum, which is in agreement with previously published studies of this patient population. Patients in the very early stages of AD (MCI, prodromal AD) have been characterized by GMV loss in specific brain areas including the inferior temporal or fusiform gyri that could also be associated with the extent of cognitive decline and the rate of conversion to AD [32–35]. Basal ganglia abnormalities and atrophic changes (including the putamen) in patients with MCI or AD were also detected [36, 37]. Neuronal changes and atrophy in the cerebellum in AD patients were identified both histopathologically [38] and by using structural MRI [39, 40]. The fact that we have not found statistically significant atrophy of the mesial temporal lobe structures may be caused by the features of SBM—a multivariate statistical method. While it sensitively identifies widespread areas of even mild GMV loss engaged within the same component that differentiates MCI/AD from HC, it may not be sensitive for detecting spatially very discreet local GMV differences that can be better detected by voxel-based morphometry. On the other hand, SBM reduces the multiple-comparison problem (that is profound in voxel-based morphometry) thus enabling the detection of differences of GMV loss patterns between smaller sample sized groups. Importantly, the brain atrophy found in our MCI/AD subjects had an important impact on STG-rTMS cognitive aftereffects. Surprisingly, the l-GMV may not play such an important role related to the cognitive rTMS outcomes in MCI/AD patients as a distinct pattern of global GMV atrophy. Nevertheless, this is in accord with the features of both methods. The SBM as a multivariate statistical method is sensitive to subtle GMV changes which are spread over brain regions. From the statistical point of view, the ROI analysis

is rather univariate method which may leave the subtle changes unrecognized (i.e., produced false negative results). Unfortunately the brain atrophy has thus far been overlooked as an important variable in rTMS studies performed in patients with neurodegenerative brain diseases (for review, see [8]) and this requires further research.

We are fully aware of some limitations of this study. In our study, it was not possible to obtain biomarkers based on cerebrospinal fluid (due to small patients' compliance) or PET (amyloid PET is not available in the Czech Republic). We did not explore the possible genetic factors that may influence rTMS outcomes. A direct comparison of the rTMS effect on cognitive functions between cognitively intact healthy seniors and MCI/AD patients would be preferable in future research. We are aware of the fact that multiple comparison testing for cognitive outcomes including both number of stimulated regions and number of cognitive tests (including subscales) would result in non-significant results. But due to the exploratory nature of the study, we report all results and uncorrected *p*-values. The additional limitation is that we did not use the sham coil for controlling of placebo effects as it was common in the previous studies. Using vertex as a control stimulation site we cannot dissociate between a real benefit of the rTMS and a blocked placebo/repetition effect.

On the other hand, to our knowledge this is the first study to explore the impact of GMV atrophy on behavioral outcomes of NIBS in patients with MCI/AD. An additional strength of this study is that we applied comprehensive neuropsychological assessment in all of our patients; HC were also screened for cognitive decline using a complex screening method. The exact location of the coil over each stimulated site was found using neuronavigation with frameless stereotaxy. The precise spot was maintained and monitored during the entire stimulation. We also very carefully controlled for possible confounding variables.

In conclusion, one session of high-frequency rTMS of the right IFG and STG enhanced attention and psychomotor speed in MCI/AD patients. Further studies should evaluate whether multiple-session rTMS protocols would induce long-term clinically relevant changes. We showed for the first time that atrophic changes impact cognitive effects induced by rTMS applied over the temporal neocortex and may at least partially explain the fact that patients with advanced AD do not profit from the stimulation [9]. Future NIBS studies in patients with neurodegenerative brain diseases should properly evaluate the brain atrophy and consider this variable as an important confounder of the rTMS outcome.

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2. Patterns of Grey Matter Atrophy at Different Stages of Parkinson's and Alzheimer's Diseases and Relation to Cognition

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Commentary.

This publication addressed the question of whether neurodegenerative diseases with different underlying pathologies show distinct patterns of structural brain damage and whether these changes are associated to cognitive impairment. The study was motivated by the need to move beyond global measures of brain atrophy and to identify disease-specific spatial patterns across different clinical stages. The findings demonstrated disease- and stage-dependent patterns of atrophy, with cortical and hippocampal changes associated with cognitive impairment and differences in sensitivity across imaging methods. The novelty of the work lies in the comparative framework, which enabled direct examination of similarities and differences between two major neurodegenerative disorders. This study developed the structural imaging approach introduced in my earlier work and shifted the focus from treatment response to the characterization of disease progression.

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ORIGINAL PAPER

Patterns of Grey Matter Atrophy at Different Stages of Parkinson's and Alzheimer's Diseases and Relation to Cognition

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Abstract

Using MRI, a characteristic pattern of grey matter (GM) atrophy has been described in the early stages of Alzheimer's disease (AD); GM patterns at different stages of Parkinson's disease (PD) have been inconclusive. Few studies have directly compared structural changes in groups with mild cognitive impairment (MCI) caused by different pathologies (AD, PD). We used several analytical methods to determine GM changes at different stages of both PD and AD. We also evaluated associations between GM changes and cognitive measurements. Altogether 144 subjects were evaluated: PD with normal cognition (PD-NC; n = 23), PD with MCI (PD-MCI; n = 24), amnestic MCI (aMCI; n = 27), AD (n = 12), and age-matched healthy controls (HC; n = 58). All subjects underwent structural MRI and cognitive examination. GM volumes were analysed using two different techniques: voxel-based morphometry (VBM) and source-based morphometry (SBM), which is a multivariate method. In addition, cortical thickness (CT) was evaluated to assess between-group differences in GM. The cognitive domain z-scores were correlated with GM changes in individual patient groups. GM atrophy in the anterior and posterior cingulate, as measured by VBM, in the temporo-fronto-parietal component, as measured by SBM, and in the posterior cortical regions as well as in the anterior cingulate and frontal region, as measured by CT, differentiated aMCI from HC. Major hippocampal and temporal lobe atrophy (VBM, SBM) and to some extent occipital atrophy (SBM) differentiated AD from aMCI and from HC. Correlations with cognitive deficits were present only in the AD group. PD-MCI showed greater GM atrophy than PD-NC in the orbitofrontal regions (VBM), which was related to memory z-scores, and in the left superior parietal lobule (CT); more widespread limbic and fronto-parieto-occipital neocortical atrophy (all methods) differentiated this group from HC. Only CT revealed subtle GM atrophy in the anterior cingulate, precuneus, and temporal neocortex in PD-NC as compared to HC. None of the methods differentiated PD-MCI from aMCI. Both MCI groups showed distinct limbic and fronto-temporo-parietal neocortical atrophy compared to HC with no specific between-group differences. AD subjects displayed a typical pattern of major temporal lobe atrophy which was associated with deficits in all cognitive domains. VBM and CT were more sensitive than SBM in identifying frontal and posterior cortical atrophy in PD-MCI as compared to PD-NC. Our data support the notion that the results of studies using different analytical methods cannot be compared directly. Only CT measures revealed some subtle differences between HC and PD-NC.

Keywords Parkinson's disease · Alzheimer's disease · Mild cognitive impairment · Voxel-based morphometry · Source-based morphometry · Cortical thickness

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Introduction

There is significant grey matter (GM) atrophy in both Alzheimer's disease (AD) and Parkinson's disease (PD), leading to gradual and irreversible cognitive decline. This GM loss is well described in both AD and amnestic mild cognitive impairment due to AD (aMCI) (Dickerson et al. 2009a, b; Ferreira et al. 2011; Chételat et al. 2005; Jack et al. 2013), in which temporal lobe atrophy, including a loss of GM in

both hippocampi, together with atrophy in the occipital and parietal lobes creates a typical pattern. Results are inconsistent in all stages of PD (Duncan et al. 2013). Some studies have shown clear atrophy already in the early stage of PD (e.g. Brück et al. 2004; Beyer et al. 2007; Madhyastha et al. 2015; Lee et al. 2013; Rektorova et al. 2014); others reported little or no change compared to HC (e.g. Dalaker et al. 2009; Weintraub et al. 2011; Agosta et al. 2013; Rektor et al. 2018). The same applies for PD-MCI: some authors did not demonstrate any changes in GM (Dalaker et al. 2010), while others report GM atrophy in various areas of the brain (e.g. Melzer et al. 2012; Weintraub et al. 2011; Gasca-Salas et al. 2017; Ye et al. 2017; Xu et al. 2016). Distinct atrophy patterns have been observed in demented PD patients by many authors, involving mainly hippocampal structures and the temporal, occipital, and frontal lobes as well as the basal ganglia and other subcortical structures (Weintraub et al. 2011; Rektorova et al. 2014; Apostolova et al. 2010; Ibarretxe-Bilbao et al. 2011; Pan et al. 2013; Burton et al. 2004). Some of these studies correlated the atrophy levels with cognitive outcomes; however, the inconsistency is even greater in these data (e.g. Almeida et al. 2003; Tinaz et al. 2011). A recently published study by Duncan et al. (2013) addressed this incongruence by evaluating different studies and suggesting issues that future studies should address, stating mainly that unified criteria for PD-MCI diagnosis should be applied. Study results are also difficult to compare due to the use of various methods such as voxel-based morphometry, source-based morphometry, cortical thickness, or graph theory-based analyses.

The aim of this paper was to use two different morphometric approaches in order to assess grey matter volumes (GMV) in the same groups of patients and healthy controls to describe GM changes at different stages of AD and PD and correlate these data with the results of cognitive domain z-scores. Like most authors, we used voxel-based morphometry (VBM); based on the results of our previous studies (Rektorova et al. 2014; Anderkova et al. 2015), we also included source-based morphometry (SBM), i.e. a multivariate technique for data analysis. This method finds independent components of changes in GMV common to a group of subjects and compares it to another group, thus displaying functionally connected areas in the brain where GMV changes occur. The method makes it possible to identify smaller changes that co-occur within larger brain networks (Xu et al. 2009).

Cortical thickness (CT) was also evaluated in order to assess between-group differences in GM when comparing early neurodegeneration cases (aMCI, PD-NC, and PD-MCI) with HC. We included this method as most researchers currently use it to measure distinct patterns of cortical atrophy in PD and AD, claiming that this method is more sensitive in depicting early GM changes, particularly where there are smaller changes occurring over a larger cortical area (Lehericy et al. 2017; Duncan et al. 2013). The presumably high sensitivity can be achieved partly through reduced registration errors across different brains; however, there is a potential for error in the segmentation process (Hutton et al. 2009).

In the current study, we specifically aimed to compare two groups with MCI as a result of different brain pathologies, i.e. PD-MCI and aMCI groups. Some authors have reported that memory-related patterns of cortical atrophy characteristic of AD may be also associated with dementia in PD (Weintraub et al. 2012; Rektorova et al. 2014; Tam et al. 2005). However, to our knowledge no study has yet directly compared the patterns of cortical atrophy between pre-dementia stages of the two neurodegenerative brain diseases.

Methods

A total of 147 participants were recruited. Of these 147 participants, three were excluded because of motion artefacts on MRI images (2 PD, 1 PD-MCI). From the 144 remaining subjects, 27 had amnestic MCI (aMCI) (Petersen et al. 2001), 12 had probable AD with documented progression of cognitive deficits (McKhann et al. 2011), 23 were clinically established PD patients (Postuma et al. 2015) with normal cognition (PD-NC), 24 were diagnosed with mild cognitive impairment in PD (PD-MCI) (Litvan et al. 2012), and 58 were age-matched healthy controls (HC). All subjects underwent MR imaging in 3 T Siemens Prisma machine (at CEITEC, Masaryk University) and were cognitively tested by experienced neuropsychologists using a cognitive test battery (Anderkova et al. 2017, see in more detail below). PD-NC and PD-MCI subjects were examined and scanned in the ON medication state without dyskinesias. Patients with major tremor and with dyskinesias were not enrolled. Subjects with any diagnosed psychiatric disorder and subjects with alcohol/drug abuse were excluded. All patients were longitudinally followed at the First Department of Neurology, Masaryk University and St. Anne's Hospital, Brno, Czech Republic. Each participant signed an informed consent form and the study was approved by the local ethics committee.

Neuropsychological Assessment

We used the cognitive test battery described in our previous publications (Anderkova et al. 2017; Nemcova-Elfmarkova et al. 2017). The complex neuropsychological testing evaluated global cognitive functions (MMSE) and five domains: memory (Rey-Osterrieth Complex Figure Test: Immediate Recall, Delayed Recall, Recognition Task; Wechsler Memory Scale III: Word List I, Word List II, Recognition Task);

attention (Stroop Color and Word Test (SCWT): Word and Color parts and Trail Making Test part A); executive functions (SCWT: Color-Word part, Trail Making Test part A, Verbal Fluency Test: semantic; lexical, and Clock test); visuospatial functions (Rey-Osterrieth Complex Figure Test: Copy, Visual Object, and Space Perception Battery—Silhouettes); language (Mississippi Aphasia Screening Test—Receptive, Expressive, and Total index), activities of daily living (Functional Activities Questionnaire); and depression (Geriatric Depression Scale). Five cognitive domains (memory, attention, executive function, visuospatial function, and language) were inspected for cognitive decline. All of the subjects who scored below − 1.5 SD in two tests in one domain compared to normative data were categorized as MCI subjects. We applied the MCI criteria of Petersen et al. (2001) and Litvan et al. (2012), according to which all subjects who have concern regarding a change in cognition, impairment in one or more cognitive domains, preservation of independence in functional abilities, and are not demented have MCI. The cognitive domain z-scores were computed as the average z-scores of the tests included in the particular domain (Anderkova et al. 2017).

MRI Data Acquisition

All subjects were scanned using MPRAGE sequence with 240 sagittal slices, TR = 2300 ms, TE = 2.36 ms, FOV = 256 mm, FA = 8°, matrix size 256 × 256, slice thickness = 1 mm, in 3 T Siemens Prisma MR scanner.

Pre-processing

SPM12 software was used to pre-process all anatomical T1 and T2 weighted images. Spatial normalization was performed to create a study-specific template so that all images could be registered in the same stereotactic space. MRI images were segmented into grey and white matter segments and spatially registered to the MNI coordinate system using the DARTEL toolbox. Images were smoothed with a spatial filter with a Gaussian kernel (FWHM = 8 mm). The resolution of the resulting GMV images was 1.5 × 1.5 × 1.5 mm.

Voxel Based Morphometry (VBM)

This morphometric method compares GMV in specific voxels of two different groups. Specific contrasts were applied using a paired T-test in SPM12—HC versus each patient group and certain patient groups among themselves. The effects of age, gender, education, and depression (using Geriatric Depression Scale) was filtered out each time. Group differences were considered significant if $p < 0.05$ after family-wise error (FWE) correction was performed. Cluster threshold was set at $t > 2.5$. Loadings from significant contrasts were extracted for further statistical correlation.

Source-Based Morphometry (SBM)

This morphometric method uses spatial Independent Component Analysis (ICA) to find naturally grouping, spatially independent sources of local GMV variability with common co-variation among subjects—components. The GM images from all subjects were concatenated and reshaped to a 2D matrix with the number of voxels and the number of subjects as matrix dimensions. Principal Components Analysis (PCA) was performed to reduce the data dimensionality together with the Minimum Description Length algorithm in order to estimate the optimal number of components (Li et al. 2007). Spatial ICA was performed on the reduced data using the Infomax algorithm. The result was a number of components that each comprised two features—subjects' loadings and a spatial map. The subjects' loadings, after filtering out the effects of age, gender, education, and depression (using Geriatric Depression Scale), were subjected to inter-group comparisons using the Mann–Whitney U test to identify significant differences between pairs of groups. There were three SBM analyses: HC versus aMCI versus AD, HC versus PD-NC versus PD-MCI, and HC versus aMCI versus PD-MCI. False Discovery Rate (FDR) was used to address the issue of multiple testing, and the significance level was set to $p = 0.05$. Significant component images were spatially normalized to MNI space with a threshold set to $Z > 2.5$. The clusters with 500 and more adjacent voxels were superimposed on the study-specific GM images to visualize regions with strong inter-group differences.

Surface-Based Morphometry—Cortical Thickness (CT) Analysis

Images were analysed using FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu). Data was first processed with the *recon-all* pipeline, resulting in segmented images and cortical thickness maps. Upon completion, grey and white matter boundaries were visually inspected for each subject. Altogether nine subjects were excluded for surface-based analysis due to abberations that were not possible to correct manually (3 HC, 2 AD-MCI, 2 AD, 1 PD-MCI, 1 PD). Small imprecisions in temporal pole segmentation were handled by excluding the temporal lobe label (Desikan-Killiany atlas—Desikan et al. 2006) from all further analyses. CT maps were then resampled onto the *fsaverage* subject and smoothed with a FWHM of 10 mm. To identify differences between relevant groups, data were entered into the Freesurfer GLM model, and age, gender, education, and GDS scores were used as covariates of no interest. Based

on previously published studies (Uribe et al. 2016; Hanganu et al. 2014; Biundo et al. 2013; Liu et al. 2010), we hypothesized that we would find more cortical thinning in the patient groups with early degeneration (aMCI, PD-NC, PD-MCI) than in HC. In line with the literature, we accepted significance at the uncorrected p-value of 0.001 (Ashburner et al. 2003; Lyoo et al. 2006; Tae et al. 2008) with additional cluster extension thresholds of 40 mm^2 to describe distinct cortical atrophy in our cohorts of interest. However, we had no a priori hypothesis when comparing both MCI groups since no such studies exist except our own VBM studies (Anderkova et al. 2017; Nemcova-Elfmarkova et al. 2017). Therefore, we used a more conservative approach to exclude false positive results. To correct for multiple comparisons, we used a precached clusterwise Monte Carlo simulation with 10,000 iterations, with the absolute vertex-wise cluster-forming threshold of p = 0.001 and cluster-wise threshold of p = 0.05.

Table 1 Demographic and clinical data of HC and patient groups

	HC; n = 58	aMCI; n = 27	AD; n = 12	PD-NC; n = 23	PD-MCI; n = 24
Age	67.5 ± 7.3	69.8 ± 6.9	72.9 ± 8.6	61 ± 8.4	65.1 ± 10.0
Education duration	15.4 ± 2.5	14.3 ± 3.0	14.6 ± 5.6	16.2 ± 3.3	14 ± 3.1
Sex. % of male	31	37	8	74	67
MMSE	28.5 ± 1.2	27.1 ± 1.3	20.9 ± 2.3	28.2 ± 1.2	27 ± 2.2
GDS	2.3 ± 2.5	3.7 ± 3.4	2.1 ± 1.8	2.8 ± 2.8	2.9 ± 2.3
LED	–	–	–	815.5 ± 21.5	909 ± 482.5
UPDRS III	–	–	–	17.6 ± 8.8	17.7 ± 8.8

MMSE mini mental state examination, *GDS* geriatric depression scale, *LED* levodopa equivalent dose, *UPDRS III* unified Parkinson's disease rating scale subsection III

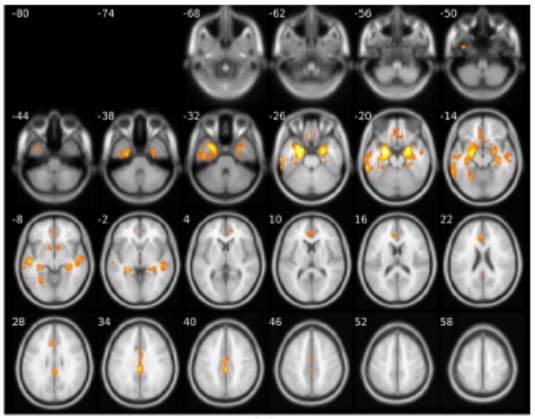
Table 2 Demographic and clinical data—differences between groups

	HC versus AD (p value)	HC versus aMCI (p value)	HC versus PD-NC (p value)	HC versus PD-MCI (p value)	aMCI versus PD-MCI (p value)	PD-NC versus PD-MCI (p value)
Age	0.15	0.57	0.02	0.87	0.23	0.43
Gender	0.53	0.98	< 0.01	0.01	0.15	0.98
Education duration	0.93	0.55	0.86	0.38	0.99	0.13
MMSE	< 0.01	< 0.01	0.96	< 0.01	0.99	0.04
GDS	0.99	0.18	0.95	0.91	0.82	0.99
LED	–	–	–	–	–	0.97
UPDRS III	–	–	–	–	–	0.54

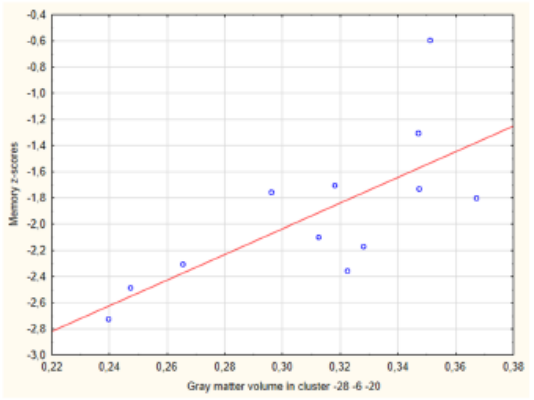
MMSE mini mental state examination, *GDS* geriatric depression scale, *LED* levodopa equivalent dose, *UPDRS III* unified Parkinson's disease rating scale subsection III

Table 3 Cognitive tests results

	Memory	Attention	Executive	Visuospatial	Language
HC	0.63 ± 0.5	−0.06 ± 0.7	0.59 ± 0.8	0.44 ± 0.6	0.44 ± 0.4
AD	−1.92 ± 0.6	−2.23 ± 1.1	1.52 ± 0.7	−0.92 ± 1.2	−1.01 ± 1.5
aMCI	−0.22 ± 0.8	−0.89 ± 0.9	−0.60 ± 0.7	0.10 ± 0.6	0.14 ± 0.6
PD-NC	0.24 ± 0.7	−0.27 ± 0.9	0.36 ± 0.7	0.44 ± 0.6	0.32 ± 0.6
PD-MCI	−0.67 ± 0.7	−1.21 ± 0.9	−0.56 ± 0.8	−0.14 ± 0.8	−0.04 ± 0.9
HC versus AD (p value)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
HC versus aMCI (p value)	< 0.01	< 0.01	< 0.01	0.36	0.54
HC versus PD-NC (p value)	0.20	0.84	0.74	1.00	0.97
HC versus PD-MCI (p value)	< 0.01	< 0.01	< 0.01	0.02	0.12
AD versus aMCI (p value)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
aMCI versus PD-MCI (p value)	0.20	0.57	0.99	0.78	0.93
PD-NC versus PD-MCI (p value)	< 0.01	< 0.01	< 0.01	0.08	0.59



(a)



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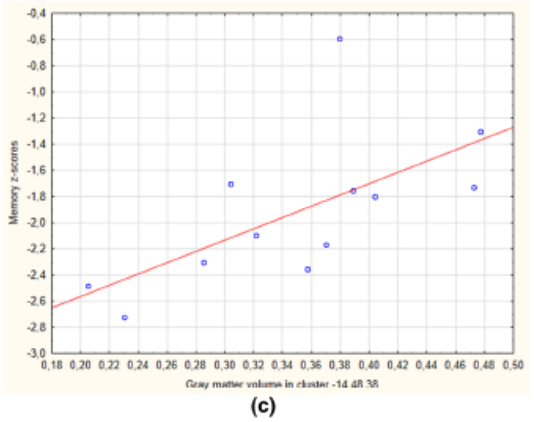

(c)

Fig. 1 **a** VBM analysis HC versus AD. **b** Correlation of cognitive z-scores in HC versus AD VBM analysis. **c** Correlation of cognitive z-scores in HC versus AD VBM

Statistics

Statistical analysis was performed in STATISTICA 12 (StatSoft, Inc.) software.

Correlation with Cognitive z-Scores

Spearman correlation was used to reveal any potential relationship between the GMV/CT regions of significant between-group differences and cognitive domain z-scores. The multiple testing problem was handled by FDR and the significance level was set to p=0.05.

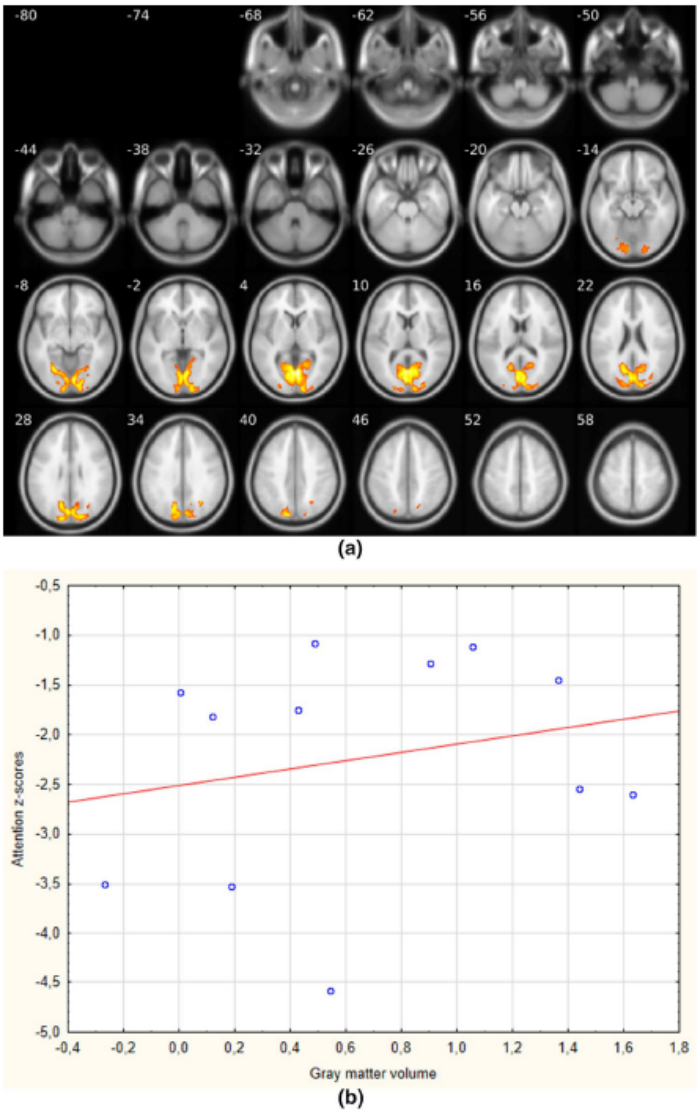

Fig. 2 **a** SBM analysis HC versus aMCI versus AD—component 2. **b** Correlation of cognitive z-scores in component 2

Results

For the demographic and clinical data of subjects, see Tables 1 and 2.

There was a significant difference in age between the groups of HC and PD-NC, due to the younger PD-NC patients enrolled. The two Parkinson disease groups (PD-NC and PD-MCI) had significantly different gender distribution compared to HC. Every cognitively impaired group (AD, aMCI, and PD-MCI) scored significantly lower in MMSE according to their diagnosis. PD-MCI also differed from PD-NC in MMSE.

For cognitive tests results, see Table 3.

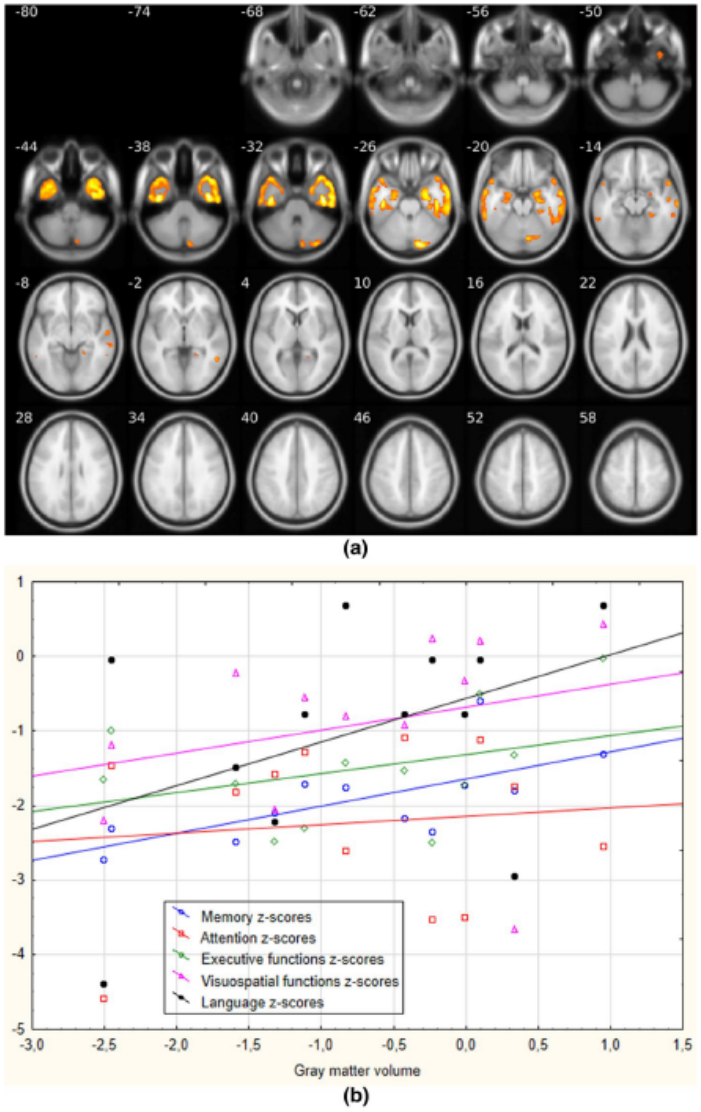

Fig. 3 **a** SBM analysis HC versus aMCI versus AD—component 8. **b** Correlation of cognitive z-scores in component 8

SBM Results

HC Versus aMCI Versus AD (Comparing Different Cognitive Stages of AD and HC)

Out of ten components, only three showed significant differences between groups not caused by artefacts as verified by visual inspection (C1, C2 and C8), see also Tables 4 and 5.

HC Versus PD-NC Versus PD-MCI (Comparing Different Cognitive Stages of PD-NC and HC)

Nine components were found in this analysis; however, none was significant.

HC Versus PD-MCI Versus aMCI: (Comparing Two Different MCI Groups and HC)

Ten components were found, of which one showed significant differences between groups (C9), see also Table 7.

Results According to Groups

HC Versus AD

The AD group presented a typical hippocampal and temporal lobe atrophy compared to HC using VBM (see Fig. 1a); GMV in these regions correlated with memory z-scores in the AD group (see Fig. 1b, c; $R_{1b}=0.71$, $p_{1b}=0.010$, $R_{1c}=0.69$, $p_{1c}=0.014$). The SBM component 2 (C2) exhibited a GMV loss in occipital regions (Fig. 2a) and differentiated AD from HC (M-W test; $p=0.0013$). This component correlated with attention domain z-scores in the AD group (Fig. 2b, $R=-0.46$, $p=0.003$). The SBM component 8 (C8) showed a hippocampal and temporal lobe atrophy (Fig. 3a), which correlated with all cognitive z-scores (Fig. 3b) (M-W test; $p=0.0047$). Surface-based analysis was not performed in the AD group because of too small sample size ($n=10$ after removing data from two subjects, see also above). For detailed results, see Table 4.

HC Versus aMCI

The aMCI patients showed more atrophy than HC in both the anterior and posterior cingulate (see Fig. 4) according to VBM. SBM revealed a temporo-fronto-parietal atrophy (see Fig. 5) in aMCI (M-W test; $p=0.0083$) when compared to HC (Component C1). Various fronto-occipital areas were affected in aMCI compared to HC (see Fig. 6a, b) using surface-based analysis. For detailed results, see Table 5.

HC Versus PD-NC

Only surface-based analysis revealed differences between HC and PD-NC: PD-NC displayed cortical thinning in the frontal and temporal areas, and in the precuneus (see Fig. 7). For detailed results, see Table 6.

HC Versus PD-MCI

Using VBM, we observed GMV differences between PD-MCI and HC in the left precuneus and bilateral orbitofrontal cortex (see Fig. 8). The SBM component 9 (C9) displayed a temporo-fronto-parietal atrophy (see Fig. 9) in PD-MCI compared to HC (M-W test; $p=0.0203$). Using CT measures, PD-MCI differed from HC by cortical thinning in the right superior frontal gyrus (Fig. 10). For detailed results, see Table 7.

AD Versus aMCI

Using VBM, the aMCI and AD patients differed in the GMV of the left hippocampus (lower in AD than in aMCI, see Fig. 11a), the GMV of this structure correlated with

Table 4 HC versus AD

Method	Cluster size	X_1	Y_1	Z_1	$Area_1$
VBM	464,216	−28	−6	−20	l.+r. Hippocampus
	2215	−14	48	38	l. Superior frontal gyrus
SBM	16,358 (C2)	4.5	−70.5	6	Cuneus, precuneus, calcarine fissure, lingual gyrus
	10,602 (C8)	39	−7.5	−37.5	ITG, MTG, STG, fusiform gyrus, hippocamus, uncus, parahipocampal gyrus
	7113 (C8)	−40.5	−13.5	−34.5	ITG, MTG, STG, fusiform gyrus, hippocamus, uncus, parahipocampal gyrus
	1338 (C8)	16.5	−87	25.5	Posterior cerebellar lobe
CT	Not performed				

C2 component 2, *C8* component 8, *STG* superior temporal gyrus, *MTG* medial temporal gyrus, *ITG* inferior temporal gyrus, X_1, Y_1, Z_1 MNI coordinates of cluster maximum intensity, $Area_1$ brain region according to automated anatomical labeling (Tzourio-Mazoyer et al. 2002)

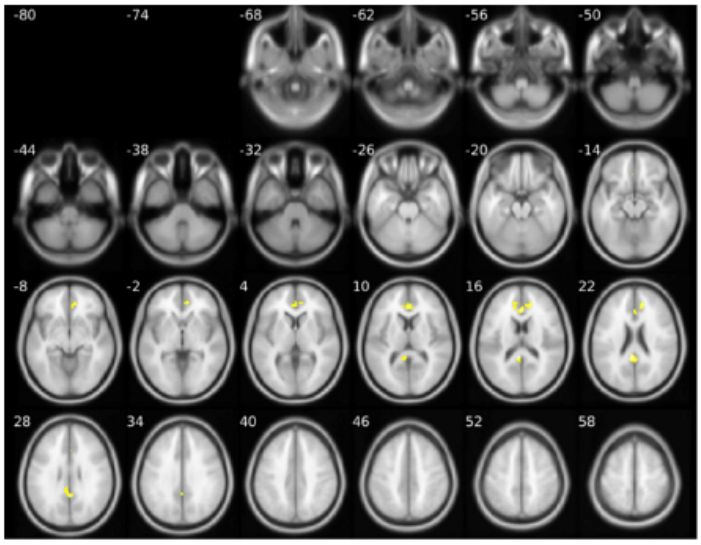

Fig. 4 VBM analysis HC versus aMCI

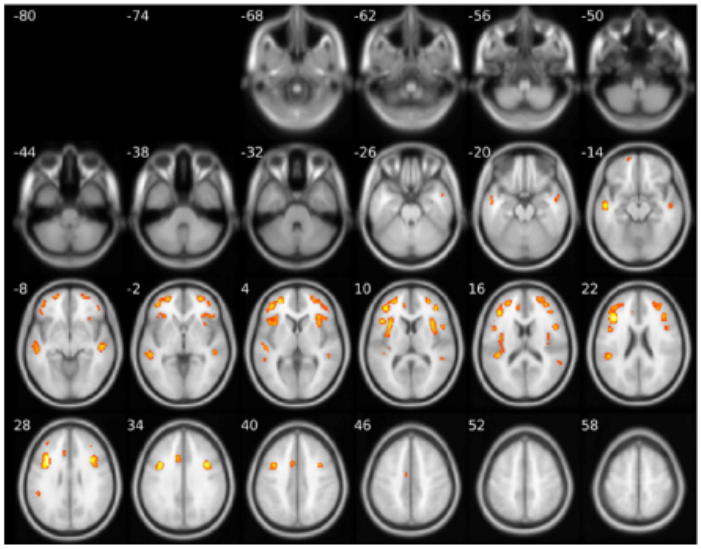

Fig. 5 SBM analysis HC versus aMCI versus AD—component 1

all cognitive domains except for visuospatial functions in the AD group (see Fig. 11b; for R and p values, see Table 8.) Surface-based analysis was not performed due to a small sample size of the AD group. For detailed results, see Table 9.

PD-NC Versus PD-MCI

Lower GMV was found in the left orbitofrontal cortex in PD-MCI than in PD-NC (see Fig. 12a) when VBM was employed. The GMV of this region correlated with memory z-scores in PD-MCI (Fig. 12b, $R = 0.42$, $p = 0.003$).

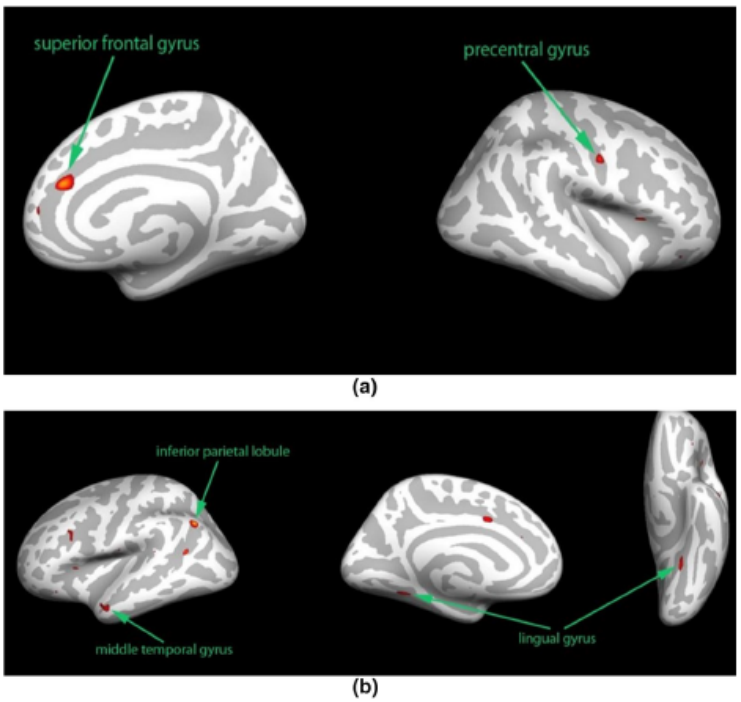

Fig. 6 **a** Cortical thickness analysis HC versus aMCI. **b** Cortical thickness analysis HC versus aMCI

Table 5 HC versus aMCI

Method	Cluster size	X_1	Y_1	Z_1	$Area_1$
VBM	1978	−4	−44	18	l. + r. Posterior cingulate
	4588	15	42	20	l. + r. Anterior cingulate
	2156	18	16	−12	r. Gyrus rectus
SBM	697 (C1)	51	−22.5	−7.5	STG, MTG
	893 (C1)	−52.5	−15	−12	MTG
	6025 (C1)	−37.5	21	25.5	SFG, MFG, IFG, Inferior parietal lobule, insula
	4163 (C1)	36	7.5	36	MFG, SFG, insula
	563 (C1)	−9	21	33	Anterior cingulate
	Size (mm^2)	X_2	Y_2	Z_2	$Area_2$
CT	126.84	−51.3	−1.7	−22.7	l. Middle temporal gyrus
	117.55	−40.4	−62.7	39.6	l. Inferior parietal lobule
	49.72	−30.8	−51.6	−3.2	l. Lingual gyrus
	98.84	14.9	34.5	16.4	r. Superior frontal gyrus (ACC)
	47.55	45.5	−5.8	26.4	r. Precentral gyrus

C1 component 1, *STG* superior temporal gyrus, *MTG* medial temporal gyrus, *SFG* superior frontal gyrus, *MFG* medial frontal gyrus, *IFG* inferior frontal gyrus, X_1, Y_1, Z_1 MNI coordinates of cluster maximum intensity, $Area_1$ brain region according to automated anatomical labeling (Tzourio-Mazoyer et al. 2002), X_2, Y_2, Z_2 Talairach coordinates of vertex with maximum p value, $Area_2$ brain region designated according to Desikan–Killiany atlas (Desikan et al. 2006), *r*/*l*. right/left, *ACC* anterior cingulate cortex

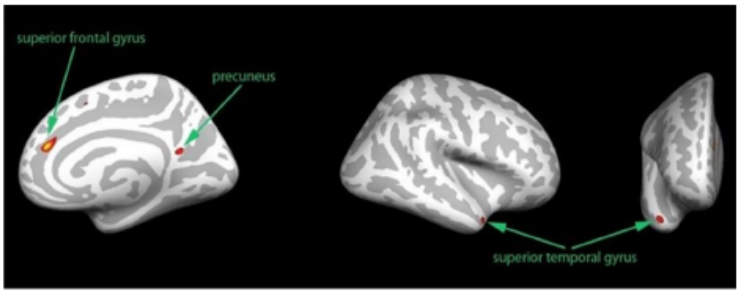

Fig. 7 Cortical thickness analysis HC versus PD-NC

Table 6 HC versus PD-NC

Method					
VBM	No significant difference				
SBM	No significant difference				
	Size (mm^2)	X_2	Y_2	Z_2	$Area_2$
CT	84.54	15.1	36.2	15.2	r. Superior frontal gyrus (ACC)
	66.35	45.6	13.9	– 23.1	r. Superior temporal gyrus
	41.57	7.2	– 55.4	16.5	Precuneus

X_2, Y_2, Z_2 Talairach coordinates of vertex with maximum p value, $Area_2$ brain region designated according to Desikan–Killiany atlas (Desikan et al. 2006), *r./l.* right/left, *ACC* anterior cingulate cortex

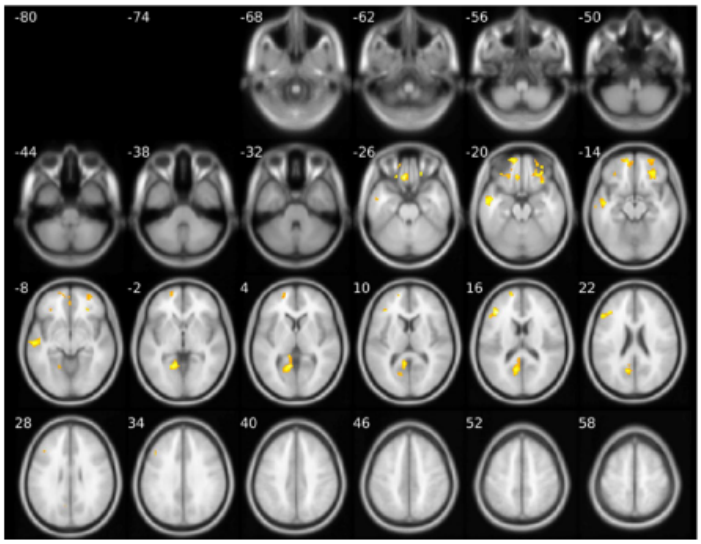

Fig. 8 VBM analysis HC versus PD-MCI

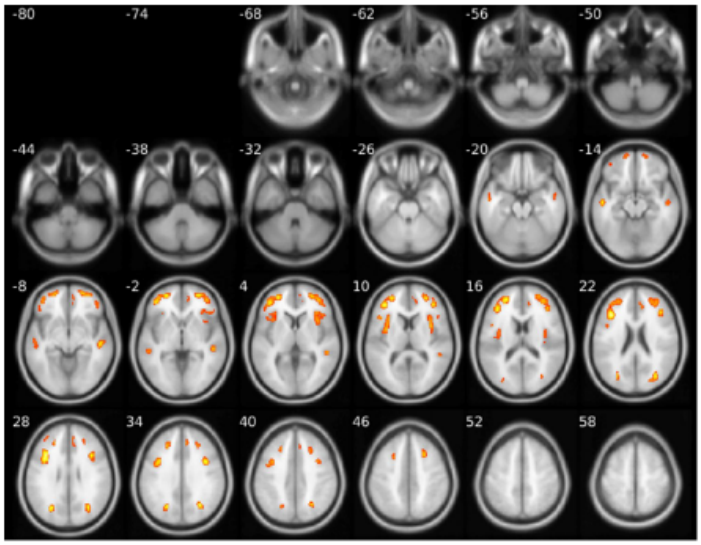

Fig. 9 SBM analysis HC versus PD-MCI versus aMCI—component 9

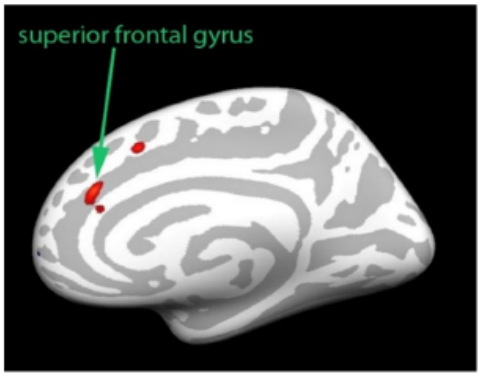

Fig. 10 Cortical thickness analysis HC versus PD-MCI

Moreover, PD-MCI showed a parietal cortical thinning when compared to PD-NC (see Fig. 13). For detailed results, see Table 10.

aMCI Versus PD-MCI

No method was able to detect any differences between aMCI a PD-MCI groups.

Discussion and Conclusion

Our study is the first one comparing multiple techniques of GM atrophy assessment in different stages of AD and PD. We looked at specific between-group differences in GM volumes using both VBM and SBM. We were particularly interested in comparing two groups with MCI caused by different brain pathologies (i.e. AD and PD pathologies). CT measures were additionally employed since some authors have asserted that CT is the most sensitive technique for describing subtle GM alterations in the early stages of neurodegenerative brain diseases (Lehericy et al. 2017; Duncan et al. 2013). Thus altogether three different approaches were used and their complementarity was assessed. VBM was used as the gold standard method for assessing localized GM changes between group pairs in a voxel-wise manner. SBM is a multivariate method which uses independent component analysis in order to target more widespread patterns of cortical atrophy, i.e. structural components of cortical regions with related GMV changes (Rektorova et al. 2014). The method makes it possible to evaluate GM alterations among multiple groups of subjects. As mentioned in "Introduction", "Methods", and "Results" sections above, cortical thickness analysis was additionally performed in all groups except for AD patients.

Using both VBM and SBM, we were able to distinguish between AD and HC. GMV loss was recorded in the temporal lobes and both hippocampi (C3), regions typically

Table 7 HC versus PD-MCI

Method	Cluster size	X_1	Y_1	Z_1	$Area_1$
VBM	1102	33	39	−16	r. Orbitofrontal cortex
	1437	−8	30	−26	l. Orbitofrontal cortex
	1486	−10	−56	3	l. Precuneus
SBM	736 (C9)	51	−24	−6	STG, MTG
	527 (C9)	−52.5	−13.5	−13.5	MTG
	3749 (C9)	37.5	6	36	SFG, MFG, IFG
	4628 (C9)	−37.5	21	25.5	SFG, MFG, IFG
	538 (C9)	10.5	36	24	MFG
	1112 (C9)	34.5	16.5	7.5	Insula, IFG
	866 (C9)	−36	0	12	Insula, IFG
	505 (C9)	−27	−67.5	30	Superior parietal lobule, precuneus
	772 (C9)	33	−73.5	21	Superior parietal lobule, precuneus
	Size (mm^2)	X_2	Y_2	Z_2	$Area_2$
CT	63.83	14.7	33.3	21.1	r. Superior frontal gyrus (ACC)

C9 component 9, *STG* superior temporal gyrus, *MTG* medial temporal gyrus, *SFG* superior frontal gyrus, *MFG* medial frontal gyrus, *IFG* inferior frontal gyrus, X_1, Y_1, Z_1 MNI coordinates of cluster maximum intensity, $Area_1$ brain region according to automated anatomical labeling (Tzourio-Mazoyer et al. 2002), X_2, Y_2, Z_2 Talairach coordinates of vertex with maximum p value, $Area_2$ brain region designated according to Desikan–Killiany atlas (Desikan et al. 2006), *r/l.* right/left, *ACC* anterior cingulate cortex

associated with Alzheimer pathology (Jack et al. 2013). This GM atrophy correlated with cognitive outcomes in each cognitive domain. Greater atrophy in the occipital lobes was seen in the AD group than in HC (C2). The latter pattern of GM loss is also in congruence with published research (Thompson et al. 2003) and was associated with lower scoring in attention domain. These regions are crucial for processing visual information; intact visual processing is required for spatial orientation, which is known to be particularly impaired in AD (Quental et al. 2013; Binetti et al. 1996).

Both VBM and SBM methods were able to detect GMV atrophy in our aMCI patients as compared to HC. VBM detected GMV loss in the limbic region within the anterior and posterior cingulate cortex. Using SBM, we observed temporo-fronto-parietal atrophy (C1) that differentiated aMCI from HC, while CT revealed additional changes in temporo-parieto-occipital regions in the aMCI group. These results are in accordance with previous findings that showed similar patterns of atrophy in the medial temporal, lateral temporoparietal, and midline parietal regions, with variable findings in frontal regions using CT (e.g. Petersen et al. 2001; Du et al. 2007) as well as using VBM and similar techniques (e.g. Apostolova et al. 2007; Whitwell et al. 2008).

Only cortical thickness measures were useful in identifying differences between HC and PD-NC. This was probably because the cortical thickness measure is more sensitive to cortex changes, possibly because it is less dependent on cortical folding and the overall brain size than VBM and SBM (Hutton et al. 2009). We found GM changes in the anterior cingulate cortex/superior frontal gyrus, right anterior temporal gyrus ,and precuneus. Previous CT studies are in line with our current data with described changes in the parietal cortices (Madhyastha et al. 2015), and in the anterior cingulate and the superior frontal gyrus (Ye et al. 2017). Moreover, longitudinal studies show that PD patients who later develop dementia have reduced cortical thickness in the right precentral and superior frontal gyri as well as in the ACC (Compta et al. 2013). Atrophy of the temporal and posterior cortical areas also seem to be predictive of cognitive decline in PD (Mak et al. 2015; Weintraub et al. 2011; Camicioli et al. 2003; Song et al. 2011; Lee et al. 2013; for review, see; Rodriguez-Oroz et al. 2015).

VBM and SBM both found a greater GMV loss in PD-MCI than in HC. VBM showed a more localized atrophy in the precuneus and orbitofrontal cortex, i.e. regions involved in visual processing and attention, memory, visuospatial functions, and other cognitive functions (Cavanna and Trimble 2006; Sack 2009). Additional atrophy was observed in the orbitofrontal cortex in PD-MCI patients as compared to HC. These GMV changes were related to declines in memory domain z-scores (Preston and Eichenbaum 2013). Using SBM we recorded GMV loss in temporo-fronto-parietal regions (C9); and using CT, in the right superior frontal region. Many authors as well as recent reviews (Pereira et al. 2014; Hanganu et al. 2014; Mak et al. 2015; Nagano-Saito

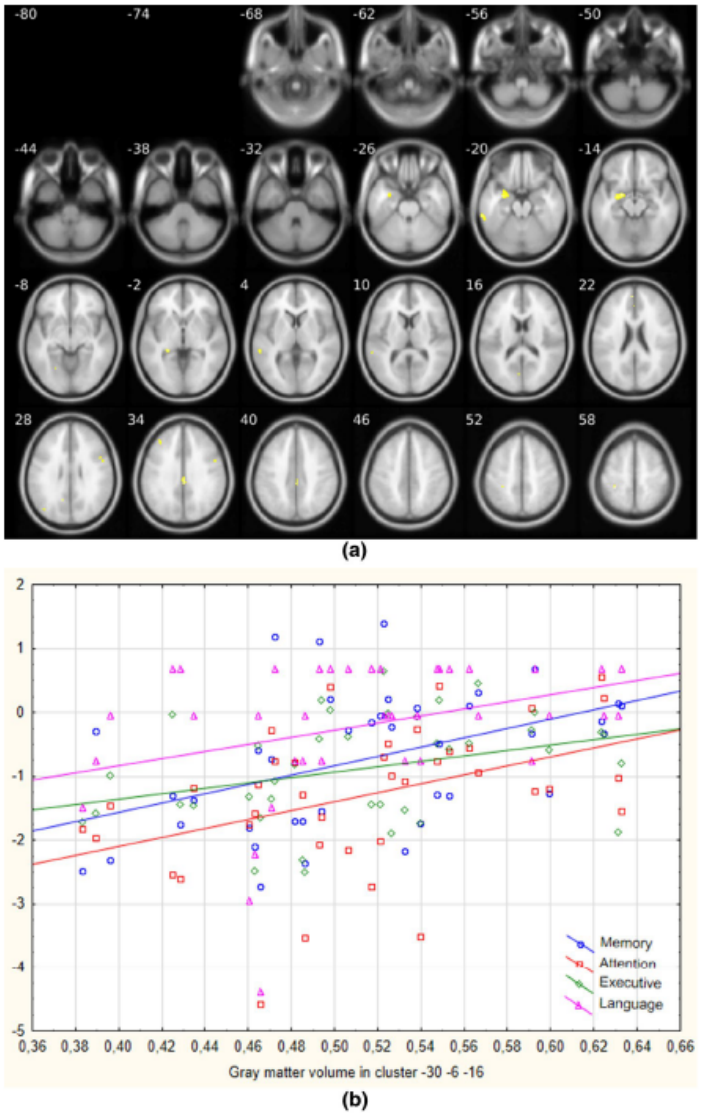

Fig. 11 **a** VBM analysis AD versus aMCI. **b** Correlation of cognitive z-scores in AD versus aMCI VBM analysis

et al. 2005; Summerfield et al. 2005; Beyer et al. 2007; Song et al. 2011; Melzer et al. 2012 for review; see also; Duncan et al. 2013; Rodriguez-Oroz et al. 2015) point out that PD-MCI patients exhibit rather heterogeneous areas of GM atrophy. similar to those in PD-dementia but to a lesser extent. The GM atrophy is particularly found in orbitofrontal and other prefrontal regions, the superior temporal and parietal

Table 8 AD versus aMCI difference in GMV: correlations with cognitive domain z-scores

Domain	R-value	p-value
Memory	0.49	< 0.01
Attention	0.48	< 0.01
Executive	0.36	0.03
Language	0.34	0.03

Table 9 AD versus aMCI

Method	Cluster size	X_1	Y_1	Z_1	$Area_1$
VBM	1382	−30	−6	−16	l. Hippocampus
SBM	No significant difference				
CT	Not performed				

X_1, Y_1, Z_1 MNI coordinates of cluster maximum intensity, $Area_1$ brain region according to automated anatomical labeling (Tzourio-Mazoyer et al. 2002)

neocortex, occipital areas, medial temporal regions, and the precuneus. Prefrontal and temporo-parietal atrophy has been the most consistent finding (Burton et al. 2004; Compta et al. 2012; Xu et al. 2016; Gasca-Salas et al. 2017; Pereira et al. 2014; Hanganu et al. 2014; Mak et al. 2015).

Neither VBM nor SBM found differences in GMV between aMCI and PD-MCI. CT did not reveal any difference between these two entities either. This result of our study supports earlier published VBM and SBM MRI data

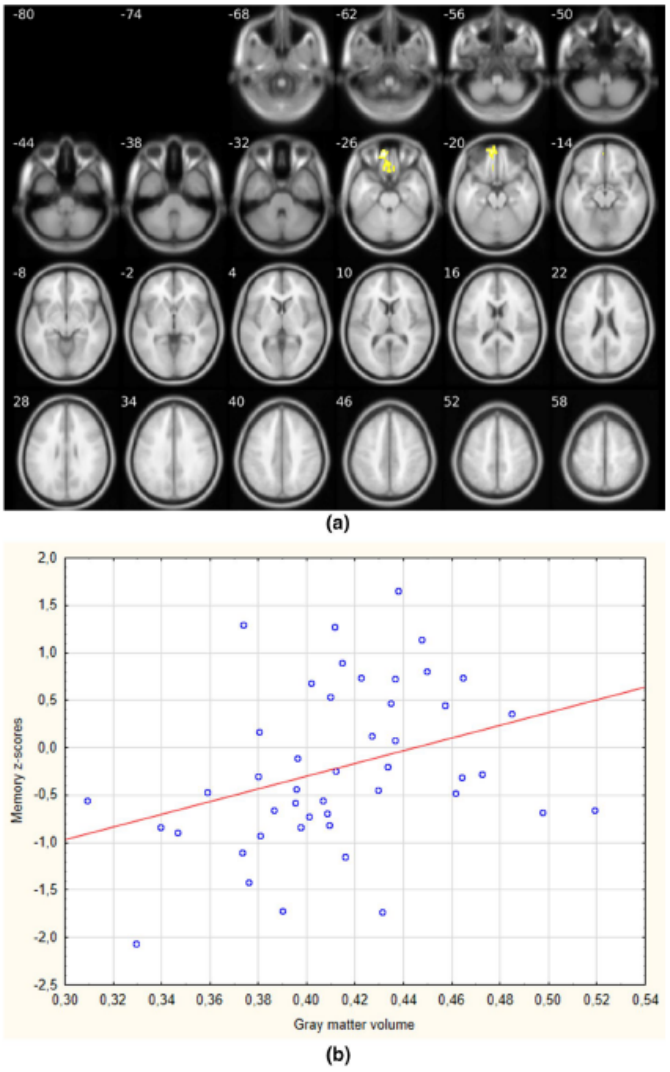

Fig. 12 **a** VBM analysis PD-NC versus PD-MCI. **b** Correlation of cognitive z-scores in PD-NC versus PD-MCI VBM analysis

(Weintraub et al. 2011; Rektorova et al. 2014) as well as functional MRI results (Anderkova et al. 2017; Nemcova-Elfmarkova et al. 2017); the result suggests that even though AD and PD are two different clinical entities (Besser et al. 2016), the distinct pathology underlying the neurodegenerative process responsible for cognitive decline development in both diseases might share a common structural pattern (Jellinger et al. 2002; Irwin et al. 2012, 2013; Compta et al. 2011, 2012; Pan et al. 2013). In this regard, a recent study showed that AD and PD cognition-related metabolic patterns as assessed by ^{18}F-fluorodeoxyglucose PET (Mattis et al. 2016) might be more sensitive than structural or functional MRI in differentiating the two patient groups.

We are aware of several limitations of our study, mainly uneven group samples and age differences between participants. We tried to minimize the impact of these discrepancies by using age, education duration, sex and GDS as covariates in second-level statistical analysis. We lacked CSF or amyloid PET biomarkers in our aMCI subjects and this may be quite a heterogenous group with respect to brain pathology and progression rates. Subjects with aMCI that lack amyloid pathology based on biomarker assessment are referred to as "SNAPs" (suspected non-Alzheimer pathology). In some, the cognitive impairment is caused by PART (primary age-related tauopathy) (Jack et al. 2016; Vos et al. 2013; Jack 2014). This entity does not progress to dementia as fast as aMCI due to typical AD pathology, but individuals with PART have increased levels of tau pathology and still bear a significant risk to dementia conversion as compared to healthy age-matched individuals (Burnham et al. 2016). Nevertheless, it was shown that 12% of all aMCI subjects progress to typical AD dementia every year and 80% of them convert into AD dementia within 6 years (Petersen et al. 2001).

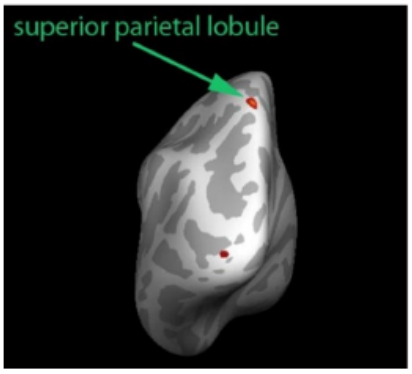

Fig. 13 Cortical thickness analysis PD-NC versus PD-MCI

In conclusion, we found distinct limbic and fronto-temporo-parietal neocortical atrophy in both MCI groups compared to HC with no specific differences between them. AD subjects displayed a typical pattern of major temporal lobe atrophy which was associated with deficits in all cognitive domains. VBM and CT were more sensitive than SBM for identifying distinct frontal limbic and superior parietal lobule atrophy in PD-MCI as compared to PD-NC. Only CT measures revealed some subtle differences between HC and PD-NC.

Our data support the notion that the results of studies using different analytical methods cannot be directly compared (Duncan et al. 2013) and may show slightly different results when comparing the same groups of subjects. CT measures seem to depict early GM changes more sensitively than VBM or SBM; all methods identify the well described spatial patterns of GM atrophy in advanced brain neurodegeneration. Further longitudinal studies should show which analytical methods are best suited for monitoring disease progression and potential treatment effects.

Table 10 PD versus PD-MCI

Method	Cluster size	X_1	Y_1	Z_1	$Area_1$
VBM	3826	−8	56	−22	l. Orbitofrontal cortex
	1091	−4	48	33	l. Superior frontal gyrus
SBM	No significant differences				
	Size (mm^2)	X_2	Y_2	Z_2	$Area_2$
CT	68.56	−13.2	−59.2	59.3	l. Superior parietal lobule

X_1, Y_1, Z_1 MNI coordinates of cluster maximum intensity, $Area_1$ brain region according to automated anatomical labeling (Tzourio-Mazoyer et al. 2002), X_2, Y_2, Z_2 Talairach coordinates of vertex with maximum p value, $Area_2$ brain region designated according to Desikan–Killiany atlas (Desikan et al. 2006), *r*/*l*. right/left

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Compliance with Ethical Standards

Conflict of Interest All author declares that they have no conflict of interest to disclose.

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3. Diffusion Tensor and Restriction Spectrum Imaging Reflect Different Aspects of Neurodegeneration in Parkinson's Disease

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Commentary.

The aim of this study was to investigate whether diffusion MRI techniques provide complementary information about neurodegeneration in PD. Conventional DTI is sensitive to microstructural alterations but has limited biological specificity. Restriction spectrum imaging was therefore examined as a potentially more specific approach to characterizing tissue changes. The central hypothesis was that these two diffusion methods would capture different components of Parkinsonian neurodegeneration rather than providing redundant information. The results supported this hypothesis by demonstrating that DTI and restriction spectrum measures reflected distinct aspects of brain microstructural pathology. The main contribution of this work was methodological as well as biological: it showed that multimodal diffusion imaging can improve the characterization of neurodegenerative processes beyond conventional structural MRI. In the context of our previous publications, this study extended the investigation from macroscopic gray matter atrophy to microstructural tissue alterations. It also established a methodological basis for subsequent studies using DKI and other advanced diffusion approaches.

PLOS ONE

RESEARCH ARTICLE

Diffusion tensor and restriction spectrum imaging reflect different aspects of neurodegeneration in Parkinson's disease

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Data Availability Statement: There are both legal and ethical reasons for not sharing the data publicly. The informed consent prohibits open data sharing. Additionally, sharing these data publicly is in violation of the Norwegian patient data protection regulations. The minimal data set may be made available upon special request for researchers who meet the criteria for access to confidential data. The data is stored on a secure server (hosted by the University of Oslo). Access can be arranged on an individual basis, following

Abstract

To meet the need for Parkinson's disease biomarkers and evidence for amount and distribution of pathological changes, MRI diffusion tensor imaging (DTI) has been explored in a number of previous studies. However, conflicting results warrant further investigations. As tissue microstructure, particularly of the grey matter, is heterogeneous, a more precise diffusion model may benefit tissue characterization. The purpose of this study was to analyze the diffusion-based imaging technique restriction spectrum imaging (RSI) and DTI, and their ability to detect microstructural changes within brain regions associated with motor function in Parkinson's disease. Diffusion weighted (DW) MR images of a total of 100 individuals, (46 Parkinson's disease patients and 54 healthy controls) were collected using b-values of 0–4000s/mm^2. Output diffusion-based maps were estimated based on the RSI-model combining the full set of DW-images (Cellular Index (CI), Neurite Density (ND)) and DTI-model combining b = 0 and b = 1000 s/mm^2 (fractional anisotropy (FA), Axial-, Mean- and Radial diffusivity (AD, MD, RD)). All parametric maps were analyzed in a voxel-wise group analysis, with focus on typical brain regions associated with Parkinson's disease pathology. CI, ND and DTI diffusivity metrics (AD, MD, RD) demonstrated the ability to differentiate between groups, with strongest performance within the thalamus, prone to pathology in Parkinson's disease. Our results indicate that RSI may improve the predictive power of diffusion-based MRI, and provide additional information when combined with the standard diffusivity measurements. In the absence of major atrophy, diffusion techniques may reveal microstructural pathology. Our results suggest that protocols for MRI diffusion imaging may be adapted to more sensitive detection of pathology at different sites of the central nervous system.

GDPR guidelines. Contact person will be the Division Director; Anita Bjørnstad (Anita.Kristin.Bjornstad@ahus.no).

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Introduction

Parkinson's disease (PD) is a slowly progressing neurodegenerative disorder, affecting both motor function and cognition. Accurate biomarkers for PD diagnosis and progression are scarce. PD is primarily diagnosed clinically after the pathology has reached an advanced stage, and confirmed post-mortem by loss of dopamine neurons in the substantia nigra and the presence of intracellular aggregates of α-synuclein called Lewy bodies [1]. Formation of Lewy bodies, loss of neurites, loss of dopamine-containing neurons and gliosis in substantia nigra is an early phenomenon in PD, whereas cortical pathology is thought to precede major subcortical affection on Dementia with Lewy Bodies [2, 3]. Lewy body pathology spreads from the brainstem and subcortical structures, to related areas with eventual allo- and neocortical involvement [4]. α-synuclein deposition is considered the core pathological substrate of PD. In addition to accumulation in Lewy-bodies, a major deposition site of central nervous system (CNS) oligomeric α-synuclein is in the presynapse, accompanied by transsynaptic dendritic spine loss [5, 6], suggesting widespread pathology. MRI studies have indicated that slight grey matter loss is involved in PD cognitive decline [7]. In PD with normal cognition, there is slight volume decrease in the frontoparietal regions and also increase in the midbrain and cerebellum. There is also a second pattern with medial temporal lobe atrophy more closely coupled to cognitive decline [8], and we recently described cortical metabolic changes associated with biomarker changes in temporo-occipital and frontal regions [9].

To meet the need for PD biomarkers and evidence for amount and distribution of pathological changes, MRI diffusion tensor imaging (DTI) techniques have been explored. By measuring diffusion of water molecules in the brain, this technique gives information on neuronal integrity which may be linked to disease state. A number of studies have reported that the DTI derived indices fractional anisotropy (FA) and mean diffusivity (MD) in the substantia nigra and other brain structures associated with PD pathology differentiate between PD and controls with decreased FA and increased MD in PD. However, there are conflicting reports. Some more recent studies have reported an increase in FA in early PD, while several other studies have not found any differences. For example; studies manually delineating regions of interest have found decreased FA in the substantia nigra compared to controls [10–16], or asymmetrical substantia nigra FA [17], whereas other studies report increased FA [18–20] or low sensitivity within the same region [21–24]. Changes in other DTI indices in PD in white matter structures of the brain associated with PD pathology have also been described previously, suggesting that white matter neurodegeneration (due to PD) may be detectable using DTI [25]. Basal ganglia group differences in DTI metrics have also been reported, some demonstrating decreased FA [13, 15], however others report no change [11]. Similarly, there are conflicting reports regarding the relationship between PD motor symptoms and DTI-derived parameters [11–13, 15, 26–28], suggesting that the DTI parameters may not be sensitive enough for characterization of PD CNS tissue changes alone.

Diffusion measurements using the DTI model relies on a Gaussian unrestricted diffusion model weighted predominantly by free and hindered diffusion. However, as the underlying microstructure within the grey matter is heterogeneous and unorganized, and diffusion uniformly restricted, the Gaussian model may have limited sensitivity in these regions. Tissue characterization of the underlying microstructure may thus benefit from a diffusion model accounting for heterogeneous compartmentalization at the sub-voxel level. Published studies using advanced modelling on PD data are few, however studies using the diffusion kurtosis imaging technique (DKI) [29], which quantifies the degree of heterogeneity of the tissue, have suggested improved MR-based diagnosis of the basal ganglia and substantia nigra of the grey matter [10, 19, 29–32].

An extended diffusion modelling technique named Restriction Spectrum Imaging (RSI) [33] uses high-angular DWI data obtained across a range of b-values to separate intracellular restricted diffusion from hindered diffusion across different length scales and tissue geometrics. RSI has been shown to give valuable information on tissue cellularity, and for neurological diseases it may allow within-voxel characterization of neurite densities and organization [34–36]. The technique has, however, never been used to study PD pathology prior to this study. As brain-stem and cortical pathology may be sites affected early in Lewy Body disease, and MRI techniques with sufficient specificity and sensitivity for PD pathology at these sites have not been developed, the aim of this study was to evaluate both DTI and RSI measurements and their ability to detect potential PD in these regions. By isolating the restricted diffusion volume fraction from the hindered diffusion, the RSI model allows us to investigate restricted compartments separately, which may hold important information about the underlying tissue microenvironment not available using DTI.

Materials and methods

Study population

The study was approved by the Ethical Committee of the Medical Faculty of Masaryk University in Brno, Czech Republic, with approval number: 32/2014. All participants signed an informed consent form.

For the purpose of this study a total of 100 individuals were enrolled based on published research criteria [1], of them 46 PD patients (age: 63.1 ± 9.4, 14 females, details in Table 1) and 54 healthy controls (HC) (age: 67.2 ± 7.2, 37 female) (Details in Table 1). All were examined by both an experienced neurologist and an experienced neuropsychologist using a detailed neuropsychological cognitive test battery [37, 38]. Subjects with any diagnosed psychiatric disorder, dementia or subjects with alcohol/drug abuse were excluded from the study. All patients were longitudinally followed at the First Department of Neurology, Masaryk University and St Anne's Hospital, Brno, Czech Republic.

MRI

MRI was carried out on a 3T Siemens Prisma scanner with a 64-channel receiving head-neck coil. T1 images were acquired by Ultrafast Gradient Echo 3D (MPRAGE) sequence with TR 2300 ms, TE 2.36 ms, TI 900 ms Echo, voxel size 1x1x1 mm, FOV 256x256x240 mm, PAT 2 GRAPPA, flip angle 8°, Turbo factor 240. For DWI EPI based sequence we used TR 9300 ms,

Table 1. Demographics and clinical characteristics.

PD	
Number of subjects	46
Age at examination, years	63.1 ± 9.4, range 43–86
Females	14 (30.4%)
Years with symptoms	5.5217, range 1–20
UPDRS3	17.7826, range 5–40
HC	
Number of subjects	54
Age at examination, years	67.2 ± 7.2, range 47–81
Females	37 (68.5%)

Demographics and clinical characteristics of the study population with n = 100

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TE 97 ms, voxel size 2x2x2 mm, FOV 228x228x130 mm (matrix 114x114x65, with fat suppression), EPI factor 114, PAT 2 GRAPPA, Diffusion mode MDDW with 1 b = 0 image and 30 non-collinear diffusion gradient directions for each diffusion weightings of b = 500, 1000, 2000, 4000 s/mm^2. In addition, a single SE-EPI volume was acquired at b = 0 and with opposite phase direction to allow for EPI-distortion correction [39].

Image processing and statistical analysis

Image processing and model estimation. All image processing, except the RSI analysis, was carried out using the FMRIB Software Library v5.0 (FSL) [40] and Matlab R2015b (MathWorks, Natick, Massachusetts).

Brain extraction, EPI-distortion, eddy-current and movement corrections were done using the *bet*, *topup* and *eddy* toolboxes in FSL. The quality of all corrected output data was evaluated by visual inspection. A standard tensor model was fit to the DW-data using *dtifit* from the FSL toolbox [41]. Here, the DTI model was modelled using b = 0 and b = 1000 s/mm^2. The descriptive diffusion parameters FA, MD, Axial (AD) and Radial Diffusivity (RD) were derived. RSI models the high-angular DW data based on the parameterization of the fiber orientation density function (FOD) using 4th order spherical harmonics, combined with an axially-symmetric Gaussian model [33]. The RSI spectrum was fit to the DW data using least-squares estimation with Tikhonov regularization. The regularization constant was determined based on careful evaluating the root mean square deviation of the modelled signal attenuation compared to the measured signal attenuation over a range of constants. The RSI spectrum was defined as a combination of anisotropic, restricted and free/hindered diffusion compartments as detailed by White et al [33]. Here, the longitudinal diffusivity (D_L) is fixed to $3.4{\cdot}10^{-3}$ mm^2/s and the transverse diffusivity (D_T) was varied from 0 mm^2/s to D_L in 10 equally spaced steps. In addition, two extra terms were added to the model; spherically restricted water diffusion defined as $D_T = 0$ and $D_L = 0$, and free water diffusion defined as $D_T = D_L = 3.4{\cdot}10^{-3}$ mm^2/s. For the purpose of this study we focused the analysis on the water signal fraction from the spherically restricted diffusion compartment (cellularity index; CI) and the water signal fraction of anisotropic restricted diffusion by summing the scales of $D_T/D_L = 0$ and $D_T/D_L = 0.225$, assumed to reflect relative density of neuronal processes (defined here as "nurite density"; ND) [33]. RSI modelling was performed in Matlab.

Co-registration. To prepare for voxel-wise and ROI based analysis, all structural and DW parametric maps were co-registered into a common space using the FSL software library. First, all T1 weighted (T1w) images were grey matter (GM) segmented and registered to the MNI 152 standard space using non-linear registration [42]. A carefully chosen selection of gender and age matched structural images from both groups of patients and controls were averaged to create a study-specific GM template [43]. All individual T1w images were subsequently registered to the GM template. All the b = 0 images from the DWI set were registered to the corresponding T1w image using affine 12 degrees of freedom registration, and further registered to the common average GM template using the same T1w-to-GM template transformation. All diffusion parametric maps derived from the DWI set (FA, MD, AD, RD, CI and ND) underwent the same transformations derived for the b = 0 images. The co-registered diffusion parametric maps were lastly smoothed using Gaussian kernel of FWHM = 3 mm.

Defining regions of interest. The main focus in this study was assessing group differences in the subcortex and brainstem. For this purpose, voxel-wise analysis was performed within the brainstem (2743 voxels), thalamus ((left; right) of 758 and 752 voxels respectively), caudate (left; right 193; 198 voxels), palladium (left; right 101; 111 voxels), putamen (left; right 443; 431

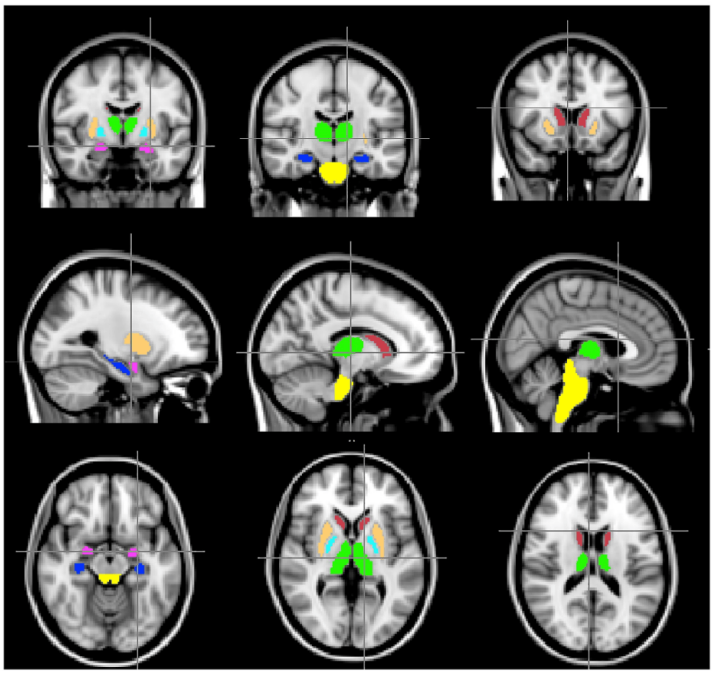

Fig 1. ROIs evaluated in the study. Thalamus (green), hippocampus (blue), brainstem (yellow), amygdala (magenta), palladium (beige), caudate (dark red) and putamen (cyan).

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voxels) and amygdala (left; right 95; 114 voxels). We also examined the hippocampus (left; right, 214; 183 voxels) as cognitive impairment is frequent in PD and we have previously demonstrated smaller hippocampi in PD as compared to controls [44]. All ROIs analysed in the study were defined and implemented by the FSL Harvard-Oxford subcortical structural atlas and are displayed in Fig 1. The ROIs were probabilistically defined and thresholded at 80% (i.e > 80% likelihood of a voxel analysed being within the specific region).

Statistical analysis. Voxel-wise group differences for each diffusion parameter were analyzed within each ROI by applying general linear modelling (GLM) using *randomise*, a FSL statistical tool. *Randomise* allows for permutation-based non-parametric testing using cluster-based thresholding to correct for multiple comparisons across space. *Randomise* was applied to the co-registered and smoothed parametric maps using 5000 permutations and a cluster-based correction threshold of $t > 2.3$. Age was regressed out for all analyses. Due to a small sample size there was a slight mismatch in the distribution of genders between the control group and the PD group (Table 1), hence gender was *not* regressed out in this analysis. We recognize this as a limitation. To account for the difference in separability between the different parameters, we estimated the degree of separability (AUC; Area Under Curve) between groups in significant regions using Receiver Operator Characteristics (ROC).

Results

We found that that both DTI-derived and RSI-derived metrics are sensitive to group effects with significant clusters ($p < 0.05$) in the thalamus, the hippocampus and the amygdala manifested as increased CI, MD, RD and AD and decreased ND in the PD group. CI was also significantly increased in the brainstem of the PD group. The AUC was largest using CI (0.66–0.69) with a larger group difference (~ 8%) compared to the remaining metrics (~ 0.55, ~ 3%), suggesting that CI may be more sensitive to pathological changes in the PD group.

The significant results of the voxel-wise analysis and corresponding AUCs are summarized in Table 2. Here, mean values of each group and relative group difference (PD/HC), absolute mean values of each group, AUC and relative number of significant voxels ($p < 0.05$) compared to the full ROI size (100 * # significant voxels/ # voxels in the ROI) within each cluster are displayed. Only ROIs with significant group difference are shown. Clusters with significant group differences after accounting for multiple comparisons ($p<0.001$) are denoted with an asterisk (*). Fig 2 displays clusters of significant group difference at $p < 0.05$, and $t > 2.3$. Only CI was sensitive to group differences in the brainstem, significant clusters are displayed in Fig 2A. Fig 2B–2E show significant clusters using ND, AD and CI for the sub cortical regions; thalamus, hippocampus and amygdala. Significant regions using the remaining metrics were similar in size to the regions depicted here (see statistical summary in Table 2).

Discussion

In this study, we investigated two diffusion-based MRI techniques and their sensitivity to microstructural changes within brain regions associated with motor function in PD. Group differences between a PD patient group and an age-matched HC group have been analyzed using both standard DTI-derived metrics (assuming Gaussian diffusion), and diffusion metrics describing the restricted non-Gaussian diffusion fraction derived from the RSI model.

In summary, we found that both diffusion-based models were sensitive to group specific differences in subcortical brain regions known to be prone to pathology in PD. Our results further indicate that CI and ND may improve detection of between group differences, and provide additional information when combined with the remaining diffusivity measurements.

DTI-derived parameters are estimated based on a Gaussian diffusion model, and is commonly associated with extracellular, hindered water diffusion. Increased RD and AD (and MD) is typically considered an effect of atrophy, demyelination or loss of structural organization due to an increased extracellular space [45–49]. Our findings are consistent with previous reports of increased diffusivity in regions known to be affected by PD pathology. In contrast, the RSI-derived parameters CI and ND are assumed to reflect the signal fraction from isotropic and anisotropic restricted diffusion pools associated with intracellular space, hence changes in these metrics may be related to microstructural and axonal changes. As ND reflect the fraction of restricted anisotropic diffusion, assumed related to the neurite density [33, 50], decreased ND might implicate microstructural damage of- or related to the neurons. Increased CI, interpreted as increased isotropic restriction, may be related to glia infiltration or activation, as previously shown in the PD brainstem [3, 51]. Although the true biological interpretation of either is currently vague, the findings fit well with the current understanding of ND and CI biological underpinnings, and the understanding of PD pathophysiology. We argue that changes measured using the two models reflect different aspects of neurodegeneration and that the RSI model hold relevant information about the underlying tissue microenvironment not available using DTI. The RSI technique (or other non-Gaussian models) may be considered a supplementary model, contributing a fuller picture of the underlying pathology.

Table 2. Differences between patients (PD) and healthy controls (HC).

	AD	MD	RD	ND	CI
Brainstem					
PD/HC	-	-	-	-	1.075* *(PD: 0.17 ± 0.03) (HC: 0.16 ± 0.03)*
AUC					0.69
%					21.60%
Right thalamus					
PD/HC	1.018 *(PD: 1.35 ± 0.30) (IIC: 1.33 ± 0.30)*	1.020 *(PD: 1.18 ± 0.30) (IIC: 1.16 ± 0.30)*	1.022 *(PD: 1.17 ± 0.35) (IIC: 1.14 ± 0.35)*	0.97* *(PD: 0.62 ± 0.13) (IIC: 0.63 ± 0.13)*	1.071 *(PD: 0.12 ± 0.03 (IIC: 0.11 ± 0.03)*
AUC	0.55	0.55	0.55	0.59	0.67
%	19.70%	16.20%	15.80%	41.10%	31.80%
Left thalamus					
PD/HC	-	-	-	0.978* *(PD: 0.69 ± 0.10) (HC: 0.70 ± 0.10)*	1.071 *(PD: 0.12 ± 0.03) (HC:0.11 ± 0.03)*
AUC				0.59	0.67
%				30.90%	27.40%
Right hippocampus					
PD/HC	1.035 *(PD: 1.35 ± 0.20) (HC: 1.31 ± 0.20)*	1.037 *(PD: 1.16 ± 0.20) (HC: 1.11 ± 0.20)*	1.040 *(PD: 1.05 ± 0.40) (HC: 1.01 ± 0.30)*	0.967* *(PD: 0.58 ± 0.11) (HC: 0.60 ± 0.10)*	-
AUC	0.55	0.54	0.54	0.55	
%	45.54%	50.82%	54.10%	48.63%	
Left hippocampus					
PD/HC	1.039 *(PD: 1.33 ± 0.30) (HC: 1.28 ± 0.20)*	1.041 *(PD: 1.14 ± 0.20) (HC: 1.09 ± 0.20)*	1.045 *(PD: 1.05 ± 0.50) (HC: 1.00 ± 0.30)*	0.964 *(PD: 0.57 ± 0.11) (HC: 0.59 ± 0.10)*	-
AUC	0.54	0.54	0.53	0.55	
%	21.03%	19.16%	17.29%	15.89%	
Right amygdala					
PD/HC	1.022* *(PD: 1.23 ± 0.20) (HC: 1.21± 0.20)*	1.022* *(PD:1.03 ±0.20) (HC:1.01± 0.20)*	1.022* *(PD:0.94 ± 0.40) (HC: 0.92± 0.40)*	0.973* *(PD: 0.63 ± 0.14) (HC: 0.64 ± 0.13)*	1.084 *(PD: 0.05 ± 0.001) (HC: 0.05 ± 0.01)*
AUC	0.55	0.55	0.55	0.57	0.66
%	47,37%	52,63%	50%	88,60%	17.54%
Left amygdala					
PD/HC	1.025* *(PD: 1.28 ± 0.20) (HC: 1.25 ± 0.20)*	1.029* *(PD:1.04 ± 0.20) (HC: 1.01± 0.20)*	1.034* *(PD: 0.85 ± 0.40) (HC: 0.92 ± 0.40)*	0.975* *(PD:0.60 ± 0.09) (HC:0.61 ± 0.09)*	-
AUC	0.58	0.59	0.60	0.57	
%	82.11%	89.47%	86.32%	37.89%	

The results of the voxel wise analysis, showing relative group difference (PC/HC), along with mean absolute value for each group, measured separability (AUC) and the percentage of significant voxels ($p<0.05$) compared to the full ROI size for each ROI and diffusion metric.

'-' denotes when there is no significant group difference.

'*' denotes parameters that were significant when accounting for multiple comparisons ($p<0.001$). The DTI-metrics are in the unit $10^{-3}mm^2/s$ while the RSI-metrics are unitless.

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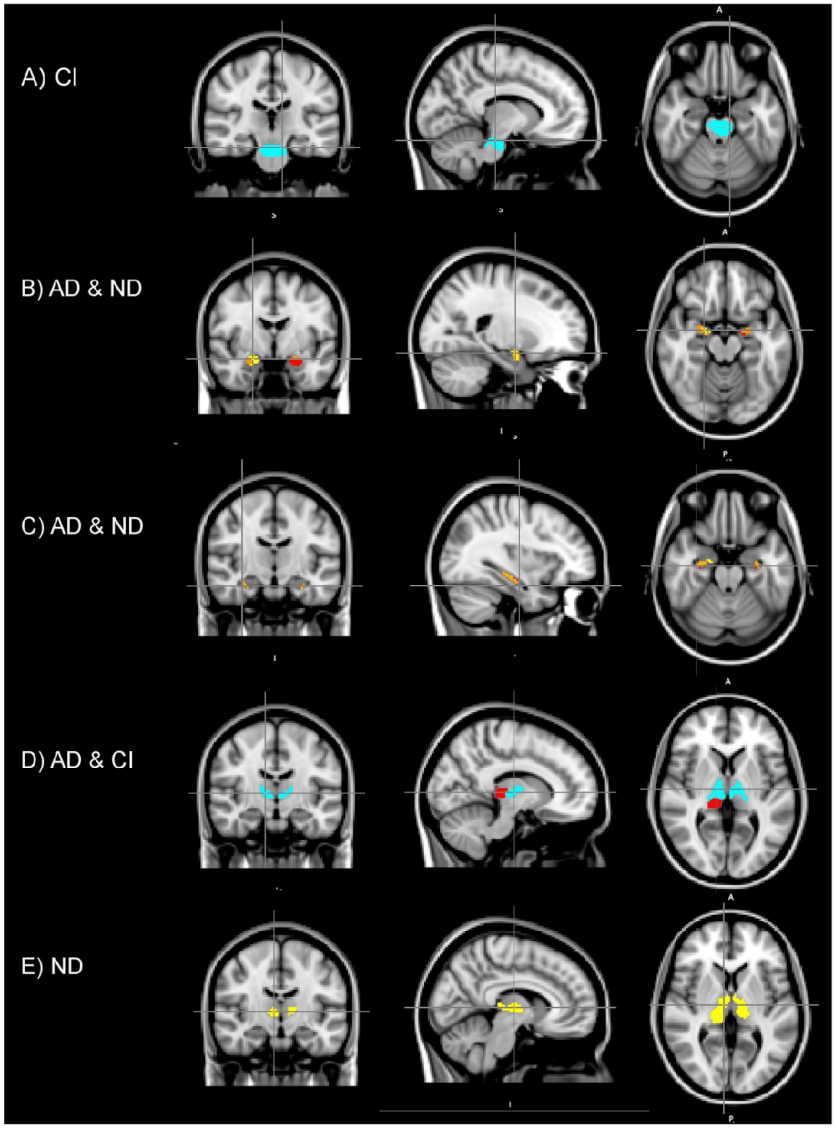

Fig 2. Results of the voxel-wise group analysis. Brainstem (A), the amygdala (B), the hippocampus (C) and the thalamus (D, E). Images display clusters of voxels where group differences are significant ($p<0.05$). Here, CI and AD were increased, and ND was decreased in the patient group in the respected clusters. A) The cluster in the brainstem where CI were increased. C) AD (red) compared with ND (yellow), overlapping regions of significance (orange). D) AD (red) compared with ND (yellow) and joint (orange) C) AD (red) and CI (magenta), no overlapping voxels. D) ND (yellow).

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Neurodegeneration of thalamic regions is known to occur in association with loss of dopaminergic function in the basal ganglia in PD and there has been substantial evidence of PD pathology within the thalamus [52]. Previous studies have reported increased MD and decreased FA as a result of PD pathology within the thalamus [13, 28]. In this study, we found no evidence that FA significantly differed between groups, however, diffusivity (MD, RD and AD) was increased in the thalamus of the PD group. Furthermore, we found that CI and ND were changed in the PD group, suggesting loss of microstructural and neuronal integrity in the thalamus. Similar results were found in the amygdala and hippocampus as well, suggesting that all these regions experience some extent of neurodegeneration at the microstructural level in addition to demyelination and loss of structural organization.

As mentioned in the introduction, despite reports suggesting that FA may be a sensitive biomarker for tracking pathological changes within e.g. the substantia nigra [10–17], other reports have contradicted these findings [18–20]. As the subcortical regions are structurally heterogeneous and unorganized, however, neurodegeneration would likely occur correspondingly. As a consequence, MD, AD and RD should be more sensitive to degeneration and damage of these regions compared to FA, which is also in accordance with our analysis. Regardless, as our results are limited by a lower image resolution of the brainstem compared to other previous studies of the substantia nigra, our study cannot confirm nor contradict previous findings. Using an adapted high-resolution DTI protocol may improve sensitivity for detecting pathology particularly within the brainstem.

No significant group difference was found within the pallidum or striatum using either of the techniques. As the organization of neurons in the basal ganglia is known to be atypical and less myelinated compared to white matter regions, the DTI-derived metrics may simply be insensitive to changes in these regions. Similarly, RSI measures did not reveal group differences in these regions either, which may be due to variations between basal ganglia neuropathology and neuropathology at other PD predilection sites, e. g. in terms of glia activation, or aspects of neurodegeneration.

Limitations of the present study include potential influence of anti-Parkinson medication and neurostructural differences related to the sex, and years with PD diagnosis which were not used as regressors due to the small sample size. Also, longitudinal clinical and imaging data is not available at present but is planned for a future work. To avoid overfitting, ROI volume size was not accounted for in the primary GLM analysis as a multivariate analysis showed that diffusion metrics were more strongly linked to group differences compared to the ROI volume, and average volume size within a group did not significantly differ between groups. In parallel, however, we repeated the GLM analysis including volume as variable. These results yielded similar results as the results stated above, but significant clusters were slightly smaller in size as a result of volume effects. Although the presented approach has potential limitation, we argue that it does not interfere with our hypothesis that RSI provides supplementary information about tissue microstructure and pathology as both RSI and DTI are introduced to the same bias, whereas differences between the two remain the same.

Parkinson pathology is generally varied, both during the disease course and between affected sites in an individual patient. In the absence of major atrophy, our study suggest that diffusion techniques may reveal microstructural pathology and protocols for MRI diffusion imaging of PD may be adapted to more sensitive detection of pathology at different CNS sites.

Conclusions

In conclusion according to our analyses, both DTI and RSI are sensitive to PD pathology. Changes measured using the two models reflect different aspects of neurodegeneration and

should be considered complimentary techniques. Adapting protocols for more sensitive detection of PD pathology may improve detection and understanding of pathology.

Author Contributions

Conceptualization: Anders Dale, Atle Bjørnerud, Tormod Fladby.

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Formal analysis: Tuva R. Hope, Per Selnes.

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Methodology: Anders Dale.

Project administration: Atle Bjørnerud, Tormod Fladby.

Resources: Irena Rektorová, Lubomira Anderkova, Nela Nemcova-Elfmarkova, Zuzana Balážová.

Software: Tuva R. Hope, Anders Dale.

Supervision: Atle Bjørnerud, Tormod Fladby.

Validation: Tuva R. Hope.

Visualization: Tuva R. Hope.

Writing – original draft: Tuva R. Hope, Per Selnes, Tormod Fladby.

Writing – review & editing: Tuva R. Hope, Per Selnes, Irena Rektorová, Lubomira Anderkova, Nela Nemcova-Elfmarkova, Zuzana Balážová, Anders Dale, Atle Bjørnerud, Tormod Fladby.

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4. Patterns of Diffusion Kurtosis Changes in Parkinson's Disease Subtypes

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Commentary.

This study continued the investigation of neuroimaging heterogeneity in PD by focusing on DKI. PD is described as a heterogeneous disorder, and the study was performed to determine whether this clinical heterogeneity is reflected in distinct patterns of microstructural brain abnormalities. We hypothesized that DKI measures, which are sensitive to non-Gaussian water diffusion and tissue complexity, would reveal subtype-specific alterations not detectable using conventional diffusion measures alone. The results showed DKI differentiated cognitively normal PD from HC with 81% accuracy and PD with MCI from cognitively normal PD with 78% accuracy, revealing distinct gray- and white-matter microstructural alterations between these groups. The novelty of the study lies in applying advanced diffusion imaging to the problem of Parkinsonian heterogeneity. In relation to previous work, it represented a direct methodological continuation of the DTI and restriction spectrum study while expanding the focus toward disease stratification. The results contributed to subsequent research aimed at identifying imaging biomarkers of clinical progression and cognitive impairment.

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Patterns of diffusion kurtosis changes in Parkinson's disease subtypes

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ABSTRACT

Background: Diffusion kurtosis imaging has been applied to evaluate white matter and basal ganglia microstructure in mixed Parkinson's disease (PD) groups with inconclusive results.
Objectives: To evaluate specific patterns of kurtosis changes in PD and to assess the utility of diffusion imaging in differentiating between healthy subjects and cognitively normal PD, and between PD with and without mild cognitive impairment.
Methods: Diffusion scans were obtained in 92 participants using 3T MRI. Differences in white matter were tested by tract-based spatial statistics. Gray matter was evaluated in basal ganglia, thalamus, hippocampus, and motor and premotor cortices. Brain atrophy was also assessed. Multivariate logistic regression was used to identify a combination of diffusion parameters with the highest discrimination power between groups.
Results: Diffusion kurtosis metrics showed a significant increase in substantia nigra ($p = 0.037$, Hedges' $g = 0.89$), premotor ($p = 0.009$, Hedges' $g = 0.85$) and motor ($p = 0.033$, Hedges' $g = 0.87$) cortices in PD with normal cognition compared to healthy participants. Combined diffusion markers in gray matter reached 81% accuracy in differentiating between both groups. Significant white matter microstructural changes, and kurtosis decreases in the cortex were present in cognitively impaired versus cognitively normal PD. Diffusion parameters from white and gray matter differentiated between both PD phenotypes with 78% accuracy.
Conclusions: Increased kurtosis in gray matter structures in cognitively normal PD reflects increased hindrance to water diffusion caused probably by alpha-synuclein-related microstructural changes. In cognitively impaired PD, the changes are mostly driven by decreased white matter integrity. Our results support the utility of diffusion kurtosis imaging for PD diagnostics.

1. Introduction

Parkinson's disease (PD) is a synucleinopathy characterized by misfolded alpha-synuclein accumulation in Lewy bodies and Lewy neurites, alpha-synuclein-related activation of microglia and neuroinflammation, and brain degeneration reflected by gross brain atrophy [1–3], although other comorbid brain pathologies such as vascular changes or Alzheimer's disease-related pathology may also co-occur [1]. Both animal and human studies [4–6] have suggested that diffusion tensor imaging (DTI), and particularly diffusion kurtosis imaging (DKI), may well serve as early biomarkers of white matter (WM) and gray matter (GM) microstructural brain changes in PD. Increased kurtosis is an indicator of the heterogeneous environment with restrictions to diffusion. The mean kurtosis (MK) is a measure of diffusion kurtosis in the voxel. The axial kurtosis (AK) is the kurtosis along the direction of axons, and radial kurtosis (RK) describes kurtosis in a perpendicular direction. A recent review and meta-analysis of the results of conventional DTI findings in PD [7] reported specific changes in fractional anisotropy (FA) and/or mean diffusivity (MD), particularly within the substantia nigra (SN), the corpus callosum, and the cingulate and temporal cortices, as well as in the corticospinal tract.

A clear limitation of a DTI model is the oversimplified assumption that the probability of water diffusion follows the Gaussian distribution. The complexity of the brain tissue leads to serious alterations from this pattern, which is particularly relevant to GM [4,8,9]. Unlike conventional DTI, DKI allows for quantification of the non-Gaussian diffusion as

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it uses multi-shell acquisition [8]. Therefore, DKI may provide more precise information about the microstructural properties and both GM and WM tissue heterogeneity [4,8,9]. Having said that, only few studies have used DKI to assess WM and subcortical GM changes in PD as compared to healthy controls (HC) so far with inconsistent results [6,10,11]. The inconsistency in the direction of DKI parameters changes may stem from the small sample sizes and from the fact that the recruited subjects were not cognitively examined in detail, and thus may have represented heterogeneous groups of PD subjects with normal cognition (PD-NC) and with MCI (PD-MCI). Another potential source of inconsistent results may arise from different statistical approaches employed, since the diffusion MRI is lacking a golden standard for the data analysis [12].

Therefore, in our cross-sectional study we focused on the evaluation of microstructural changes in PD with various clinical subtypes (PD-NC, PD-MCI) as compared to HC. Having been motivated by our translational DKI research in animal models of PD [4,13,14] we used the DKI parameters to measure changes particularly in GM within the basal ganglia, hippocampus and motor (M1)/premotor (PMC) cortical areas, i.e. the cortical output areas of the motor striato-thalamo-cortical loop [15]. Diffusion changes in the WM skeleton were also assessed. In order to keep the results comparable with previous DTI studies, we additionally used the conventional DTI approach with single-shell acquisition.

The main goal was to describe specific patterns of DKI/DTI changes in PD-NC and to evaluate whether DKI can serve as a diagnostic biomarker of this PD phenotype. We hypothesized that we would be able to identify specific patterns of DKI changes particularly in our subcortical and cortical GM regions of interest (ROIs). The secondary objective was to describe microstructural correlates of MCI in PD and evaluate how accurately diffusion MRI can identify the PD-MCI subtype in the PD population. This is clinically relevant since MCI in PD is a clear risk factor for PD-dementia and a more malignant disease course [1,16]. In line with the literature, we suspected decreased integrity of WM along with distinct cortical brain atrophy in PD-MCI [2,7].

2. Patients and methods

2.1. Subjects in the cohort

We analyzed DKI, DTI, and structural MRI data of PD patients and HC from a previously published cohort [17–19]. In brief, PD patients were recruited based on criteria of established PD-MCI [16] and PD [20]. All subjects underwent cognitive testing covering all cognitive domains and an MRI examination (for details see below). Exclusion study criteria included alcohol/drug abuse, hallucinations or visual misperceptions, and any diagnosed psychiatric disorder. None of the patients was taking any psychoactive drugs. Each subject signed an informed consent form and the study was approved by the local ethics committee.

2.2. Neuropsychological assessment

A cognitive test battery described in our previous publications [18,19] was used. For a description of the cognitive test battery, see Supplementary Materials. The cognitive domain Z-scores were computed as the average Z-scores of the tests included in the particular domain [19] and each domain was inspected for cognitive decline. Subjects who scored below −1.5 SD in two tests in at least one cognitive domain but with preserved independence in functional abilities were categorized as PD-MCI.

2.3. MR acquisition

Imaging was performed on a 3T Siemens Prisma scanner (Siemens Corp., Erlangen, Germany) at CEITEC, Masaryk University, Brno, Czech Republic. T1 imaging was performed with MPRAGE sequence with 240 sagittal slices, TR = 2300 ms, TE = 2.36 ms, FOV = 256 mm, FA = 8°, matrix size 256 × 256, voxel size 1 × 1 × 1 mm. DKI sequence with the following parameters was used: 30 non-colinear diffusion directions for each of b-values 500, 1000, 2000, and 4000 s/mm^2 and 10 acquisitions without diffusion weighting (b = 0 s/mm^2), TR = 9300 ms, TE = 97 ms, FOV = 228 mm, acquisition matrix 114 × 114 × 64, voxel size 2 × 2 × 2 mm. Three acquisitions with b = 0 s/mm^2 with opposite phase polarity were acquired. The total acquisition time for the diffusion MRI was 21:31.

2.4. DKI/DTI data preprocessing

FSL software version 5.0.9 was used for the preprocessing. Data were first corrected for susceptibility-induced distortions using the topup tool and for movement and eddy current artifacts using the eddy tool. Non-brain tissue voxels were automatically excluded using the Brain Extraction Toolbox. The output brain masks were checked one by one and corrected manually when needed.

To model the kurtosis tensor in DKI data, the Diffusional Kurtosis Estimator software (https://www.nitrc.org/projects/dke) implemented in MATLAB (The MathWorks, Inc.) was used. The robust fitting option using the robust estimation of tensor by outlier rejection algorithm was used. Parameters of interest were fractional anisotropy (DKI_FA), MK, RK, and AK.

The DTI dataset contained single-shell data (b = 1000 s/mm^2). FSL function DTIFIT was used to model the diffusion tensor of each voxel in the DTI data and parametric maps were calculated. Parameters of interest were DTI_FA, MD, radial diffusivity (RD), and axial diffusivity (AD).

2.5. DKI/DTI data analysis in our subcortical and cortical GM ROIs

Masks of our subcortical ROIs, i.e. bilateral: putamen, caudate nucleus, globus pallidus and thalamus, and of the hippocampus were calculated using T1-weighted images in FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu). The mean SN mask was also created, for details see Supplementary Materials and Figure S1. The mask of cortical GM was obtained using the SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12) segmentation tool. Masks of the cortical ROIs were obtained from the Juelich histological atlas. For each subject, the mask of cortical GM was multiplied by masks of M1 and PMC. All subjects' resulting masks were visually inspected for segmentation errors and the resulting mask was registered to each subject's native diffusion-weighted space.

To identify differences between group pairs (PD-NC vs. HC, PD-MCI vs. PD-NC, PD-MCI vs. HC), the non-parametric permutation based approach using the permutation analysis of linear model (PALM) tool in FSL and general linear model was used. The number of permutations was set at 5000. Data were controlled for the effects of age, gender, education, and LED (just for the PD groups). The $\alpha = 0.05$ was set as the significance level. Effect sizes (ES) were additionally calculated using the Hedges' g (for details about the formula, see Supplementary Material).

2.6. DKI/DTI data analysis of WM: exploratory analysis using TBSS

After the above-mentioned preprocessing and quality control, the data underwent tract-based spatial statistics (TBSS) analysis [21].

We first performed a voxel-wise nonlinear registration of all subjects' FA maps to the chosen target. The FMRIB58_FA_1 mm image was used as a target. The whole dataset was then affine-transformed to MNI152 standard space with 1x1x1 mm resolution. The FA image was created and the skeleton of WM tracts common for all subjects was calculated at a threshold of 0.2. Individual FA maps of all subjects were projected onto the skeleton. Other parametric maps were handled similarly. They were nonlinearly registered to a common space and then projected onto the FA skeleton.

The voxel-wise statistics across subjects were calculated using the general linear model and non-parametric permutation test by the FSL Randomise tool. The number of permutations was set at 5000 and the threshold-free cluster enhancement method was used. Data were controlled for the effects of age, gender, education, and LED (just for the PD groups). The $\alpha = 0.01$ was set as the significance level. Regions showing significant differences between groups were defined using the JHU ICBM-DTI-81 W M labels atlas and the Juelich histological atlas provided by FSL.

2.7. Deformation-based morphometry

The DBM analysis [22] was conducted using the Computational Anatomy Toolbox (CAT 12, http://dbm.neuro.uni-jena.de/cat/) and SPM12 software running under MATLAB2014b. The Jacobian determinant for each voxel in normalized space was calculated for each subject and the resulting maps were spatially smoothed using the Gaussian filter kernel with a FWHM of 6 mm. Smoothed Jacobian determinant volumes were compared across groups by applying a two-sample *t*-test. Results were controlled for age, gender, education and LED (just for the PD groups). The FDR correction was used and the $p_{FDR} = 0.05$ was set as the significance level.

2.8. Differential analysis

To identify an information-rich, parsimonious combination of parameters with the highest discrimination power between the groups, we employed the multivariate class-weighted logistic regression with L1 regularization (LASSO). The DKI and DTI parameters from all GM ROIs and WM skeleton served as the input features. Before training, all features were standardized on a per feature basis to have zero mean and the standard deviation of one. To get the optimal trade-off between the models' complexity and the ability to generalize, we fine-tuned the models' regularization strength parameter via the grid-search technique over a grid of 100 numbers evenly distributed on a logarithmic scale: <log10(-2)– log10(0.25)>. The grid-search was set-up to use the stratified 5-fold cross-validation with 20 repetitions aiming at optimizing Matthew's correlation coefficient. Finally, we used the Receiver Operator Characteristics (ROC) curve to fine-tune the trained models' decision thresholds to achieve the optimal trade-off between the models' sensitivity and specificity. Using the fine-tuned decision thresholds, we evaluated the models' classification performance using the stratified 5-fold cross-validation. The ROC curves were plotted using the predicted labels' probabilities obtained via the cross-validation procedure employed during the final evaluation of the trained models. Resulting regression coefficients were considered as feature importances. For the analysis, we used the Python programming language (v. 3.7) and the scikit-learn (https://scikit-learn.org/stable/) machine learning library (v. 0.22.2).

3. Results

3.1. Demographic, clinical, and cognitive data

The cohort including HC and PD subjects consisted of 107 participants. Altogether 15 subjects were excluded from the diffusion data analysis: 12 subjects (10 HC, 2 PD-MCI) were excluded because of excessive movement or susceptibility artifacts in the MR data; 3 subjects (1 HC, 2 PD-MCI) did not finish the MR protocol. For detailed demographic and clinical information on the final dataset, which consists of 92 subjects, see Table 1.

There was a significant difference in age and gender distribution between HC and PD groups. No difference was observed in time from diagnosis between the PD-NC and PD-MCI groups. Therefore, our PD groups reflect different PD phenotypes (with and without MCI) rather than different stages of PD progression.

Table 1
Demography and clinical characteristics: all values are depicted as mean ± standard deviation. MMSE – Mini mental state examination; GDS – Geriatric depression scale; UPDRS III - Unified Parkinson's Disease Rating Scale, part III (Motor Examination); LED – Levodopa equivalent dose; H&Y score - Hoehn & Yahr scale; * marks differences that remained significant after FDR correction.

	HC	PD-NC	PD-MCI	PD-NC versus HC (p-value)	PD-MCI versus PD-NC (p-value)	PD-MCI versus HC (p-value)
N	48	23	21	–	–	–
Gender (M/F)	16/32	17/6	16/5	0.001*	0.862	0.001*
Age	66.7 ± 7.5	61.0 ± 8.4	64.8 ± 10.7	0.008	0.145	0.648
Years of education	15.6 + 2.6	16.2 + 3.3	14.5 + 3.0	0.472	0.045	0.046
MMSE	28.5 ± 1.2	28.2 ± 1.2	26.9 ± 2.0	0.234	0.018*	<0.001*
GDS	2.46 ± 2.7	2.82 ± 2.9	2.76 ± 2.2	0.549	0.774	0.357
UPDRS III	–	17.6 ± 8.7	17.5 ± 8.6	–	0.832	–
LED	–	822.5 ± 536.2	861.5 ± 486.2	–	0.655	–
H&Y score	–	1.82 ± 0.46	1.76 ± 0.53	–	0.745	–
Side of symptom onset (L/R/B)	–	13/9/1	6/14/1	–	0.167	–
Years from diagnosis	–	5.0 ± 3.1	5.5 ± 4.5	–	0.981	–
Cognitive domain Z-scores						
Memory	0.59 ± 0.49	0.24 ± 0.74	−0.63 ± 0.73	0.052	0.001*	<0.001*
Attention	−0.04 ± 0.65	−0.27 ± 0.91	−1.15 ± 0.94	0.212	0.004*	<0.001*
Executive	0.52 ± 0.75	0.35 ± 0.67	−0.60 ± 0.91	0.403	<0.001*	<0.001*
Visuospatial	0.41 ± 0.59	0.44 ± 0.55	0.01 ± 0.80	0.614	0.244	0.0826
Language	0.45 + 0.42	0.31 + 0.62	0.03 + 0.82	0.050	0.076	0.0032*

3.2. MRI data results

3.2.1. Evaluation of GM microstructure

Only differences between groups that revealed both statistical significance and large ES (Hedges' $g > 0.8$) or medium ES (Hedges' $g > 0.5$) are described in the text below. For all p-values, FSs and DKI/DTI values, see Supplementary Materials, Table S1 and Table S2.

3.2.1.1. PD-NC versus HC. In PD-NC as compared to HC we found increases of DKI_FA in the SN ($p = 0.037$, Hedges' $g = 0.89$), and AK in M1 and PMC ($p = 0.033$, Hedges' $g = 0.87$ and $p = 0.009$, Hedges' $g = 0.85$, respectively).

3.2.1.2. PD-MCI versus PD-NC. In PD-MCI as compared to PD-NC we found decreases of AK in M1 ($p - 0.01$, Hedges' $g - 0.85$), DKI_FA in thalamus ($p = 0.007$, Hedges' $g = 0.55$), MK in thalamus, M1 and hippocampus ($p = 0.015$, Hedges' $g = 0.50$, $p = 0.013$, Hedges' $g = 0.62$ and $p - 0.011$, Hedges' $g - 0.62$, respectively), RK in thalamus, M1 and hippocampus ($p = 0.006$, Hedges' $g = 0.57$, $p = 0.026$, Hedges' $g = 0.50$ and $p = 0.006$, Hedges' $g = 0.70$, respectively), AK in PMC ($p = 0.034$, Hedges' $g = 0.66$) and DTI FA in thalamus and hippocampus ($p = 0.002$, Hedges' $g = 0.76$ and $p = 0.018$, Hedges' $g = 0.60$, respectively).

Boxplots for significant differences with large ES (Hedges' $g \geq 0.8$) are shown in Fig. 1. Of note, in the PD-MCI group, the values of DKI parameters of interest were between those in the PD-NC and HC groups;

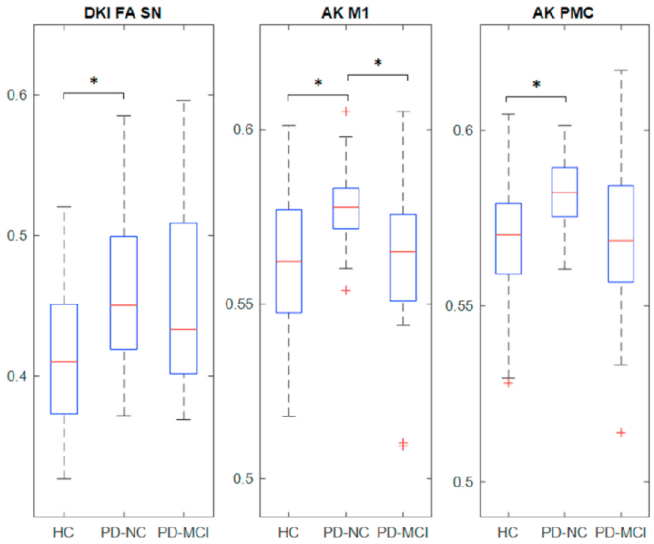

Fig. 1. Visualization of markers in GM; * marks comparisons that revealed both statistical significance and large effect size (Hedges' g > 0.8).

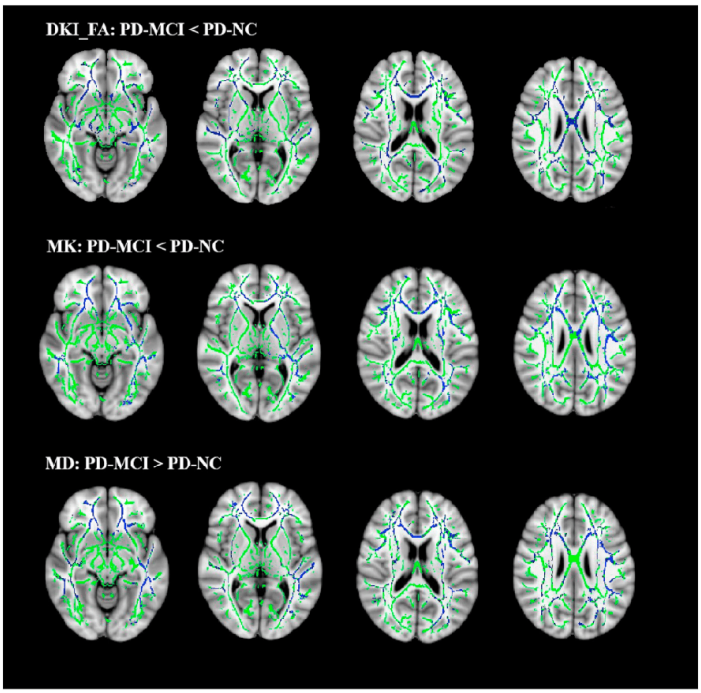

Fig. 2. TBSS results, contrast PD-MCI to PD-NC; blue – significant differences (level of significance 0.01); green – FA skeleton. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

see Fig. 1 and Supplementary Materials, Table S1.

3.2.1.3. PD-MCI versus HC. No differences in subcortical or cortical structures were detected.

3.2.2. Evaluation of WM microstructure

3.2.2.1. PD-NC versus HC. The TBSS revealed no significant differences between the two groups.

3.2.2.2. PD-MCI versus PD-NC/HC. TBSS revealed widespread DKI/DTI changes in the PD-MCI as compared to the PD-NC (see Fig. 2) and HC groups. See Supplementary Materials for all results.

3.2.3. Evaluation of brain atrophy using deformation-based morphometry

DBM revealed brain atrophy in the PD-MCI as compared to HC in the frontal pole bilaterally ($p_{FDR} = 0.05$), see Figure S2 in Supplementary Materials. No other differences were detected between the groups.

3.3. Differential analysis

We achieved 81% accuracy (ACC), 92% sensitivity (SEN), and 71% specificity (SPE) in discriminating between PD-NC and HC, the following features were selected by the logistic regression (feature importance/weights are in parentheses): L1_thalamus (2.53), DTI_FA_-palidum (2.49), DKI_FA_caudate (2.05), DTI_FA_putamen (1.72), AK_PMC (1.66), DTI_FA_caudate (1.49), RK_thalamus (1.26), DKI_FA_-putamen (1.20).

For discriminating PD–NC and PD–MCI we achieved 78% ACC, 83% SEN, and 74% SPE. The following features were selected by the logistic regression: MK_WM (0.43), DTI_FA_WM (0.33), AK_M1 (0.05). For ROC curves, see Fig. 3

4. Discussion

The PD pathology is complex and affects the brain tissue structure by various processes that are difficult to separate with MRI methods. In early PD, these include particularly alpha-synuclein accumulation [3], microglial activation [23], and axonal sprouting [24], all of which are processes that increase the restrictions to the diffusion in the environment. On the other hand, a more malignant course of the disease linked with early cognitive impairment is characterized by major neurodegeneration that presents with gross cortical brain atrophy and altered WM integrity [1–3,7] i.e. processes that lead to decreased heterogeneity of the brain tissue. Having said that, we propose DKI parameters as valuable translational diagnostic markers that seem to closely reflect distinct patterns of ongoing brain changes in various cognitive PD subtypes.

We first demonstrated that DKI reflects specific pathological processes in the brain of two different animal models of PD: a TNWT-61 genetic mouse model overexpressing human alpha-synuclein [4], and a toxic methamphetamine-induced PD mouse model [14]. The former model is characterized by alpha-synuclein accumulation in subcortical and cortical brain regions and microglial activation with no brain atrophy [25]; the latter model is characterized particularly by early degeneration of dopaminergic cells [26]. In the first model, we previously detected increased MK in subcortical GM structures and in the motor cortex [4]. These results are in line with the current findings of increased kurtosis of both subcortical and cortical GM in the PD-NC as compared to HC. Immunohistochemical examination of the GM structures of the scanned TNWT-61 mice revealed alpha-synuclein accumulation-induced changes [4]. Moreover, kurtosis parameters were positively correlated with the total alpha-synuclein signal in the thalamus [13]. On the contrary, early losses of dopaminergic cells induced by intraperitoneal administration of methamphetamine induced decreased heterogeneity of the brain tissue that were reflected by a decrease in kurtosis in the SN, striatum, and sensorimotor cortex [14]. This accords well with the current result of decreased M1 kurtosis in the PD-MCI as compared to the PD-NC group.

Using DKI in human PD, Kamagata et al. [6] were the first to show widespread decreases of MK in WM in a small sample of PD compared to HC (10 HC, 12 PD). Surova et al. [10] observed a similar direction of MK changes (decreases) in both WM and the subcortical GM, namely in the putamen. Conversely, Wang et al. [11] reported increased MK of the SN, caudate, putamen, and globus pallidus in the PD group as compared to HC. Of note, the PD subjects were not cognitively tested and therefore, the authors could not compare PD-NC and PD-MCI groups. As mentioned above, our patterns of kurtosis changes within cortical and subcortical ROIs in PD-NC and PD-MCI accord well with the DKI results in animal models of PD although results in mice models of PD and in humans cannot be directly compared. Our findings at least partially shed light on the inconsistencies of previous studies' results in PD patients.

In the PD-NC group, as compared to the HC group, we demonstrated significant increases of kurtosis parameters in the SN as well as in premotor and motor cortices, i.e. areas engaged within the motor basal ganglia-thalamo-cortical loop. Lewy body pathology in the SN that is prominent already at Braak stages III [27], or neural sprouting [24] may all play roles as potential causes of increased hindrance to water diffusion in the GM tissue. On the other hand, the motor cortex is not affected by Lewy body pathology until stage VI [27]. Nevertheless, early cellular alterations with alpha-synuclein aggregation without the formation of Lewy bodies [28] and increased neuronal spine turnover within M1 pyramidal neurons [29] may lead to increased heterogeneity of the tissue and account for kurtosis increases.

In PD-MCI, kurtosis values of the SN were between those in PD-NC and HC and did not significantly differ from either of the groups. In

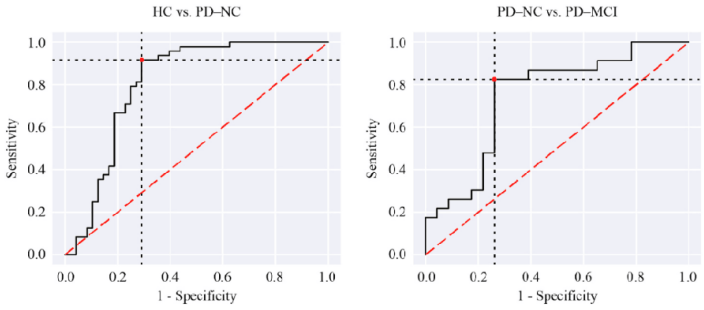

Fig. 3. Differential analysis results, ROC curves.

M1, the PD-MCI group showed significant decrease of kurtosis parameters as compared to PD-NC but they did not differ from the kurtosis values of HC. Similar results were detected also for PMC, thalamus and hippocampus with medium to large ES. These results may reflect an initial neurodegeneration of these areas in MCI patients that co-occurs with the abovementioned cellular changes, such that the positive and negative kurtosis deflection of each balances the other, resulting in kurtosis having almost "normal" values, comparable to those of HC. We cannot exclude other potential explanations for the findings such as an under-detection of kurtosis changes in PD-MCI in the present study, but this seems less probable.

Whereas no significant changes in WM were detected in the PD-NC group, the cognitively altered PD phenotype showed prominent changes in WM as compared to PD-NC and to HC. Changes in the PD-MCI group are not surprising as they suggest altered WM integrity in a more malignant disease subtype. Previous studies also described DTI changes of WM in PD-MCI as compared to HC and/or to PD-NC pointing to decreased WM integrity in the anterior and superior corona radiata, genu, and body of the corpus callosum, anterior and inferior fronto-occipital fasciculus, uncinated fasciculus, and superior and inferior longitudinal fasciculi [30,31]. Note that DBM revealed GM atrophy in frontal regions of PD-MCI only when compared to HC while no significant results were obtained from the PD-MCI and PD-NC comparison. Of note, some authors found WM changes already in early stages of PD [7] and subcortical atrophy in PD as compared to HC using the DBM [22]. Unlike in most of previous studies in the current work we thoroughly examined our groups using a comprehensive cognitive test battery which is our study strength. Nevertheless, we cannot exclude a possibility that the results are specific patient groups-driven and cannot be fully generalized.

All in all, our results reflect deterioration of GM and WM microstructure in different PD phenotypes with and without MCI. Of note, only DKI but not DTI metrics showed significant differences in GM across groups. In PD-NC, increased kurtosis in GM structures including the premotor and motor cortices reflects increased hindrance to water diffusion probably due to early alpha-synuclein-induced microstructural changes. Our PD-MCI group was characterized by profound changes in WM microstructure and gross cortical atrophy. The differential analysis of combined DKI/DTI measures of our GM ROIs showed reasonable accuracy for identifying both PD-NC and PD-MCI subjects.

4.1. Study limitations

The microstructural mechanisms underlying the changes in DKI parameters are discussed in the light of pathological studies in animal models. The human clinico-pathologic studies are needed to further determine neural underpinnings of the DKI changes in the human PD brain. Motivated by our animal studies we focused on the motor basal ganglia-thalamo-cortical loop, including the PMC and M1 cortical regions. Future studies should explore the whole cerebral cortex using the DKI. The study participants were recruited as consecutive cases and we were not successful in perfect matching of all three groups for age and gender. Despite the fact that all analyses were controlled for age, gender, years of education (and LED in the PD groups) we cannot fully exclude a possible effect of unequally distributed participants in respective groups. Since we compared the PD groups with HC only, we cannot conclude on the specificity of our PD-MCI results and further research should focus on comparing MCI groups with various brain pathologies. In the differential analysis, we used a classical cross-validation scheme during the hyper-parameters search. The use of nested cross-validation would be more appropriate for studies with larger datasets.

Disclaimer

The article reflects only the author's view and the Research Executive Agency (REA) is not responsible for any use that may be made of the information it contains.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.parkreldis.2020.10.032.

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5. Early Changes in the Locus Coeruleus in Mild Cognitive Impairment with Lewy Bodies

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Commentary.

This publication marks an important transition toward the investigation of pre-dementia stage of DLB. The LC is an early site of pathological involvement and plays a central role in noradrenergic modulation, attention, cognition, and brain network regulation. The study was motivated by the need for sensitive biomarkers capable of detecting neurodegenerative changes before the development of established dementia. We investigated whether individuals with MCI associated with Lewy body disease exhibit early structural abnormalities of the LC and examined their associations with cognitive performance. Neuromelanin-sensitive MRI revealed reduced LC integrity in these subjects, particularly in its caudal portion, with right-sided abnormalities associated with impaired visuospatial memory, suggesting early LC involvement in Lewy body disease. The findings provided evidence of LC alterations at the prodromal stage, supporting its potential role as an imaging biomarker of early Lewy body pathology. The novel contribution was the focus on a small but neurobiologically critical brainstem structure in a clinically challenging prodromal population. This work connects our earlier structural imaging research with our later studies of functional connectivity and cognitive impairment in Lewy body diseases. It also opens a translational perspective by identifying a potential target for early diagnosis, disease stratification, and future intervention studies.

RESEARCH ARTICLE

Early Changes in the Locus Coeruleus in Mild Cognitive Impairment with Lewy Bodies

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ABSTRACT: Background: Although neuromelanin-sensitive magnetic resonance imaging (NM-MRI) has been used to evaluate early neurodegeneration in Parkinson's disease, studies concentrating on the locus coeruleus (LC) in pre-dementia stages of dementia with Lewy bodies (DLB) are lacking.
Objectives: The aims were to evaluate NM-MRI signal changes in the LC in patients with mild cognitive impairment with Lewy bodies (MCI-LB) compared to healthy controls (HC) and to identify the cognitive correlates of the changes. We also aimed to test the hypothesis of a caudal-rostral α-synuclein pathology spread using NM-MRI of the different LC subparts.
Methods: A total of 38 MCI-LB patients and 59 HCs underwent clinical and cognitive testing and NM-MRI of the LC. We calculated the contrast ratio of NM-MRI signal (LC-CR) in the whole LC as well as in its caudal, middle, and rostral MRI slices, and we compared the LC-CR values between the MCI-LB and HC groups. Linear regression analyses were performed to assess the relationship between the LC-CR and cognitive outcomes.
Results: The MCI-LB group exhibited a significant reduction in the right LC-CR compared to HCs ($P = 0.021$). The right LC-CR decrease was associated with impaired visuospatial memory in the MCI-LB group. Only the caudal part of the LC exhibited significant LC-CR decreases in MCI-LB patients compared to HCs on both sides ($P < 0.0001$).
Conclusions: This is the first study that focuses on LC-CRs in MCI-LB patients and analyzes the LC subparts, offering new insights into the LC integrity alterations in the initial stages of DLB and their clinical correlates. © 2024 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: mild cognitive impairment with Lewy bodies; neuromelanin-sensitive magnetic resonance imaging; locus coeruleus

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A new classification of Parkinson's disease (PD) with the SynNeurGe research diagnostic criteria was recently published, suggesting the documentation of pathological α-synuclein in tissues or cerebrospinal fluid and/or pathogenic gene variants that induce or cause a strong predisposition to PD.[1] These results provide only "yes" or "no" answers; the evaluation of neurodegeneration using neuroimaging tools has also been recommended to better characterize the spatial distribution, temporal evolution, and magnitude of the changes. Another paper[2] directly referred to neuronal α-synuclein diseases. The authors provided a staging system for research based on the detection of pathological α-synuclein, dopaminergic neuronal dysfunction, the presence of specific clinical symptoms, and functional impairment. Although the two aforementioned papers presented important breakthroughs for diagnosing Lewy body diseases in research, a mechanistic understanding of how the temporal spread of α-synuclein pathology in the brain affects motor symptoms and cognitive and other nonmotor symptoms of the disease is also warranted for developing disease-modifying treatment. In this regard, brain alteration patterns, as assessed using noninvasive and widely available MRI, may provide important insights into the development of distinct clinical phenotypes, their temporal evolution, and disease prognosis.[3-5]

An interesting hypothesis has been formulated concerning the temporal spread of α-synuclein pathology[6] originating either in the peripheral nervous system and gastrointestinal tract in particular, spreading in a prion-like manner toward the brain following Braak staging ("body-first" subtype), or starting asymmetrically in the amygdala and limbic system and from there following a rostral-caudal pattern of Lewy body pathology and spreading to the peripheral system ("brain-first" subtype). In line with this, the authors suggested the presence of early cognitive decline and an overall malignant course of the disease in the former (dementia with Lewy bodies [DLB]) subtype and an earlier more pronounced asymmetric parkinsonism (typical for less-malignant PD phenotype) in the latter subtype.[7-9]

Probably the best-described phenotype of pre-dementia DLB is mild cognitive impairment with Lewy bodies (MCI-LB), which can be distinguished from other causes of MCI by the presence of core clinical features of DLB and diagnostic biomarkers such as reduced dopamine transporter uptake in the basal ganglia as assessed using dopaminergic single-photon emission computed tomography/positron emission tomography (SPECT/PET), reduced cardiac metaiodobenzylguanidine uptake on myocardial scintigraphy, and polysomnography-diagnosed rapid eye movement (REM) sleep without atonia.[10] Other supportive features and biomarkers have also been documented.[10-13]

However, a recent meta-analysis revealed that sensitivity of FP-CIT SPECT (2β-carbomethoxy-3β-(4-iodophenyl)-*N*-(3-fluoropropyl) nortropane was rather low for diagnosing MCI-LB (65%–66%).[11] This could be because in early MCI-LB, the α-synuclein pathology affects the locus coeruleus (LC) prior to the substantia nigra pars compacta (SNpc, in accordance with the aforementioned "body-first" hypothesis), which is consistent with a typical cognitive profile of MCI-LB, that is, executive and attention deficits in particular.[14]

To test this hypothesis, we used neuromelanin-sensitive magnetic resonance imaging (NM-MRI) of the LC and its caudal, intermediate, and rostral subparts. The LC produces norepinephrine, a neurotransmitter that regulates arousal, attention, autonomic functions, and the sleep–wake cycle, and that also plays a crucial role in stress response and depression, emotional and episodic memory, and working memory and executive functions.[15,16] Neuromelanin (NM), as a by-product of the catecholamine metabolism, is a biochemical-insoluble pigment of dopaminergic neurons in the SNpc and in the noradrenergic neurons in the LC. The NM bound to the metal is paramagnetic, and paramagnetic substances shorten the T1 relaxation time.[17] This allows the visualization of structures containing NM, such as the SNpc and the LC, as high-intensity areas; the signal from the surrounding brain tissue is suppressed. NM levels are known to decline with neurodegeneration,[18] and NM-MRI of the SNpc has been particularly useful for evaluating SNpc neurodegeneration early in the course of PD,[19,20] and in patients with REM sleep behavioral disorder (RBD).[21,22] Moreover, NM-MRI seems suitable for monitoring disease progression.[23,24]

Although NM-MRI of the LC has been used in PD,[16,25,26] to the best of our knowledge no study has yet used NM-MRI to study LC changes in clinically well-defined MCI-LB patients. We tested the utility of NM-MRI as a potential marker of the early neurodegeneration of the LC in MCI-LB compared to healthy controls (HC). We specifically concentrated on studying the LC for two reasons: (1) pathological changes affect this area early according to the body-first hypothesis; (2) LC disruption is related to cognitive, autonomic, and psychiatric functions,[15,27] which are predominant in DLB patients, whereas motor symptoms dominate in early PD and are rather closely related to SNpc disruption.[28] We hypothesized a measurable reduction in LC signal in MCI-LB compared to age-matched HCs. In line with the body-first hypothesis, we predicted that the differences between MCI-LB and HC are more pronounced in the caudal subparts of the LC. We also hypothesized that in the MCI-LB group, the MRI signal in the LC correlates with specific MCI-LB cognitive deficits.

In a majority of DLB patients, Alzheimer's disease (AD) brain pathology may coexist with α-synuclein pathology and may influence its clinical manifestation.[29] AD-related pathology has been described in the

LC of AD patients.[30] To evaluate whether any significant correlations between the LC MRI signal and cognitive outcomes are modified by AD-related co-pathology in our MCI-LB cohort, we used a validated blood-based immunoassay measuring plasma pTau181 as a biomarker of AD-related brain pathology[31 33] in individual subjects.

Patients and Methods

Participants

This study included 38 participants who met the criteria for MCI-LB.[10] We included 59 participants as HCs. For recruitment procedures and inclusion and exclusion criteria, see Supplementary Material. During the in-person screening visit, we collected a detailed medical history and conducted a neurological and cognitive examination; for details, see Supplementary Material.

All participants were informed in detail about the study and signed an informed consent form approved by the local ethics committee. All data were handled in line with the General Data Protection Regulation (GDPR). Based on research criteria for diagnosis of MCI-LB,[10] a cognitive complaint was required from the patient or from an informant or clinician, and a cognitive profile of MCI-LB was evaluated by MDS (the International Parkinson and Movement Disorder Society) level II criteria for PD-MCI.[34] We included MCI subjects with two neuropsychological tests with a cutoff score under −1 standard deviation (SD) below the age-appropriate norms or with one test with a cutoff score under −1 SD below age-appropriate norms plus a Montreal Cognitive Assessment cutoff score of 26 with the coexistence of at least two core clinical features of DLB according to the results of a detailed clinical examination, patient interview, and cutoff scores of validated scales/questionnaires evaluating fluctuating cognition, visual hallucinations, RBD, and one or more spontaneous cardinal features of parkinsonism[10,35] (see Supplementary Material for details). Based on the clinician's interview with a patient and a caregiver, the cognitive deficits did not interfere with patients' daily functioning. HCs did not have MCI or meet any core clinical symptoms of MCI-LB. All of our participants underwent plasma sample collection through venipuncture. Plasma pTau181 was evaluated by our partner institution (Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden) in a blinded manner, on the Simoa HD-1 (Quanterix, Billerica, MA, USA) as described previously.[32]

Image Acquisition

All magnetic resonance images were acquired using the 3 T Siemens Magnetom Prisma. A three-dimensional brain volume imaging sequence was performed for the whole brain T1 mprage structural data with the following parameters: repetition time (TR) = 2400.0 ms, echo time (TE) = 2.27 ms, inversion time (TI) = 1150 ms, field of view (FOV) = 218 × 218 mm, matrix size = 256 × 256, flip angle = 8°, bandwidth = 210 Hz/Px, averages = 1, in-plane resolution = 0.85 × 0.85 mm, slice thickness = 0.85 mm with no intersection gap, and number of slices = 192. The LC NM-MRI scans were performed using a T1-weighted fast spin-echo sequence with the following parameters: TR = 890.0 ms, TE = 13 ms, in-plane resolution = 0.4 × 0.4 mm, slice thickness = 3.0 mm, matrix size = 512 × 464, nine slices, FOV = 220 × 200 mm, flip angle = 180°, Magnetization Transfer Contrast (MTC) on, bandwidth = 160 Hz/Px, and number of averages = 3.

Image Processing and Accessing the Neuromelanin Signal

To process the data, we employed a combination of software tools, including Advanced Normalization Tools (ANT, version 2.2.0), Matlab (MATLAB R2017b), and the FMRIB Software Library. Initially, T1-weighted images were skull stripped using ANTs' antsBrainExtraction.sh. This was followed by spatial normalization to the MNI152NLinAsym template space using antsRegistrationSyN.sh (incorporating rigid, affine, and deformable syn transformations). Subsequently, we coregistered the NM-MRI image to the T1w (in native space) image using antsRegistrationSyN.sh with rigid transformations. Finally, we executed antsApplyTransforms to integrate the previously derived transformations, facilitating a one-step normalization of the LC and pontine tegmentum (PT) masks to the NM-MRI image.

To access the LC signals, we adopted a methodology comparable to one used in previous research.[36-38] A manually delineated overinclusive mask served as the search area for the LC (see Fig. 1). For the reference region, we drew the region of interest (ROI) in the central PT (see Fig. 1). This reference area was selected based on past studies due to its high signal-to-noise ratio. It is thought to remain unaffected by any pathogenesis, making it appropriate for intensity normalization to counteract variances between subjects and slices.[39] Within each slice, the intensity was linearly modified so that the mean intensity of the reference region was uniform across all images and set to the same predefined value. For further analyses, we used the contrast ratio (CR) between the LC and the central PT using formula (1):

$$\mathrm{LC-CR} = \left(\mathrm{LC}_{\mathrm{intensity}} - \mathrm{PT}_{\mathrm{intensity}}\right)/\mathrm{PT}_{\mathrm{intensity}} \quad (1)$$

In these overinclusive masks on both the right and left sides, we localized the voxel with the highest

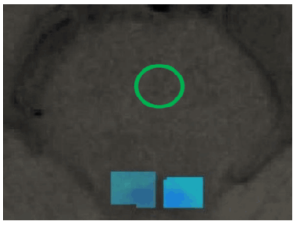

FIG. 1. The manually drawn overinclusive mask (two rectangular shapes) projected on a sample participant's neuromelanin (NM) image; the circle is a reference region of the pontine tegmentum. [Color figure can be viewed at wileyonlinelibrary.com]

intensity that aligned with the anatomical position of the LC. A cross of five voxels was then placed at this peak voxel to cover the LC's neuronal distribution of 1 to 2 mm. If the peak voxel was adjacent to the fourth ventricle, we shifted the center voxel one step away to avoid underestimating the signal intensity. This ensured the peak voxel stayed within the LC cross ROI. The signal was then measured in three slices denoting the rostral, middle, and caudal LC (see Supplementary Material), with the middle slice positioned 7 mm below the inferior edge of the inferior colliculus, in line with the existing literature.[37,40]

Statistical Methods

All statistical analyses were performed using software from MATLAB, Python, and SPSS, and R. Demographic, clinical data, and LC-CR (controlling for the effect of age) were expressed as the mean and SD and were analyzed using the 1-tailed Mann-Whitney U test. The χ^2 test was used to compare categorical variables such as sex. To investigate the association between LC-CR and cognitive performance (see Table 1), linear regression analyses were used, controlling for the effect of age and education and adhering to the assumptions of linear regression (see Supplementary Material). In addition, using Spearman's correlations we exploratively assessed the correlation between LC-CR and other nonmotor scores that might have revealed significant associations based on the literature,[16] such as the Geriatric Depression Scale (GDS), Epworth Sleepiness Scale (ESS), and REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ). Finally, to assess whether any significant associations between LC-CR and cognitive outcomes of interest were modified by AD brain co-pathology, we additionally regressed out the effect of plasma pTau181 levels.[32]

Results

There were no significant sex differences between the MCI-LB patients and the HC group, but there was a significant difference in age and education. Table 1 presents the demographic, clinical, and cognitive data for both groups.

Neuromelanin-Sensitive magnetic resonance imaging

We observed a significantly reduced signal in the right LC of the MCI-LB group compared to the HC group ($P = 0.021$); there was a similar nonsignificant

TABLE 1 *Demographic, clinical, and cognitive data*

	HC mean ± SD	MCI-LB mean ± SD	*U*	*P*
Sex (female:male)	20:18	39:20		0.185
Age	65.72 ± 7.14	69.65 ± 5.86	1514	0.004
Education (y)	16.14 ± 0.45	13.83 ± 0.58	651	<0.001
MoCA	27.38 ± 1.39	23.92 ± 3.06	324	<0.001
Visuospatial functions z score	0.17 ± 0.77	−0.50 ± 1.12	724	0.004
Memory z score	0.58 ± 0.64	−0.37 ± 0.83	387.5	<0.001
Attention z score	0.26 ± 0.59	−0.23 ± 0.68	652.5	<0.001
Executive functions z score	0.32 ± 0.75	−0.41 ± 0.84	602	<0.001
GDS score	6.88 ± 5.11	8.21 ± 6.26	1220	0.376
RBDSQ score	2.86 ± 2.16	4.16 ± 2.59	1420.5	0.016
ESS score	7.50 ± 4.34	8.03 ± 3.98	1223	0.363
UPDRS score	2.58 ± 3.48	6.37 ± 4.39	1750	<0.001

We used the Mann-Whitney U test for age and the χ^2 test for sex comparison between the MCI-LB and HC groups.
Abbreviations: HC, healthy control; SD, standard deviation; MCI-LB, mild cognitive impairment with Lewy bodies; MoCA, Montreal Cognitive Assessment; GDS, Geriatric Depression Scale; RBDSQ, Rapid Eye Movement Sleep Behavior Disorder Screening Questionnaire; ESS, Epworth Sleepiness Scale; UPDRS, Unified Parkinson's Disease Rating Scale.

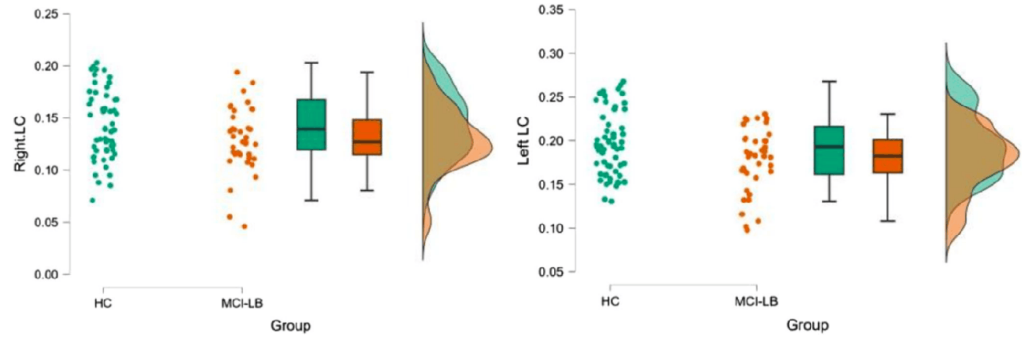

FIG. 2. Raincloud plots of the differences between both groups for both the left and the right LC-CR (locus coeruleus-contrast ratio). [Color figure can be viewed at wileyonlinelibrary.com]

trend on the left side ($P = 0.055$); see Figure 2. When the LC subparts were analyzed in separate slices (see Patients and Methods), we found a significant signal reduction on both sides only for the caudal part of the LC in MCI-LB compared to the HC group ($P < 0.0001$); see Figure 3. Additional results are presented in detail in the Supplementary Material.

Linear Regressions

Linear regression analyses, controlling for age and education, were conducted to examine the associations between LC-CR and various cognitive domains. The right LC-CR significantly predicted the memory domain ($F(3, 33) = 3.49$, $\beta = 14.72$, $P = 0.01$), as shown in Figure 4 and the Supplementary Material, which includes regression parameters and diagnostics. However, the right LC-CR only marginally predicted visuospatial functions ($F(3, 36) = 3.38$, $\beta = 11.46$, $P = 0.053$). Additionally, the left LC-CR did not significantly predict either the memory or visuospatial domains.

Further inspection of the memory domain revealed that the right LC-CR specifically predicted the sum of immediate recall trials, as measured by the Brief Visuospatial Memory test (BVMT)_2 z score ($F(4, 33) = 2.88$, $\beta = 19.38$, $P = 0.011$); this relationship remained significant even when accounting for the levels of plasma pTau181 ($P = 0.514$; see Supplementary Material). Other scales within the memory domain were not significantly predicted.

Exploratory analyses of LC subparts indicated a positive association between visuospatial function z scores and both the right-middle LC ($F(1, 36) = 4.77$, $\beta = 8.31$, $P = 0.036$) and the left-middle LC ($F(1, 36) = 4.21$, $\beta = 6.52$, $P = 0.048$). Furthermore, the right-middle LC significantly predicted the memory z score ($F(1, 33) = 7.96$, $\beta = 9.44$, $P = 0.008$).

Correlation Analyses

Regarding the exploratory analyses of other non-motor scores (GDS, ESS, RBDSQ), we found a

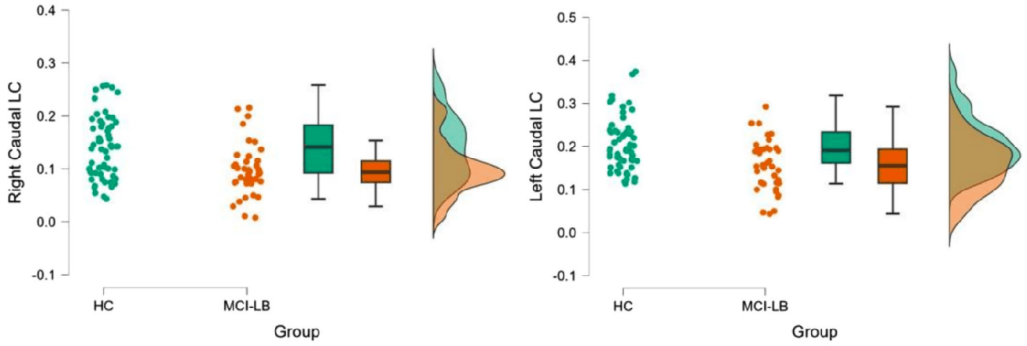

FIG. 3. Raincloud plots showing the difference between mild cognitive impairment with Lewy bodies (MCI-LB) and healthy controls (HC) for the right- and the left-caudal LC-CR (locus coeruleus-contrast ratio). [Color figure can be viewed at wileyonlinelibrary.com]

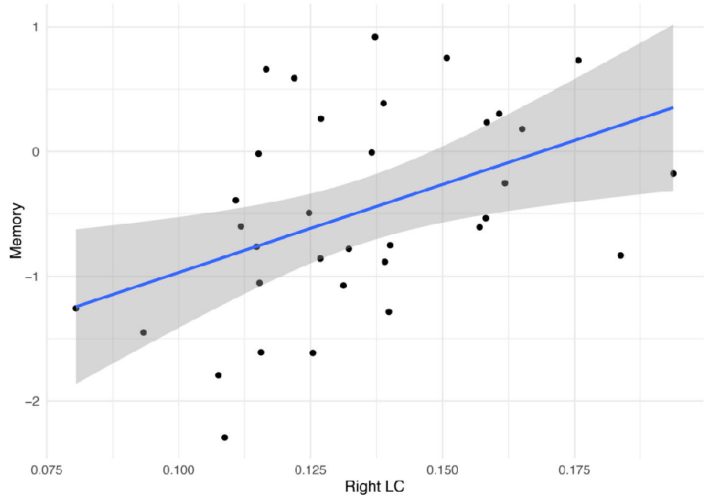

FIG. 4. Scatterplot of significant association between the right LC-CR (locus coeruleus-contrast ratio) and the memory *z* score of the mild cognitive impairment with Lewy bodies (MCI-LB) group. [Color figure can be viewed at wileyonlinelibrary.com]

significant negative correlation between the left LC-CR and GDS (rho = − 0.303, $P = 0.032$); see figure in Supplementary Material. Exploratory correlations with the LC subparts exhibited a negative correlation between the right caudal LC and RBDSQ (rho = −0.309, $P = 0.029$) and between the right-middle LC and GDS (rho = −0.378, $P = 0.01$) and the left-middle LC and GDS (rho = −0.357, $P = 0.014$).

Discussion

This is the first study that focuses on LC integrity and its clinical correlates specifically in MCI-LB patients compared to HCs, including an evaluation of distinct LC subparts using NM-MRI. Moreover, plasma pTau181 values were implemented in data analysis to provide information on the influence of AD-related co-pathology on associations between LC-CR and cognitive outcomes of interest in the MCI-LB cohort.

We observed decreased integrity of the right LC in the MCI-LB group compared to the HC group with a similar nonsignificant trend on the left side. When we zoomed in on the LC-CR in separate slices, we found only the caudal part of the left and right LC significantly affected in MCI-LB compared to HCs. Moreover, the exploratory analysis showed that the caudal part of the right LC was negatively correlated with the RBDSQ scores, meaning that the more pronounced the degeneration of the caudal LC, the more pronounced the symptoms of RBD. Although we found no studies investigating NM in different parts of the LC in DLB, similar findings of caudal LC alterations were mentioned in previous studies of PD,[25,26,41,42] which reported that the decrease in LC-CR was not evenly distributed throughout the LC and that the middle and caudal parts of the LC were more affected than the rostral part. In line with our study results, Nobileau and colleagues[43] demonstrated that LC signal intensity was reduced in PD with RBD but not in PD without RBD. In the same vein, the disruption of LC-related functional networks was seen in PD with RBD; these networks were less affected in PD without RBD.[44] Overall, our result of the caudal LC degeneration in MCI-LB further supports the "body-first" hypothesis in this more malignant neuronal α-synuclein disease population[8,45] and underscores the potential of NM-MRI as a biomarker for identifying candidates for specific strategies of disease modification and prevention.[9]

Specific disruptions of LC segments may be disease specific; for example, in the early stages of AD, the rostral portions of LC are the most affected.[46] In the current work, the right LC-CR exhibited significant association with memory functions, especially with the sum of BVMT immediate recall trials, which is a measure of visual and spatial short-term memory and the learning abilities of the subject.[47] Of note, the exploratory analyses revealed that the visuospatial functions and memory LC-CR associations were particularly with the middle subpart of the LC, which is in accordance with the functional organization of the LC.[15] Visual memory and visuospatial ability impairments are

characteristic of MCI-LB,[48] and visual memory performance in DLB is poorer than in Parkinson's disease dementia[49] and equally poor[50,51] or even worse than in AD.[52]

The current results are in line with the suggestion that noradrenergic neurons in the LC play a critical role in learning and memory,[53,54] due to its well-described neural projections to the amygdala, hippocampus, and prefrontal cortex.[15,55] Because AD-related brain pathology may co-occur in DLB[29] and may also be associated with visuospatial memory impairment,[56] we assessed the impact of pTau181 plasma levels, that is, a biomarker of AD brain pathology,[32] on the association between LC-CR and BVMT. We did not observe a major impact of the AD plasma biomarker levels on the relation, suggesting that the association of the NM-MRI results with cognitive outcomes of interest remains rather specific for the neuronal α-synuclein disease.

We also conducted an exploratory analysis focused on depressive symptoms. The results showed a significant negative correlation between the LC-CR and GDS score in the MCI-LB group; depressive symptoms tended to increase with LC neurodegeneration. The LC is known to play a key role in mood regulation, and its degeneration has been implicated in various mood disorders, including major depression.[57,58] Some works have indicated increased vulnerability of LC neurons to stress and damage.[57-59] Our findings support the notion that LC dysfunction could contribute to the development of depressive symptoms in the early stages of DLB.[59]

Finally, we found significantly higher asymmetry of the LC-CR between the left and right hemispheres in MCI-LB patients compared to HCs, with a more pronounced LC degeneration on the right side. In the literature, LC-CR asymmetry was associated with higher age and worse behavioral performance, such as low emotional memory and regulation, higher anxiety, and depression.[60] Here we show that the neurodegeneration process may even enhance such asymmetry. Notably, the right-sided but not left-sided LC-CR signal alteration was also described in PD patients compared to HCs,[26,61] and the LC degeneration was associated with motor symptoms (bradykinesia, motor fluctuations, tremor) and nonmotor symptoms (fatigue, apathy, orthostatic dysregulation, constipation, RBD).[26,43,61,62] Notably, we did not compare the LC-CR asymmetry between MCI-LB and PD patients with or without RBD, and therefore, we cannot comment on the level of LC-CR asymmetry between potentially more malignant ("body-first") and less malignant ("brain-first") neuronal Lewy body disease subtypes.

Conclusion

This study contributes to an in-depth understanding of the neural underpinnings and specific neurotransmitter involvement of the pre-dementia stage of DLB. Our findings revealed a significant reduction in the LC integrity of MCI-LB patients compared to HCs and more involvement of the caudal part of the nucleus in the patient group. This supports a caudal-rostral direction of pathological α-synuclein spread. Our results suggest a role for NM-MRI in identifying early neurodegenerative changes in the LC associated with MCI-LB. Furthermore, the study highlights the relationship between LC neurodegeneration and cognitive decline. Future research should focus on longitudinal studies to monitor the progression of LC changes over time and investigate the potential of NM-MRI as a diagnostic or/and prognostic tool for DLB. Further exploration of the relationship between LC neurodegeneration and other nonmotor symptoms in DLB in longitudinal studies may provide valuable insights into the pathophysiology and progression of the disease and guide the development of targeted therapeutic strategies. ■

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Part II. Brain Network Dysfunction in Neurodegenerative Disorders

6. Striato-cortical Connections in Parkinson's and Alzheimer's Diseases: Relation to Cognition

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Commentary.

This study marked a major shift in my research from regional structural abnormalities toward the investigation of large-scale brain networks. The study aimed to determine whether alterations in striato-cortical connectivity are associated with cognitive impairment in PD and AD. We hypothesized that disease-specific disruption of striato-cortical networks would be related to cognitive performance. The findings demonstrated that functional connectivity alterations were associated with cognitive deficits and differed between PD and AD. In both PD and AD, striato-cortical connectivity was reduced compared with HC; middle striatum–precuneus connectivity was associated with executive dysfunction in PD and memory impairment in AD, while reduced connectivity between the dorsal striatum and frontal and sensorimotor cortices in PD was associated with greater motor symptom severity and higher dopaminergic medication requirements. In relation to previous publications, this work complemented structural MRI findings by demonstrating that cognitive dysfunction cannot be explained solely by regional atrophy. It provided an important foundation for subsequent studies of functional connectivity in prodromal Lewy body disease and for investigations of dynamic brain network organization.

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Supporting Data

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Striato-cortical Connections in Parkinson's and Alzheimer's Diseases: Relation to Cognition

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ABSTRACT

Background: Functional connectivity is abnormal in PD and in early Alzheimer's disease.
Objectives: The objective of this study was to evaluate resting-state striato-cortical connectivity in PD and Alzheimer's disease and assess their relation to cognitive outcomes. Groups with mild cognitive impairment as a result of different pathologies (PD vs. Alzheimer's disease) were also compared.
Methods: Seed-based connectivity of the dorsal, middle, and ventral striatum was analyzed in 111 patients using functional MRI. The correlation between connectivity at regions of between-group differences and clinical outcomes was assessed.
Results: Patients showed lower striatal connectivity than controls. Connectivity between the middle (associative) striatum and precuneus negatively correlated with executive functions in PD and with memory performance in Alzheimer's disease. PD with cognitive impairment showed decreased connectivity of the dorsal (motor) striatum when compared with early Alzheimer's disease.
Conclusions: Striatal connectivity was reduced in patients when compared with controls. Similar compensatory mechanisms were employed to overcome various cognitive deficits in PD and Alzheimer's disease. © 2017 International Parkinson and Movement Disorder Society
Key Words: striatum; Parkinson's disease; Alzheimer's disease; resting state functional connectivity; cognition

Some abnormalities in resting state functional connectivity (rs-FC) have been documented in both Alzheimer's disease (AD) and Parkinson's disease (PD), mainly in the default mode network.[1-6] However, a recent meta-analysis reported additional rs-FC differences between patients with amnestic mild cognitive impairment (MCI) and healthy controls (HC) in regions of the cortico-striato-thalamic loop.[7] Previous studies have also shown a selective loss of cholinergic neurons and amyloid deposition in the ventral striatum of AD patients.[8,9] Striatal rs-FC changes in PD patients were attributed to a loss of dopaminergic neurons that was related particularly to motor symptoms of PD.[10-12] A recent study showed that cognitive decline and motor deficit in PD are each associated with a differentiable pattern of rs-FC of striatal subregions (caudate and putamen).[13] Moreover, the study results demonstrated that specific striatal (caudate) rs-FC changes might reflect brain compensation strategies employed in an attempt to overcome cognitive deficits in PD. It is not known whether these patterns of brain changes are general and common for pathological aging caused by various pathologies or whether this is a unique mechanism for the PD population.

Therefore, we focused on subcortical-cortical rs-FC differences in both PD and AD-MCI when compared with HC. In addition, we studied the differences between PD-MCI and AD-MCI, that is, MCI groups caused by different pathologies. Moreover, we explored the associations between clinical variables and the rs-FC of specific subdivisions of the striatum parcellated according to the probabilistic tractography

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results[14] and thus more closely reflecting the anatomical structures of the well-known basal ganglia circuitries (sensorimotor, associative, and limbic).[15] We hypothesized the following: the most dorsal part of the striatum, linked particularly to the primary motor cortex and premotor regions including the supplementary motor area (SMA) and pre-SMA, would be related mostly to motor symptoms in PD; the middle striatum, connected with the dorsolateral prefrontal cortex, would be particularly related to executive functions and goal-directed behavior in both diseases; and the ventral striatum, connected with limbic cortical regions in the orbitofrontal cortex and ventral anterior cingulate, would be related to reward-related behavior as well as to cognition in both PD and AD.

Methods

A total of 141 participants were enrolled in our study based on published clinical and research criteria: 41 PD,[16] 42 AD-MCI,[17] and 58 HC. All participants were examined by an experienced neurologist who also evaluated hippocampal volume losses on coronal MRI slices in AD-MCI patients and by an experienced neuropsychologist using a detailed neuropsychological cognitive test battery[18] (see supplementary material). Patients were scanned in the ON medication state without dyskinesias. We acquired 200 resting-state fMRI scans in a 3T Siemens Prisma MR scanner (Siemens Corp., Erlangen, Germany) and the extent of motion was carefully controlled (see supplementary material). Voxel-based morphometry from the magnetization-prepared rapid gradient-echo (MPRAGE) images was used to assess brain atrophy.[3] A seed-based functional connectivity method with preselected subcortical striatal regions was used (for details about the parcellation,[14] see supplementary material). The maps of striatal subregions served as seeds for whole-brain functional connectivity calculated in a voxel-wise manner. Connectivity of the dorsal, middle, and ventral part of striatum was studied, which are involved in sensorimotor, executive, and limbic basal ganglia circuits, respectively. Data were analyzed using a one-way analysis of variance model with subsequent Pearson correlation analysis (for details see supplementary material).

Results

Altogether 111 (32 PD, 31 AD-MCI, 48 HC) of the initial 141 participants were analyzed; fMRI data from 30 participants were removed as a result of motion artefacts. See Table 1 for demographic and clinical characteristics of the final dataset. Side of symptom onset in PD was equally distributed between the left and right sides, and mild cognitive impairment was found in 50% of the PD patients.[19] AD-MCI displayed significant gray matter volume decreases in the hippocampus bilaterally; there was no atrophy in PD

TABLE 1. Demographic and clinical variables

	HC, n = 48	AD-MCI, n = 31	PD, n = 32	HC vs AD-MCI, *P* value	HC vs PD, *P* value	AD-MCI vs PD, *P* value
Age in years (SD)	66.9 (7.6)	70.6 (7.7)	62.9 (9.6)	**<.05**	**<.05**	**<.05**
Education in years (SD)	15.6 (2.6)	14.0 (4.0)	15.2 (3.4)	ns	ns	ns
Sex, % of male	31	29	72	ns	**<.05**	**<.05**
Disease duration in years (SD)	–	3.5 (3.5)	4.6 (2.8)	–	–	ns
Levodopa daily equivalent dose	–	–	814.1 (553.2)	–	–	–
UPDRS III (motor examination)	–	–	18.5 (8.9)	–	–	–
Hoehn and Yahr stage	–	–	1.8 (0.5)	–	–	–
Clinical subtypes of PD	–	–	Tremor-dominant (n = 10) Rigidity + bradykinesia dominant (n = 3) Postural instability gait difficulty dominant (n = 3) Po predominance (n = 16)	–	–	–
MMSE	28.4 (1.3)	25.9 (2.5)	27.5 (2.1)	**<.05**	**<.05**	**<.05**
Memory z-score	0.6 (0.5)	−0.6 (1.0)	−0.1 (0.8)	**<.05**	**<.05**	**<.05**
Attention z-score	0.0 (0.6)	−1.1 (1.0)	−0.8 (1.1)	**<.05**	**<.05**	ns
Executive functions z-score	0.5 (0.8)	−0.8 (0.8)	−0.2 (0.9)	**<.05**	**<.05**	**<.05**
Visuospatial functions z-score	0.5 (0.6)	−0.1 (0.9)	0.2 (0.8)	**<.05**	ns	ns
Language z-score	0.5 (0.4)	−0.1 (0.9)	0.1 (0.8)	**<.05**	**<.05**	ns
Geriatric Depression Scale	2.3 (2.7)	3.5 (3.4)	2.8 (2.3)	ns	ns	ns
Functional Activities Questionnaire	98.7 (2.3)	91.6 (11.2)	94.1 (10.3)	**<.05**	**<.05**	ns

AD-MCI, Alzheimer's disease–mild cognitive impairment; HC, healthy controls; ns, nonsignificant; significant differences are displayed in bold.

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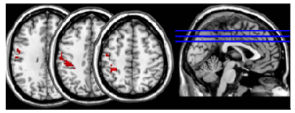
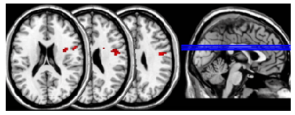
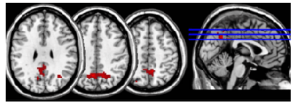
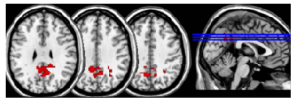
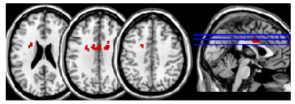
TABLE 2. rs-FC in patients when compared with HC and correlations between rs-FC strength in ROIs and composite cognitive domain z-scores in individual groups ($P < .05$) [Color table can be viewed at wileyonlinelibrary.com]

Dorsal striatum PD vs HC	Cluster	MNI-coordinates of local cluster max (XYZ), mm			No. of voxels	Correlation coefficient in significant clusters for group in bold	Clinical outcomes/ LED	Cluster image
	Right middle frontal g.	36	53	13	46			
	Left sensorimotor cortex	−36	-40	58	176	$R = -0.42$ $P = .016$ and $R = -0.35$ $P = .046$ **PD** vs HC	LED and UPDRS III, respectively	
	Right precentral g./ inferior frontal g.	48	2	22	67	$R = -0.31$ $P = .032$ PD vs **HC**	Executive	
	Sulcus calcarinus/middle occipital g./cuneus	−21	−76	19	882			
Dorsal striatum PD-MCI vs AD-MCI	Right precentral g./ right postcentral g.	−57	−19	25	82			
	Sulcus calcarinus/ lingual g.	9	−76	4	183			
Middle striatum PD vs HC	Left inferior frontal g./insula/ putamen	−27	8	7	242			
	Left precentral/ postcentral g.	−54	−16	40	668			
	Precuneus/posterior cingulate g.	−6	−58	37	283	$R = -0.44$ $P = .011$ **PD** vs HC	Executive	
	Cuneus/sulcus calcarinus/middle occipital g.	−15	−94	10	642			
Middle striatum AD-MCI vs. HC	Precuneus/posterior cingulate g.	9	−58	37	229	$R = -.49$ $P = .005$ **AD-MCI** vs HC	Memory	
	Middle cingulate g.	−18	5	43	172	$R = -0.31$ $P = .029$ AD-MCI vs **HC**	Executive	

AD-MCI, Alzheimer's disease–mild cognitive impairment; HC, healthy controls; rs-FC, resting state functional connectivity; LED, levodopa equivalent dose; ROI, region of interest; MNI-coordinates, coordinates from the Montreal Neurological Institute; g., gyrus.

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when compared with HC using a voxel-based morphometry approach.

PD patients showed lower rs-FC than HC between the dorsal/middle striatum and the sensorimotor, prefrontal, and parieto-occipital regions. As for significant correlations between the rs-FC strength in our cortical clusters and cognitive domain z-scores/levodopa equivalent dose (LED)/UPDRS-III in the PD group, we found associations between the rs-FC of the dorsal striatum–left primary sensorimotor cortex and LED ($R = -0.42$, $P = .016$)/UPDRS-III ($R = -0.35$, $P = .046$), and for the middle striatum-precuneus and executive functions ($R = -0.44$, $P = .011$). Partial correlations between LED and striato-sensorimotor connectivity were no longer significant when the effect of disease duration and/or UPDRS-III were regressed out. The same was true for the partial correlation between the rs-FC and UPDRS-III after regressing out an effect of LED and/or disease duration. On the other hand, the partial correlation between middle striatum–precuneus rs-FC and executive functions remained significant after regressing out the effect of LED and UPDRS-III ($R = -0.43$, $P = .014$). We did not find any significant difference in rs-FC between PD and PD-MCI. PD-MCI demonstrated lower rs-FC than AD-MCI between the dorsal striatum and left primary sensorimotor cortex and bilateral primary visual areas.

AD-MCI demonstrated lower rs-FC than HC only between the middle striatum and precuneus/cingulate gyrus. In the AD-MCI group, an association between the rs-FC of the middle striatum-precuneus and memory was present ($R = -0.49$, $P = .005$). Using the same cortical clusters, in HC we found an association between executive functions and the rs-FC of the dorsal striatum–right precentral gyrus ($R = -0.31$, $P = .032$), and between executive functions and the rs-FC of the middle striatum–middle cingulate gyrus ($R = -0.31$, $P = .029$; see Table 2). No significant differences in connectivity were found with the seed placed in the ventral striatum.

Discussion

The rs-FC has been studied extensively in PD and AD-MCI. However, it is difficult to compare the results of studies when different MRI machines, protocols, and analytical methods are used. In our study, we focused on subcortical-cortical rs-FC changes in patients with both PD and AD-MCI when compared with HC and their relation particularly to cognitive functions using the same data acquisition and methodological approaches. All participants were examined by a detailed cognitive battery, and their cognitive status was carefully assessed and identified based on recent diagnostic criteria.[17,19] The parcellation of the striatum was uniquely based on its structural connectivity and its involvement in specific basal ganglia circuitries.[14]

In PD, we found decreased connectivity from the dorsal and middle striatum to the primary sensorimotor areas that are typically associated with motor impairment. Similar results were reported by others, where reduced rs-FC from the dorsal striatum in PD was mostly present in the OFF medication state; L-dopa reversed these changes at least in the early stages of PD.[10,12,20-22] We found a negative correlation between the rs-FC of the dorsal striatum–left sensorimotor cortex and both LED and UPDRS-III in consecutive patients ON dopaminergic medication with mild to moderate PD (mean Hoehn and Yahr stage = 1.8 [0.5]). The partial correlation between LED and rs-FC strength lost significance when UPDRS-III and disease duration were regressed out, suggesting that in mild to moderate PD, patient dopaminergic medication does not reverse connectivity abnormalities caused by basal ganglia degeneration but, rather, the amount of LED acts (together with UPDRS-III score and disease duration) as a measure of the disease severity.

Moreover, we found decreased connectivity between the middle striatum and precuneus in both PD and AD-MCI, which was negatively correlated with executive functions and memory performance, respectively. Moreover, no significant differences in the middle striatal connectivity with precuneus were detected when PD-MCI and AD-MCI were compared directly. The patients who showed prominent deficits in the described cognitive domains were the ones with less prominent rs-FC decreases within the relevant patient groups, that is, the magnitude of their rs-FC almost reached the connectivity strength of HC. Of note, the connectivity between the middle striatum and precuneus was positive in all groups. Therefore, the speculation about decreased anticorrelation between areas engaged in the dorsal attentional (caudate nucleus) and default mode (precuneus) networks suggested by others[13] cannot be applied here to explain our results. We suggest that negative correlations between the clusters' rs-FC strength between the middle striatum and precuneus and cognitive performance in patient groups may reflect compensatory attempts to overcome basal ganglia and/or hippocampal degeneration. As a posterior cortical hub, the precuneus is tightly interconnected with both the hippocampus and middle striatum, and its connectivity and integrity have been linked to memory performance and to other cognitive (dys)functions.[1,23-26] In PD, posterior cortical deficits were related to more rapid cognitive decline according to longitudinal studies[27,28] and to cholinergic deficits that are common to both PD-dementia and AD.[29-31] Of note, negative correlations were also observed between the magnitude of striato-prefrontal connectivity and executive functions in HC, indicating the presence of rather general than disease-specific compensatory mechanisms already in healthy aging, taking place within the

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associative basal ganglia circuitry. Decreased rs-FC between the dorsal striatum and primary sensorimotor and visual areas in PD-MCI when compared with AD-MCI might be a PD-specific feature reflecting dopaminergic neurodegeneration that is more pronounced in the dorsal part of the striatum than in the ventral parts.

In conclusion, PD and AD-MCI patients showed lower striato-cortical rs-FC than HC. In PD, the clinical correlates of these changes varied depending on the involvement of the motor or associative basal ganglia circuitries. In the motor circuit, reduced connectivity correlated with the severity of parkinsonism; in the associative circuit, there was an effort to compensate for the deficit in executive functions as a result of basal ganglia degeneration by increasing connectivity between the middle striatum (mostly caudate) and precuneus. We did not observe any changes in connectivity patterns from the ventral striatum–limbic basal ganglia circuit. This result is in line with the known striatal dorsal-ventral gradient for dopamine loss. In AD-MCI, the deeper memory deficits because of hippocampal atrophy were also accompanied by increasing connectivity between the middle striatum and precuneus. The limitations of this study are scanning in the ON medication state, not controlling for apathy or fatigue, the clinically given age difference in our groups (PD younger than AD-MCI patients), and the fact that because of the exploratory nature of the analysis, we reported the correlations without corrections for multiple comparisons. In sum, similar compensatory mechanisms were demonstrated in patients with distinct neurodegenerative brain diseases and in aged HC. These general mechanisms employed in an attempt to overcome various cognitive deficits in AD and PD and variable (but still normal) cognitive performance in aged HC may be caused by cholinergic losses present already in healthy aging[32,33] and more pronounced in AD/PD.[9,29-31]

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's website.

In Vivo Cortical Tau in Parkinson's Disease Using 18F-AV-1451 Positron Emission Tomography

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ABSTRACT

Background: Alzheimer's disease copathology is common in PD at autopsy. In non-PD subjects with mild cognitive impairment, tau depositions can be detected using 18F-AV-1451 PET. We hypothesized that 18F-AV-1451 PET would show tau aggregation in PD with mild cognitive impairment and correlate with cognitive dysfunction.

Objectives: To describe tau aggregation in PD patients.

Methods: Twenty-six PD patients and 23 controls had 18F-AV-1451 PET and neuropsychological assessment to detect mild cognitive impairment.

Results: Nine PD patients (35%) were identified with mild cognitive impairment. Regional analyses showed no significant differences between groups. Voxel-wise analyses showed no correlation with cognitive domain z-scores within patients. One patient with mild cognitive impairment was estimated Braak tau stage 5; all other patients were stage 0.

Conclusion: Our results indicate that tau pathology, as detected by 18F-AV-1451, is uncommon in PD with mild cognitive impairment and shows no significant correlation with cognitive dysfunction at this stage. © 2017 International Parkinson and Movement Disorder Society

Key Words: Tau; AV-1451; imaging; MCI; Parkinson's disease

Parkinson's disease (PD) is associated with the presence of Alzheimer's disease (AD) pathology at autopsy, particularly in patients with PD dementia (PDD),[1,2] which 80% of PD patients eventually develop.[3,4] Mild cognitive impairment (MCI) in otherwise healthy subjects progresses to dementia in 60% of cases, and AD pathology can be found in vivo at the MCI stage, using the tau neurofibrillary tangle (NFT) tracer, AV-1451.[5-8]

We hypothesized that tau aggregation would be increased in PD with MCI (PD-MCI) compared to PD without (PD-nonMCI). To test this, we divided PD patients into MCI and non-MCI groups using comprehensive neuropsychological assessment as recommended by Litvan and colleagues,[9] scanned them using the AV-1451 NFT tracer, and compared them with healthy controls.

Patients and Methods

Study Subjects

A total of 26 patients with idiopathic PD and 23 age-matched control subjects were recruited and gave

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7. Striato-cortical Functional Connectivity Changes in Mild Cognitive Impairment with Lewy Bodies

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Commentary.

This study extended the network-based approach to MCI with Lewy bodies, a clinically important prodromal stage of DLB. Building on our previous findings of striato-cortical dysfunction in PD and AD, we investigated whether similar or distinct connectivity alterations were already present at the prodromal stage of Lewy body disease. We hypothesized that MCI with Lewy bodies would be associated with early alterations in striato-cortical communication related to cognitive function. Patients with MCI with Lewy bodies showed increased connectivity between the dorsal striatum and bilateral anterior temporal cortex, with greater connectivity associated with better executive function. These findings demonstrated that functional network alterations are already detectable before the development of dementia and extend the evidence for striato-cortical dysfunction from established neurodegenerative diseases to prodromal Lewy body disease. The study therefore provided an important link between my earlier work in PD and AD and subsequent investigations of dynamic functional connectivity, supporting the potential of functional network measures as biomarkers of early Lewy body disease.

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Striato-cortical functional connectivity changes in mild cognitive impairment with Lewy bodies

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A B S T R A C T

Background: Functional connectivity changes in clinically overt neurodegenerative diseases such as dementia with Lewy bodies have been described, but studies on connectivity changes in the pre-dementia phase are scarce.
Objectives: We concentrated on evaluating striato-cortical functional connectivity differences between patients with Mild Cognitive Impairment with Lewy bodies and healthy controls and on assessing the relation to cognition.
Methods: Altogether, we enrolled 77 participants (47 patients, of which 35 met all the inclusion criteria for the final analysis, and 30 age- and gender-matched healthy controls, of which 28 met all the inclusion criteria for the final analysis) to study the seed-based connectivity of the dorsal, middle, and ventral striatum. We assessed correlations between functional connectivity in the regions of between-group differences and neuropsychological scores of interest (visuospatial and executive domains z-scores).
Results: Subjects with Mild Cognitive Impairment with Lewy Bodies, as compared to healthy controls, showed increased connectivity from the dorsal part of the striatum particularly to the bilateral anterior part of the temporal cortex with an association with executive functions.
Conclusions: We were able to capture early abnormal connectivity within cholinergic and noradrenergic pathways that correlated with cognitive functions known to be linked to cholinergic/noradrenergic deficits. The knowledge of specific alterations may improve our understanding of early neural changes in pre-dementia stages and enhance research of disease modifying therapy.

1. Introduction

Dementia with Lewy bodies (DLB) is the second most common neurodegenerative disorder associated with dementia after Alzheimer's disease (AD). It is characterized by α-synuclein accumulation leading to the formation of Lewy bodies (LB) and Lewy neurites resulting in cell death. From a clinical perspective, it typically manifests with distinct psychiatric disturbances, isolated REM sleep behavioral disorder (iRBD), fluctuation of cognition and alertness, and motor symptoms of parkinsonism [1]. No disease-modifying treatment yet exists for this condition; the accurate diagnosis of the early pre-dementia stages of DLB is essential for enhancing research that might lead to novel potential therapies and enhance understanding of the cascade of pathological events occurring during the disease progression [2]. However, DLB is especially difficult to diagnose in the early pre-dementia stages because of the varied clinical presentation including rather non-specific features such as constipation, sleep problems, mood and behavioral disturbances, smell changes, and altered attention and executive functions [3–5]. McKeith et al. [4] described three various subtypes of prodromal DLB with regard to their early clinical presentation: Mild Cognitive Impairment with Lewy bodies (MCI-LB) with a characteristic cognitive profile (impairment in one or more cognitive domains, usually attention-executive and/or visual processing domains are compromised), delirium onset (occurrence of delirium as a one of the first features of DLB), and psychiatric symptoms onset (late-onset major depressive disorder and late-onset psychosis amongst predominant symptoms) presentations.

Approximately 80% of people with iRBD [6] will develop neurodegenerative disease within ten years. While iRBD is already regarded as a synucleinopathy, it may take many years before other symptoms develop so that probable DLB can be diagnosed [5–7]. Of note, both iRBD [6] and MCI-LB [4] may also progress to Parkinson's disease (PD). When this happens, it is likely a more malignant PD subtype with a faster cognitive decline and other non-motor features that resemble those of

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DLB [5,8]. Therefore, some researchers have preferred to write in terms of Lewy body disease spectrum [9,10]. However, not all patients with DLB or PD manifest MCI or iRBD symptoms in the early stage of the disease [4,11]. Neuroimaging has the potential to identify early brain changes from both the temporal and spatial perspectives. Moreover, neuroimaging can help to elucidate the neural underpinnings of the early DLB clinical subtypes and can serve as a surrogate biomarker for predicting and tracking the disease progression [3,9].

A diagnosis of probable MCI-LB is defined by the presence of concern regarding cognitive decline, objective evidence of impairment in one or more cognitive domains and presence of at least two core clinical features (fluctuating cognition, recurrent visual hallucinations, REM sleep behavior disorder (RBD), parkinsonism) or one core clinical feature and at least one indicative biomarker (clear deficit on dopaminergic imaging, polysomnographic confirmation of REM sleep without atonia, reduced meta-iodobenzylguanidine uptake on myocardial scintigraphy) [4].

Unlike in MCI-AD, MCI-LB typically do not show major brain atrophy on MRI [9,12]), although some distinct gray matter (GM) alterations may occur in MCI-LB such as insular GM volume loss and cortical thinning [12–14]. On the other hand, resting-state functional connectivity (FC) disruptions, as measured by MRI, may be of particular importance and specifically in early DLB stages. As compared to PET, SPECT, and polysomnography, MRI is a widely available, well tolerated, and relatively inexpensive method. Alterations of large-scale brain networks reflect brain plasticity changes on the synaptic level, and they seem to be associated with clinical symptoms [15] and disease-specific molecular changes [16]. While alterations in numerous large-scale brain networks have been described in iRBD [17–19], Parkinson's disease with and without cognitive impairment (PD [20–23]), and DLB [24–26], studies on connectivity changes in MCI-LB have been scarce. A systematic review [24] of connectivity studies in DLB showed that most consistent disruptions concern the frontoparietal default mode network (DMN) and visual networks and their connections to other brain regions; however, Schumacher et al. [27] found no significant changes in the MCI-LB group as compared to controls or to MCI due to AD pathology. The authors used both static and dynamic fMRI connectivity measures and compared the groups with respect to within- and between-network functional connectivity using an independent component analysis (ICA) method and applied two different approaches to study dynamic functional connectivity.

In the current study, we focused on the striatal FC in drug-naïve MCI-LB patients in terms of any dopaminergic, noradrenergic, and cholinergic medications. The reasons for this focus were: 1. FDG-PET study showed that DLB is associated with major metabolic connectivity alterations in the nigro-striato-cortical dopaminergic network, which is a hallmark in the DLB disease process [28]; 2. Some authors proposed that fMRI-based functional connectivity is an adequate measure for assessing pathology spread across the neural network [29,30], and the substantia nigra within the nigro-striato-cortical pathway is one of the main sites of α-synuclein aggregation [31]; 3. Striatal frontal and perisylvian projections have also been major parts of cholinergic and noradrenergic networks [28] that have been collectively implicated in the pathophysiology of cognitive and other non-motor features of DLB [32].

Based on the results in subjects with early drug-naïve PD [33] or with MCI due to AD pathology [34–36] we hypothesized alterations in striato-cortical FC either in an attempt to compensate for brain pathology and/or reflecting brain maladaptation and behavioral deficits. To further elucidate this issue, we explored the association between FC and cognitive outcomes and the direction of such correlations. We hypothesized that distinct alterations in the striato-cortical FC would be particularly associated with visuospatial [37,38] and executive functions [39,40], as these cognitive domains are disrupted in DLB and MCI-LB patients [37–40].

2. Methods

2.1. Subjects

Altogether, 47 MCI-LB subjects and 30 HC were enrolled in the study. All subjects were examined by an experienced neurologist who included MCI-LB subjects in the study based on published criteria [4] (for details about the recruitment, the examination and the questionnaires and scales used, see supplementary material) and by an experienced neuropsychologist using a detailed neuropsychological cognitive test battery evaluating four cognitive domains: memory, attention, executive and visuospatial functions. The complex neuropsychological cognitive battery was used to evaluate four cognitive domains: memory (The Brief Visuospatial memory test revised [41], Philadelphia Verbal Learning Test [42]); attention (Wechsler Adult Intelligence Scale-III: Letter-Number Sequencing, Digit Symbol Substitution [43]); executive functions (Semantic and phonemic verbal fluency [44], Picture arrangement test [43]) and visuospatial functions (Judgment of Line Orientation [45]). The cognitive domain Z scores were computed as the average Z scores of the tests included in the particular domain.

MCI-LB subjects were diagnosed as MCI and had at least two core clinical features of MCI-LB (fluctuating cognition, visual hallucinations, or RBD, and one or more spontaneous cardinal features of parkinsonism). Exclusion criteria were serious brain injury, major psychiatric disorder, and central nervous system disease (other than MCI-LB in the patient group), alcohol/drug abuse, or any contraindications for MRI; HC did not have MCI or meet any core clinical symptoms of MCI-LB [4] or have any other neurological disorder or any potential genetic risk factors for neurogenerative disorders. All subjects signed an informed consent form that was approved by the local ethics committee.

2.2. MRI data acquisition

Subjects were scanned with the 3T Siemens Prisma MR scanner. The measurement consisted of one functional multi-echo RS run and a structural scan. The fMRI acquisition parameters: TR = 980 ms, TE_1 = 14 ms, TE_2 = 34.63 ms, TE_3 = 55.26 ms, flip angle = 50°, voxel size = 2.5 x 2.5 × 2.5 mm^3, no gap between slices, 60 transversal slices with in-plane matrix size 80 x 80 voxels, 580 scans were collected for each echo. A multiband factor of 5 and a PAT factor of 2 were used. High-resolution anatomical T1-weighted images were acquired using the MP-RAGE sequence with 192 sagittal slices, matrix size 256 x 256, voxel size 0.85 x 0.85 × 0.85 mm^3, TR = 2400 ms, TE = 2.27 ms, flip angle = 8°.

2.3. fMRI data preprocessing

The fMRI data preprocessing and analysis was done in the SPM12 toolbox. The standard preprocessing pipeline consisted of the following steps: realign & unwarp, weighted multiecho mean composite fMRI scan computation [46], spatial nonlinear registration to MNI template (using the T1 image aligned via rigid registration to mean realigned fMRI scan), and 3D smoothing using Gaussian kernel with 5 mm FWHM. All included subjects' data had sufficient fMRI data quality; i.e. data were without excessive spatial dropouts, and every subject had less than 2.5% of scans with framewise displacement (FWD [47]) above 0.75 mm, and no between-scan FWD above 2.5 mm (i.e., voxel size). Echoes were merged based on contrast-to-noise ratio criterion [46] using our own in-house implementation of the procedure in MATLAB. We have used this processing step in previous studies [48,49].

The data were decomposed to ICA components using the GIFT toolbox. The group ICA was computed and the number of components was estimated using the minimum descriptive length criterion [50]. All 37 estimated ICA components were assessed by visual inspection of their spatial patterns, and the component time-courses were then back-reconstructed for individual subjects using the GICA3 algorithm [51]. Twelve noise components were identified and used for later for

filtering as confound variables in seed analysis, performed with general linear model (GLM). ROI signals were extracted as the first eigenvariates [52–54] from the three striatal masks based on the FSL atlas by Tziorzi et al. [55]. The same procedure was used in our previous study [15] in patients with PD and AD as compared to HC; for details about the parcellation, see the supplementary material (Fig. S1). The three subdivisions spanned: 1. the dorsal striatum as the most dorsal part of the caudate and putamen; 2. the middle striatum as the more anterior part of the caudate and putamen; and 3. the ventral striatum as the most ventral part of the striatum (mostly the putamen). Based on the segmented T1 images, for every subject, the voxels containing CSF were excluded from striatal ROI masks. All signals were bandpass filtered with a low cutoff of 0.01 Hz and a high cutoff of 0.1 Hz.

2.4. fMRI data analysis

The functional connectivity analyses were performed with seed GLM analysis in a whole-brain voxel-wise manner for all subjects for all three striatal ROIs using the SPM 12 toolbox in MATLAB. The GLM contained the extracted ROI signal and 20 confound variables (12 ICA noise components, 6 movement regressors for head translations and rotations, 1 regressor with framewise displacement metric, and 1 regressor for constant; regressors for movement and framewise displacement were bandpass filtered with a low cutoff of 0.01 Hz and a high cutoff of 0.1 Hz). Subsequently, the between-group analyses were performed with GLM for each ROI separately including regressors representing age, sex, length of education, number of voxels in individual mask, and index of brain atrophy. The index of brain atrophy was computed from the whole-brain segmented T1 images as a division of the sum of probabilities:

$$\boldsymbol{AT} = \left(\sum pGM + \sum pWM\right) / \left(\sum pGM + \sum pWM + \sum pCSF\right)$$

where $\sum pX$ is the sum of probabilities of a given type of compartment X in all brain voxels, GM is gray matter, WM is white matter, and CSF is cerebrospinal fluid. To mitigate the effect of different ROI sizes and brain atrophy, we decided to model its related variability.

Spatial maps were initially thresholded at $p < 0.001$ uncorrected for multiple comparisons, and then thresholded at $p < 0.05$ family-wise error (FWE) corrected at the cluster level [56,57].

For every connection that survived correction, the beta values representing the individual connectivity strength were correlated using Spearman's correlation coefficient (with the significance level set to $p < 0.05$ uncorrected; due to the exploratory nature of this analysis, no corrections for multiple comparisons were made as in our previous study [15]) separately in the MCI-LB and HC groups with the neuropsychological scores of interest (visuospatial and executive domains z-scores); the age, sex, education length, ROI mask size, and atrophy index were regressed out of the original beta values across all subjects prior to the correlation analyses as well as during the between-group comparisons.

3. Results

3.1. Sample characteristics and behavioral differences in clinical characteristics

Out of 47 MCI-LB subjects, 7 MCI-LB patients did not finish the whole set of examinations, 5 MCI-LB patients and 1 HC did not pass the fMRI data quality check due to excessive motion, and 1 HC did not pass the fMRI data quality check due to insufficient spatial coverage/excessive dropouts (criteria are described in Section 2.3). Therefore, 35 MCI-LB patients (69.6 ± 6 years, 17 males, 18 females) and 28 HC (67.7 ± 7 years, 13 males, 15 females) met all the criteria to be included in the study. Regarding the core clinical features in the 35 MCI-LB patients: 4 were above the cut-off score for fluctuating cognition, 5 for recurrent visual hallucinations, 16 for RBD, and 26 for parkinsonism. The MCI-LB and HC groups did not differ in age or sex; see Table 1 for all demographic and clinical characteristics of the final dataset with comparisons between MCI-LB and HC.

3.2. Between-group striatal connectivity differences and correlations of striatal connectivity measures with neuropsychological scores of interest (visuospatial and executive domains z-scores)

The only significant between-group differences were observed for the dorsal striatum ROI, and all these differences reflected MCI-LB > HC, i.e., stronger connectivity in the MCI-LB group than in the HC group (to see the connectivity of the dorsal striatum in healthy participants see Fig. S2 in the supplementary materials). The results were localized in three connections linking dorsal striatum and: 1. left middle temporal gyrus, 2. right middle temporal gyrus, 3. inferior part of the right postcentral gyrus; – see Fig. 1 and Table 2.

Subsequently, connectivity of the dorsal striatum in the MCI-LB group was correlated with neuropsychological scores of interest (visuospatial and executive domains z-scores). Connection 1 (L temporal regions) positively correlated with the executive domain z score ($r = 0.34$; $p = 0.046$), see Fig. 2. Connection 2 (R temporal regions) showed a trend toward similar positive correlation with the executive domain z score ($r = 0.32$; $p = 0.058$).

4. Discussion

While striatal connectivity has been studied in PD and DLB by several authors [15,58,59] our work is concentrated specifically on basal ganglia subcortical-cortical FC in drug-naïve MCI-LB as compared to HC. We found increased connectivity in MCI-LB from the dorsal part of the

Table 1
Demographic and clinical characteristics of the subjects. Mann-Whitney *U* test was used to compare MCI-LB to HC (chi-squared test for sex). The cognitive domain Z scores were computed as the average Z scores of the tests included in the particular domain: Memory (the Brief Visuospatial Memory Test revised, Philadelphia Verbal Learning Test); Attention (Wechsler Adult Intelligence Scale-III: Letter-Number Sequencing, Digit Symbol Substitution); Executive Functions (semantic and phonemic verbal fluency, picture arrangement test) and Visuospatial Functions (Judgment of Line Orientation). Legend: Unified Parkinson's Disease Rating Scale: Motor Examination (UPDRS III), Montreal Cognitive Assessment (MoCA) REM sleep behavior disorder screening questionnaire (RBDSQ), Mayo Fluctuation Scale (MFS), Neuropsychiatric Inventory (NPI) – hallucinations subscore, Geriatric Depression Scale (GDS), Epworth Sleepiness Scale (ESS).

	HC (N = 28) - mean, std	MCI-LB (N = 35) - mean, std	U	P
Age	67.65; 7.13	69.56; 6.3	569	0.275
Sex	13 M, 15 F	17 M, 18 F	chi = 0.0286	0.8656
Education	17.23; 3.48	13.76; 3.43	217	**< .001**
UPDRS III	0.93; 1.51	6.54; 4.65	921.5	**< .001**
ESS	5.46; 3.76	7.97; 3.85	687.5	**0.006**
RBDSQ	1.93; 1.51	3.94; 2.54	716	**0.002**
GDS	4.25; 3.7	8.5; 6.4	717	**0.002**
MFS	0.11; 0.32	0.89; 1.08	714	**< .001**
NPI	0; 0	0.14; 0.36	546	**0.036**
MoCA	27.68; 1.28	23.69; 3.5	122.5	**< .001**
Visuospatial functions Z score	0.36; 0.7	−0.49; 1.1	266.5	**0.002**
Memory Z score	0.59; 0.63	−0.41; 0.92	188.5	**< .001**
Attention Z score	0.42; 0.53	−0.3; 0.79	227	**< .001**
Executive functions Z score	0.31; 0.78	−0.43; 0.89	268	**0.002**

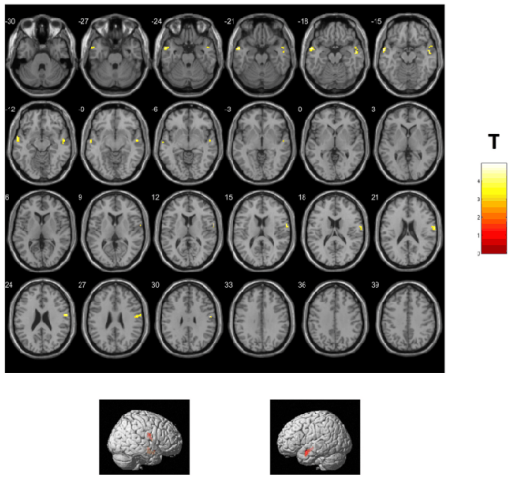

Fig. 1. Statistically significant between-group effect of dorsal striatum connectivity (T-statistics). Stronger connectivity in MCI-LB group only as compared to HC (warm color). Initially thresholded at p = 0.001 uncorrected for multiple comparisons at the voxel level with cluster level threshold p = 0.05 FWE corrected. Positions of slices in the z-axis in MNI space are indicated above the slices. The scale is valid for the slice view representation of the statistical comparison. In the bottom part, we show a rendering of the statistical comparison. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

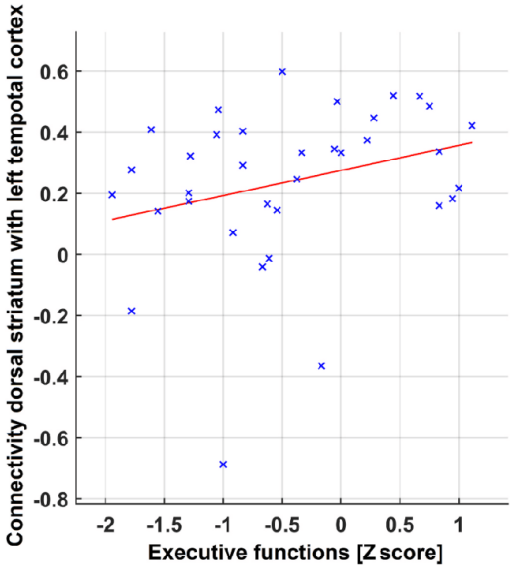

Fig. 2. Scatter plot showing correlation between functional connectivity for connection 1 (dorsal striatum to left temporal cortex) and executive functions z-score. To visualize the data, we used a linear trend (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Listing of connections from dorsal striatum with a statistically significant between-group effect (T-statistics) – only stronger connectivity in the MCI-LB group than in the HC group. Initially thresholded at p = 0.001 uncorrected for multiple comparisons at the voxel level with cluster level threshold p = 0.05 FWE corrected. For every connection, a cluster level pFWE value and the number of voxels with a significant effect (k) are reported. For each local maxima peak T-statistics (max T) with corresponding x, y, z MNI coordinates and anatomical label are reported.

Connections from dorsal striatum to:	k	$p_{FWE\ corrected}$	max T	X	Y	Z	Anatomical Label
1. left temporal cortex	59	0.003	4.93	−57	2	−19	L middle temporal gyrus
			3.96	60	10	10	L middle temporal gyrus
2. right temporal cortex	41	0.020	4.55	54	−7	−19	R middle temporal gyrus
			3.61	54	−16	−13	R middle temporal gyrus
			3.59	51	5	−19	R temporal pole: superior temporal gyrus
3. postcentral gyrus	35	0.039	4.38	63	−1	11	R Rolandic operculum
			4.05	54	−10	29	R postcentral gyrus
			3.96	63	−4	23	R postcentral gyrus

striatum, particularly to the bilateral temporal regions and to the right postcentral gyrus near the Rolandic operculum.

A recent systematic review demonstrated that structural and functional connectivity characteristics in DLB may be particularly valuable at early disease stages, before overt brain atrophy and dementia can be observed [24]. However, functional connectivity studies in the early stages of DLB (performed in either iRBD or MCI-LB) have not returned consistent results [16–19,27,60,61]. fMRI and PET studies in iRBD have collectively reported reduced functional connectivity in the executive control network, basal ganglia and sensorimotor networks [60], as well as the posterior part of the DMN, attention, and limbic networks [16], resembling overt DLB stages [24]. Another group [61] found functional and metabolic changes in the occipital and parietal areas in iRBD with MCI, and hypometabolism in these brain regions was a predictor of phenoconversion to PD or DLB. In contrast to that, in the MCI-LB group as compared to HC, Schumacher et al. [27] did not find any resting-state FC changes. The authors studied whole-brain resting-state FC using static and dynamic connectivity measures. Their sample consists of 31 MCI-LB patients compared to 28 MCI-AD patients and 24 HC. The groups were compared with respect to within- and between-network functional connectivity. In addition, dynamic functional connectivity was also studied using sliding-window analysis and eigenvector dynamic analysis. We believe that in early phases of DLB the changes in the whole-brain resting-state FC may not show major significant changes as compared to HC, while the alterations can be more prominent in more advanced stages of the disease. In our work we instead concentrated on subcortical structures (striatum, our region of interest) and its connectivity where the connection strength deviations might specifically reflect early pathological changes and behavioral deficits in drug naïve subjects (28–32).

Our result of increased connectivity goes in the opposite direction of our previous study findings in the PD population with or without MCI

[15]. In that study, we used the same methods of basal ganglia parcellation and connectivity assessment, and we observed significantly decreased dorsal striatal connectivity with prefrontal motor regions that correlated negatively with UPDRS III scores such that lower connectivity was associated with worse motor impairment. The dorsal part of the striatum includes the most dorsal part of the caudate and putamen and is located mostly in the post-commissural parts of both. This "motor" basal ganglia loop has anatomical connections particularly to the rostral- and caudal sensorimotor part of the frontal lobe and parietal lobes, and it is mainly related to motor function control [55]. Therefore, the motor basal ganglia circuitry may be less affected in MCI-LB as compared to PD or PD-MCI subjects who manifest clear motor parkinsonism. In line with this, our MCI-LB patient group consisted of subjects with no symptoms or minimal symptoms of parkinsonism based on UPDRS III testing. Therefore, it is plausible to interpret increased connectivity of the motor basal ganglia circuitry in our study as compensatory. In support of this notion, we clearly demonstrated that increased FC in the MCI-LB group was positively correlated with our cognitive outcomes. Notably, one study has shown that compensatory changes may occur on the level of the basal ganglia even in PD patients with early and very mild motor involvement [58].

We found increased FC of the dorsal basal ganglia to the perisylvian temporal regions, temporal poles, and the postcentral gyrus, that belong to both cholinergic and noradrenergic cortical output areas [28,62–66]. In synucleinopathies, very early cholinergic alterations occur of the nucleus basalis of Meynert (NBM) as well as of the cholinergic projections particularly into limbic regions and the temporal cortex [28,63,64,67–72]. For example, Schumacher et al. [63] showed specific cholinergic white matter disruptions particularly in the lateral NBM pathway in patients with DLB and MCI-LB. The lateral NBM pathway is known to have a perisylvian division supplying the frontoparietal operculum, insula, superior temporal gyrus and temporal poles; and a capsular division traveling in the external capsule and uncinate fasciculus, supplying the remaining parts of the parietal, and temporal neocortex [65,66,73,74]. In addition to cholinergic denervations, early region-specific noradrenergic alterations have also been implicated in synucleinopathies [28]. In patients with iRBD, reduced noradrenergic transporter density has been observed in sensorimotor regions including the postcentral gyrus [75], and disruptions of noradrenergic locus coeruleus (LC)-related functional networks particularly within the temporal neocortex and temporal poles were reported in PD patients with iRBD as compared to those without iRBD [76]. Notably, MRI of the LC contrast ratio showed more pronounced global and bilateral involvement of the structure in patients with DLB as compared to AD [77].

The spatial distribution of our findings is further supported by recent MRI literature in synucleinopathies. For example, increased resting-state FC in the postcentral gyrus has been a hallmark of PD as compared to HC as revealed by a recent meta-analysis [78]. This abnormality of the postcentral gyrus was unaffected by medication and cross-validated by an independent data set. Another meta-analysis of resting-state fMRI studies in PD patients with cognitive impairment as compared to PD without cognitive impairment or HC also reported abnormal (decreased) connectivity of the temporal regions of the auditory network [79]. Temporal brain region involvement has been reported in DLB subjects [25,80,81] as well as in PD with cognitive decline [82–84]. For example, a decreased within-network connectivity in temporal areas and increased between-network connectivity (between the frontal and temporal networks) was observed in DLB [81]. DLB subjects also showed altered structural connectivity: decreased clustering in bilateral temporal regions using graph analysis [25].

As already mentioned above, we showed that in the MCI-LB group increased dorsal basal ganglia connectivity, particularly to the left-sided left temporal lobe, was positively correlated with executive functions z-score. The finding could fit with attempts to compensate particularly for cholinergic and noradrenergic dysfunctions early in the course of the disease. These neurotransmitter alterations have been associated with early attention and executive function deficits that characterize the MCI-LB state [4]. We specifically included semantic and phonemic verbal fluency tests [44] and picture arrangement test [43] in our cognitive battery for evaluation of the executive function domain. Our result is in line with previous research that showed that semantic verbal fluency engages the left temporal lobe as a part of the ventral language pathway [85,86], and that temporal lobe hypometabolism seems to be one of the best predictors of DLB conversion [80]. An example of possible compensation within the temporal regions in synucleinopathy was provided by De Micco et al. [58]. In their work, the temporal network efficiency as assessed by resting-state fMRI was crucial for maintaining an effective cognitive outcome in drug naïve PD patients at baseline and for predicting slower cognitive deterioration over time. On the other hand, it has also been hypothesized that brain atrophy progression in synucleinopathies is shaped by connectivity strength and local vulnerability, with α-synuclein spreading *trans*-synaptically, via hyperconnected brain networks [58,87,88]. In this regard, we cannot rule out that hyperconnectivity between the dorsal striatum and cortical cholinergic and noradrenergic output regions in our study (albeit associated with better cognitive outcomes) may eventually lead to acceleration of neurodegeneration and faster disease progression. We plan to study this in an ongoing longitudinal study.

The study limitations include the lack of indicative and supporting biomarkers. Information on dopamine transporter imaging by SPECT/PET, polysomnographic confirmation of RBD, meta-iodobenzylguanidine uptake on myocardial scintigraphy or EEG [4] would have been beneficial for supporting early diagnosis of prodromal DLB. Unfortunately, we did not have access to study these biomarkers in our individuals. We used clinical diagnosis following the clinical diagnostic recommendations [4]. Cognitive fluctuations are one of the core clinical features of MCI-LB [4] and although the average Mayo fluctuation scale score was rather low in our MCI-LB group, it was significantly lower than HC. Therefore, it might have been beneficial to perform multiple neuropsychological testing in each individual to calculate true cognitive deficit. We tried to overcome this problem by performing cognitive testing and fMRI examination on the same day, thus eliminating day-to-day variability of the level of cognitive performance. Performing cognitive testing multiple times on the same day would have not been feasible. Another limitation is that our groups were statistically significantly different in years of education. Neuropsychological test raw scores of the subjects were always corrected for the educational level of each person. Education length was also regressed out of the original beta values across all subjects prior to the correlation analyses as well as during the between-group comparisons. Although we took these steps, we cannot fully exclude some influence of varying educational levels on our results.

In summary, we were able to capture early abnormal connectivity of the motor part of basal ganglia with posterior cortical regions involved in cholinergic and noradrenergic pathways in MCI-LB that was associated with cognitive functions also linked to cholinergic/noradrenergic deficits. We demonstrated increased connectivity from the dorsal part of the striatum to the right inferior part of the postcentral gyrus and the anterior part of the temporal cortex bilaterally; the latter finding was positively correlated with executive function performance. We believe that our results might broaden the knowledge about alterations in the early pre-dementia subtype of DLB that might help in early diagnosis and enhance our understanding of early brain compensatory attempts. On the other hand, increased basal ganglia connections to temporoparietal regions might also fit with the connectome-spreading model of brain pathology [30,58,87–91], in which early increased connection between the basal ganglia and other cortical areas may be related to more rapid and severe symptom development later on. Further longitudinal studies are warranted to prove this assumption.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reason-able request.

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CRediT authorship contribution statement

Lubomira Novakova: Conceptualization, Project administration, Writing – original draft, Writing – review & editing. **Martin Gajdos:** Data curation, Formal analysis, Methodology, Visualization, Writing – review & editing. **Marek Barton:** Data curation, Formal analysis, Methodology. **Lubos Brabenec:** Conceptualization, Data curation, Investigation. **Zaneta Zeleznikova:** Data curation, Formal analysis. **Ivona Moravkova:** Conceptualization, Investigation, Project administration. **Irena Rektorova:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the writing process

No AI or AI-assisted technologies was used in the writing process.

Declaration of competing interest

None of the authors have any conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2024.106031.

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8. Dynamic Functional Connectivity is Related to Cognitive Performance of Prodromal Lewy Body Dementia

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Commentary.

While previous functional connectivity studies generally treated brain networks as temporally stable, this publication addressed the dynamic nature of functional brain organization. The study was performed to determine whether temporal fluctuations in connectivity are altered in prodromal Lewy body dementia and whether these dynamic properties are related to cognitive performance. In these subjects visuospatial performance was associated with the occurrence and duration of specific dynamic connectivity states involving DAN, SMN, VN, and FPN. The results demonstrated relationships between dynamic connectivity characteristics and cognitive performance, indicating that temporal network organization provides information beyond conventional static functional connectivity. This publication represents a methodological progression from the static striato-cortical analyses of previous work and provides a bridge toward more individualized and sensitive network biomarkers. Its impact lies in supporting the view that neurodegeneration affects both the spatial architecture and temporal flexibility of brain networks.

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RESEARCH

Dynamic functional connectivity is related to cognitive performance of prodromal Lewy body dementia

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Abstract
Brain connectivity dynamics in mild cognitive impairment with Lewy bodies (MCI-LB) is largely unknown. We aimed to identify brain connectivity dynamics related to cognitive performance in drug naïve MCI-LB. Healthy (55 participants, age 67.7±6.4, 33 females) and MCI-LB subjects (30 participants, age 68.5±6.0, 16 females) underwent cognitive testing and fMRI. The fast eigenvector centrality dynamic functional connectivity was employed to detect connectivity states. Mean state duration and occurrence were correlated to cognitive measures. In the MCI-LB group only, the occurrence and mean duration of the state characterized by between-network connectivity of the dorsal attention, sensorimotor, and visual networks correlated with visuospatial function (VSF) domain z-scores (r=0.68, p=0.003, and r = -0.68, p=0.003, respectively). The VSF also correlated with the occurrence of the state characterized by intra-network connectivity of the frontoparietal control network (r = -0.55, p=0.041). We identified specific mechanisms that seem to facilitate visuospatial performance in MCI-LB.

Keywords Dynamic functional connectivity · Resting state fMRI · Dynamic connectivity states · Cognitive domains · Prodromal Lewy body dementia · Fast eigenvector centrality

Introduction

Lewy body dementia (LBD) is the second-most common degenerative dementia after Alzheimer's disease (AD) (Goodman et al. 2017) which is characterized by core clinical features including parkinsonism, REM sleep behavioral disorder, fluctuating cognition and visual hallucinations, and dementia dominated by dysexecutive syndrome, attentional deficits and visuospatial dysfunction, particularly early during the disease progression, at the stage of mild cognitive impairment (Donaghy et al. 2018; McKeith et al. 2020; van de Beek et al. 2020; Jellinger 2023).

Resting-state functional magnetic resonance imaging (rs-fMRI) is an effective tool for determining changes in brain networks related to LBD (Scamarcia et al. 2021). Using static and dynamic functional connectivity (dFC) (Schumacher et al. 2021), the authors were not able to uncover specific changes in mild cognitive impairment with Lewy bodies (MCI-LB) patients as compared to healthy controls (HC) and mild cognitive impairment due to AD (MCI-AD). However, distinct alterations of the spatiotemporal dynamics of whole-brain resting states were found by using a microstate analysis of EEG data (Lamoš et al. 2021).

It has been already shown that graph properties and dynamic graph properties of functional connectivity as evaluated by eigenvector centrality may reflect pathological changes related to brain neurodegeneration (Lorenzini et al. 2023). In LBD, Schumacher et al. (Schumacher et al. 2019a) reported an increased time in a state of low inter-network connectivity and difficulties in switching into densely

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connected networks. Furthermore, the authors observed reduction in the temporal variability of global network efficiency, similarly to other works performed in Parkinson's disease dementia (PDD) (Díez-Cirarda et al. 2018; Fiorenzato et al. 2019), which shares with LBD significant symptomatic and pathological overlap (Aarsland et al. 2009; Friedman 2018; Jellinger and Korczyn 2018).

Fast eigenvector centrality (FEC) is a whole-brain, voxel-wise approach designed to detect dominant patterns of fluctuations (Preti and Van De Ville 2016). By operating at the voxel level, FEC obviates the need for subjective parcellation schemes, which influence observed connectivity dynamics. The identified representative dominant patterns (RDPs) reflect established large-scale resting-state networks (RSNs), including the default mode (DMN), fronto-parietal (FPN), salience, and attention networks (Shirer et al. 2012). Given that RSN disruptions are present in both LBD(Schumacher et al. 2019a; Matar et al. 2022; Firbank et al. 2024; Onofrj et al. 2026) and PD (Onu et al. 2015; Caspers et al. 2021). Using eigenvector centrality on selected clusters, Lorenzini et al. reported reduction of functional integration between RSN in the early stages of Alzheimer's disease (AD) which further promoted cognitive deterioration (Lorenzini et al. 2023). FEC extends this metric with whole brain and parcellation independent methodology that capture dynamic network-level connectivity in spatially unconstrained manner. Therefore, it offers a promising framework for investigating the prodromal stages of these disorders, such as MCI-LB. We employed rs-fMRI and the FEC to uncover dFC states and their relation to early cognitive deficits in drug-naïve MCI-LB patients. We aimed to enhance our understanding of the role of the brain's (mal)adaption and compensation related to brain pathology and its impact on cognitive performance, particularly on the cognitive domains that are known to be impaired in the pre-dementia stage, including attention, executive and visuospatial functions. The rationale for using dFC was to assess dynamics of brain network patterns that may be well suited to reflect fluctuating cognition and lapses of attention characteristic of LBD and its prodromal stages. We hypothesized alterations in dynamics of the RSN involved in the LBD development and progression (Chabran et al. 2020), including the sensorimotor network (SMN), dorsal attention network (DAN), DMN, visual network (VN), and frontoparietal control network (FPCN), particularly in their relation to cognitive performance in the cognitive domains of interest.

Methods

Participants

Altogether, 37 participants diagnosed with MCI-LB and 65 healthy controls (HC) entered the study. A participant was assigned to the MCI-LB group based on a neuropsychological examination and the simultaneous occurrence of two or more core clinical symptoms of LBD as described previously (McKeith et al. 2020; Novakova et al. 2024; Železníková et al. 2024). All MCI-LB participants were newly diagnosed and medication-naïve. The HC group was matched for age and gender and underwent the same examinations. All subjects were informed about the scientific nature of this study and signed informed consent forms. The study received the approval of the local ethics committee.

Neuropsychological data and clinical scales and questionnaires

Global cognitive status was assessed by the Montreal Cognitive Assessment (MOCA) (Nasreddine et al. 2005) and depression was evaluated by the Geriatric Depression Scale (GDS) (Yesavage and Sheikh 1986). All MCI-LB patients were examined by the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) III Motor Examination scores (Goetz et al. 2008), while all participants underwent the Neuropsychiatric Inventory (Cummings et al. 1994), Epworth Sleepiness Scale (Johns 1991), REM Sleep Behavior Disorder Screening Questionnaire (Stiasny-Kolster et al. 2007), and Mayo Fluctuation Scale (Ferman et al. 2004). All subjects were examined by a neuropsychological examination using a cognitive battery to assess attention (Digit Symbol Substitution and Letter-Number Sequencing (Wechsler 2006), Wechsler Adult Intelligence Scale, [WAIS-III] (Wechsler 2006), executive function (semantic and phonemic verbal fluency (Nikolai et al. 2015), Picture arrangement test as part of WAIS-III (Wechsler 2006), and visuospatial function (Judgment of Line Orientation (JLO) (Benton et al. 1994). Z-scores were calculated for each cognitive test based on normative data provided in the official test manuals, which account for age, ensuring that each participant's score was standardized relative to an appropriate reference population. The cognitive domain scores (attention, executive functions) were then computed as the average of the z-scores of the tests within each domain. As we used only one test (JLO) for evaluation of the visuospatial domain, we refer to the test and not to the visuospatial domain z-score. To define mild cognitive impairment (MCI), we applied a threshold of −1 standard deviation (SD) below the normative mean for each test. Participants were classified as having MCI if they scored at least 1 SD below the expected score in at least

two tests in one or more cognitive domains (Aarsland et al. 2010).

MRI and ECG data acquisition

MRI data were acquired with a 3 T MRI scanner Siemens Prisma. The imaging protocol involved anatomical sequence T1 MPRAGE (iso-voxel size 0.85 mm; FoV 218 × 218 mm; TE 2.27 ms; flip angle 8°; 192 transversal slices; TR 2400 ms) and eyes-closed resting-state sequence (rs-fMRI) multi-echo multiband BOLD fMRI (multiband factor 5; TR 980 ms; flip angle 50°; TE [14.00, 34.63, 55.26] ms; FoV 200 mm; iso-voxel size 2.5 mm; 60 slices in transversal plane; 580 scans). When scanning rs-fMRI data, subjects kept their eyes closed and tried to relax and not to fall asleep.

Simultaneously to rs-fMRI data acquisition electrocardiographic data (ECG) were recorded with MR compatible BrainProducts BrainAmp (sampling frequency 5000 Hz).

MRI data processing

Rs-fMRI data were processed with a combination of SPM12 toolbox (Penny et al. 2011) and in-house MATLAB scripts running in MATLAB 2019a with Statistics and machine learning toolbox. The preprocessing pipeline consisted of realigning, multi-echo data merging based on contrast-to-noise ratio; CNR (Poser et al. 2006; Mikl et al. 2026), spatial normalization to MNI, spatial smoothing (Gaussian kernel with FWHM 6 mm) (Preti and Van De Ville 2016; Preti and Ville 2017; Candemir 2023), motion censoring (replacing scans with FD > 1.5 mm and one preceding and two following scans using interpolation (Power et al. 2012), and regressing out nuisance regressors for white matter (first PCA component out of the thresholded probabilistic mask of white matter with a threshold > 0.9), for cerebrospinal fluid (first PCA component out of the thresholded probabilistic mask of cerebrospinal fluid with a threshold > 0.9), and 24 movement regressors (translations, rotations, their temporal derivatives, their squares, and their squares of temporal derivatives). The data were then filtered with a high pass filter (cutoff 1/128Hz); finally, the first 10 scans were excluded to eliminate artifacts related to filtering present at the beginning of the signal.

The quality of fMRI data was checked for signal dropouts with the Mask Explorer tool (Gajdoš et al. 2016) and movement-related artifacts were checked with the framewise displacement (FD) metric (Power et al. 2012). Data where FD > 0.5 mm was exceeded in more than 20% of the scans (threshold based on other studies e.g. (Power et al. 2014; Šimko et al. 2021; Schumacher et al. 2021)), were excluded from the analysis to minimize spurious motion-related correlations in the dFC results. Four HC and 6 MCI-LB subjects were excluded due to the mentioned FD criterion, and 6 HC and 1 MCI-LB were excluded due to signal dropouts and random radiological findings. Additionally, Cohen d effect sizes of mean FD were evaluated between groups and before and after motion correction procedure.

Dynamic functional connectivity

FEC is a template-free method working with whole-brain data that computes voxel-wise eigenvector centrality to measure functional connectivity (Preti and Van De Ville 2016). Preprocessed data were analyzed based on the sliding window approach. In this study, we used ~ 60 s (61 scans) rectangular windows (Preti et al. 2017) with a step of 10 scans in the temporal domain. Shorter windows are more prone to introduce spurious fluctuations (Shakil et al. 2016). The eigenvector centralities were computed in each temporal window. MATLAB implementation of the FEC method based on the work of Preti et al.(Preti and Van De Ville 2016) was used. The computation of eigenvector centralities used the ARPACK software library implemented in MATLAB.

The representative dominant patterns (Preti and Van De Ville 2016), capturing brain states, were subsequently estimated by principal component analysis of the FECs. Mean duration and occurrence were used to quantify functional connectivity dynamics (Lamoš et al. 2021; Schumacher et al. 2021). Here the mean duration is an average duration of a continuous presence of the state in seconds and occurrence is a number of presences of the states per minute. Coverage was used as a descriptive parameter capturing percentual distribution of the states.

Elbow criterion on the sum of squared errors and second derivative of Silhouette criterion (Michel and Brunet 2019) were used to decide the optimal number of representative dominant patterns, using range 2 to 8 patterns.

Representative dominant patterns were characterized based on their local maxima and their relevance to the large-scale resting state brain networks. Hubs of the DMN, SMN, DAN, VN and the FPCN were defined with coordinates based on Gao and Lin 2012 (Gao and Lin 2012). Local maxima of a representative dominant pattern were assigned to the network if its coordinate was located within 20 mm of a network hub (Stanley et al. 2013). Moreover, Automated anatomical labelling atlas was used for characterization of the local maxima coordinates (Tzourio-Mazoyer et al. 2002).

Correlation analyses

The occurrence and mean duration of the detected brain states entered correlation analyses with z-scores of attention

and executive function domains and with a z-score of the task evaluating visuospatial functions (represented with JLO z-scores). All of these cognitive functions are known to be impaired early in the course of the disease (Scamarcia et al. 2021; Schumacher et al. 2021). Analyses were performed separately for HC and MCI-LB groups, using the Spearman correlation coefficient, $p<0.05$, false discovery rate (FDR). Subsequently, effect of age was regressed out from FEC parameters with general linear model and partial Spearman correlation to mentioned cognitive z-scores was computed with sex and education as covariates. Cognitive z-scores already reflected age when preparing composite z-score. Non-parametric method was selected because of observed outliers in the analyzed data. Wilcoxon ranksum test was used for testing differences in continuous variables between groups. Fisher's exact test was used for testing differences in gender. Linear regression model (LRM) was used to evaluate between-groups interactions in identified significant relations. Analyses were performed with inhouse MATLAB scripts including Statistics and machine learning toolbox.

Analysis of vigilance

Because fluctuation of vigilance is characteristic for MCI-LB, we used power spectral density analysis of ECG as an indirect measure of vigilance. Notably, power spectral density of ECG was reported to display a high correlation with the Psychomotor Vigilance Task (Chua et al. 2012). ECG data were processed in the tool Brain Vision Analyzer. After removal of gradient artifacts, peaks of R waves were detected. Subsequent RR intervals were processed in the MATLAB toolbox HRVTool (Vollmer 2019) (v1.07) with computation of power spectral density in the 0.02 to 0.08 Hz frequency (Chua et al. 2012). Power spectral density was analyzed in a sliding window of 300 s, similarly to the work of Chua et al.(Chua et al. 2012). Mean of power spectral density across the signal was subsequently evaluated.

Table 1 Demographic and clinical data of HC and MCI-LB including cognitive domains and core clinical features of MCI-LB

	HC*	MCI-LB*	*p* value
Age	55; 67.7±6.4 (67.3)	30; 68.5±5.9 (68.8)	$p=0.585$
Gender	55; 22 M/33 F	30; 14 M/16 F	$p=0.648$
Education [years]	55; 16.5±3.2 (17.0)	30; 14.0±3.7 (13.0)	$P=0.001$
Attention	55; 0.6±0.6 (0.5)	30; −0.2±0.7 (−0.2)	$p<0.001$
Executive functions	55; 0.3±0.5 (0.3)	30; −0.4±0.9 (−0.5)	$p<0.001$
Visuospatial functions	55; 0.5±0.6 (0.6)	30; −0.4±1.1 (−0.3)	$p<0.001$
Montreal Cognitive Assessment (MOCA)	55; 27.5±1.1 (27.0)	30; 24.1±3.2 (25.0)	$p<0.001$
MDS-UPDRS III Motor Examination scores	N/A	30; 5.9±4.1 (5.0)	N/A
Neuropsychiatric Inventory	55; 0.0±0.0 (0.0)	29; 0.2±0.4 (0.0)	$p=0.002$
REM Sleep Behavior Disorder Screening Questionnaire	55; 1.7±1.4 (2.0)	30; 4.1±2.5 (4.5)	$p<0.001$
Mayo Fluctuation Scale	55; 0.3±0.5 (0.0)	30; 0.9±1.0 (1.0)	$p<0.001$

*Number of valid observations; [mean ± standard deviation (median)]

Results

Altogether 7 MCI-LB and 11 HC were excluded based on the low quality of the MRI data. The final dataset thus involved 30 participants with MCI-LB (age 68.5±6.0; 16 females) and 55 HC (age 67.7+6.4; 33 females). Demographic and clinical data are summarized Table 1. The groups did not differ in age or gender, but they significantly differed in all three assessed cognitive domains.

The elbow criterion (see Supplementary Material for details) and second derivative of Silhouette criterion (see Figure S8) identified four states as optimal; their spatial patterns are visualized in Supplementary Material Figures S1-S4. The local maxima of state #1 corresponded to the hubs of the SMN (4 maxima in the bilateral postcentral gyri and in the right precentral gyrus), DAN (3 maxima in the right precuneus, left fusiform gyrus, and right middle temporal gyrus), and VN (2 maxima bilaterally in the cuneus). The local maxima of state #2 corresponded to the hubs of FPCN (12 maxima in the bilateral inferior parietal lobule, bilateral middle frontal gyrus, and bilateral superior frontal gyrus) and DMN (2 maxima in the right angular gyrus and left superior frontal gyrus, medial part). State #3 corresponded to the hubs of FPCN (10 maxima in bilateral supramarginal gyrus, bilateral insula, middle frontal gyrus right, and middle cingulum right) and SMN (supplementary motor area left). State #4 corresponded to the hubs of FPCN (3 maxima in the inferior frontal gyrus, pars orbitalis left, and precentral gyrus right), DMN (2 maxima in the superior frontal gyrus, medial part right, and precuneus left), and SMN (postcentral gyrus left). The states' hubs are specified in Supplementary Materials Tables S2-S5. The mean time coverage of the states 1–4 were [39.2, 18.9, 20.5, 21.3] in MCI-LB and [43.2, 16.2, 18.3, 22.3] in HC, with no significant differences between both groups.

The correlation analysis revealed a significant relationship between the occurrence and of state #1 and visuospatial function *z*-scores ($r(24) = 0.54$, $p= 0.005$, *95% CI [0.08, 0.80]*, with significant LRM interaction $p=0.010$) and between the mean duration od state #1 and visuospatial

function z-scores ($r(22) = -0.61$, $p = 0.002$, *95% CI [−0.83,−0.14]*) in the MCI-LB group. The LRM interaction between MCI-LB and HC was significant in both cases with $p = 0.010$. After controlling the analysis to the effect of age, sex and education significant relationship between the occurrence of state #1 and visuospatial function z-scores ($r(22) = 0.68$, $p = 0.003$, *95% CI [0.23, 0.89]*) and between the mean duration of state #1 and visuospatial function z-scores $r(22) = -0.68$, $p = 0.003$, *95% CI [−0.89, −0.24]*) remained, see Fig. 1. The LRM interaction between MCI-LB and HC was significant in both cases with $p = 0.019$ in first case and with $p = 0.003$ in second case. We also found a statistically significant correlation between the mean duration of state #3 and visuospatial function z-scores ($r(24) = 0.54$, $p = 0.004$, *95% CI [0.21, 0.73]*, with LRM interaction $p = 0.487$) in MCI-LB (unlike in HC), but after controlling the analysis for effect of age, sex and education, this result did not survive FDR correction, with the opposite direction

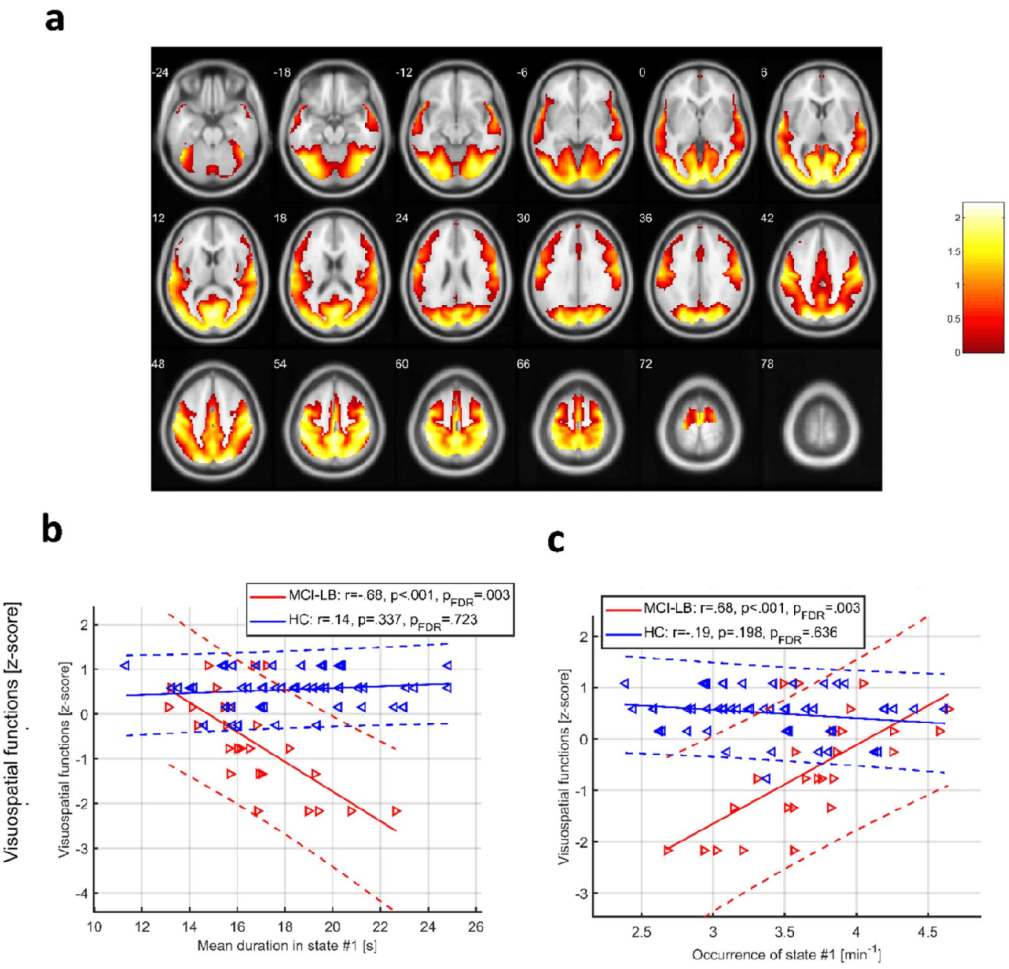

Fig. 1 Characteristics of FEC state #1. **a** The spatial distribution of the representative dominant pattern's eigenvector centralities. **b** Scatterplots represent the relation of visuospatial function z-scores to the mean duration of state #1 with linear trends (solid line) and 95% confidence intervals (dashed line). Correlation value is for partial correlation of Spearman R. Red is for MCI-LB and blue is for HC. **c** Scatterplots represent the relation of visuospatial function z-scores to the occurrence of state #1 with linear trends (solid line) and 95% confidence intervals (dashed line). Correlation value is for partial correlation of Spearman R. Red is for MCI-LB and blue is for HC

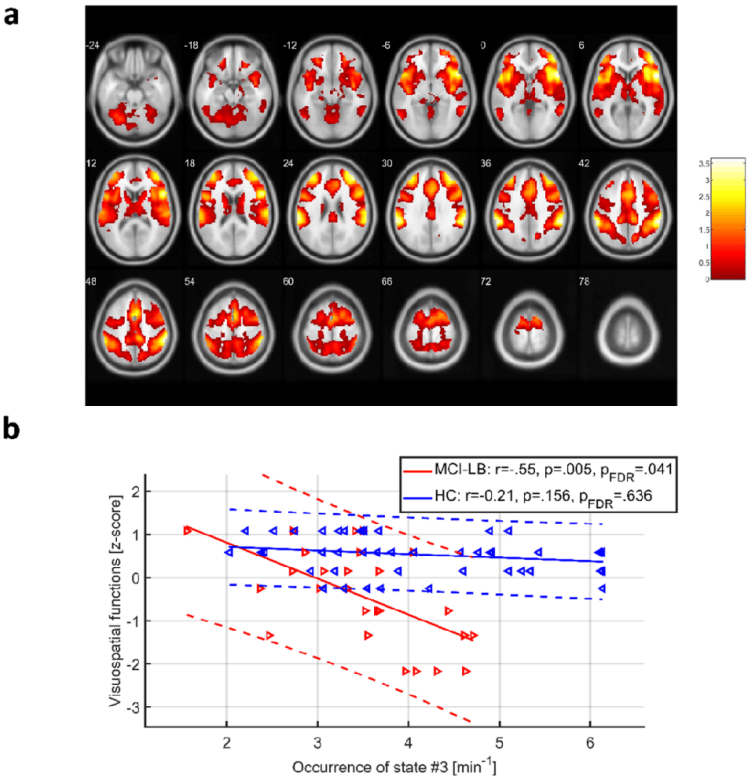

Fig. 2 Characteristics of FEC state #3. **a** The spatial distribution of the representative dominant pattern's eigenvector centralities. **b** Scatterplots represent the relation of visuospatial function z-scores to the occurrence of the state #3 with linear trends (solid line) and 95% confidence intervals (dashed line). Correlation value is for partial correlation of Spearman R. Red is for MCI-LB and blue is for HC

of correlation. However, a significant association emerged between the occurrence of state #3 and visuospatial z-scores after FDR correction (*r(22) = −0.55, p = 0.041, 95% CI [−0.83, −0.15]*, with non-significant LRM interaction *p = 0.504*), see Fig. 2. All results of the correlation analyses are presented in Supplementary Material Table S7 and Table S10. To assess whether results are related to education as a proxy of cognitive reserve/brain resilience, we further divided the MCI-LB group according to the education length mean. Notably, results remained significant in the MCI-LB individuals with lower education while they lost significance in the higher education subgroup. For detailed results, see Suppl. Material., section Effect of education.

The HC and MCI-LB groups did not differ in any of our dFC parameters of interest (Wilcoxon rank sum test, $p_{FDR}<0.05$); all dFC parameters and p-values of between-group differences are presented in Supplementary Material Table S6.

The analysis of power spectral density in 0.02 to 0.08 Hz of RR intervals revealed no significant differences in mean power spectral density (Wilcoxon rank sum $p = 0.694$, Cohen d = 0.109) between MCI-LB and HC group. The power spectral density values are depicted in Table S9.

The effect size of movement correction on mean FD is negligible within both groups ($HC_{Cohen\ d} = 0.016$ and in MCI-LB $_{Cohen\ d} = 0.009$) and the difference between both groups in mean FD has also very small effect size (Cohen d < 0.2). Details of this comparison are depicted in Supplementary Material Table S8.

Discussion

In the present study, we report that dynamics in fluctuations of BOLD fMRI capture variations in cognitive performance in drug-naïve MCI-LB. It was shown that data-driven representative dominant patterns of the FEC method can represent the dynamics of large-scale RSN (Preti and Van De Ville 2016). In MCI-LB, unlike in IIC, FEC revealed increased temporal engagement of the state characterized by positive between-network connectivity of the DAN, SMN, and VN in relation to visuospatial performance, such that

the domain z-scores were positively correlated with the state occurrence and negatively correlated with its mean duration. Interaction between MCI-LB and HC group was significant in both dFC parameters. Shorter dwell time and a more frequent state occurrence can be interpreted as a measure of (enhanced) temporal between-network flexibility (10, 11), here of the brain networks primarily engaged in LBD neurodegeneration (8), and underlying visuospatial processing control (Garcia-Diaz et al. 2018). The DAN primarily engages bilateral frontal eye fields, intraparietal sulci, and motion-sensitive middle temporal areas subserving visuospatial function, eye movements, and hand-eye coordination (Corbetta and Shulman 2002). The DAN regions involved in the dFC state #1 included the right precuneus, left fusiform gyrus, and right middle temporal gyrus. The middle temporal area and precuneus have also been identified as components of the ventral and dorsal visual streams, respectively. The dorsal visual stream has been characterized as the 'where' pathway, responsible for spatial attention, whereas the ventral visual stream has been identified as the 'what' pathway, associated with object recognition, both located particularly in the right hemisphere (Kravitz et al. 2011). The left fusiform gyrus has been implicated in visual mental imagery (Spagna et al. 2021). All the hubs of the DAN, SMN, and VN engaged in this dFC state have been reported as structural brain correlates of impaired visuospatial processing in PD subjects (Garcia-Diaz et al. 2018). We may therefore speculate that the level of network flexibility of the three major task-positive networks may facilitate visuospatial processing in early stages of LBD. Similar to our results, Asendorf et al.(Asendorf et al. 2024) showed that specific high network dynamics are relevant in motor control in cognitively unimpaired Parkinson's disease (PD) patients and associated with dopamine transporter availability in the striatum. Indeed, visuospatial attention and processing control in LBD is related to various neurotransmitters far beyond the dopaminergic system (Kanel et al. 2020; Ophey et al. 2023). Regarding cognitive functions, Nomi et al.(Nomi et al. 2017) demonstrated that the frequency of specific brain-state occurrences is important for executive function tasks in healthy individuals. This was particularly true for configurations enabling cognitive flexibility as compared to states associated with low arousal. In the same vein, Kim et al.(Kim et al. 2017) demonstrated that interconnected between-network state occurrence encompassing the central executive network, VN, and SMN was increased in PD patients. The authors interpreted this result as a potential compensatory mechanism of intrinsic brain networks resulting in stronger synchrony. The current results can also be interpreted as increased adaptive network integration which was positively associated with a better cognitive performance. Loss of adaptive integration was previously described in fully blown LBD using dynamic EEG microstate measures in relation to fMRI dynamic connectivity magnitude between basal ganglia and cortical networks (Schumacher et al. 2019b).

On the other hand, visuospatial functions in MCI-LB were negatively correlated with occurrence of the state representing mostly within-network connectivity of the FPCN hub nodes. This result was mirrored by positive correlation between the cognitive outcome and the state dwell time, although the latter result was not significant when age, sex and education were regressed out. In other words, decreased occurrence (in this case a measure of temporal stability) of the primarily intra-network connectivity state served as another mechanism to facilitate cognition. The frontoparietal control system includes the anterior prefrontal, dorsolateral prefrontal, dorsomedial superior frontal/anterior cingulate, anterior inferior parietal lobule, and anterior insular cortex. Our dFC state #3 included particularly the anterior inferior parietal lobule, known to be involved in control of spatial attention (Sapir et al. 2005), and the insula and anterior cingulate, engaged in conflict monitoring, information integration, and response selection (Christopher et al. 2015).

Increased dwell time in the state dominated by within-network connectivity is usually regarded as network segregation, and as such it has been repeatedly related to impaired clinical symptoms of PD (Kim et al. 2017; Fiorenzato et al. 2019). However, the FPCN has a unique role in facilitating switching between the DAN and the "task-negative" DMN, thus supporting on-demand brain network dynamics and guiding cognitive processing by integrating information from the external environment with stored internal representations (Vincent et al. 2008; Cascone et al. 2021). Notably, in the study of (Firbank et al. 2024), patients with advanced LBD/PD-D group with hallucinations showed reduced connectivity of the network hubs involving ACC, bilateral anterior insula and parietal regions, i.e. the regions involved in our FPCN. This was related to worse visuospatial performance. In the current study, prodromal LBD subjects reveal increased temporal stability and network segregation of the FPCN, which was was related to better visuospatial performance.

A limitation of our study is a smaller sample size in the MCI-LB group, which could have resulted in lower statistical power for detecting between-group differences in the temporal parameters of the studied states. Another limitation is representation of visuospatial functions by a single JLO test. Additionally, the MCI-LB group was characterized clinically while no suggestive biomarkers of dementia with Lewy bodies were used such as dopaminergic imaging or polysomnography. Of note, dopaminergic imaging has low sensitivity in prodromal DLB (McKeith et al. 2020)

and neither polysomnography nor α-synuclein examination using seed amplification assay from CSF or skin biopsy is broadly available in our country.

As the paradigm of the rs-fMRI measurement involved eyes closed condition, we used power spectral density of RR intervals as an indirect measure of clinical vigilance. We did not observe any differences between both groups and therefore we do not assume that the observed FEC results in the MCI-LB group were caused by the decreased levels of vigilance during MRI data acquisition. As power spectral density of RR intervals is an indirect measure, the influence of fluctuations in vigilance cannot be entirely excluded.

Taken together, our findings indicate that MCI-LB subjects engage distinct brain networks related to visuospatial processing control. Due to positive correlations with cognitive z-scores, this could be interpreted as a compensatory mechanism that seems to lead to visuospatial function facilitation in LBD subjects in the early phase of the disease, while the networks dynamics may change over time as the disease progresses to fully blown LBD. In this regards, only longitudinal studies may shed further light on this hypothesis. While compensation refers to adaptive processes in the brain that dynamically stabilize task performance despite the presence of pathology (Cabeza et al. 2018), we have to acknowledge that interpretation of the current results as "compensatory" is very speculative and much larger sample sizes would be needed and ideally task-based fMRI or an interventional condition would have to be employed to further support this notion. Based on our study design we were not able to relate the identified adaptive changes with the degree of brain pathology (e.g. demonstrating the magnitude nigrostriatal loss or degeneration of cholinergic tracts or nucleus basalis of Meynert). In fact, our results could be also related to brain resilience which refers to a cumulative capacity of neuronal resources that enables individuals to sustain function despite progressive pathology. Unlike brain compensation, this capacity develops as a consequence of lifestyle-related exposures such as e.g. education. To further address to which extend the (positive) relationship between dynamic connectivity of the states 1 and 3 and cognitive outcomes of interest is dependent on brain resilience, we divided the MCI-LB group according to their median length of education. We found that the results remained significant only in the subgroup of MCI-LB subjects with lower length of education while the correlations with cognitive outcomes were lost in the subgroup with higher length of education. In other words, the relationship between the engagement of specific dynamic states and cognitive outcomes of interest was "buffered" by cognitive reserve. Therefore, it seems plausible to state that the degree of dynamic flexibility and stability of distinct functional connectivity states at rest is (positively) associated with cognitive performance in MCI-LB and it is highly dependent on the brain resilience.

In conclusion, using dFC analysis, we were able to identify two mechanisms that were related to visuospatial variability in MCI-LB, including the network flexibility and adaptive integration of the networks involved in visuospatial function processing, and the network stability and segregation of the network particularly engaged in switching between task-positive and task-negative brain networks.

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Data availability The data supporting the findings of this study are not openly available. Anonymized data are, however, available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare no competing interests.

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9. Language Impairment in Parkinson's Disease: fMRI Study of Sentence Reading Comprehension

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Commentary.

This study expanded my research toward the neural basis of language impairment in PD. Although language deficits are increasingly recognized as part of Parkinsonian cognitive dysfunction, their underlying neural mechanisms remain incompletely understood. The study aimed to investigate neural mechanisms of sentence reading comprehension and to identify functional alterations associated with language impairment. We hypothesized that PD would be associated with impaired sentence comprehension and altered task-based functional connectivity. Patients with PD showed impaired comprehension of syntactically complex, non-canonical sentences and disrupted task-dependent connectivity between the right striatum and supplementary motor area, with greater connectivity disruption associated with poorer comprehension. The novel contribution was the use of an ecologically relevant sentence comprehension paradigm to investigate language dysfunction in PD. This work broadened my previous focus on resting-state functional connectivity by introducing task-based fMRI and provided the methodological and conceptual basis for subsequent studies comparing language processing across Lewy body diseases.

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Language impairment in Parkinson's disease: fMRI study of sentence reading comprehension

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Parkinson's disease (PD) affects the language processes, with a significant impact on the patients' daily communication. We aimed to describe specific alterations in the comprehension of syntactically complex sentences in patients with PD (PwPD) as compared to healthy controls (HC) and to identify the neural underpinnings of these deficits using a functional connectivity analysis of the striatum. A total of 20 patients PwPD and 15 HC participated in the fMRI study. We analyzed their performance of a Test of sentence comprehension (ToSC) adjusted for fMRI. A task-dependent functional connectivity analysis of the striatum was conducted using the psychophysiological interaction method (PPI). On the behavioral level, the PwPD scored significantly lower (mean ± sd: 77.3 ± 12.6) in the total ToSC score than the HC did (mean ± sd: 86.6 ± 8.0), $p = 0.02$, and the difference was also significant specifically for sentences with a non-canonical word order (PD-mean ± sd: 69.9 ± 14.1, HC-mean ± sd: 80.2 ± 11.5, $p = 0.04$). Using PPI, we found a statistically significant difference between the PwPD and the HC in connectivity from the right striatum to the supplementary motor area [SMA, (4 8 53)] for non-canonical sentences. This PPI connectivity was negatively correlated with the ToSC accuracy of non-canonical sentences in the PwPD. Our results showed disturbed sentence reading comprehension in the PwPD with altered task-dependent functional connectivity from the right striatum to the SMA, which supports the synchronization of the temporal and sequential aspects of language processing. The study revealed that subcortical-cortical networks (striatal-frontal loop) in PwPD are compromised, leading to impaired comprehension of syntactically complex sentences.

KEYWORDS

Parkinson's disease, language impairment, sentence reading comprehension, task fMRI, functional connectivity, striatum

Introduction

The hallmark of Parkinson's disease (PD) is the degeneration of dopaminergic neurons in the basal ganglia; it affects the motor system and may also affect language processes. Most of the attention in the field of communication disorders in Parkinson's disease (PD) is paid to motor speech changes. Hypokinetic dysarthria (decreased variations in pitch and

loudness and imprecise articulation) can be found using advanced analytic techniques on speech recordings, and non-invasive brain stimulation can be used as a modifying therapy for it (Brabenec et al., 2019, 2021; Klobusiakova et al., 2021).

Apart from the difficulties in speech, language problems also occur in patients with PD (PwPD). Language is one of the main cognitive domains and language performance might be influenced by overall cognitive functioning. Executive and visuospatial domain is typically impaired in early stages of cognitive decline in PD (Aarsland et al., 2021), but other domains including language might be impaired as well.

In the language production component there are deficits in grammatical formation, syntactical complexity, content/informativeness of narrative speech, specific problems with words representing actions (action naming/fluency)/manipulable objects, and non-verbal communication (Smith and Caplan, 2018). Recent study (Lowit et al., 2022) showed higher rate of grammatical and lexical errors in sentence production tasks in PwPD and even though the authors found the link between executive function and language performance in grammar tasks but performance in Sentence Generation and Narrative was independent of it. Two fMRI studies focused on brain activation in PD during action naming and processing found that PwPD seemed to process action verbs *via* the non-motor cortical networks (Péran et al., 2009; Abrevaya et al., 2016). In the language comprehension component, processing complex grammatical and syntactical structures, and narrative comprehension seem to be deficient in PwPD (Ash et al., 2012; Gross et al., 2012, 2013; Grossman et al., 2012, 2017). PwPD, as compared to healthy controls (HC), were impaired in syntactic comprehension and judgment, and at naming manipulated objects but not at naming non-manipulated objects (Johari et al., 2019).

Most authors attribute the sentence comprehension deficits in PD to limited cognitive resources (Grossman et al., 2000) and problems mostly in the executive domain, such as difficulties in set-shifting, attention, inhibition, and working memory (Grossman et al., 2002; Lee et al., 2003; Hochstadt et al., 2006; Hochstadt, 2009; Colman et al., 2011; Marková et al., 2018). Angwin et al. (2005) reported deficits in lexical-semantic processing during sentence comprehension. Their results suggest that some sentence comprehension deficits in PD may be related to a reduction in information processing speed. By contrast, Skeel et al. (2001) found deficits in processing both semantically and syntactically loaded sentences in PD, but no deficits in working memory and no significant impact of dopaminergic medication. Some authors also found that linguistic impairments might be independent of cognitive deficits, such as in executive functioning (Troche and Altmann, 2012) or link between executive function and language performance might be task dependent (Lowit et al., 2022).

Comprehension of non-canonical, passive, or object-relative structures seems to cause the most problems in PD (Lee et al., 2003; Angwin et al., 2005). Non-canonical order refers to sentences in which the *patient* (affected by the action or object) precedes the *agent* of the action (subject). In English, a rigid word order encodes the grammatical relations between words (if the word order of a sentence–e.g., The mother is kissing the daughter–is changed, the meaning of the sentence also changes: The daughter is kissing the mother). Non-canonical order in English is accomplished mostly by using passive structures (The mother is kissed by the daughter). By contrast, languages such as Slovak or Czech have a more flexible word order and these grammatical relations are indicated by case markings on the nouns (nominative–NOM vs. accusative–ACC). An example of a sentence with a canonical order in Slovak: Dcéra (NOM) bozkáva mamu (ACC), meaning "The daughter is kissing the mother"; and with non-canonical order: Mamu (ACC) bozkáva dcéra (NOM). This type of non-canonical word order sentence does not have the same function in English; the meaning is: "The mother is kissed by the daughter" but it is not passive in Slovak. Both sentences are active and typical for Slovak language but canonical word order has a processing advantage over non-canonical word order (for more details see **Supplementary Material** or Marková et al., 2017).

The neural network for sentence comprehension in healthy subjects (Walenski et al., 2019), calculated using activation likelihood estimation based meta-analyses, showed a significant involvement of regions such as the inferior frontal gyrus, insula, temporal pole, precentral gyrus, supplementary motor area (SMA), superior and middle temporal gyrus, angular gyrus, and temporal occipital fusiform cortex. The results from an analysis of non-canonical sentences showed the engagement of the frontal and posterior temporal regions as well as the left angular gyrus and the right insula.

Few neuroimaging studies using fMRI have been concentrated on language comprehension in PD. Grossman et al. (2003) found that impaired sentence comprehension in PD was associated with changes in a large-scale network important for cognitive resources during sentence processing (decreased activation mainly of the striatum and increased compensatory activation of structures associated with working memory). In accordance with these results, another group found that dysfunction of the caudate nucleus networks underlies PwPD' difficulty in dealing with complex sentence structures (before vs. after sentences) as measured in fMRI (Ye et al., 2012).

Although sentence comprehension was shown to be compromised in PD, the pathogenic role of abnormalities affecting functional integration between large-scale brain networks has not been demonstrated conclusively and a limited number of studies have focused on disturbed sentence comprehension using fMRI. We specifically focused on the striatum, because the loss of dopaminergic neurons and their projections to the striatum produces the core motor symptoms of PD and contributes to some of the cognitive and behavioral features, such as dysfunction of the basal ganglia thalamocortical network (McGregor and Nelson, 2019). We studied the striatal connectivity because previous research hinted that its alterations were connected with language impairment in PD (Grossman et al., 2003; Péran et al., 2009; Ye et al., 2012; Abrevaya et al., 2016). Our aim was to describe specific alterations in the comprehension of syntactically complex sentences in PD as compared to HC and to identify the neural underpinnings of these deficits using a functional connectivity analysis of the striatum. Our hypothesis was that PwPD show deficits in their comprehension of syntactically complex sentences (especially of non-canonical structures) with altered task-dependent functional connectivity from the striatum as compared to HC.

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Methods

Participants and examination protocol

We included clinically diagnosed non-demented PwPD (Postuma et al., 2015) and HC with the exclusion criteria of serious brain injury, major psychiatric disorder or central nervous system disease (other than PD in PwPD group), DBS or pump-delivered therapy, alcohol/drug abuse, and any contraindications for MRI. To evaluate cognitive functions, we used the following neuropsychological tests (as described in Marková et al. (2018)], in which we also indicate the cognitive domain that they primarily measure: Montreal Cognitive Assessment (MOCA)–general cognitive functioning [a cut-off score for a normal performance and inclusion in our protocol was ≥24 points as suggested by Lucza et al. (2015)], Rey's figure–visuo-spatial functions, visual memory; repetition of numbers and letters–verbal working memory and working memory capacity; Stroop test–processing speed, sensitivity to interference, cognitive flexibility; Trail Making Test–processing speed (Part A), cognitive flexibility and task switching (Part B); semantic and phonemic verbal fluency – initiation, language skills, verbal executive; auditory verbal learning test (AVLT)—memory learning test – immediate and delayed verbal memory. The neurocognitive battery was administered only in the group of PwPD. Beck Depression Inventory-II (BDI-II) was used as a subjective measure of depressive symptoms (no cut-off score was used, which is mentioned as a limitation of our study in the Section"Discussion").

Our cohort consisted of 20 PwPD (age 60.4 ± 7.6 years; 60% men; disease duration: 5.5 ± 3.1 years; The Unified Parkinson's Disease Rating Scale–UPDRS III 27.8 ± 10.2; Hoehn and Yahr–H and Y score 2 for all subjects except one, who had 2.5; Levodopa Equivalent Daily Dose–LEDD 1,156 ± 603.1 mg/day; 10 patients had right-sided PD symptom predominance, 8 had left-sided symptom predominance and 2 were without predominance; MOCA 27.4 ± 2.1; BDI-II 13.6 ± 8.6) and 15 HC (age 64.5 ± 4.3 years; 40% men; MOCA 27.9 ± 1.5; BDI-II 4.0 ± 2.3). The groups did not significantly differ in age, gender, or education, but they differed in BDI-II ($p = 0.0002$).

Experimental design–the test of sentence comprehension

All participants were right-handed Slovak native speakers and we analyzed the performance of a language-specific Slovak version of the test of sentence comprehension (ToSC) adjusted for fMRI (**Figure 1**) (for more details Marková et al., 2018, 2017). The ToSC consists of black-and-white pictures depicting an action (the roles of the main participants may reverse and they differ visually from each other, e.g., in the shade of their clothes) and semantically reversible sentences in which the main participants (subjects and objects) can exchange their thematic roles and the sentence still remains meaningful. These syntactically complex sentences contain transitive verbs and two nouns (person/animal), one functioning as a subject and the other as an object, both of which can perform the action expressed by the verb. In sentences with a "canonical order" of thematic roles, the first noun in the sentence is assigned the role of an agent (i.e., the doer of an action); in sentences with a non-canonical order of thematic roles, the first noun in the sentence plays the role of the patient (i.e., the entity that undergoes or is affected by some action). Participants quietly read the sentence below the picture on the screen during fMRI and their task was to decide whether the sentence matched the scene in the picture by pressing a YES or NO button. **Figure 1** shows an example of a picture that was presented together with the sentence "The mother (ACC) is kissing the daughter (NOM) in the white dress." All tested sentences were active and all of them were unambiguous because of the morphological cue at nouns and pronouns (for more details see **Supplementary Material** or Marková et al., 2017). The control task used the same picture material; there was a syntactically simple sentence below "The scene is taking place indoors." Or the scene is taking place outdoors.' and the participants had to decide whether decide whether the sentence matched the scene by pressing a YES or NO button. All participants were properly instructed and practiced the task before they were scanned. Only participants who understood the instructions were eligible for the study. All patients were tested in their best ON state in the morning i.e., patients took their regular morning dopaminergic medication 1 h before the data acquisition. They had to be without any changes in dopaminergic treatment in the last 3 months. All subjects signed an informed consent form that was approved by the local ethics committee.

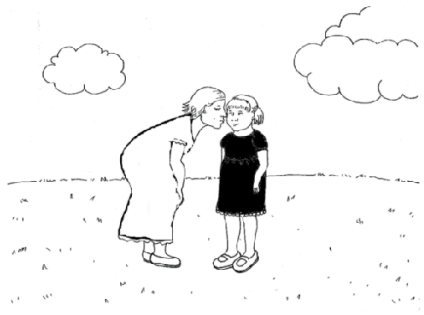

FIGURE 1
Test of sentence comprehension, sample picture, English translation: The mother in a white dress is kissing the daughter.

MRI examination

3T Siemens Prisma MR scanner (Siemens Corp., Erlangen, Germany) was used for data acquisition at the Central European Institute of Technology (CEITEC), Masaryk University in Brno, using the T1 MPRAGE sequence (TR 2,300 ms; TE 2.33 ms; voxel size 1 × 1 × 1 mm; FoV 224 × 224 mm; flip angle 8°; 240 sagittal slices) and multiecho BOLD fMRI sequence for two sessions of the task fMRI [TR 987 ms; TE (14.2, 35.4) ms; voxel size 2.561 × 2.561 × 2.5 mm; FoV 210 mm; flip angle 45°; 64 axial slices; 500 scans per session; multiband factor 4]. The fMRI task consisted of 96 trials, divided into 2 sessions (48 trials each).

During the trial (10 s), a picture was presented with a sentence describing the picture (until the participant responded by pressing a YES or NO button, up to 5 s), followed by a screen with a fixation cross (minimally 5 s). The duration time of the whole fMRI task (2 sessions together) was 16 min. The sentences were characterized by the condition of canonicity (canonical, non-canonical, and control; 32 trials each). The rate of correct and false statements was 1:1 (48 to 48). Conditions were randomly intermixed and the number of correct responses (accuracy of ToSC) was an outcome measure.

Data analyses

The fMRI data were preprocessed using the SPM 12 toolbox running under Matlab 2019a (MathWorks, Inc.). Preprocessing included realignment, multiecho merging based on CNR (Poser et al., 2006), spatial normalization, and smoothing (5 mm FWHM Gaussian filter). We excluded subjects for whom the framewise displacement metric exceeded 0.5 mm in more than 20% of the scans in each of the sessions. For fMRI analyses, four PwPD were excluded from analysis due to excessive movements. We controlled data for spatial abnormalities (e.g., dropouts) with the Mask Explorer tool (Gajdoš et al., 2016). We prepared masks of the striatum based on the structural anatomical striatal atlas (Tziortzi et al., 2011) available within the FSL software package. This atlas is subdivided into three striatal subregions–caudate, putamen, and ventral striatum. We prepared masks of the left and right striatum consisting of all three subregions present in the left and right hemispheres.

The Mann–Whitney U-test was used to calculate the differences in accuracy between groups. Spearman correlation coefficients were calculated between behavioral outcomes (accuracy of the ToSC fMRI task) and neuropsychological scores in patients. We used general linear model (GLM) to filter out the effect of those neuropsychological measures that at least moderately correlated with our behavioral outcomes (accuracy of the ToSC fMRI task) to find out how much language domain (in our case comprehension of syntactically complex sentences) might be independently impaired of other cognitive domains in PD.

A task-dependent functional connectivity analysis from the striatum was studied using the psychophysiological interaction method (PPI) (O'Reilly et al., 2012). We used whole-brain seed PPI computed with GLM. We modeled regressors for the activity of the seed (physiological regressor), for the task conditions (psychological regressors: canonical, non-canonical and control condition convolved with hemodynamic response function) and interaction of physiological and psychological regressors (PPI regressors). The model also involved 24 confound regressors for movement parameters (translations and rotations, their squares, their differences, and squares of their differences), 2 confound regressors with signals originating in white matter and cerebrospinal fluid and confound regressors for scans exceeding 1.5 mm of framewise displacement. All reported results had a statistical significance threshold set to $p < 0.05$ with FWE correction at the cluster level with initial cut $p = 0.005$ uncorrected. All reported coordinates are in MNI space. Spearman partial correlations, controlling for the effects of age and the BDI-II were calculated between the PPI parameters of interest (i.e., with significant differences between both groups) and behavioral outcomes (accuracy of the ToSC fMRI task in non-canonical sentences). Only medium to strong correlations with $|r| > 0.4$ were reported.

Results

On the behavioral level, the PwPD scored significantly lower (mean ± sd: 77.3 ± 12.6) on total score of ToSC fMRI task than HC (mean ± sd: 86.6 ± 8.0), $p = 0.02$, and the difference was significant for both canonical (PD-mean ± sd: 84.6 ± 12.7, HC-mean ± sd: 92.9 ± 5.8, $p = 0.03$) and non-canonical sentences (PD-mean ± sd: 69.9 ± 14.1, HC-mean ± sd: 80.2 ± 11.5, $p = 0.04$). Compared to a control task, the difference between PwPD and HC in comprehending syntactically complex sentences nearly reached the level of significance ($p = 0.05$). In PwPD, the overall accuracy positively correlated with phonemic fluency [$r(14) = 0.49$, $p = 0.03$] and the accuracy of non-canonical sentences positively correlated with both phonemic fluency [$r(14) = 0.40$, $p = 0.08$] and interference of Stroop task [$r(14) = 0.43$, $p = 0.06$], but p-values were non-significant, for more details see **Supplementary Table 1** in the **Supplementary Material**. After filtering the PwPD data from the effect of these two neuropsychological measures that correlated with the accuracy (phonemic fluency and interference of Stroop task) the differences between groups in accuracy was even more prominent in both total score of ToSC fMRI task ($p = 0.01$) and specifically for non-canonical sentences ($p = 0.03$).

Using PPI, we found that there was a statistically significant difference between PD and HC in connectivity from the right striatum to the SMA [4 8 53] for non-canonical sentences (the connectivity was higher in PD than in HC), **Figure 2**, for more details on PPI connectivity see **Supplementary Table 2** and **Supplementary Figures 1–4** in the **Supplementary Material**. This PPI connectivity was negatively correlated with the accuracy of the comprehension of non-canonical sentences in PwPD [$r(14) = -0.64$, $p = 0.01$], see **Figure 3**.

Discussion

Primary findings of this study showed worse performance of sentence comprehension in PwPD as compared to HC. A significant increase of functional connectivity between the right striatum and the SMA was also found in PwPD, which was negatively correlated with their impaired accuracy of non-canonical sentence comprehension. These findings suggest a compromised striatal-frontal loop in PwPD that may be related to their diminished comprehension of syntactically complex sentences.

Behavioral results that revealed problems in the PwPD with comprehension of syntactically complex sentences are in line with previous research (Lee et al., 2003; Angwin et al., 2005). Marková et al. (2018), using the same test material (ToSC) as was adapted in our fMRI task, showed that PwPD were significantly worse than HC in decoding sentences with non-canonical order. In other words, we replicated this behavioral outcome in a different sample under different data collection conditions (reading/visual

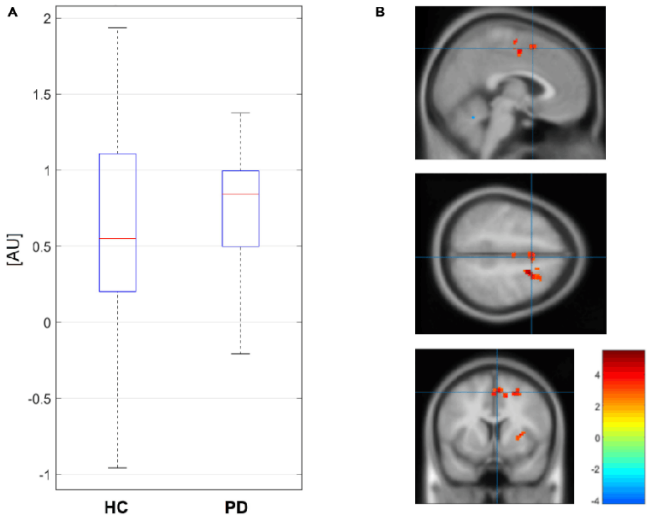

FIGURE 2
Boxplots of values of seed PPI connectivity in contrast for non-canonical stimuli from right striatum to SMA (3.73, 8.37, and 52.5) are depicted on the left **(A)**. Localization of the position in parametric map of the two sample t-test between PD and HC (three orthogonal views) with initial cutoff $p = 0.005$ for cluster level inference is on the right **(B)**.

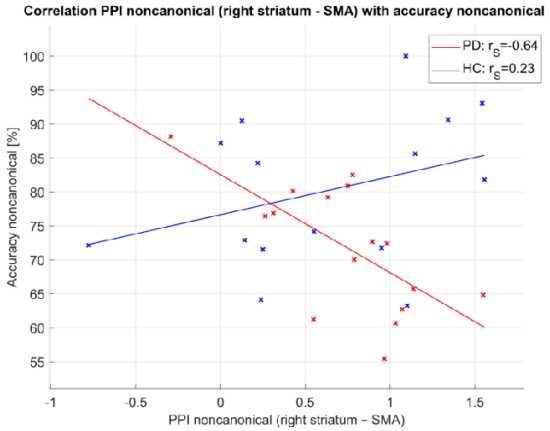

FIGURE 3
Correlation of PPI connectivity seed striatum right to SMA and accuracy of non-canonical sentences. Here r_S for Spearman correlation coefficient.

sentence comprehension inside of MRI as compared to auditory sentence comprehension in ambulance settings). In our study all patients were tested in their best ON medication state which might influence their performance in sentence comprehension task. Previously it was suggested that dopamine supports sentence comprehension in PD and that PwPD OFF levodopa have even poorer comprehension of syntactically complex sentences (Grossman et al., 2001) and slower lexical activation (Angwin et al., 2007) as compared to ON state. Studying a different neurodegenerative disease group and using ToSC, Marková et al. (2017) showed that patients with even mild Alzheimer's disease (AD) have more serious problems processing sentences with a non-canonical order.

Our results indicated that overall accuracy in an fMRI task and accuracy for non-canonical sentences correlated mostly with executive functions (in this study related to phonemic fluency,

interference of Stroop task), such as difficulties in set-shifting, mental search and monitoring, and inhibition. Similarly, executive functioning was previously described as being associated with impaired language comprehension difficulties in PD (Grossman et al., 2002; Lee et al., 2003; Hochstadt et al., 2006; Hochstadt, 2009; Colman et al., 2011; Marková et al., 2018). After filtering out the effect of executive functions, the differences between groups did not disappear; instead, they were even slightly more prominent. Thus, impaired executive functioning does not represent the main cause of the problems with comprehension of syntactically complex sentences in PD. The fact that we did not find the link between executive functioning and language deficits might be task-dependent as was shown previously (Lowit et al., 2022), in our study related to choice of phonemic fluency, interference of Stroop task and sentence reading comprehension task. Alternatively we might as other authors (Troche and Altmann, 2012) also speculate that language domain (in our case comprehension of syntactically complex sentences) might be independently impaired of other cognitive domains (e.g., executive functions). For the sentence comprehension in Slovak it is important to perceive and recognize the morphological cue and to suppress the strategy "the first noun in a sentence is the agent." The reason of worse performance of PwPD in non-canonical sentences might be the "insensitivity" to a morphological cue, or the insufficient sources for its processing.

In our research we aimed to describe subcortical-cortical effective functional connectivity differences in PwPD compared to HC while performing a sentence comprehension task. We found an increase of connectivity between the right striatum and the SMA in the PwPD group, but this increase was not associated with a better performance; instead, we found a negative correlation with task accuracy. The SMA is connected with the basal ganglia (striatum) circuit that supports the synchronization of temporal and sequential aspects (i.e., syntax) in language processing (syntactic processes under attentional control) (Kotz et al., 2009). The SMA has many other roles in PD with regard to motor aspects; it has also been shown to be a part of the neural network for sentence comprehension in healthy subjects (Walenski et al., 2019).

Maladaptation in an effort to compensate in neurodegenerative disorders has been described previously (Cramer et al., 2011). Neuroplasticity is a life-long process that intermediates the structural and functional response of dendrites, axons, and synapses to new experience or some pathological changes (Mesulam, 1999). It was described previously in mild cognitive impairment preceding AD that there is a phase of increased hippocampal activation early in the course of the disease followed by a subsequent decrease as the disease progresses (Dickerson et al., 2005). Similarly, in PD it was shown that the decline in heightened plasticity that was present at onset of the disease may reflect failure of compensatory mechanisms that maintained function in the preclinical state (Kojovic et al., 2015). In degenerative diseases with increasing pathology over time causes compensatory mechanisms to fail or become pathogenic in their effects on vulnerable neuronal populations, thereby destabilizing networks (Mesulam, 1999).

The limitations of this study are relatively small sample size, examining in the ON medication state, the lack of neuropsychological evaluation of HC and no cut-off score in BDI-II applied. In sum, we believe that subcortical-cortical networks (especially striatal-frontal loop) in PwPD are compromised, leading to deficits in comprehension of syntactically complex sentences. In the future, more studies are needed to explore the functional and structural (Hope et al., 2019; Sejnoha Minsterova et al., 2020) connectivity alterations that may be responsible for the language impairment in PD and the therapeutical approaches to address these language dysfunctions. Non-invasive brain stimulation techniques that showed potential to be modifying therapy for disturbed speech in PwPD (Brabenec et al., 2019, 2021) might also explain the causality of the altered connectivity of distinct cortical regions with the basal ganglia on specific language task performance and might modify task-related connectivity for improved language task performance (Novakova et al., 2020). But this approach for language impairment in PD needs further research and validation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the University Hospital Bratislava. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LN, IR, ZC, PV, and JM: study conception and design. AM, ZK, and JS: data collection. LN and MG: data analysis. LN, MG, PV, ZC, and IR: interpretation of results. LN: manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2023.1117473/full#supplementary-material

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10. Sentence Comprehension in Lewy Body Diseases: A Functional Magnetic Resonance Imaging Study

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Commentary.

This study extended the investigation of language impairment from PD to the broader spectrum of Lewy body diseases. It was motivated by the need to understand whether language dysfunction reflects shared or disease-specific neural mechanisms across related synucleinopathies. We hypothesized that patients with Lewy body diseases would demonstrate altered activation of language and associated cognitive networks during sentence comprehension and that these abnormalities would be related to cognitive impairment. Patients with mild cognitive impairment with Lewy bodies showed impaired comprehension of syntactically complex sentences, accompanied by reduced striatal connectivity with the cuneus, precuneus, and left supramarginal gyrus, particularly within the dorsal language pathway. The findings demonstrated that sentence comprehension difficulties are associated with distributed network dysfunction rather than abnormalities confined to isolated language regions. In relation to the 2023 PD study, this publication broadened the clinical scope while maintaining a consistent experimental framework, thereby establishing a coherent line of research on the neural mechanisms of language dysfunction across Lewy body diseases.

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BRAIN COMMUNICATIONS

Sentence comprehension in Lewy body diseases: a functional magnetic resonance imaging study

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Lewy body diseases, including Parkinson's disease and dementia with Lewy bodies, often involve mild cognitive impairment at diagnosis (mild cognitive impairment with Lewy bodies (MCI-LB). Language dysfunction in MCI-LB patients is often unrecognized. This study aimed to assess syntactic comprehension deficits in MCI-LB patients and to explore their neural correlates. A total of 25 MCI-LB patients (mean ± sd: 72 ± 5.6 years old, 10 women) and 25 healthy controls (HC, mean ± sd: 66 ± 4.0 years old, 12 women) performed task functional MRI Test of Sentence Comprehension (ToSC). Functional connectivity was analysed using psychophysiological interaction (PPI) method, focusing on the striatum and language networks. MCI-LB patients had lower ToSC scores than HC (MCI-LB: 74.7 ± 15.7, HC: 88.5 ± 9.0, $P < 0.001$) and their PPI analysis revealed decreased connectivity from the striatum to the cuneus, precuneus, and left supramarginal gyrus, and reduced connectivity particularly in the dorsal pathway during noncanonical (syntactically more complex) sentence processing. Taken together, in this cross-sectional study MCI-LB patients showed impaired sentence comprehension related to decreased subcortical-cortical and dorsal language network connectivity. Specific changes in frontotemporal connectivity in MCI-LB might be a promising indicator of language related cognitive impairment in these a-synucleinopathies.

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Keywords: language dysfunctions; mild cognitive impairment; Lewy body diseases; functional MRI; brain connectivity

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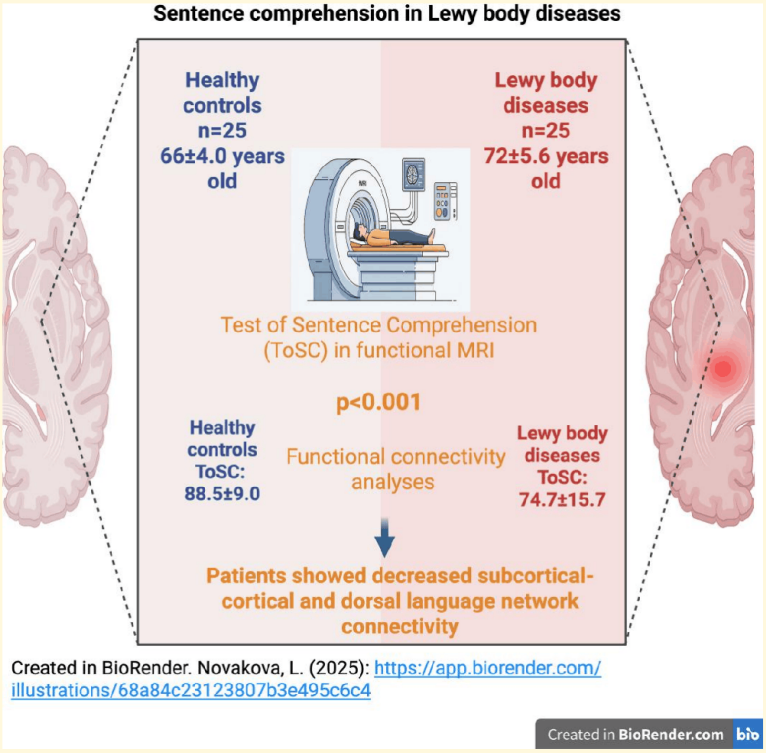
Graphical Abstract



Introduction

One of the most important human functions is the ability to communicate; the loss or limitation of this ability has devastating impacts on the individual and those around them. Language processing and the use of complex sentence structures are key features that distinguish human language from other forms of communication observed in non-human species. Syntactic comprehension is a hallmark of human communication, enabling the understanding and interpretation of how words are arranged in a sentence to express specific meanings.[1] In English, grammatical relations between words are primarily determined by a fixed word order. Changing the order of the nouns in a sentence, such as from *The mother is kissing the daughter* to *The daughter is kissing the mother* alters the meaning entirely. Noncanonical sentences (The daughter is kissed by the mother, non-canonical order in English is accomplished mostly by using passive structures) include syntactically complex sentences in which the first noun is a receiver of action (not a doer), making them more difficult to understand. In contrast, Slavic languages like Czech rely on a more flexible word order, with grammatical relations indicated by case markings on nouns (e.g. nominative–NOM and accusative–ACC). For instance, a Czech sentence in a canonical word order might be *Máma (NOM) líbá dceru (ACC)*, meaning *The mother is kissing the daughter*. A noncanonical equivalent, *Dceru (ACC) líbá mámu (NOM)*, conveys the same meaning—*The mother is kissing the daughter*—despite the different word order.[3] For an example of the test task see Fig. 1.

Functional magnetic resonance imaging enables the study of the neural correlates of language function in living humans. The functional connectivity of the language network (domain-specific areas) consists of two basic pathways: dorsal (connecting the inferior frontal gyrus and the premotor area with the posterior superior temporal gyrus and inferior



Figure 1 Test of sentence comprehension, sample picture. English translation: The daughter (accusative) is kissed by the mother (nominative) in the white dress (noncanonical word order in Czech, active). We have the permissions from the original authors of the images to include it in our manuscript (Nohová et al. 2022).[2]

parietal lobe via the arcuate fasciculus), which is mainly involved in auditory-motor integration, speech production and repetition, and phonological processing; and ventral (connecting the superior and middle temporal gyrus, inferior parietal lobe, and occipital lobe with the inferior frontal gyrus via the inferior fronto-occipital fasciculus), which is mainly involved in speech comprehension and semantic processing.[4] In addition to domain-specific areas, a domain-general network related to language is also involved: the bilateral fronto-insular-parietal system, which reflects general cognitive efforts proportional to the difficulty of the task.[5]

Neuronal Lewy body diseases (LBDs) consist of two major clinical entities – Parkinson's disease (PD) and dementia with Lewy bodies (LB). The vast majority of patients with LBDs already have mild cognitive impairment (MCI) at the time of the diagnosis. Language dysfunctions in patients with LBDs with MCI (MCI-LB) are often unrecognized and negatively affect the patient's quality of life. Behavioural and neuroimaging studies previously demonstrated that patients with LBDs exhibit deficits in sentence comprehension, particularly when processing complex syntactic structures.[6-11] In healthy subjects, a meta-analysis of functional imaging studies has suggested the dorsal language pathway as critical for processing syntactically complex sentences.[12] Complementary, evidence from patients with post-stroke aphasia and primary progressive aphasia further shows that damage to the dorsal pathway strongly predicts deficits in syntactic comprehension.[13-16]

We previously found that Slovak patients with PD without MCI already have problems with sentence reading comprehension with altered task-dependent functional connectivity.[3] We aimed to continue our previous research[3] and describe specific alterations in comprehending syntactically complex sentences in LBDs that already have MCI (MCI-LB patients) as compared to healthy controls (HC) and identify the neural underpinnings of these deficits using functional connectivity analysis from the striatum (the area involved in syntactic sentence processing and affected by the disease) and in language areas (divided into dorsal and ventral pathways[4]). We hypothesized that MCI-LB patients demonstrate greater behavioural impairments in comprehending syntactically complex sentences than HC. These deficits would be especially apparent for noncanonical sentences (our sentences of interest), and we would be able to identify neural correlates of behavioural deficits in MCI-LB as compared to HC in striato-cortical and language-related domain-specific networks. We further hypothesized that MCI-LB patients would show reduced functional connectivity of these large-scale brain networks compared to HC.

Materials and methods

Participants

A total of 25 MCI-LB (mean ± sd: 72 ± 5.6 years old) and 25 HC (mean ± sd: 66 ± 4.0 years old) participated in the fMRI study. Inclusion criteria were right-handedness, Czech as their first language, age (60–80 years), presence of PD-MCI[17] or MCI-LB.[18] We recruited participants from our study cohorts.[19,20] All participants underwent a neuropsychological examination prior to fMRI scanning that consisted of a short neuropsychological battery[21] (testing six cognitive domains: short-term memory, visuospatial functions, language functions, attention, executive functions, and long-term memory), the Montreal Cognitive Assessment test (MoCA),[22] the Boston Naming Test (BNT),[23] the Geriatric Depression Scale (GDS)[24] and the Czech version of the Functional Activities Questionnaire (FAQcz).[25] MCI subjects had subjective cognitive complaints and scored with at least two neuropsychological tests with a cutoff score under −1.5 standard deviation (SD) below the age-appropriate norms or with one test with a cutoff score under −1.5 SD below the age-appropriate norms plus a MoCA cutoff score of 26. PD-MCI patients had MCI and clinically established PD, were longitudinally followed by a neurologist, and were on a stable dopaminergic medication (mean Levodopa Equivalent Dose = 942 ± 399.6) at least 4 weeks prior to the baseline assessment and during the whole study and were tested in the ON medication state without dyskinesias. MCI-LB subjects were diagnosed with MCI and had at least two core clinical features of MCI-LB (fluctuating cognition, visual hallucinations, or REM sleep behaviour disorder, and one or more spontaneous cardinal features of parkinsonism). The exclusion criteria were a cardiac pacemaker or any MRI-incompatible metal in the body, epilepsy, any diagnosed psychiatric disorder, alcohol/drug abuse, and for the HC group additionally the presence of LBDs or other neurogenerative disorder or MCI/dementia. For more details see Table 1. The Mann-Whitney U-test was used to calculate the differences between MCI-LB and

Table 1 Characteristics of the sample

Factor	MCI-LBD/PD	HC	Test statistics of the between-group difference
N	25	25	
Demographic variables			
Sex			X^2 (1, N = 50) = 0.33
Female (*n*)	10	12	
Male (*n*)	15	13	
Age (M ± SD)	72 (± 5.54)	66.2 (± 3.94)	**U = 114.5, Z = −3.85**[a]
Education Length (M ± SD)	14.84 (± 3.81)	16.2 (± 2.78)	U = 274.5, Z = −0.737
Neuropsychological Tests (M ± SD)			
MoCA score	23.56 (± 2.45)	27.28 (± 1.34)	**U = 121.5, Z = −3.71**[a]
Short-term memory z-score	−1.04 (± 0.76)	−0.08 (± 0.75)	U = 165, Z = −2.86
Visuo-spatial functions z-score	−0.01 (± 0.5)	0.65 (± 0.53)	**U = 143, Z = −3.29**[a]
Attention z-score	−0.77 (± 0.68)	−0.32 (± 0.76)	U = 291, Z = −0.42
Executive functions z-score	−1.22 (± 0.65)	−0.12 (± 0.63)	**U = 134, Z = −3.46**[a]
Long-term memory z-score	−1.39 (± 0.64)	−0.49 (± 0.59)	U = 163, Z = −2.9
Language functions z-score	−0.67 (± 0.55)	1.12 (± 0.4)	**U = 145, Z = −3.25**[a]
Boston naming test score	25.76 (± 2.95)	28.56 (± 1.58)	U = 207, Z = −2.05
GDS score	3.12 (± 3.13)	0.84 (± 1.14)	U = 226.5, Z = −1.67
FAQcz score	1.92 (± 2.97)	0 (± 0)	U = 189.5, Z = −2.39

Note: Education Length is quantified as the total number of years of formal education completed. Due to the distribution of most variables not meeting the assumption of normality, non-parametric tests were performed. MoCA, Montreal Cognitive Assessment; GDS, Geriatric Depression Scale; FAQcz, Functional Activities Questionnaire, Czech version; *M*, mean; SD, standard deviation. [a]Values in bold indicate that the difference is significant at the $P < 0.05$ level after adjusting the variables for the effect of age and applying the Bonferroni correction.

Table 2 PPI significant differences in connectivity in non-canonical condition, the dorsal pathway, statistical significance thresholds of *t*-tests were set to $P < 0.05$, FDR corrected

Sign. diff. in	Connection	HC mean ± std	MCI-LB mean ± std	original *P* value *t*-test
noncanonical condition	T1p - T1a	0.223 ± 0.179	0.077 ± 0.243	0.021
	T1a - PMd	0.175 ± 0.182	0.031 ± 0.169	0.007
	T1a - F3op	0.165 ± 0.264	−0.035 ± 0.179	0.004
	FOP - F3op	0.198 ± 0.202	0.016 ± 0.198	0.003
	PMd - F3op	0.264 ± 0.303	0.049 ± 0.275	0.013

T1a/p, anterior/posterior superior temporal gyrus; F3op, pars opercularis of the inferior frontal gyrus; FOP, deep frontal operculum; PMd, dorsal premotor cortex.

HC. All subjects signed an informed consent form that had been approved by the local ethics committee.

MRI examination

The 3T Siemens Prisma MR scanner (Siemens Corp., Erlangen, Germany) was used for data acquisition at the Central European Institute of Technology (CEITEC), Masaryk University in Brno, using the T1 MPRAGE sequence (TR 2.400 ms; TE 2.27 ms; voxel size 0.85 × 0.85 × 0.85 mm; FoV 218 × 218 mm; flip angle 8°; 192 sagittal slices) and multiecho BOLD fMRI sequence for two sessions of the task fMRI [TR 980 ms; TE (14.0, 34.6, 55.3) ms; voxel size 2.5 × 2.5 × 2.5 mm; FoV 200 mm; flip angle 50°; 60 axial slices; 500 scans per session; multiband factor 5].

fMRI task

We analysed the performance of the Czech version of the ToSC[2] adjusted for fMRI. This was a slightly modified version of the fMRI task used in our previous research, in which the sentences were in Slovak.[3] Subjects viewed pictures in conjunction with the sentences from the ToSC on the screen during fMRI and decided whether the sentences matched the pictures by pressing a YES or NO button. The fMRI task consisted of 96 trials, divided into 2 sessions (48 trials each); the duration of the whole fMRI task was 16 min. Canonical and noncanonical word order sentences were used (ratio of use: 1:1). The rate of correct and false statements was 1:1 (48 to 48). All participants were properly instructed and practiced the task before they were scanned. The percentage of correct answers, reflecting accuracy, was our outcome measure.

Data analysis

The fMRI data was preprocessed using the SPM 12 toolbox running under Matlab 2017b (MathWorks, Inc.); this included realignment, multiecho merging based on CNR,[26] spatial normalization, and smoothing (5 mm FWHM Gaussian filter). Levels of excessive motion set to the framewise displacement metric exceeded 0.5 mm in more than 20% of the scans in each of the sessions. Two MCI-LB subjects were excluded from analysis due to excessive movements. We then controlled the data for spatial abnormalities (e.g. dropouts) with the Mask Explorer tool.[27]

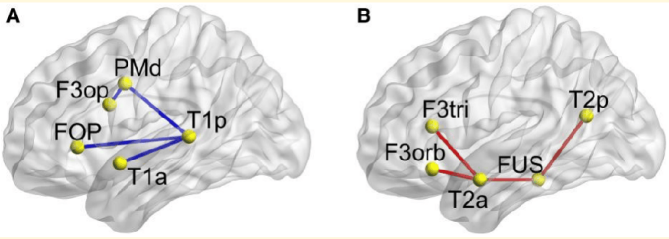


Figure 2 Dorsal and ventral language pathways. Dorsal (**A**) and ventral (**B**) pathways for language with region of interests (ROIs) that were used for the psychophysiological interaction (PPI) analysis; T1a/p: anterior/posterior superior temporal gyrus; T2a/p: anterior/posterior middle temporal gyrus; FUS: fusiform gyrus; F3orb/tri/op: pars orbitalis/triangularis and opercularis of the inferior frontal gyrus; FOP: deep frontal operculum; PMd, dorsal premotor cortex. Figure was created using BrainNet Viewer, Xia *et al.* (2013), MNI coordinates for ROIs were adapted from Sauer *et al.* 2008.

We used a mask of the whole striatum based on the structural anatomical striatal atlas[28] available within the FSL software package. We used five peak coordinates for the ventral and the dorsal language pathways, according to previously published work,[4] see Fig. 2. Spheres with the centroid at the corresponding peak voxel (radius = 6 mm) were created and intersected with the fMRI group mask. The final masks then served as ventral and dorsal language network region of interests (ROIs) and were used in the subsequent analyses.

Two task-dependent functional connectivity analyses (from the whole striatum to the rest of brain and in between the ROIs of language areas[4]) were studied using the psychophysiological interaction method (PPI)[29] with age (which differed between both groups) used as a nuisance regressor. We used a general linear model with regressors for PPI interactions for canonical and noncanonical conditions, effects of seed, effects of task conditions (canonical, noncanonical), and nuisance regressors (24 regressors for movement, nuisance regressors for white matter signal and for signal from cerebrospinal fluid). T-tests were used to calculate the differences between MCI-LB and HC. For PPI analyses from the striatum to the whole brain, we reported results with a statistical significance threshold set to $P < 0.05$ with FWE correction at the cluster level with an initial cutoff of $P = 0.001$. For PPI analyses between ROIs separated into dorsal and ventral language networks, we reported results with statistical significance threshold set to $P < 0.05$ with FDR correction. ROIs were selected as spheres with a radius of 6 mm with the first eigenvariate as a representative signal.

Results

Behavioural results

On the behavioural level, the MCI-LB patients had significantly lower total ToSC scores (mean ± sd: 74.7 ± 15.7) in fMRI than HC (mean ± sd: 88.5 ± 9.0), $P < 0.001$, and the difference was significant for both canonical (MCI-LB-mean ± sd: 82.2 ± 15.6, HC-mean ± sd: 94.0 ± 7.6, $P = 0.001$) and noncanonical sentences (MCI-LB-mean ± sd: 67.5 ± 18.0, HC-mean ± sd: 82.9 ± 12.7, $P < 0.001$).

Connectivity results

Using PPI from the striatum, we found that there was a statistically significant decrease in connectivity to the cuneus/precuneus/lingual gyrus for both canonical and noncanonical sentences in MCI-LB as compared to HC (the decrease in noncanonical sentences was more prominent) and to the left supramarginal gyrus for noncanonical sentences only, see Fig. 3 and Fig. 4. Second PPI of the language networks showed decreased connectivity in MCI-LB subjects as compared to HC during noncanonical condition between ROIs in the dorsal pathway, see Table 2. For all PPI scores, see Supplementary material.

Discussion

This study aimed to assess syntactic comprehension deficits in MCI-LB patients and to explore the neural correlates using functional connectivity analysis of the striatum and language networks. Our behavioural results showed disturbed sentence comprehension in MCI-LB patients with altered task-dependent functional connectivity from the striatum to the cuneus/precuneus/lingual gyrus and to the left supramarginal gyrus. We also found decreased connectivity in dorsal language network in MCI-LB patients as compared to HC. Changes in noncanonical sentences were related specifically to the dorsal pathway disturbances.

The cuneus/precuneus/lingual gyrus are posterior cortical regions that seem to be heavily affected in DLB patients, with these regions showing cortical thinning compared to both HC and patients with Alzheimer's disease.[30] Posterior cortical hypometabolism involving the above-mentioned regions and in the supramarginal gyrus was also present in fully blown DLB patients on FDG PET.[31,32] These areas

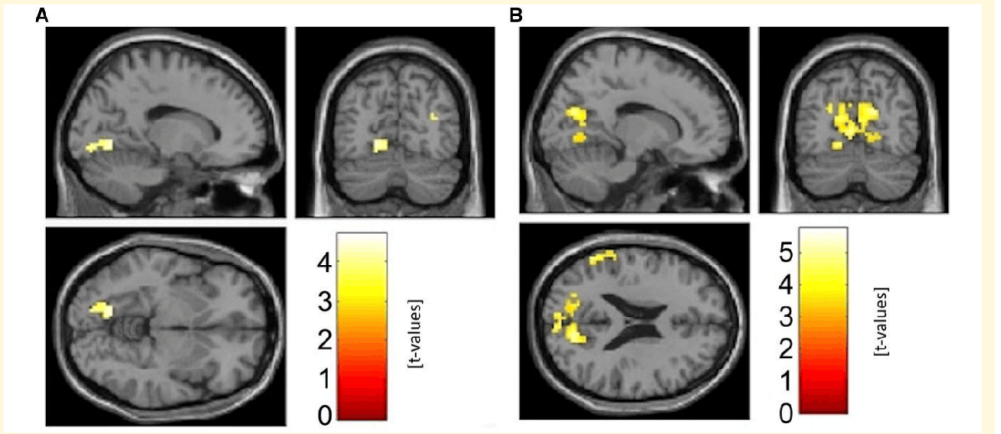


Figure 3 Connectivity differences, seed striatum, comparing patients versus healthy controls. Results of the psychophysiological interaction method (PPI) analysis using t-tests comparing patients with mild cognitive impairment with Lewy bodies (MCI-LB, $N = 25$) versus healthy controls (HC, $N = 25$), seed striatum, A: canonical B: noncanonical. In both cases were used two sample t-tests with t-values thresholded with $P < 0.001$.

showed hypometabolism even in the prodromal stages and this was associated with a higher phenoconversion rate to PD/DLB.[33]

A meta-analysis of resting-state functional connectivity in patients with a-synucleinopathy (PD, DLB, multiple system atrophy) showed hypoconnectivity between subcortical regions and the posterior default mode network (precuneus) as compared to HC.[34] This is in line with our previous study, where we found decreased resting-state functional connectivity between middle striatum and precuneus in PD, which was negatively correlated with executive functions.[35] Functionally posterior cortical regions are connected with visuospatial processing, executive functions, memory, word processing, and phonological processing.[36-39] Specifically, the supramarginal gyrus plays an important role in visual word recognition and in phonological decoding, as was seen using transcranial magnetic stimulation.[38,39]

In our study, reduced connectivity between the striatum and dorsal language pathway regions in MCI-LB were not observed in the whole-brain connectivity analyses, apart from the supramarginal gyrus, which can indeed be considered a dorsal pathway component. One possible explanation is that the strong involvement of occipital and ventral visual association cortices in our visually presented sentence comprehension task may have dominated the connectivity patterns. Prior studies have shown that sentence comprehension tasks involving picture stimuli engage rapidly and extensively visual networks in addition to classical language regions.[40,41] This may have made it more difficult to detect subtle decreases between the striatum and dorsal language areas.

Regarding the results of a PPI analysis reviewing task-related changes of specific seeds of the dorsal and ventral language pathways, we found decreased connectivity in our patient group as compared to HC, such that syntactically more complex sentences were connected to disruptions of dorsal pathway connections. This is in line with previous literature linking syntax specifically to this dorsal language pathway. The processing of complex syntax relies on the posterior superior temporal gyrus/sulcus and BA 44/45 via the dorsal pathway, but its engagement may partly reflect increased working memory demands from greater syntactic complexity.[4] Other authors[42] similarly associated higher working memory loads with the dorsal stream. Interestingly, there is also ontological differentiation: while the ventral pathway is present at birth, the dorsal pathway, critical for processing complex syntax, undergoes prolonged maturation and remains incomplete at age seven.[43] In an awake language mapping study, direct subcortical stimulation showed that the dorsal pathways are critical for organizing words in a sequence necessary for sentence generation.[44] In fact, these cortico-striatal language pathways are related to complex syntax[45] and can dynamically adjust for syntactic complexity (canonical versus noncanonical sentences).[46]

Specifically, we have seen that connectivity between anterior part of the superior temporal gyrus and the language areas in the frontal lobule (F3op: pars opercularis of the inferior frontal gyrus; PMd: dorsal premotor cortex) was lower in MCI-LB patients than in HC during noncanonical condition. Specific changes in frontotemporal connectivity were described previously in LBDs and might be a promising indicator of cognitive impairment in these a-synucleinopathies.[47-49]

In our previous study,[3] we found that patients with PD without MCI performed worse in sentence comprehension than HC and exhibited increased PPI functional connectivity

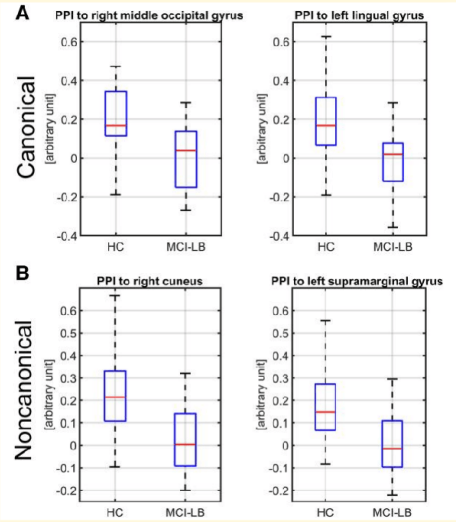


Figure 4 Boxplots of the psychophysiological interaction (PPI) analysis, seed striatum. (A) canonical—here difference between groups in connectivity to right middle occipital gyrus has on cluster level inference pFWE = 0.031, and to the left lingual gyrus pFWE = 0.019, (B) noncanonical—here difference in connectivity between groups connectivity to right cuneus has on cluster level inference pFWE < 0.001 and to the left supramarginal gyrus pFWE = 0.007. Difference is computed with two sample *t*-test on patients with mild cognitive impairment with Lewy bodies (MCI-LB, *N* = 25) versus healthy controls (HC, *N* = 25), significant clusters.

between the right striatum and the supplementary motor area (SMA), which was related to reduced accuracy in non-canonical sentence comprehension. In comparison to our previous study,[3] the current study with cognitively impaired patients reflects the disease stage when connectivity within specific language networks is decreased. Similar changes, from connectivity increases to decreased connectivity, were previously described in both AD[50] and PD.[51]

The study limitations include a relatively small sample size, the lack of indicative biomarkers, the possible role of medications used by participants, and the age difference between the patient group and HC, although age was used as a covariate of no interest in our further data analyses.

In summary, MCI-LB patients demonstrated impaired comprehension of syntactically complex sentences as compared to HC. We identified task-related reduced functional connectivity in these patients within specific subcortical-cortical and language networks compared to HC. We identified distinct alterations in the dorsal pathway that were linked to processing of syntactically complex sentences in MCI-LB.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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Competing interests

All authors declare no competing interests.

Data availability

Anonymized data will be shared with qualified researchers upon reasonable request to the corresponding author. Code used for data analysis is available in the supplementary materials.

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11. Language Network Disruption in Patients with Lewy Body Diseases

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Commentary.

This publication represents the network-level culmination of my work on language dysfunction in Lewy body diseases. After identifying abnormalities during sentence comprehension, the next question was whether these deficits were reflected in the organization of the underlying language networks. The study aimed to characterize disruptions in functional interactions among brain regions supporting language and related cognitive processes. We hypothesized that language impairment would be associated with altered rs-FC within and beyond the classical language networks. In our patients, language impairment was particularly associated with reduced efficiency and functional connectivity of the dorsal language network, with weaker frontotemporal coupling correlating with poorer language performance. These findings supported the view that language impairment in Lewy body diseases reflects distributed network dysfunction rather than isolated abnormalities of individual language regions Building on the 2023 and 2025 studies, this work demonstrates the progression of my research from identifying abnormalities during language processing to characterizing the network architecture underlying language impairment using rs-FC.

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RESEARCH

Language network disruption in patients with Lewy body diseases

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Abstract
Lewy body diseases, including Parkinson's disease and dementia with Lewy bodies, are marked by neuronal α-synuclein aggregation, motor parkinsonism, cognitive impairment and diverse non-motor symptoms including communication impairments. Compared to other symptoms, non-motor communication impairments remain under-explored, especially outside English-speaking cohorts. The aim of this study was to elucidate the neural underpinnings of linguistic deficits as assessed by fMRI in Czech-speaking patients diagnosed with neuronal Lewy body disease and mild cognitive impairment (LBD-MCI). Scores from the Short Neuropsychological Battery and resting-state functional MRI data were analyzed in 26 Czech-speaking patients with LBD-MCI and 24 healthy controls. Analyses targeted regions of interest within the dorsal and ventral language networks. We applied graph theory metrics, within-network connectivity and seed-based functional connectivity analysis. Graph analysis revealed dorsal-stream disruption in LBD-MCI: reduced clustering coefficient, increased path length, and diminished node strength, each correlating with language functions; ventral-stream topology remained intact. Within-network analyses showed impaired connectivity across both pathways, but weaker coupling between the opercular inferior frontal gyrus and posterior superior temporal gyrus predicted behavioral scores in language functioning, further highlighting dorsal vulnerability. Seed-based analysis identified reduced frontotemporal connectivity in the dorsal stream and decreased fronto-occipital and temporo-cerebellar connections in the ventral stream. LBD-MCI is characterized particularly by network-specific reductions in dorsal language stream efficiency and functional connectivity, underpinning syntactic and phonological processing deficits. These findings offer novel insights in the neural basis of language impairment in LBD-MCI.

Keywords Lewy body diseases · Mild cognitive impairment · Language network · Functional connectivity · Network efficiency

Introduction

The capacity to communicate is one of the most vital skills in humans, and its loss can profoundly impact both the individuals and their environment. In patients with Lewy body diseases (LBDs), communication impairments might not always be profoundly manifested, yet even minor deficits can significantly disrupt social interactions, ultimately diminishing the patient's quality of life. LBDs, including Parkinson's disease (PD) and dementia with Lewy bodies (DLB), are progressive neurodegenerative conditions marked by the accumulation of α-synuclein protein aggregates into Lewy bodies and neurites, resulting in widespread neuronal loss (Goedert et al. 2013). As the second-most prevalent cause of neurodegenerative dementia after Alzheimer's disease, LBDs affect millions worldwide, with significant effects

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on motor and non-motor functions (Boström et al. 2007; Walker et al. 2015).

Non-motor deficits, most notably in the domains of language and communication, are frequently underappreciated as significant contributors to the burden experienced by patients and caregivers (Aarsland et al. 2017) and have been relatively overlooked in research. The majority of PD patients develop dementia as part of the course of the disease (Hely et al. 2008); however, the cognitive decline may already be present at the time of diagnosis, concomitant with impairments in communication (Miller 2017; Smith and Caplan 2018). In DLB, mild cognitive impairment (MCI) or dementia are present from the onset of the disease, as is their adverse impact on communication (McKeith et al. 2017). Notably, recent evidence suggests that language deficits in DLB may even precede the clinical manifestation of this broader cognitive impairment (Watanabe et al. 2023). It is clear that the communication deficits observed in both PD and DLB extend beyond motor speech challenges, such as hypokinetic dysarthria, and likely encompass difficulties in higher-order language processes. Consequently, these language difficulties can have a profound impact on patients' social engagement and quality of life (Smith and Caplan 2018).

Language processing is a complex task requiring the integration of syntactic, semantic, and phonological information; it is heavily reliant on cognitive resources such as working memory, attention, and executive function (Caplan and Waters 2013; Yoon et al. 2015). In LBDs, these cognitive functions are frequently impaired, leading to difficulties in language processing including comprehension, production, and high-level language tasks (e.g., making inferences, comprehending ambiguous sentences and metaphors). This phenomenon may be especially true when processing syntactically complex sentences, which require substantial cognitive effort (Murray 2008).

Studies in PD have shown that patients exhibit deficits in processing complex sentences, a phenomenon tied to their reduced working memory and attention (Grossman et al. 2002, 2003; Bocanegra et al. 2015). Similarly, Hochstadt et al. (2006) and Colman et al. (2011) reported that language impairments in PD correlate with challenges in sequencing, switching, and manipulating linguistic information; these challenges are closely tied to impaired executive functioning in patients, highlighting the interplay between language and broader cognitive decline. In DLB, where cognitive impairment emerges early (McKeith et al. 2017), language deficits may be even more severe, though their nature in early-stage LBDs remains relatively underexplored.

There are two essential neural pathways supporting language processing (Saur et al. 2008; Nasios et al. 2019; Bartoň et al. 2023). The dorsal language network, which involves the posterior superior temporal gyrus, inferior parietal lobe and connections through the arcuate fasciculus and superior longitudinal fasciculus to the premotor cortex, Brodmann Area 44 and left inferior frontal cortex, is primarily responsible for phonological and syntactic processing (Friederici 2011). This network facilitates the structural analysis of linguistic stimuli, enabling the decoding of grammatical relationships (Saur et al. 2008). Conversely, the ventral language network, connecting the superior temporal gyrus and the middle temporal gyrus, inferior parietal lobe, and occipital lobe to the inferior frontal gyrus through the inferior fronto-occipital fasciculus, underpins semantic processing and comprehension (Hickok and Poeppel 2007). Both language pathways have rich, effective inter-regional connections with other brain areas and networks (Forkel and Hagoort 2024), including the visual network (particularly its occipital regions; Perrone-Bertolotti et al. 2017; Yablonski et al. 2023; Zhang et al. 2018; the frontoparietal network; Soshi 2023; and the cerebellum; Habas 2021; Jobson et al. 2024; King et al. 2022). In healthy brains, these networks and related brain regions are engaged in an orchestrated manner to support language processing. However, in LBDs, it is not entirely clear whether and to what extent neuronal Lewy body disease pathology disrupts language networks' integrity and their inter-regional connectivity with other brain areas, potentially impairing linguistic function.

The aim of the present investigation was to examine alterations in the efficiency and connectivity of the dorsal and ventral language networks in early LBDs as compared to healthy controls (HC) and to explore their association with language functions. By examining the dorsal and ventral language networks through graph-theory-based network analysis and seed-based functional connectivity analysis, our aim was to characterize the neural underpinnings of linguistic difficulties in LBD-MCI patients. We aimed to enhance the understanding of how alterations in these networks drive language impairments in LBDs, potentially laying the groundwork for future interventions.

In accordance with the research aims, we expected that LBD-MCI patients display disrupted network topology in conjunction with altered functional connectivity within core language networks, as evidenced by graph theory metrics and seed-based analyses. Furthermore, we anticipated that resting-state fMRI reveals altered functional connectivity between language networks and other related brain areas in LBD-MCI patients. Collectively, these hypotheses suggest novel findings to facilitate a deeper understanding of the neural mechanisms that underlie language and communication impairments in LBD-MCI.

Methodology

Participants

Participants were recruited through the CEITEC research group database and public advertisements. Initially, 60 participants were enrolled: 27 LBD-MCI and 33 HC. Written informed consent, approved by the local ethics committee, was obtained from all participants for the conduct of the study and the subsequent publication of the results, based on the collected and anonymized data.

The inclusion criteria were as follows: right-handedness (evaluated with the Edinburgh Handedness Inventory; Oldfield 1971), Czech as the first language, age between 60 and 80 years, and a diagnosis of Parkinson's disease and MCI (MCI-PD; as per Litvan et al. 2012) or the MCI stage of dementia with Lewy bodies (MCI-LB; as per McKeith et al. 2020). The evaluation of MCI, characterized by the presence of subjective cognitive complaints in conjunction with objective deterioration in cognitive functioning, was undertaken in accordance with Level I criteria (Goldman et al. 2015). To this end, the Montreal Cognitive Assessment was used, with a cut-off score of 26 plus a cut-off score of 1.5 standard deviations (SD) below the age-appropriate norms in any of the neuropsychological tests used. Alternatively, a patient had to score 1.5 SD below the age-appropriate norms in at least two tests. Exclusion criteria included the presence of a cardiac pacemaker or any MRI-incompatible metal implants, a history of epilepsy, diagnosed psychiatric disorders, alcohol or drug abuse, and for the HC group, the presence of LBD, other neurodegenerative disorders, or MCI/dementia.

Patients with clinically established PD were longitudinally followed by a neurologist and kept on a stable dopaminergic medication regimen for at least 4 weeks prior to baseline assessment and throughout the study. They underwent testing in the ON medication state without experiencing dyskinesias. The levodopa equivalent dose was calculated to quantify dopaminergic medication usage. The levodopa equivalent dose was determined based on established conversion factors for antiparkinsonian medications, following the methodology outlined by Tomlinson et al. (2010), with the average levodopa equivalent dose being 942.72 mg (SD = 399.65) for the whole subgroup.

Following the initial screening at Level I, which was used to categorize the HC group, the more stringent Level II criteria were also assessed as a part of the neuropsychological evaluation in the course of the study (Goldman et al. 2015). In this detailed evaluation, each cognitive domain was probed with two standardized neuropsychological tests, and impairment was defined as scoring 1.5 SD below age-adjusted norms in one or more domains. Eight individuals who were initially classified as HC by Level I criteria approached the Level II criteria (Goldman et al. 2015) for MCI and were consequently excluded from the HC group and all subsequent analyses. Additionally, 1 HC and 1 LBD-MCI participant were excluded from the study due to their inability to complete either the magnetic resonance imaging (MRI) protocol or the neuropsychological examination, resulting in missing critical data.

The final sample comprised 26 LBD-MCI subjects (of whom 18 had been diagnosed with MCI-PD and 8 with MCI-LB) and 24 HC participants (see Fig. 1). Goldman et al. (2014) concluded that the MCI stage, being a transitional phase preceding dementia, blurs the diagnostic boundaries between Parkinson's disease and dementia with Lewy bodies. Accordingly, MCI-PD and MCI-LB were treated as a single LBD-MCI group. Both conditions share a common alpha-synuclein–based neuropathology and exhibit highly overlapping clinical and cognitive profiles in prodromal stages (Ciafone et al. 2020).

The LBD-MCI and HC groups were statistically matched for demographic variables such as sex and education. A significant discrepancy in age was observed (see Results); consequently, the effect of age was controlled in all subsequent analyses. To ensure that the observed functional connectivity alterations were not driven by gross anatomical atrophy, we performed a control analysis of global grey matter volume (GMV; see Supplementary Text S1)

Neuropsychological evaluation

Prior to fMRI scanning, all participants underwent a comprehensive neuropsychological examination lasting approximately 60 min, which included the Montreal Cognitive

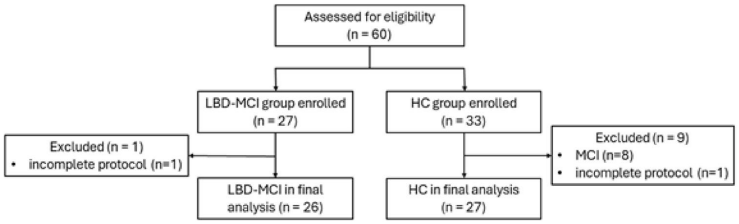

Fig. 1 Flow diagram of the participants

Assessment (Nasreddine et al. 2005), the Boston Naming Test (Zemanová et al. 2016), the Geriatric Depression Scale (Yesavage et al. 1982), the Functional Activities Questionnaire (Bezdicek and Lukavsky 2010), validated for the Czech population, and the Czech versions of Short Neuropsychological Battery (assessing six cognitive domains: short-term memory, visuospatial functions, language functions, attention, executive functions, and long-term memory; Straková et al. 2020).

The assessment of language functions comprised a naming task, in which patients identified objects based on verbal descriptions, and tests of verbal fluency. The latter included a semantic fluency task (naming as many animals as possible in one minute) and a phonemic fluency task (generating words beginning with the letters 'M' and 'B' within one minute). Language functions as assessed by the Short Neuropsychological Battery should rely on interactions between both language streams (Nasios et al. 2019). Specifically, in the naming task, the dorsal stream is important for transforming semantic representations into phonological forms (phonological encoding) and for motor planning stages of naming; the ventral stream supports semantic representations and the retrieval of conceptual knowledge necessary for accurate naming (McKinnon et al. 2018; Nasios et al. 2019). The category-based fluency tasks are commonly considered to have a close relationship with the ventral language pathway, as the semantic aspects of fluency performance rely heavily on accessing semantic categories and retrieving exemplar knowledge (Blecher et al. 2019). In contrast, phonemic fluency primarily recruits the dorsal stream, as it requires the strategic search and retrieval of words based on their phonological properties rather than their meaning - a process heavily dependent on the left inferior frontal gyrus and its dorsal connections (Costafreda et al. 2006; Hickok and Poeppel 2004). Additionally, the executive control required for both fluency tasks further recruits dorsal frontal circuits (Gonzalez et al. 2021). Consequently, this combination of tasks ensures that the functional integrity of both the dorsal and ventral language pathways is examined.

MRI sequences

Data acquisition was performed using a 3 T Siemens Prisma MR scanner (Siemens Corp., Erlangen, Germany) at the Central European Institute of Technology (CEITEC), Masaryk University in Brno. The following MRI sequences were used: magnetization-prepared rapid gradient-echo high-resolution T1-weighted images (192 sagittal slices, slice thickness = 0.85 mm, TR = 2400 ms, TE = 2.27 ms, FA = 8°, FOV = 218 mm, voxel size 0.85 × 0.85 × 0.85 mm) and multiecho gradient-echo echo-planar imaging sequence (500 scans, 60 transversal slices, slice thickness = 2.5 mm, TR = 980 ms, TE = {14; 34.63; 55.26} ms, FA = 50°, FOV = 200 mm, voxel size 2.5 × 2.5 × 2.5 mm) acquired during resting-state condition (eyes closed, subjects instructed to lie still and not to fall asleep).

Preprocessing

Resting-state fMRI data were pre-processed using MATLAB 2019a (MathWorks, Inc.) and SPM12 software. The preprocessing consisted of realignment, multi-echo data merging based on the contrast-to-noise ratio (Poser et al. 2006), normalization into standard anatomical space (Montreal Neurological Institute coordinates), and spatial smoothing. After the registration of T1 subject image to mean resliced fMRI image (coregistration), the warp (deformation) was computed for T1 subject image that best aligned the SPM MNI space template. This warp was then applied in order to register fMRI images to MNI space. Regarding distortion correction neither field mapping nor any other special method/tool was used.

We checked for motion artifacts using framewise displacement (Power et al. 2014). There were no subjects with excessive motion artifacts based on our criteria of less than 10% of scans with framewise displacement > 0.75 mm or at least one scan with framewise displacement > 3 mm. A high-pass filter with a cut-off of 1/128 Hz was applied. The six-motion parameter time series, framewise displacement, and the signals from white matter and cerebrospinal fluid were regressed out of the data in subsequent analyses.

Regions of interest

The analysis of the obtained fMRI data focused on specific regions of interest (ROIs) drawn from the study by Saur et al. (2008) describing dorsal and ventral brain pathways involved in language processing. For the dorsal pathway, the ROIs included the posterior superior temporal gyrus (T1p; 57, 36, 6), anterior superior temporal gyrus (T1a; 57, 3, 9), frontal operculum (FOP; 42, 27, 0), dorsal premotor cortex (PMd; 48, 0, 36), and inferior frontal gyrus (F3op; 45, 9, 24). For the ventral pathway, the ROIs comprised the posterior middle temporal gyrus (T2p; 48, 60, 18), anterior middle temporal gyrus (T2a; 51, 0, 18), fusiform gyrus (FUS; 30, 33, 18), inferior frontal gyrus pars triangularis (F3tri; 48, 27, 12), and inferior frontal gyrus pars orbitalis (F3orb; 45, 27, 12).

Rather than using a standard anatomical atlas, spheres with the centroid at the corresponding peak voxel (radius = 6 mm) were created and intersected with the fMRI group mask. This mask is a group mask created using mask explorer toolbox (Gajdoš et al. 2016). Firstly, individual subjects' binary masks are calculated using the mentioned

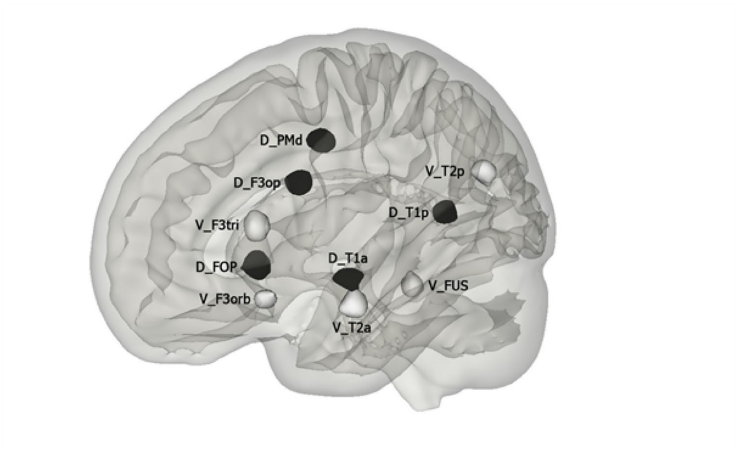

Fig. 2 Illustration of the ROIs used in our analyses, with dorsal pathway regions indicated in light grey and ventral pathway regions indicated in dark grey

Table 1 Selected areas within the dorsal and ventral language network [a]

	ROI label in Schaefer atlas	MNI coordinates			Corresponding ROIs from Saur et al. (2008) [b]
		R	A	S	
Dorsal					
	85	−50	3	38	PMd
	131	−45	20	27	F3op
	186	−48	28	0	FOP
	195	−53	6	−11	T1a
	198	−52	−43	5	T1p
Ventral					
	128	−48	35	10	F3tri
	170	−57	−9	−14	T2a
	173	−45	−58	21	T2p
	185	−46	32	−10	F3orb
	193	−30	−33	−18	FUS

[a] The table presents the ROIs from the Schaefer atlas (2018) that were used in our analyses with their Montreal Neurological Institute coordinates (centroids) based on the final masks and corresponding area labels used by Saur et al. (2008).

[b] PMd - dorsal premotor cortex, F3op - opercular part of the inferior frontal gyrus, FOP - frontal operculum, T1a - anterior superior temporal gyrus, T1p - posterior superior temporal gyrus, F3tri - triangular part of the inferior frontal gyrus, T2a - anterior middle temporal gyrus, T2p - posterior middle temporal gyrus, F3orb - orbital part of the inferior frontal gyrus, FUS - fusiform gyrus.

toolbox as following: a threshold of 80% of the image mean intensity is used in order to differentiate between brain tissue (value 1) and background noise (value 0). Then, a group mask is calculated as a conjunction of subjects' masks. The final masks then served as dorsal and ventral language network ROIs and were used in the subsequent analyses. All the regions in question are depicted in Fig. 2 and listed in Table 1, which details the correspondence between these coordinate-based regions and the atlas-based parcels used for graph analysis.

Graph metrics

Pre-processed resting-state fMRI data were further processed using MATLAB 2019a and the Brain Connectivity Toolbox (NITRC: Brain Connectivity Toolbox: Tool/Resource Info, n.d.). The whole brain was parceled into 400 regions of interest according to the 400-region Schaefer atlas (Schaefer et al. 2018). These ROIs were intersected with individual subject binary masks (described in Sect. 2.5; Gajdoš et al. 2016) to ensure high signal quality in every subject. There were 19 ROIs that had more than 50% of signal dropouts (Gajdoš et al. 2018) in more than 10% of subjects; these ROIs were excluded from subsequent analyses. The time series of each ROI was averaged and cross-correlated using Pearson's correlation coefficient to form a correlation matrix.

(381 × 381) for each subject. For further analyses, Fisher's z-transformed Pearson's correlation coefficients were used. Weighted networks were analyzed so that useful information about the strength of connections was preserved. Negative correlations were replaced with zeros.

We computed the following global metrics: average clustering coefficient, characteristic path length, average node strength, modularity, and global efficiency. The local clustering coefficient, nodal path length, and node strength were calculated for ROIs of the whole brain. Next, the average value of each measure was computed specifically for the dorsal and ventral language network as a representative local measure. The selection of specific parcels from the Schaefer atlas that correspond to the dorsal and ventral

language networks defined by Saur et al. (2008) is detailed in Table 1.

Seed-based analysis

Seed-based functional connectivity analysis was performed on pre-processed resting-state data. Specifically, the abovementioned final masks serving as dorsal and ventral language network ROIs (see Saur et al. 2008) were generated and their corresponding mean time series were then extracted and entered in the SPM first-level model as a regressor. The resulting contrasts were then entered into a two-sample t-test to identify brain areas with connectivity differences between LBD-MCI and HC groups, with age used as covariate of no interest. Clusters that survived an initial voxel-wise threshold of $p<.001$ (uncorrected) and a cluster-level FWE correction at $p<.05$ were defined as significant.

For post-hoc characterization of these effects, mean beta values from their first-level seed-to-voxel maps were extracted for each participant and averaged within these significant clusters, resulting in one average connectivity value for each seed-cluster pair. These per-subject average connectivity values were compared between the LBD-MCI and HC groups using Analysis of Covariance (ANCOVA) with age as a covariate. To ensure robustness against non-normal distributions, parameter estimates were calculated using bias-corrected and accelerated (BCa) bootstrapping with 1000 resamples. The overall statistical threshold was set at $p<.05$ FWE-corrected at the cluster level, with an initial voxel-wise cut of $p<.001$ uncorrected.

ROI analysis (dorsal/ventral LN)

In order to analyze resting-state functional connectivity between individual ROIs in dorsal and ventral language network separately, the time series of each ROI (abovementioned final sphere masks; Saur et al. 2008) were averaged and cross-correlated using Pearson's correlation coefficient. Thus, a correlation matrix for each subject for dorsal and ventral language network separately was created and Fisher's z-transformed. Z values then served as representative resting-state connectivity measures.

Statistical analysis

All statistical analyses were performed using software from MATLAB and SPSS. Group comparisons between LBD-MCI and HC in demographic variables not accounting for age were performed using the Mann-Whitney U test. The χ^2 test was used to compare categorical variables such as sex. For neuropsychological domains, graph metrics and connectivity metrics, group differences between LBD-MCI and HC were assessed using Analysis of Covariance (ANCOVA) with age included as a covariate. To account for the non-normal distribution of the data, significance was determined using bias-corrected and accelerated (BCa) bootstrapping with 1000 resamples; we also tested group × age interactions to verify the homogeneity of regression slopes. To ensure that the observed significant between-group differences were not influenced by demographic variables or depressive symptoms, we performed a sensitivity analysis using ANCOVA models that included Sex, Education, and GDS as additional covariates.

In order to further investigate the roles of specific language networks, an additional correlation analysis was performed, focusing on network-specific graph theory metrics and resting-state connectivity values for both the dorsal and ventral language networks and their associations with neuropsychological domains. In addressing the association between cognitive performance, language functions, graph metrics and seed-to-seed connectivity, partial correlations were employed, with the effect of age being controlled for. The subsequent objective was to determine the predictive capacity of both the language network efficiency disruption and the connectivity disruption on behavioral scores of language function. To this end, two hierarchical linear regression analyses were performed to determine the predictive value of network alterations while controlling for the effect of age. In the first analysis, language functions were designated as the dependent variable; age was entered in the first block, while dorsal and ventral network graph metrics were entered stepwise in the second block. In the second analysis, the same hierarchical procedure was applied, with seed-to-seed connectivity values defined as the independent variables in the second step.

Results

Sample characteristics

The LBD-MCI group was, on average, 6.2 years older than the HC group ($p<.05$). The LBD-MCI group achieved significantly lower scores than the HC group in language functions and in all tested domains except for attention.

We assessed global grey matter volume to control for potential atrophy-related confounds. After controlling for age, no significant group differences in GMV were observed and structural volume did not correlate with functional connectivity metrics (for full details and statistics, see Supplementary Text S1). Consequently, age was included as the sole nuisance covariate in all statistical models. For a detailed comparison of the groups, please refer to Table 2.

To validate the combination of MCI-PD and MCI-LB patients into a single LBD-MCI cohort, we performed a comprehensive comparison of these subgroups. the only significant between-subgroup difference was observed on the Functional Activities Questionnaire (U=29,5, Z = −2.363, p=.032). On average, MCI-PD patients scored 3.125 points lower on the 30-point scale, indicating slightly reduced independence in instrumental activities of daily living compared with MCI-LB patients. No other significant differences were found in demographics, neuropsychological performance (see Supplementary Table S2 for a detailed comparison of the MCI-PD and MCI-LB subgroups), graph metrics or connectivity measures. Furthermore, a sensitivity analysis excluding the MCI-LB subgroup yielded results consistent with the primary analyses. Consequently, the LBD-MCI sample was treated as a single group for the purposes of the present analyses.

Graph analysis

Graph theory analysis demonstrated significant disruptions in global network efficiency among LBD-MCI relative to HC. ANCOVA tests controlling for age disclosed that LBD-MCI patients exhibited a significantly lower average global clustering coefficient [F(1,47)=6.23, η^2_p=0.12, p =.016] and global node strength [F(1,47)=6.81, η^2_p=0.13, p =.012], alongside a greater average global path length [F(1,47)=7.87, η^2_p=0.14, p =.007], compared to HC.

To delineate network-specific effects, graph analyses were conducted separately for the dorsal and ventral language networks. Within the dorsal language network, significant alterations were observed in LBD-MCI patients, with a decreased clustering coefficient [F(1,47)=6.16, η^2_p=0.12, p =.017], an elevated average path length [F(1,47)=8.58, η^2_p=0.15, p =.005] and a reduced node strength [F(1,47)=6.86, η^2_p=0.13, p =.012]. The between-group differences persisted after covarying for Sex, Education, and GDS scores. In contrast, parallel analysis of the ventral language network revealed no significant differences in these graph metrics between LBD-MCI and HC.

With regard to the associations observed with neuropsychological domains, the correlational analysis indicated a significant correlation between the metrics and language function, especially within dorsal network. Nevertheless, a distinction was identified between the two networks: metrics from the dorsal language network were also correlated with executive function and long-term memory; those from the ventral language network demonstrated correlations with attention. The detailed results of this analysis are presented in Table 3.

In terms of the regression analysis investigating the graph metrics disruption as a predictor of language functions

Table 2 Characteristics of the sample

Factor	LBD-MCI [a]	HC	Test statistics of the between-group difference [a]
N	26	24	
Participant characteristics			
Sex			X^2 (1, N=50)=0.28
Female (n)	10	12	
Male (n)	15	13	
Age (M±SD [b])	72.31 (±5.65)	66.08 (±3.98)	U=109, Z=−3.95*
Education length [c] (M±SD)	15.04 (±3.87)	16.33 (±2.76)	U=246.5, Z=−1.29
Grey matter volume proportion (M±SD	0.37 (±0.03)	0.4 (±0.03)	F (1,47)=1.2, η^2_p=0.03
Neuropsychological examination (M±SD)			
Montreal Cognitive Assessment score	23.38 (±2.56)	27.25 (±1.36)	F (1,47)=25.2, η^2_p=0.35*
Short-term memory z-score	−1.12 (±0.81)	−0.07 (±0.76)	F (1,47)=13.34, η^2_p=0.22*
Visuospatial functions z-score	−0.03 (±0.5)	0.65 (±0.54)	F (1,47)=14.92, η^2_p=0.24*
Attention z-score	−0.76 (±0.67)	−0.3 (±0.77)	F (1,47)=1.16, η^2_p=0.02
Executive functions z-score	−1.28 (±0.7)	−0.13 (±0.64)	F (1,47)=19.35, η^2_p=0.29*
Long-term memory z-score	−1.42 (±0.64)	−0.51 (±0.6)	F (1,47)=14.89, η^2_p=0.24*
Language functions z-score	−0.73 (±0.68)	0.14 (±0.4)	F (1,47)=12.12, η^2_p=0.21*
Geriatric Depression Scale score	3.08 (±3.07)	0.87 (±1.15)	F (1,47)=5, η^2_p=0.1*
Functional Activities Questionnaire score	2.54 (±4.29)	0 (±0)	F (1,47)=4.2, η^2_p=0.08*

[a] Due to the non-normal distribution of most variables, group comparisons were performed using either non-parametric tests (Mann-Whitney U) or ANCOVA with age included as a covariate. For ANCOVA, parameter estimates were calculated using bias-corrected and accelerated (BCa) bootstrapping with 1000 resamples.

[b] Values in the table represent unadjusted means±standard deviation.

[c] Education length is quantified as the total number of years of formal education completed.

* The difference is significant at the p <.05 level after applying the Holm-Bonferroni FWE correction

decline, when controlled for age, the model proved to be significant, F(1,48) = 5.735, p =.021, explaining 10.7% of the variance beyond what age explained (R^2 = 0.107). The metric of dorsal path length was a sole significant negative predictor of language function z-score (B = −0.370, SE = 0.154, β = −0.327, t = −2.4, p =.021).

Within-network seed-based analysis

From a total of 20 pre-specified pairwise combinations examined−10 within the dorsal language network (comprising PMd, F3op, FOP, T1a, and T1p) and 10 within the ventral language network (comprising F3tri, T2a, T2p, F3orb, and FUS) - four connections exhibited statistically significant reductions in LBD-MCI as compared to HC. Specifically, within the dorsal language network, connectivity between the opercular part of the inferior frontal gyrus (F3op) and the posterior superior temporal gyrus (T1p) was markedly diminished in LBD-MCI [F(1,47) = 9.41, η^2_p = 0.17, p =.004]. This link within the dorsal network was significantly correlated with language functions (r =.283; p =.04). Including Sex, Education, and GDS scores did not alter the overall pattern of findings.

Within the ventral language network, significant reductions were observed in connectivity between the triangular part of the inferior frontal gyrus (F3tri) and the anterior middle temporal gyrus [T2a; F(1,47) = 6.36, η^2_p = 0.12, p =.015]. However, this link was not correlated with language functions. There was also a significant reduction in connectivity between F3tri and the posterior middle temporal gyrus (T2p; F(1,47) = 5.45, η^2_p = 0.1, p =.024], yet again without correlation with the language function. Lastly, the connection was reduced between T2a and T2p [F(1,47) = 5.44, η^2_p = 0.1, p =.024], also with no significant correlation with language function. Sensitivity analysis adjusting for sex, education length, and GDS scores failed to confirm the robustness of group difference for the T2a–T2p functional connection (p =.051). In fully adjusted model, neither the group effect nor any of the additional covariates reached statistical significance, suggesting that the originally observed difference was likely driven by shared variance among demographic and clinical factors rather than a specific disease effect. It is also important to note that none of the within-network connectivity differences retain their significance after applying a multiple-comparisons correction. For the complete list of all pairwise comparisons, see Supplementary Table S3.

With regard to the regression analysis, the investigation into the potential predictive capacities of resting-state connectivity impairments within language networks on behavioral language functioning, when controlled for age, yielded a significant model (F(1,47) = 4.1, p <.04). The model accounted for 6.4% of the variance above that explained by age (R^2 = 0.064). The value of the resting-state connectivity between the opercular part of the inferior frontal gyrus (F3op) and the posterior superior temporal gyrus (T1p) emerged as a significant positive predictor of language function z-score (B = 1.269, SE = 0.627, β = 0.253, t(47) = 2.025, p =.04).

Whole-brain seed-based analysis

In LBD-MCI patients as compared to HC, the seed-based analysis of resting-state functional connectivity revealed significant reductions in the connectivity of the language network ROIs with other areas in the rest of the brain. As illustrated in Table 4, significant differences were identified between the LBD-MCI group and the HC group for multiple seed-cluster pairs. The results remained stable after adjusting for Sex, Education, and GDS scores. Of the connections showing significant group differences, three involving dorsal network nodes and two involving ventral network nodes exhibited significant correlations with language function.

In summary, the ROIs of the dorsal language network demonstrated significant decreases in left-sided fronto-temporal connectivity, connectivity with the left posterior cerebellum (declive) and the bilateral cuneus. In the ventral network ROIs, a reduction in connectivity was observed, particularly with occipital regions, the posterior and anterior parts of the cerebellum (declive with culmen in the left hemisphere and declive with cerebellar tonsil in the right hemisphere), the right caudate nucleus, and the left superior temporal gyrus.

Table 3 Correlations between network-specific graph metrics and examined neuropsychological domains

Variable	Dorsal clustering coefficient	Dorsal path length	Dorsal node strength	Ventral clustering coefficient	Ventral path length	Ventral node strength
Language functions	0.316*	−0.367*	0.319*	0.267	−0.315*	0.247
Short-term memory	0.181	−0.227	0.165	0.170	−0.232	0.151
Visuospatial functions	0.142	−0.157	0.144	0.086	−0.131	0.073
Attention	0.261	−0.266	0.276	0.285*	−0.286*	0.319*
Executive functions	0.301*	−0.320*	0.301*	0.261	−0.296*	0.231
Long-term memory	0.325*	−0.332*	0.347*	0.260	−0.265	0.251

* p <.05

Table 4 Reduced functional seed-to-seed connectivity in LBD-MCI patients compared to healthy controls

Seed region [a]	Target region[b]	U	Z	p	r[c]
Dorsal Language Network					
F3op	left superior temporal gyrus	163	−2.893	0.004	0.347*
F3op	bilateral cuneus	99	−4.136	<0.001	0.288*
T1p	left superior and middle frontal gyrus	130	−3.534	<0.001	0.437*
T1p	left cerebellum (declive)	139	−3.359	<0.001	0.241
Ventral Language Network					
F3tri	left middle occipital gyrus	166	−2.835	0.005	0.379*
T2a	bilateral middle occipital gyrus, cuneus and precuneus	54	−5.01	<0.001	0.268
T2p	left cerebellum (declive, culmen)	129	−3.554	<0.001	0.258
T2p	right cerebellum (declive, cerebellar tonsil)	117	−3.787	<0.001	0.228
T2p	right caudate and putamen	108	−3.961	<0.001	0.237
T2p	left superior temporal gyrus and putamen	167	−2.816	0.005	0.488**

[a] The seed region shows the starting point of the connectivity analysis.

[b] The target region denotes the brain region with which the seed region shows reduced connectivity in LBD-MCI patients.

[c] The *r* column shows partial correlations of connections with language functions, age controlled.

* $p<.05$ and ** $p<.001$.

Discussion

The present study examined language processing deficits in Czech-speaking patients with LBD-MCI, highlighting the interplay between language functioning, network efficiency, and functional connectivity alterations in the dorsal and ventral language networks. Graph theory analysis indicated particularly impaired efficiency and integration in the dorsal language network. The results of the functional connectivity analyses revealed disruptions across both networks, while only a single dorsal connectivity metric emerged as a predictor of language functioning. The findings of this study indicate that the observed variations in language functions among the study groups can be partially attributable to these dorsal network differences. The findings focus specifically on the neural properties of language networks and serve to supplement and expand upon the results of earlier studies examining language processing and production in patients with PD and LBD. These earlier studies demonstrated that patients with LBDs experience progressive deterioration in brain connectivity, with the result that both their motor and cognitive functions are impacted in the context of language processing (Cotelli et al. 2007; Péran et al. 2009). The pattern of broad disruption in language networks and its association with various cognitive domains uncovered in this study is also consistent with earlier resting-state fMRI studies in LBDs, which have reported connectivity changes associated with cognitive decline (Baggio et al. 2014; Peraza et al. 2014).

Graph theory analysis revealed significant reductions in network efficiency in LBD-MCI patients, particularly within the dorsal language network, which is critical for phonological and syntactic processing. The path length showed the strongest association with behavioral measures of language functioning and emerged as a significant negative predictor. In contrast, the ventral language network, responsible for semantic processing, showed no significant disruptions in any of the network efficiency properties. These findings highlight a selective disruption of language network properties in LBD-MCI. This could potentially reflect disease-specific impairments in the neural architecture supporting phonological and syntactic processing (Friederici 2011), suggesting its vulnerability, while the ventral network's global organization appears relatively preserved.

The correlational analysis further showed that apart from association with language functions, the dorsal network's metrics also correlated with executive functions and long-term memory. The dorsal network efficiency and executive functioning were both diminished in LBD-MCI; the correlation between these might suggest that syntactic processing encompasses higher-order executive processes. Therefore, it could be hypothesized that the decline in executive functioning gives rise mainly to the syntactic aspect of linguistic difficulties. Similarly, the association with long-term memory implies that the dorsal network supports the retrieval of associations, patterns or schemas, further contributing to linguistic abilities (Caplan and Waters 2013). Overall, these findings may explain why LBD-MCI patients exhibit particular linguistic difficulties such as comprehension and naming deficits (Murray 2008), as these tasks demand both executive resources and memory retrieval – areas often impaired early in LBDs (Aarsland et al. 2017). Problematic syntactic processing is also supported by studies in PD or MCI-LB showing difficulties with complex sentence structures (Hochstadt et al. 2006; Novakova et al. 2023, 2025). Furthermore, this pattern of dorsal stream vulnerability mirrors the network degeneration observed in non-fluent/agrammatic primary progressive aphasia (Henry et al.

2016). While LBD-MCI is clinically distinct from PPA, this shared anatomical disruption likely underpins the syntactic difficulties observed in both conditions, implying a link between dorsal tract integrity and grammatical processing (Mandelli et al. 2016).

The identification of within-network frontotemporal connectivity disturbances provides insight as to which segments of the language networks are most affected. The connectivity between the inferior frontal gyrus and the posterior superior temporal gyrus appears to be the most negatively impacted within the dorsal network; in the ventral network, the connections between the triangular part of the inferior frontal gyrus, the anterior middle temporal gyrus, and the posterior middle temporal gyrus appear to be weakened. However, the regression analysis demonstrated that only the connectivity within the dorsal network significantly predicts scoring in behavioral measures of language functioning. Consequently, the seed-based connectivity analysis yielded further evidence of widespread network alterations in LBD-MCI, unveiling diminished functional connectivity and its potential influence on language deficits – particularly with regard to the effect of frontotemporal connectivity within the dorsal language network. This finding further corroborates the aforementioned results, indicating disrupted neural communication within the dorsal network.

The observed reductions in dorsal network whole-brain resting-state connectivity with other key language-related regions are consistent with previous studies highlighting the critical role of these regions in syntactic processing and phonological working memory (Friederici 2011). In particular, the frontotemporal connectivity in the dorsal language pathway, such as the opercular part of the inferior frontal gyrus (F3op; a part of Broca's area) and the left superior temporal gyrus, is hypothesized to facilitate top-down processes that are instrumental in the determination of grammatical relations (Friederici 2011). The diminished connectivity between the posterior superior temporal gyrus (T1p) and regions including the left superior frontal gyrus and the cerebellum might suggest a broader disruption in the integration of linguistic and executive mechanisms, as the T1p is traditionally considered a neurological basis for language comprehension (Buchsbaum et al. 2001; Turken and Dronkers 2011), while the left superior frontal gyrus is linked to higher cognitive and executive functions (du Boisgueheneuc et al. 2006). The cerebellum is traditionally linked to motor aspects of language deficits (De Smet et al. 2007); however, it has been increasingly recognized for its role in language processing, particularly in coordinating lexical retrieval and syntactic structuring (Murdoch 2010; Highnam and Bleile 2011; Mariën et al. 2014). van Dun et al. (2016) argued that the cerebellum is involved in language in a similar manner as it is involved in motor functions – through monitoring/coordinating cortical functions. Nasios et al. (2019) stated that the cerebellum ought to be included in an updated "multiple stream model" of language processing, as its targeting with transcranial direct current stimulation proved to have enhancing effects on verbal fluency (Turkeltaub et al. 2016). Thus, reduced connectivity with the cerebellar subparts – such as the declive, culmen, and cerebellar tonsils – may at least partially underlie deficits in language processing in LBD-MCI.

With regard to the ventral network, which is principally responsible for semantic processing and lexical access (Hickok and Poeppel 2004, 2007), between-network connectivity was also disrupted. This was particularly evident in the anterior part of the middle temporal gyrus (T2a), which exhibited significantly reduced connectivity with the middle occipital gyrus and precuneus. The middle temporal gyrus has been identified as a crucial hub for semantic memory and control with a role in accessing and integrating word meanings (Binder and Desai 2011; Ralph et al. 2017); the precuneus activation has also been observed in processing sentence semantics (Price 2010), particularly those involving spatial linguistic information and relations (Wallentin et al. 2008). However, it is important to note that unlike the dorsal network findings, these specific ventral connectivity reductions did not correlate with language function scores in our sample. While the reduced connectivity implies a compromise in the neural architecture supporting conceptual retrieval and spatial integration (Grossman et al. 2002), the lack of behavioral association suggests that these deficits may not yet be the critical limiting factor for language performance at this disease stage. The diminished connection between the posterior part of the middle temporal gyrus (T2p) and the cerebellum then points to a similar pattern of disrupted connectivity as the one observed in the dorsal network, indicating that specific cerebellar subparts might play a coordinating role (van Dun et al. 2016) in the semantic aspect of language processing as well as in the syntactic one. Yet the absence of a correlation with language scores further indicates that the observed communication deficits are predominantly driven by dorsal stream dysfunction.

Several limitations should be noted when interpreting the findings of this study. The relatively small sample size may constrain statistical power and limit the ability to generalize the results to a broader population. We addressed this by employing rigorous diagnostic criteria to ensure sample homogeneity and by using bootstrapping to ensure statistical robustness. Second, while we utilized standardized tests to assess language function, these tasks inevitably recruit domain-general cognitive processes such as attention and executive control. The correlations observed between dorsal network metrics and executive function scores likely reflect the intrinsic functional overlap of these

domains – specifically, the dorsal stream's role as an interface between syntactic processing and executive resources – rather than a lack of test specificity. Future studies with larger cohorts and more granular linguistic tasks are needed to further disentangle these components. Furthermore, the potential influence of medications used by participants presents another challenge; while patients were on stable regimens, we cannot fully rule out the possibility that these medications affected cognitive or language performance, potentially confounding the results. Additionally, a limitation of this study is the pooling of MCI-PD and MCI-LB patients into a single LBD-MCI cohort. The clinical distinction between these diagnoses is largely operational and does not necessarily reflect a clear biological separation, resulting in highly overlapping features at the MCI stage. Accordingly, our supplementary analyses confirmed the subgroups were comparable across all demographic, cognitive, and network measures, differing only in Functional Activities Questionare. Nevertheless, we acknowledge that subtle clinical differences, such as motor symptom burden, could influence resting-state dynamics or task execution in ways that might be obscured when the diagnostic subgroups are combined. And finally, the patient group was older than the HC group, which could influence the outcomes. We tried to limit this potential influence by adjusting for the effect of age in the analyses. These limitations highlight the need for careful consideration of the results and suggest directions for future studies to address these gaps.

In essence, these findings contribute to the understanding of language impairments in LBDs, extending beyond motor speech deficits such as hypokinetic dysarthria (Smith and Caplan 2018). The graph theory and seed-based analyses of resting-state fMRI data revealed significant disturbances in both the efficiency and connectivity of the dorsal language network, significantly correlating with language functioning. Notably, the path length metric and within-network connectivity between F3op and T1p areas predicted the scores in language processing tasks. Thus, the selectively impaired dorsal network properties suggest that syntactic processing may be particularly vulnerable in early-stage LBDs. The additional involvement of the ventral network connectivity indicates that semantic processing is also altered (Goedert et al. 2013), although its impact on certain language processing tasks might not be behaviorally manifested. The whole-brain connectivity analyses further confirm the findings of language network deficiency, confirming the inter-regional connectivity disturbances with other language-related brain areas. These results have the potential to enhance the current understanding of the underlying mechanisms that lead to language impairments in LBDs, thereby paving the way for the development of future intervention strategies.

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Author contributions All authors contributed to the study conception and design. Daniel Carbol conducted the formal analysis, interpreted the results and wrote the original draft of the manuscript. Lubomira Novakova conceptualized the study, designed the methodology and collected the data. Patricia Klobusiakova preprocessed and curated the data and created the visualizations. Irena Rektorova supervised the project and performed critical review and editing of the manuscript. All authors reviewed and approved the final manuscript.

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Data availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was approved the Research Ethics Committee of Masaryk University (Approval No. EKV-2022-081; Project: Jazyk a onemocnění s Lewyho tělísky: Problémy s porozuměním větám a modifikující terapie pomocí neinvazivní stimulace mozku; Proposal No. 0780/2022; Principal Investigator: Mgr. et Mgr. Ľubomíra Nováková, Ph.D.; Affiliation: CEITEC) on 27 June 2022. All participants provided written informed consent, which had been approved by the abovementioned ethics committee, prior to enrolment in the study. This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

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Part III. Modulation of Brain Plasticity and Translational Neuroscience

12. Non-invasive Brain Stimulation of the Right Inferior Frontal Gyrus May Improve Attention in Early Alzheimer's Disease

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Commentary.

This study represents the beginning of my research interest in NIBS and translational neuroscience. It was motivated by the limited therapeutic options available for cognitive impairment in AD and by evidence that cortical networks may remain modifiable despite neurodegeneration. We investigated whether stimulation of the right inferior frontal gyrus could influence attention in patients with early AD. The main hypothesis was that modulation of a cortical region involved in attentional control would lead to measurable cognitive improvement. The findings suggested a potential beneficial effect on attention, providing preliminary support for targeted neuromodulation in early AD. The principal contribution was the exploration of a network-informed stimulation target rather than a purely symptomatic intervention. This study established the conceptual basis for later work examining predictors of stimulation response and the neural mechanisms of induced plasticity. My contribution should emphasize involvement in the clinical study, cognitive assessment, interpretation of stimulation effects, and manuscript editing.

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Short communication

Non-invasive brain stimulation of the right inferior frontal gyrus may improve attention in early Alzheimer's disease: A pilot study

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ABSTRACT

Introduction: Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive tool for modulating cortical activity.
Objectives: In this pilot study, we evaluated the effects of high frequency rTMS applied over the right inferior frontal gyrus (IFG) on cognitive functions in patients with amnestic mild cognitive impairment (MCI) or incipient dementia due to Alzheimer's disease (AD).
Methods: Ten patients (6 men; 4 women, mean age of 72 ± 8 years; MMSE 23 ± 3.56) were enrolled in a randomized, placebo-controlled study with a crossover design. All participants received 2 sessions of 10 Hz rTMS over the non-dominant right hemisphere in random order: IFG (active stimulation site) and vertex (control stimulation site). Intensities were adjusted to 90% of resting motor threshold. A total of 2250 pulses were applied in a session. The Trail Making Test (TMT), the Stroop test, and the complex visual scene encoding task (CVSET) were administered before and immediately after each session. The Wilcoxon paired test was used for data analysis.
Results: Stimulation applied over the IFG induced improvement in the TMT parts A ($p = 0.037$) and B ($p = 0.049$). No significant changes were found in the Stroop test or the CVSET after the IFG stimulation. We observed no significant cognitive aftereffects of rTMS applied over the vertex.
Conclusions: High frequency rTMS of the right IFG induced significant improvement of attention and psychomotor speed in patients with MCI/mild dementia due to AD. This pilot study is part of a more complex protocol and ongoing research.

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1. Introduction

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder leading to total helplessness in the patients. It represents a great socioeconomic problem in developed countries. Despite extensive research, any successful neuroprotective or causal treatment is not yet available for AD. Non-invasive brain stimulation (NIBS) such as repetitive transcranial magnetic stimulation (rTMS) modulates cortical activity and thus offers an opportunity both to investigate the pathophysiological mechanisms of specific cognitive deficits in patients with degenerative brain diseases and to provide specific long-lasting aftereffects. So far, most studies have focused on high frequency rTMS of the left dorsolateral prefrontal cortex (DLPFC) in these patient populations (for review, see [2]). However, using frameless stereotaxy for the coil navigation, rTMS of other cortical areas might create further relevant effects in cognitive rehabilitation [41]. With only 2 cm or less of direct rTMS effect, we are unable to access deep brain structures such as the hippocampus or posterior cingulate cortex, i.e. regions that are affected early by the disease pathology and are known to be associated with preclinical and early clinical cognitive (particularly episodic memory) deficits [23]. However, attentional and visual processing deficits may also occur early during the course of AD (e.g. [16,25,27,32]). Based on our previous functional magnetic resonance imaging (fMRI) and rTMS results in patients with degenerative brain diseases, we chose two cortical areas to study: the right inferior frontal gyrus (IFG) in order to modulate attention and cognitive speed [4,43,44,46] and the right superior temporal gyrus (STG) in order to manipulate visual processing, visual memory encoding, and recognition [7,27] in AD population. These brain areas are easily accessible by rTMS when the coil is navigated by a frameless stereotaxy.

The current pilot study is part of a more complex ongoing research. We report here the preliminary results focused solely on the effects of right IFG stimulation in AD subjects. We previously showed that rTMS applied over the IFG enhanced cognitive speed in a group ($n = 10$) of non-demented Parkinson's disease (PD) subjects regardless of cognitive load [46]. In the same vein, rTMS applied over the right IFG decreased the latency of P3 waves recorded in the subthalamic nucleus of PD subjects during both simple and dual executive tasks [4]. We hypothesized that similar aftereffects would be achieved in our AD group. Since it has

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Table 1
Demographic characteristics.

Number	Patient initials	Gender	Education	Age (years)	Duration of illness (years)
1	FB	M	Medium	82	5
2	JC	M	Medium	77	3
3	MF	F	Higher	75	4
4	JF	F	Medium	73	2
5	MG	M	Medium	84	4
6	PH	F	Medium	84	1
7	JN	M	Medium	60	3
8	VP	M	Higher	76	6
9	MP	F	Medium	64	6
10	JS	M	Medium	75	5
Mean				75	3.900
SD				7.520	1.568

Education: medium (9–12 years, completion of final primary school level, secondary school, or vocational education) and higher (>12 years of education).

been shown that advanced AD subjects might no longer benefit from rTMS [1,15], we performed our study in subjects with amnestic MCI and early AD.

2. Patients and methods

In this pilot study, 10 patients (6 male and 4 female) were included. Of those, 7 subjects had a mild form of Alzheimer's disease dementia [21] and 3 subjects had an amnestic mild cognitive impairment [36]. Two subjects (JF, PH) had single domain MCI (deficit in memory) and one had multiple domain MCI (MF; deficits in both memory and attention). All patients were followed in the Center for Cognitive Impairment and Dementia, First Department of Neurology, Masaryk University and St Anne's Hospital, Brno, Czech Republic.

The mean patient age was 75 (SD = 7.52) years. The median disease duration was 3.9 years. The level of education was based on the highest level attained, and was categorized as short (eight years of mandatory primary school education), medium (9–12 years, completion of final primary school level, secondary school, or vocational education), and higher (>12 years of education). Eight patients had a medium education; two patients had a higher education. All patients underwent a battery of complex neuropsychological tests prior to the study commencement evaluating global cognitive functions, memory, attention, psychomotor speed, working memory, executive functions, visuospatial functions, language, daily living activities, and depression (Table 2, parts 1 and 2).

3. rTMS protocol

TMS was applied using a Magstim Super Rapid Stimulator (Magstim Company, Whitland, UK) with a focal 70 mm figure-eight-shaped air-cooled coil. Prior to the start of the experiment, high-resolution anatomical T1-weighted images were acquired for each subject using 1.5T Siemens Symphony MR machine and MPRAGE sequence with 160 sagittal slices, matrix size of 256 × 256 resampled to 512 × 512, and slice thickness = 1.17 mm, TR = 1700 ms, TE = 3.96 ms, FOV = 246 mm, and FA = 15°. These scans were imported into Brainsight TMS neuronavigation software. Neuronavigation was used to position the TMS coils and maintain their exact location and orientation throughout the experiment. All participants received two active stimulations (high-frequency rTMS of the right IFG and high-frequency rTMS of the right STG) and one control stimulation over the vertex. The stimulations were performed in a randomized order in three different sessions with a one-day interval between each session. Three regions were stimulated for each subject and three tests were evaluated in each session. For each subject, the order of stimulated regions was chosen randomly from six possible combinations. For each session, the order of tests was chosen randomly from six possible combinations. In both cases (i.e. region order and test order) the possible combinations were roughly counterbalanced. The stimulation parameters for all three stimulated regions were 90% MT, 10 Hz, 45 trains of 4.9 second duration with an interval of 25 s, resulting in 2250 stimuli in one session. For the right IFG, the MNI coordinates of the cortical sites underlying the coil were estimated for each subject according to our previous studies: R_IFG 48, 21, 3 [BA 45] [43]. The vertex was used as the control site, and it was identified as the point midway between the inion and the nasion, equidistant from the left and right intertragal notches [38]. We imported this point into Brainsight TMS neuronavigation software for online control of the trajectory of the stimulation parameters.

For purposes of this study, we chose the Trail Making Test (TMT) and the Stroop test in evaluating sustained attention, psychomotor speed, set shifting, and inhibition of habitual responses. Attentional visual processing, encoding, and recognition were evaluated by the complex visual scene encoding task (CVSET) [42]. We have successfully used this task in our previous fMRI studies [28,43].

Cognitive tests were used prior to and immediately after (within 30 min) each stimulation: Trail Making Test (TMT), Stroop test, and CVSET test, performed in a random order. All tests were practiced once prior to the study commencement in order to reduce the practice effects which are most prominent between the first and the second sessions [12]. The Wilcoxon paired test was used for data analyses.

4. Results

Repetitive transcranial magnetic stimulation has been a safe and well-tolerated procedure with only a few side effects (three patients had painful scalp sensations during the IFG stimulation).

Behavior data and results are presented in Tables 1 and 3. Two patients were not able to complete the TMT part B due to the higher cognitive demands as compared to the TMT part A.

Stimulation applied over the IFG induced significant improvement in the TMT part A ($p = 0.037$) and in the TMT part B ($p = 0.049$). A clinically relevant change was observed in four patients (1.5 SD or

Table 2, part 1
Neuropsychological assessment (part 1).

	Global cogn. functions		Memory								Attention, psychomotor speed, working memory				
	ACE-R	MMSE	RCFT Immed. recall	RCFT Delayed recall	RCFT Recog. task	WMSIII Word list I	WMSIII Logical memory I	WMS III Word list II	WMS III Recog. task	WMS III Logical memory II	WAIS-III Digit span	WAIS-III Symbol search	Stroop W	Stroop C	TMT A
	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Age-adj. Raw sc.	Age-adj. Raw sc.	Time (s)
Mean	67.4	23	3.69	3.63	13.9	12.9	17	0.2	15.4	5.5	11.6	19.4	71.2	56	140.2
SD	9.48	3.38	2.31	2.7	4.64	3.86	9.54	0.6	3.07	5.28	3.66	10.85	13.99	15.83	136.6

ACE-R — Addenbrooke's Cognitive Examination revised [22], MMSE — Mini-Mental State Examination [18], RCFT — Rey–Osterrieth Complex Figure Test (immediate recall, delayed recall, recognition task) [33], WMSIII — Wechsler Memory Scale III (Word list I, Logical memory I, Word list II, Recognition task, Logical memory II) [49], WAIS III — Wechsler Adult Intelligence Scale III (Digit span, Symbol search) [20], Stroop — Stroop Color and Word Test (W—word, C—color) [29], TMT A — Trail Making Test, part A [40].

Table 2, part 2
Neuropsychological assessment (part 2).

Executive functions						Visuospatial functions		Language impairment			ADL	Depression
								MAST	MAST	MAST		
Stroop WC	Interfer.	VFT sem.	VFT lex.	TMT B	Clock Test	JLO	RCFT copy	Total index	Expres. index	Recep. index	FAQ	MADRS
Age-adj. Raw sc.	Age-adj. Raw sc.	Raw Scores	Raw Scores	Time (s)	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores	Raw Scores
34.9	6.2	19.5	29.7	385.4	2.8	19.3	22.8	95.1	46.9	48.2	7.6	6.6
11.97	5.19	12.03	9.63	381.6	1.4	7.35	10.96	3.62	2.59	2.75	7.42	4.01

Stroop — Stroop Color–Word Test (WC — Word–Color Interference) [29], VFT — Verbal Fluency Test (sem. — semantic, lex. — lexical) [39], Trail Making Test, part B [40], Clock Test [22], JLO — Judgment of Line Orientation Test [6], RCFT — Rey–Osterrieth Complex Figure Test (copy) [33], MAST — Mississippi Aphasia Screening Test (Expressive, Receptive, and Total indexes) [26], ADL — Activities of Daily Living (FAQ — Functional Activities Questionnaire) [5], MADRS — Montgomery–Asberg Depression Rating Scale [34].

more) for the TMT part A and in two patients (1.5 SD or more) for the TMT part B. There was no significant difference in the Stroop test or in the CVSET after the rTMS of the right IFG. Stimulation applied over the vertex induced no significant changes in any of the used tests.

5 . Discussion

Our results demonstrate that the right IFG enhanced attention and psychomotor speed in a group of early AD and MCI-AD subjects as assessed by the TMT; we did not observe any changes induced by the vertex (i.e. a control site) stimulation. Although we observed improved performance on the TMT part B (i.e. a tool to measure executive functions), further investigations using more specific psychological tests (e.g. the Wisconsin Card Sorting Test or the Tower of London task) are needed to conclude on the impact of IFG stimulation on executive functions in this patient population.

Our findings are in line with others who also showed association between IFG and process such as attention and executive function in healthy subjects and in different patient groups [3,24,37,45,47,48,51]. Corbetta and Shulman [13] and Corbetta et al. [14] reported two distinct frontoparietal networks involved in visual attention, the dorsal and the ventral attention networks, with the right IFG being a component of the right-lateralized ventral attention network that governs reflexive reorienting. The right IFG has been shown to be activated across a wide variety of task demands, including attentional reorienting and shifting [13,19], and is often co-activated with the parietal cortex in neuroimaging studies of executive function [17]. The go/no-go task, which effectively taps the ability to inhibit prepotent motor response tendency, has consistently activated the lateral prefrontal cortex, particularly the right IFG [11]. Chambers et al. [10] showed that rTMS is applied over the right IFG modulated stop-signal reaction time.

In our study, the Interference part of the Stroop task, which evaluates inhibition of habitual responses, was not influenced by rTMS. However, the inhibition of habitual verbal responses is different from the inhibition of a prepotent motor response. In our previous study of cognitively normal PD subjects, we also did not observe any rTMS-induced changes on the Stroop Interference [46].

Weissman et al. [50] used fMRI to investigate trial-by-trial relationships between brain activity and response time in humans. They determined that attentional lapses are associated with reduced prestimulus activity in the anterior cingulate and in the right IFG, i.e. in areas involved in controlling attention. The right IFG coordinate in their study (R_IFG 49, 25, 8) was very similar to our coordinate of stimulation (R_IFG 48, 21, 3). Moreover, the same area (48, 21, 3) revealed decreased connectivity with the posterior cingulate/precuneus (i.e. the major node of the default mode network) in patients with PD with dementia as compared with healthy age-matched controls [43,44]. In non-demented PD patients, rTMS of the IFG increased the speed of cognitive processing in both the congruent and incongruent conditions of the Stroop test [46]. In the same way, PD patients with implanted DBS electrodes showed the shortening of cognitive event-related potential (ERP of P3) latency after rTMS was applied over the right IFG. This result reflects an increase in the cognitive processing speed. The connection between the IFG and subthalamic nucleus, referred to as a hyperdirect

Table 3
Results using neuropsychological age-adjusted and raw tests scores.

Localization of stimulation		Stroop W Age-adj. sc.	Stroop C Age-adj. sc.	Stroop WC Age-adj. sc.	TMT A Time (s)	TMT B Time (s)	CVSET Raw scores
IFG (before stimulation)	Minimum	57	37	20	38	75	10
	Maximum	94	74	62	368	427	29
	Mean	77.7	60.4	39.4	123.6	146.25	22.8
	SD	11.75	11.97	11.51	109.22	80.45	5.65
IFG (after stimulation)	Minimum	65	39	13	27	72	14
	Maximum	92	77	65	231	423	30
	Mean	80.7	62.6	40.2	90.8	137.5	21.5
	SD	8.97	13.73	13.36	68.37	53.95	5.5
	p	Insignif.	Insignif.	Insignif.	**0.037**	**0.049**	Insignif.
Vertex (before stimulation)	Minimum	63	32	13	40	97	16
	Maximum	92	75	46	292	487	29
	Mean	80.1	59.9	36.1	106.1	153.75	21.9
	SD	9.5	12.14	9.47	95.45	34.94	4.57
Vertex (after stimulation)	Minimum	55	35	13	41	134	12
	Maximum	92	72	55	247	443	30
	Mean	78.9	60.3	37.5	95	185.5	21
	SD	10.78	13.07	11.53	72.87	60.82	5.22
	p	Insignif.	Insignif.	Insignif.	Insignif.	Insignif.	Insignif.

IFG — inferior frontal gyrus, Stroop — Stroop Color–Word Test; W — word; C — color; WC — word–color; TMT — Trail Making Test (parts A and B); CVSET — complex visual scene encoding task recognition (we evaluated the number of correctly identified scenes from 60 photographs), age-adj. sc. — age-adjusted scores. Bold values indicate significance at $p < 0.05$).

pathway, can play a role in executive processing [4] in the PD population and also in AD patients.

In AD and MCI-AD patients, there is evidence of an important role of the IFG in attention and executive functions. Generally, findings from neuropathologic, metabolic, and resting state fMRI brain connectivity studies of AD suggest that attention decline in mild AD might depend on multifocal degeneration in a cerebral network for attention that comprises cortical and subcortical brain areas including the IFG [8,9,30,31,35].

Taken together, we have shown that modulating the IFG excitability with rTMS may lead to clinically relevant improvement in attentional task performance in early AD patients. The major limitation of the current pilot study is the small sample size. A direct comparison between the effects of active and control stimulations was not possible. In this exploratory study, we did not control for multiple testing and for other important variables such as brain atrophy, age, or age at disease onset.

Despite the study limitations, we were able to show for the first time that right IFG stimulation may induce positive effects on attention and cognitive speed in early AD patients. We were able to replicate our results obtained in a non-demented PD cohort in an MCI/mild AD patient population. The stimulation protocol was safe, and rTMS was well tolerated and did not induce any serious side effects. We plan to continue with this research, and we hope to be able to overcome the aforementioned drawbacks in the project's final data analysis.

Conflict of interest

The authors declare no conflict of interest.

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13. Cognitive Effects of Repetitive Transcranial Magnetic Stimulation in Patients with Neurodegenerative Diseases – Clinician's Perspective

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Commentary.

This publication placed early experimental findings on NIBS within a broader clinical and translational context. The review was motivated by growing interest in rTMS as a potential intervention for cognitive symptoms in neurodegenerative disorders, while recognizing the methodological and clinical challenges associated with this approach. The available evidence was critically evaluated from a clinical and translational perspective. The review found that rTMS can induce beneficial cognitive effects in AD, PD, and MCI, but the evidence remains preliminary due to substantial variability in stimulation protocols, target regions, study designs, outcome measures, and individual responses. Its principal contribution was to connect experimental neuroscience with practical considerations relevant to clinical implementation and to highlight the need for individualized approaches and mechanistic neuroimaging biomarkers. My contribution included the clinical interpretation and synthesis of the evidence, development of the translational perspective, and substantial involvement in manuscript preparation and writing.

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Review article

Cognitive effects of repetitive transcranial magnetic stimulation in patients with neurodegenerative diseases — Clinician's perspective

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ABSTRACT

Repetitive transcranial magnetic stimulation (rTMS) represents a promising tool for studying and influencing cognition in people with neurodegenerative diseases. This procedure is noninvasive and painless, and it does not require the use of anesthesia or pharmacological substances. In this systematic critical review we report outcomes from research focused on behavioral cognitive effects induced by rTMS in patients with Alzheimer's disease (AD), Parkinson's disease (PD), and mild cognitive impairment (MCI) preceding AD. There are still major limitations to rTMS use, such as a poor understanding of its after-effects and inter-individual variability in their magnitude, discrepancies in stimulation protocols and study designs, varied selection of the specific stimulated areas and control procedures, and neuropsychological methods for assessment of after-effects; hence, the results of the present research can only be considered preliminary. The future directions are discussed.

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1. Introduction

A number of studies have investigated repetitive transcranial magnetic stimulation (rTMS) as a potential therapeutic tool. rTMS is a noninvasive method that is able to modulate brain function. Lasting effects on brain plasticity can be observed after application of rTMS [1]. Two common neurodegenerative diseases, Alzheimer's disease and Parkinson's disease, are diagnosed in millions of people worldwide [2]. The diseases share several characteristics: they target predominantly the aging population and show gradual progression, individual disease-specific histopathological brain changes, and molecular mechanisms of pathogenesis. An increasing number of people are diagnosed as a result of population growth and prolonged life expectancies. Alzheimer's disease (AD) and Parkinson's disease (PD) are associated with impaired cognitive functions, reduced independent functioning, and psychological and behavioral problems. The increasing cumulative prevalence of these diseases has a huge economic impact in developed countries throughout the entire world.

Mild Cognitive Impairment (MCI) is a term used for people with impaired memory and/or other cognitive functions beyond the expected

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outcomes for the age and education but not yet within the diagnostic criteria for dementia [3]. MCI patients do not show signs of significant functional impairment but they are at high risk for dementia conversion [2].

The key principle of rTMS is based on regularly repeated stimulation of the focal cortical area by a train of magnetic pulses. Stimulating coils of different shapes induce electric currents in neurons (secondary conducting material). The effect of stimulation decreases with the distance from the stimulating coil. There is an immediate effect on underlying brain tissue to a depth of approx. 2 cm beneath the scalp as well as possible changes in distant interconnected regions. Generally, low-frequency rTMS (≤1Hz) reduces cortical excitability when applied over the motor cortex, and high-frequency rTMS increases it [1,4]. Online stimulation refers to the condition in which a person is executing a task (motor, cognitive, etc.) while receiving rTMS. The offline approach is when stimulation occurs before a task but some rTMS after-effects may interrelate with the final results [5]. There is no universal agreement on the rTMS parameters to be used or on the overall effect of therapeutic rTMS, mainly due to a lack of understanding of the mechanisms responsible for the lasting modifications of cortical excitability induced by stimulation and due to within-subject and between-subject variability of rTMS-induced effects. There is evidence that rTMS induces some after-effects that outlast the period of stimulation and depend on the number of pulses applied, the rate of application, and the intensity of each stimulus [6].

Several neurotransmitter systems can be modulated by rTMS with effects that are measurable for longer periods of time. Application of rTMS to the motor cortex or dorsolateral prefrontal cortex may increase or decrease the release of monoamines (particularly dopamine) in different cortical and subcortical areas of the brain interconnected with the stimulated area [7–9]. In addition, high-frequency rTMS applied over the prefrontal cortex may act via the stimulation of the glutamatergic prefrontal neurons [10] and may increase neurotrophic factors in the brain [11].

Other mechanisms of action have also been proposed, including changes in the effectiveness of synapses between cortical neurons (long-term potentiation and long-term depression). Positive changes can also be measured in behavior [1,5,6].

Since the first introduction in the late 1980s, rTMS has been used as a potential treatment for a variety of neurological and psychiatric disorders [1]. Studies and meta-analyses have included the therapeutic use of rTMS in depression (FDA approved since 2008) [12–14], schizophrenia [15–17], stroke [18,19], tinnitus [20–22], addiction [23,24], obsessive–compulsive disorder [25], Tourette's syndrome [26] and many other diseases.

The fundamental rationale for therapeutic use of rTMS is the fact that the effect of rTMS on cerebral cortex outlasts the duration of stimulation. Moreover, the changes induced by rTMS are not restricted to the stimulated region and possible effect may also occur in the distant functionally connected areas [8,9,27]. Another important issue is the fact that protocols using multiple sessions of stimulation may lead to long-lasting modulation of the brain plasticity [1,5,28]. It is probably not realistic to assume direct neuroprotective effects of rTMS on pathophysiological mechanisms involved in neurodegenerative diseases such as Alzheimer's disease (AD) or Parkinson's disease (PD). However, rTMS may interact with the normal processes of brain plasticity and induce or enhance compensatory mechanism leading to increase of the brain reserve; hence interfere with temporal evolution of clinically relevant cognitive symptoms of neurodegenerative diseases [29]. Postponing the clinical manifestation of fully blown dementia may be the major goal since we still do not have any causal treatment for neurodegenerative diseases.

It has been clearly shown that rTMS induces changes of cortical plasticity of motor cortices [28]. The positive effects of TMS on cognitive P300 event-related potential have also been well documented [27,30]. Although this may provide a rationale for using the rTMS as a therapeutic tool it is still not easy to estimate how and if the altered mechanism of brain plasticity can serve as a model for developing effective protocols [31]. Results of the studies employing functional MRI (fMRI), fluoro-deoxy-glucose PET (FDG PET), and EEG in different patient groups may help in formulating hypotheses to be tested by using rTMS. However, on the whole it is more difficult to induce clear behavioral and clinically relevant benefits of rTMS than to induce changes of cortical plasticity or brain activation/ network connectivity changes [28].

The limited effects of accessible pharmacological treatment for cognitive impairment in AD, PD, and MCI preceding AD has led to an increased interest in research on alternative therapeutic strategies and non-pharmacological interventions [32,33]. This review presents the results of current studies that have used rTMS in people with AD or PD and assessed its effect on cognitive functions. MCI preceding AD is included in the review because of the aim to treat impaired cognitive functions as early as possible.

2. Method

A systematic literature search of articles written in English before June 2013 was conducted in the Web of Science and PubMed databases. A wide range of keywords was used: "rTMS", "TMS", "magnetic stimulation", "cognitive", "cognition", "Alzheimer", "Parkinson", "MCI", "mild cognitive impairment", and "neurodegenerative". By combining the aforementioned keywords we identified 224 articles retrieved by the Web of Science and 67 papers collected from PubMed databases. We carefully reviewed all titles and abstracts and excluded the meeting abstracts, editorial material and non-english written articles. Then we focused on our predefined selection criteria: application of rTMS, involvement of human subjects, inclusion of patients with MCI, AD or PD, and evaluation of cognitive domains. Articles that did not meet these criteria were excluded. Our aim was to include original studies dealing with the therapeutic application of rTMS and assessing cognitive after-effects as one of the major outcomes. Having said that, we were able to identify 19 articles meeting our focus and field of interest.

Articles were divided into three groups according to the diagnosis (MCI preceding AD, AD, or PD). We found nine studies that included patients with PD, seven articles on patients with AD, and three articles that concern people with MCI. Articles were listed from the most recent to the oldest.

3. Results

We identified 19 studies published before June 2013. The mean clinical population was about 17 people per study (range of 1–45 participants). This review includes 315 people altogether, of which 48 were with MCI, 118 with AD, and 149 with PD. The publications consist of two case studies [34,35], three open studies [36–38] and 14 controlled studies [39–52]. In the text of this review, we focus only on the controlled studies, but information about all 19 studies is displayed in Tables 1–3.

As a sham condition in seven studies [39,40,42,44,45,48,52] active coils were tilted so that no magnetic stimulation reached the brain; in six studies [41,43,46,47,50,51] sham coils were used; and in one study, stimulation of the occipital cortex was used as a control site of stimulation [49].

There was certain uniformity in the selection of the targeted areas. The dorsolateral prefrontal cortex (DLPFC) was stimulated in nearly all of the studies. In 12 controlled studies, researchers stimulated this area either bilaterally [39,41,42,44–46], or unilaterally with a left-sided preponderance [43,48–51]; in one study solely the right DLPFC was stimulated [52]. Other cortical regions were stimulated less frequently, including the inferior frontal gyri [47], dorsal premotor cortex [49], supplementary motor area [52], parietal somatosensory association cortex, and Broca's and Wernicke's areas [41]. The high occurrence

Table 1
rTMS in patients with MCI and its effect on cognition.

Study	Type of study/sham	n	Target area	Number of pulses, sessions	Frequency, intensity	Localization of the area	Test	Results
Turriziani et al. (2012) [34]	Controlled study, sham (TC)	8	Left/Right DLPFC	600/day, 2 sess. separated by 3 weeks (Left DLPFC + sham, 6 h apart; Right DLPFC + sham, 6 h apart)	1 Hz, 90% MT	Template MRI guiding	Verbal and nonverbal recognition memory tasks	↑Memory after R-DLPFC rTMS
Cotelli et al. (2012) [29]	Case study	1	Left IPL	2000/day, 10 sess. over 2 weeks	20 Hz, 100% MT	Template MRI guiding	FNAT, MMSE, RCPM, story recall, AVLT, spatial span, digit span, token test, fluency, ROCF, serial position curve task, De Renzi imitation test, TMT, WCST	↑Memory in FNAT, ↑AVLT delayed recall, ↑Primary effect of serial position curve task
Solé-Padullés et al. (2006) [35]	Controlled study, sham (TC)	39	PFC	10 trains for 10 s each over 5 min. period, 1 sess.	5 Hz, 80% MT	5 cm anterior method	Associative memory task	↑Associative memory

Legend: n = number of subjects; TC = tilted coil; MT = motor threshold; (DL)PFC = (dorsolateral) prefrontal cortex; IPL = inferior parietal lobules; sess. = session/s; FNAT = face-name association task; MMSE = Mini-Mental State Examination; RCPM = Raven Colored Progressive Matrices; AVLT = Auditory–Verbal Learning Test; ROCF = Rey-Osterrieth Complex Figure; TMT = Trail Making Test; WCST = Wisconsin Card sorting test; ↑improvement of.

of DLPFC stimulation might be due to fact that this coil localization has been identified to have antidepressant effects and has therefore been intensively studied in the last years [14,53–58]. The traditional localization of the coil over the DLPFC is quite easy but imprecise (10–20 EEG system, 5 cm anterior to the motor hand area method). These methods are described in more detail below. Stimulation parameters vary from study to study.

Usually offline stimulation is used to measure cognitive effects, but Cotelli also used an online approach [44,45]. The number of pulses ranged from 100 to 2000 per day. In some studies, the effect of single session rTMS was measured [39,40,44,45,52]; other researchers assessed rTMS effects after up to 54 days of stimulation [41], see also Tables 1–3 for the details in stimulation paradigms. In nearly all of the publications high-frequency rTMS (ranged from 5 to 25 Hz) was used. In one controlled study [39] low-frequency rTMS (1Hz) was applied. The intensity of rTMS pulses was between 120% and 80% of the resting motor threshold, which correspond to the lowest intensity capable of inducing motor evoked potentials (MEPs) of the relaxed hand muscle in at least five out of ten trials.

The method of localizing the targeted area is a very important aspect of the protocol. There is no direct evidence in patients with neurodegenerative brain diseases that the neuronavigated coil would result in better cognitive effects. However, there are supporting arguments for the importance of precise coil neuronavigation in depressed patients' studies. Only 7 out of 22 patients showed proper localization of the DLPFC by moving the stimulation coil 5 cm anterior to the hand area hot spot; the rest had the center of the coil located in the premotor cortex [59]. Similar results were replicated by another study [60]. Inaccurate location of the coil was associated with poor antidepressant effects [61]. Some authors prefer "10–20 EEG system" to "5 cm method" in case those neuronavigation methods are not available [62]. But it has again been shown that individualized neuronavigated rTMS has better treatment effects than "10–20 EEG system" localization for the treatment of auditory hallucinations in schizophrenic patients [63]. In the presented review, one controlled study used a 10–20 EEG system [52]; in five controlled studies, researchers moved the stimulating coil 5 cm anterior from the primary motor cortex to localize the DLPFC [40,42,48,50,51]; in four controlled studies, specific brain regions were targeted on the basis of a reconstruction of cerebral cortex in the Talairach coordinate system using the SofTaxic Evolution navigator system and an estimated brain MRI template (www.emsmedical.net) [39,43–45]; and four controlled studies used individual subject's brain MRI data and frameless stereotaxy for the precise coil navigation [41,46,47,49]. Individualized targeting of the coil based on intrinsic functional brain connectivity as assessed by fMRI seems to be an interesting technique which needs further testing in clinical trials in different patient groups [64].

There were no severe adverse effects mentioned in the reviewed literature apart from minor tiredness in one study [36] and mild transient headaches [46–48] in the total of six patients. In four studies [37,39,40,52] the authors did not mention the side effects at all. Safety guidelines [4,65] were followed by ten studies [34,36,37,40–45,51].

The cognitive effects of rTMS are described in the next three sections, divided according to the main diagnosis. On the whole, small sized samples ($n \leq 15$) have been included [39,41,43,45–47,49,52] in most of the controlled studies. The use of improper blinding (e.g. tilted coil) [39,40,42,44,45,48,52] in addition to the use of imprecise coil localization techniques is another major limitation of some studies which will be discussed further in the Discussion section.

4. Mild cognitive impairment

The effects of brain stimulation on cognition were described in two controlled studies and one case study, summarized in Table 1. Turriziani et al. (2012) carried out three experiments that included healthy subjects and one experiment with MCI patients that were characterized mostly by memory impairment and met the diagnostic criteria for MCI

Table 2
rTMS in patients with Alzheimer's disease and its effect on cognition.

Study	Type of study/sham	n	Target area	Number of pulses, sessions	Frequency, intensity	Localization of the area	Test	Results
Rabey et al. (2012) [36]	Controlled study, sham (SC)	15	Left/Right DLPFC, Broca, Wernicke, Left/Right pSAC	1300/day, 54 sess. (5 sess./ weeks for 6 weeks, 2 sess./week for 3 months)	10 Hz, 90/110% MT	Subject MRI guiding	ADAS-cog, CGIC, NPI	↑ADAS-cog, CGIC
Haffen et al. (2012) [30]	Case study	1	Left DLPFC	2000/day, 10 sess. over 2 weeks	10 Hz, 100% MT	5 cm anterior method	MMSE, TMT, MIS, Free and Cued Recall Test, Isaacs Set Test, Picture naming, Copy	↑Episodic memory and speed processing
Ahmed et al. (2012) [37]	Controlled study, sham (TC)	45	Left/Right DLPFC	2000/day, 5 sess. in 5 consecutive days	20 Hz, 90% MT, 1 Hz, 100% MT	5 cm anterior method	MMSE, IADL, GDS	high frequency rTMS improves more than low frequency or sham in MMSE,GDS,IADL
Cotelli et al. (2011) [38]	Controlled study, sham (SC)	10	Left DLPFC	2000/day, 20 sess. over 4 weeks for real–real group, 10 sess. of sham over 2 weeks + 10 sess. of real over 2 weeks for placebo–real group	20 Hz, 100% MT	Template MRI guiding	MMSE, B/IADL. Picture naming, BADA, AAT. Serial position curve task, Cognitive estimation test	↑Auditory sentence comprehension
Bentwich et al. (2011) [31]	Open study	8	Left/Right DLPFC, Broca, Wernicke, Left/Right pSAC	1200/day, 54 sess. (5 sess./ weeks for 6 weeks, 2 sess./week for 3 months)	10 Hz, 90/110% MT	Subject MRI guiding	ADAS-cog, CGIC, NPI, HAMD, MMSE, ADAS-ADL	↑ADAS-cog. MMSE, ADAS-ADL
Cotelli et al. (2008) [39]	Controlled study, sham (TC)	24	Left/Right DLPFC	1-Day sess. divided into 3 blocks (Left DLPFC, Right DLPFC, sham)	20 Hz, 90% MT	Template MRI guiding	Picture naming	↑Object naming only in moderate to severe group, action naming in all patients
Cotelli et al. (2006) [40]	Controlled study, sham (TC)	15	Left/Right DLPFC	1-Day sess. divided into 3 blocks (Left DLPFC, Right DLPFC, sham)	20 Hz, 90% MT	Template MRI guiding	Picture naming	↑Action naming

Legend: n = number of subjects; DLPFC = dorsolateral prefrontal cortex; pSAC = parietal somatosensory association cortex; SC = sham coil; TC = tilted coil; MT = motor threshold; sess. = session/s; MMSE = Mini-Mental State Examination; B/IADL = Basic/Instrumental Activities of Daily Living; BADA = Battery for Analysis of Aphasic Deficits; AAT = Aachener Aphasie Test; ADAS-cog = Alzheimer Disease Assessment Scale-Cognitive ; CGIC = Clinical Global Impression of Change scale; NPI = Neuropsychiatric Inventory; TMT = Trail Making Test; MIS = Memory Impairment Screen; GDS = Geriatric Depression Scale; HAMD = Hamilton Depression Scale; ↑improvement of

Table 3
rTMS in patients with Parkinson's disease and its effect on cognition.

Study	Type of study/sham	n	Target area	Number of pulses, sessions	Frequency, intensity	Localization of the area	Test	Results
Srovnalova et al. (2012) [41]	Controlled study, sham (SC)	10	Left/Right DLPFC	600/day, 4 sess. (real Left/Right DLPFC, sham Left/Right DLPFC)	25 Hz, 80% MT	Subject MRI guiding	TOL	↑Total problem solving time after active Right DLPFC rTMS
Srovnalova et al. (2011) [42]	Controlled study, sham (SC)	10	Left/Right IFG	600/day, 2 sess. (real Left/Right IFG, sham Left/Right IFG)	25 Hz, 80% MT	Subject MRI guiding	Stroop test, FAB	↑All Stroop subsets
Pal et al. (2010) [43]	Controlled study, sham (TC)	22	Left DLPFC	600/day, 10 sess.	5 Hz, 90% MT	5 cm anterior method	MMSE, BDI, MADRS, Stroop test, TMT, UPDRS, HYS, TUG, ADL, VAS, ESS	↑Depression rating scales (MADRS, BDI), ↑accuracy of Stroop test
Sedlackova et al. (2009) [44]	Controlled study, controlled by OCC rTMS	10	Left PMd, Left DLPFC, Left OCC	1350/day. 3 sess.	10 Hz, 100% MT	Subject MRI guiding	VFT, TMT, Digit Span	No significant effect of rTMS
Furukawa et al. (2009) [32]	Open study	6	Frontal region	100/day, 12 sess. over 3 months	0,2 Hz, 120% MT	10–20 EEG system	TMT-B, WCST, WAIS-R, SDS, FIM	↑Scores in TMT-B and WCST
Epstein et al. (2007) [33]	Open study	14	Left DLPFC	2000/day. 10 sess. over 2 weeks	10 Hz, 110% MT	5 cm anterior method	HAMD, BDI, BPRS, HAMA, PDQ-39, RBANS, BTA, DRS, UPDRS	↑ Depression, anxiety, motor and other scales (HAMD, BDI, HAMA, DRS, UPDRS)
Boggio et al. (2005) [45]	Controlled study, sham (SC)	25	Left DLPFC	40 trains/5 s, 10 sess. over 2 weeks	15 Hz, 110% MT	5 cm anterior method	TMT-B, WCST, COWA-FAS, Stroop test, HVOT, digit span, RCPM	↑WCST, Stroop test, HVOT, ↑depression scales (HAMD, BDI)
Fregni et al. (2004) [46]	Controlled study, sham (SC)	42	Left DLPFC	40 trains/5 s, 10 sess. over 2 weeks	15 Hz, 110% MT	5 cm anterior method	MMSE, HAMD, BDI, HY, UPDRS, ADL	↑depression scales (HAMD, BDI), ↑MMSE, ADL
Koch et al. (2004) [47]	Controlled study, sham (TC)	10	Right DLPFC, SMA	250, 1 sess.	5 Hz, 100% MT	10–20 EEG system	Time reproduction task	↑Time perception after Right DLPFC rTMS

Legend: n = number of subjects; DLPFC = dorsolateral prefrontal cortex; IFG = Inferior Frontal Gyri; PMd = dorsal premotor cortex; OCC = occipital cortex; SMA = supplementary motor area; SC = sham coil; TC = tilted coil; MT = motor threshold; sess. = session/s; TOL = Tower of London; FAB = Frontal Assessment Battery; MMSE = Mini-Mental State Examination; BDI = Beck Depression Inventory; MADRS = Montgomery-Asberg Depression Rating Scale; TMT(−B) = Trail Making Test(−part B); WCST = Wisconsin Card sorting test; UPDRS = Unified Parkinson's Disease Rating Scale; HYS = Hoehn-Tahr Scale; TUG = timed up and go test; ADL = Activities of Daily Living; VAS = Visual Analog scale; ESS = Epworth Sleepiness Scale; VFT = Verbal Fluency Test: GDS = Geriatric Depression Scale; HAMD = Hamilton Depression Scale; WAIS-R = Wechsler Adult Intelligence Scale Revised; SDS = Self rating Depression Scale; FIM = Functional Independence Measure; HAMD = Hamilton Depression Scale; BPRS = Brief Psychiatric Rating Scale; HAMA = Hamilton Anxiety Rating Scale; PDQ-39 = Parkinson's Disease Quality of Life; RBANS = Repeatable Battery for Assessment of Neuropsychological Status; BTA = Brief Test of Attention; DRS = Mattis Dementia Rating Scale; COWA-FAS = Controlled Oral Word Assosiation Test: HVOT = Hooper Visual Organization Test; RCPM = Raven Colored Progressive Matrices; ↑improvement of.

described by Petersen et al. [3]. Low-frequency rTMS (1 Hz) of the left and right DLPFC was used in a study with MCI subjects to interfere with performance in a non-verbal recognition memory task. The authors demonstrated that rTMS of the right DLPFC improved recognition memory performance in healthy subjects and in MCI patients without any significant impact on the speed of response [39]. There are some additional study flaws that should be mentioned such as involvement of unbalanced samples with regard to their sizes: 100 healthy controls and only 8 patients with MCI. Moreover, different incomparable variants of the cognitive tests were used (a paper version versus a computerized version).

Elderly people with memory dysfunction (who scored at least −1 SD below standardized age-matched norms in at least one of the memory tests: Rey Auditory Verbal Learning Test, Visual Reproduction of Wechsler Memory Scale Revised or Benton Visual Retention Test) received a single session of rTMS in a study conducted by Solé-Padullés et al. (2006). Participants were randomly divided into active and sham rTMS group. Association memory performance improved significantly only in the active rTMS group after high-frequency stimulation of the prefrontal cortex site (5 cm anterior to the primary motor cortex) above the interhemispheric fissure [40]. Of note, association memory was studied by the task comprising only 10 pairs of name and face which might have been insufficient.

5. Alzheimer's disease

The number of studies that explore cognitive effects of rTMS in AD has recently increased. The characteristics of these studies are presented in Table 2.

In a randomized, double-blind study, Rabey et al. [41] combined high-frequency rTMS of six brain regions (left and right DLPFC, Broca's and Wernicke's areas, left and right parietal somatosensory association cortex) with cognitive training (rTMS-COG therapy) for a total of 54 sessions over four and half months. This stimulation period was divided into two phases: an intensive treatment phase (5 days/week for 6 weeks) and a maintenance treatment phase (biweekly for 3 months). Each day three brain regions were stimulated (Broca's and Wernicke's areas and right DLPFC or left and right parietal somatosensory association cortex and left DLPFC) and total number of pulses per day was 1300. In the placebo group, patients underwent the same procedure but the sham coil was used and nature movies were projected as a control activity instead of cognitive training. The results reveal significant improvement in the Alzheimer's Disease Assessment Scale, cognitive subsection (ADAS-cog) scores (primary outcome measure) and the Clinical Global Impression of Change (CGIC) score (secondary outcome measure) in the treatment group compared to the placebo group. The average ADAS-cog scores in the treatment group improved by 3.76 points compared to the placebo group (0.47 points) at six weeks. In the same way, at 4.5 months, the treatment group improved by 3.52 points compared to a deterioration (0.38) in the placebo group. An improvement of about four points is considered to be a clinically significant result [41]. The results of this study are very promising; however, it is difficult to distinguish between the pure effect of rTMS and the effect of cognitive therapy, since the authors focused on the synergistic effects of both treatments. On the other hand, it has to be said that the placebo group which received sham rTMS together with active cognitive training did not show any significant treatment effects. There are additional methodological points that have to be pointed out here. Stimulating more than one brain area at the same time might induce additional beneficial after-effects as compared to stimulating just one brain area [66] as well as no additional effect to the single-site rTMS stimulation [67,68]. Since stimulation of one brain area might change excitability (increase as well as decrease) of different brain areas that are functionally/anatomically interconnected with the stimulated area [8,9] the outcome of multi-site stimulation is uncertain. There is also an intercallosal effect that may increase or decrease excitability in the opposite brain hemisphere [69,70] and thus performing the stimulation bilaterally may be problematic. Moreover, in the methods section it is not clearly described how the cognitive stimulation was combined with TMS and how the coil was located over different brain regions in individual subjects. It is not indicated whether the order of stimulated regions was randomized. The size of the patients sample is very small. Taken together, results of this study are very preliminary. Hopefully other groups will be able to replicate these impressive results.

Ahmed et al. (2012) studied a larger sample of patients with AD ($n = 45$) who were divided into three main groups according to the type of stimulation they received (low-frequency rTMS, high-frequency rTMS, sham rTMS). Stimulation was applied bilaterally over the DLPFC. The authors demonstrated that the high-frequency rTMS group improved significantly more than the other groups in MMSE, Instrumental Daily Living Activity scale (IADL), and Geriatric Depression Scale (GDS). Interestingly, there was a difference between the subgroups of the mild/moderate and the severe AD patients in the achieved improvement of all rating scales. While the mild/moderate subgroup improved, no significant improvement was seen in the severe AD group [42]. Although clinically relevant scales/tests were used by the authors, the MMSE seems to be rather an unspecific instrument for detecting treatment-induced cognitive changes.

Cotelli et al. dealt with the effects of rTMS on language performance and naming in people with AD. A recent offline study by Cotelli et al. (2011) showed a significant effect of high-frequency rTMS to the left DLPFC on auditory sentence comprehension, with no effect on other cognitive functions. The authors claim that facilitation effect of left DLPFC rTMS on patients with AD seems specific to the language domain [43]. However, previous online studies demonstrated the positive effect of high-frequency rTMS over the left and right DLPFC on accuracy of action naming in AD patients [45]. These effects apply to the patients at different stages of cognitive decline in general, but object naming accuracy after left and right DLPFC rTMS improved only in the moderate to severe (MMSE < 17) group of patients [44]. Unfortunately, some important information is missing in the later studies such as the number of pulses used in a session.

6. Parkinson's disease

Several articles deal with different cognitive effects of rTMS in patients with PD. Some focus on depression as a primary outcome [48,51], assessing the cognitive functions of rTMS only as a secondary outcome.

Srovnalova et al. recently published two randomized, sham-controlled studies with a cross-over design that focused solely on cognitive functions in PD. The authors showed that high-frequency rTMS of the right DLPFC induced significant improvement in the Tower of London task performance, as expressed by total problem-solving time. No significant change was detected after left DLPFC rTMS or sham stimulation [46]. In a second study, the researchers demonstrated improvement in all Stroop subtests after active high-frequency rTMS applied sequentially over both the left and right inferior frontal gyri (IFG). The results indicated a nonspecific enhancement of the cognitive processing speed. No significant change was induced by active or sham rTMS on Frontal Assessment Battery scores [47]. However, the problem of bilateral stimulation and interpretation of the stimulation results have already been mentioned above. Previously, authors from the same lab had found no significant effect of left high-frequency stimulation of the dorsal premotor cortex (PMd) and DLPFC on cognitive functions as measured by the Verbal Fluency Test-category, Trail Making Test, or the Digit span in cognitively normal PD patients [49].

Koch et al. (2004) demonstrated that high-frequency rTMS improves time perception as measured by time reproduction task in patients with PD [52]. Pal et al. (2010) studied the effects of left high-frequency DLPFC rTMS in PD patients with depression. Significant improvement in depression rating scales – Beck Depression Inventory

(BDI) and Montgomery–Asberg Depression Rating Scale (MADRS) – and in Stroop test accuracy was identified. Other cognitive tests such as MMSE or TMT revealed no change after the stimulation [48]. Similar sample groups (depressed PD patients) were used in two other studies that compared the effects of rTMS and fluoxetine [50,51]. High-frequency rTMS of the left DLPFC significantly improved Stroop test performance after two weeks of treatment. There was also a significant improvement in Hooper Visual Organization Test (HVOT) after two weeks of stimulation that persisted for up to eight weeks after the initial stimulation. In eight weeks after stimulation, there was a significant decrease in perseverative errors in the Wisconsin Card Sorting Test (WCST) compared to the initial values [50].

In the other study [51], significant change was found eight weeks after rTMS in Activities of Daily Living (ADL) in comparison with baseline. There was also a significant difference in MMSE scores after two weeks of treatment, with more improvement in the group that received rTMS than the group that had taken fluoxetine [51]. The authors concluded that rTMS is effective in treating depression (BDI, Hamilton Depression Scale — HAMD) and improving some aspects of cognition in PD patients with depression and the effect seems to be similar to or even better than the effects of fluoxetine.

In the studies of Pal et al. [48], Boggio et al. [50] and Fregni et al. [51] the effect of rTMS was focused on depression in PD (a primary endpoint) while cognitive after-effects were also studied in these depressed PD cohorts. Another major limitation of studies of Boggio et al. [50] and Fregni et al. [51] is the lack of an appropriate placebo group.

7. Discussion

This review summarizes articles that study the cognitive effects of rTMS in patients with AD and PD and in patients with MCI preceding AD. We are aware of the existence of other reviews that partially address this issue [32,33,71–76], but this is the first comprehensive review that covers rTMS-induced cognitive changes focused on patients with the two most common neurodegenerative diseases, including MCI patients, and captures recent developments in this particular field.

On the whole results of the studies presented in this review show partial effect of rTMS on the cognitive performance of patients with neurodegenerative diseases including MCI. It has been repeatedly shown that rTMS has a potential to induce measurable behavioral effects that outlast the period of stimulation. However, the currently reviewed papers have many flaws. Almost all studies included in our review share one major limitation which is an inadequately powered sample size. Therefore, none of the studies can be assessed as Class I or Class II with regard to evidence of the treatment effect [77,78] despite the fact that some studies are randomized and controlled. Only few Class III studies have been available so far [39,44–47,49] and even this class evidence can be debated particularly due to small sample sizes included in majority of these studies. Thus no clear level of recommendation (effective, probably effective, possibly effective) can be proposed at the moment for the use of rTMS as a treatment for cognitive deficits in AD/PD based on the current reviewed literature.

We still do not understand the exact mechanisms accountable for these effects. Therefore, rTMS studies using electrophysiological methods (EEG, evoked potentials) as well as multimodal advanced imaging techniques (fMRI, FDG PET) prior to and after rTMS treatment are urgently needed to shed further light on mechanisms underlying the rTMS after-effects.

Another major problem is that we do not know which patients are more susceptible or more likely to profit from rTMS with respect to cognitive outcomes. There is high between-subject and within-subject variability in rTMS after-effects. It has been shown that a ceiling effect may occur in very mildly affected patients e.g. [48,49]. Moreover, it has been demonstrated that brain plasticity may no longer be modifiable in severely cognitively impaired patients e.g. [42,43]. Association between the AD/PD severity and motor cortex excitability has been clearly shown e.g. [79–81]. In AD patients and in healthy elderly participants, bilateral stimulation was needed to produce measurable rTMS effects [44,45,82], while only left DLPFC stimulation was necessary to produce similar effects in younger healthy participants [83]. This is consistent with the model of hemispheric asymmetry reduction in older adults (HAROLD) introduced by Cabeza [84].

But there are other important potential confounders that have to be considered such as patient's age, age at the disease onset, brain atrophy or genetic factors.

To our knowledge groups of AD, PD or MCI patients with different age at disease onset were not directly compared with respect to the therapeutic outcomes. But this parameter varies largely across reviewed studies which make the direct comparison of different studies difficult. In the reviewed articles patients with AD were older (in their mid-seventies) than patients with PD or MCI (in their mid-sixties). Although both age and disease duration were usually reported [34–37,49], the age of the disease onset was not indicated in some studies [38–41,43–45]. Indeed, early onset PD patients have different clinical symptoms as compared to the patients with late disease onset and a higher risk of bearing a known genetic mutation. In the same vein, in AD the likelihood of genetic cause (genes such as PSEN1, PSEN2, and APP) and non-memory symptoms is higher in early-onset, whereas memory problems are typical for late-onset AD [85]. Difference in glucose metabolism connected with age of onset was confirmed by several studies [86–88]. In PD, the side of the disease onset should also be considered particularly in patients at early stage of the disease [81]. Taken together, the age and side of disease onset in a very important variable that may influence the rTMS outcomes and should be either controlled for where possible or considered for the interpretation of the results.

There is also a potential influence of brain atrophy on rTMS results especially when dealing with patients with dementia. The decrease in magnitude of the current densities induced on the cortex depends on the distance to scalp, but also on anatomical and electrical features of the tissues [89]. The distance between the stimulated cortex and coil increases and the effects of rTMS may thus decrease dramatically [28,90]. Some authors even suggest higher intensities of stimulation in older patients with cerebral atrophy [91]. Prefrontal atrophy is also listed as a negative predictor of the prefrontal rTMS response in depressed patients [92] especially in patients with late-life depression. However, in the reviewed studies the brain atrophy as measure by MRI was mentioned in 6 studies [35,36,41,42,44,45] where it served for the probable diagnosis of Alzheimer's disease and for exclusion of other potential causes of dementia. This variable was not taken into account for further analysis of rTMS after-effects. In the future rTMS studies the intracranial/gray matter volume as measured by MRI or individual distance to coil as measured using the frameless stereotactic coil navigation should be carefully considered.

Differences in genetic factors may also play an important role in rTMS results. In fact, a recent study showed that apolipoprotein E (APOE) status modulates the changes in network connectivity induced by the high frequency rTMS in non-demented elderly with memory complaints [93]. Variability of response to rTMS could also result from polymorphism of the BDNF gene. The response of the Val66Met allele carriers to stimulation was different compared to the response of Val66Val individuals — the Val66Met allele carriers showed reduced or absent stimulation after-effects compared to Val66Val subjects [94,95]. Therefore, the impact of genetic factors on cortical plasticity should be considered in future research.

Taken together, the effect of rTMS may differ depending on the stage of the disease, age of the disease onset, the amount of brain atrophy and underlying brain changes, and the individual cognitive reserve. Other important confounders include genetic factors. Therefore, future studies should focus on these specific issues and explore possible confounders in more detail and in larger well described and thoroughly examined patient cohorts.

Of note, great variability in simulation parameters and study protocols designs was observed. Unlike in their online rTMS studies [44,45], Cotelli et al. showed [43] that rTMS of the left DLPFC did not have any significant effect on action or object naming when an offline approach was used. Conversely, the authors demonstrated that auditory sentence comprehension improved significantly [43]. The results of these studies teach us that the effects of online and offline rTMS may significantly differ and the outcomes of online stimulation may not predict the possible after-effects of repeated-sessions stimulation. This result has a practical consequence for designing future studies. Also, mechanisms underlying rTMS effects should be studied separately for online and offline approaches.

The duration of stimulation and the study follow-up varied a lot too. There were single session studies and studies that lasted for several months. Most studies exploring the stimulation effect duration lasted approximately 10 days. A limited number of studies have explored long-term effects based on follow up visits after the treatment. Some studies [42,43,48,50,51] included a follow-up measurement one to three months after the end of the treatment and showed that the positive effects on cognitive functions were maintained. The number of pulses ranged from a few hundred to a couple thousand. Researchers used high-frequency (25 Hz) and (although very rarely) low-frequency (1 Hz) stimulation. Intensity of stimulation ranged from 80% to 120%. There was no general agreement in the selection of the targeted area, although most of the researchers chose the DLPFC.

To summarize from all the studies included in this review, the parameters of stimulation that most likely result in significant cognitive improvement in patients with neurodegenerative diseases include high frequency (10–20 Hz) rTMS applied over the left DLPFC for at least 10 multiple sessions with the intensity between 80 and 110% of the individual motor threshold. These results are similar to the outcomes of the systematic review by Guse et al. [71], but those authors included a different range of population groups (mostly patients with depression and schizophrenia). Unfortunately, at the moment there is still no clear evidence for the superiority of specific protocols for inducing cognitive after-effects in patients with AD, PD or MCI since the direct comparisons of different protocols performed in one patient group sample are lacking. Some authors rather compare effects of rTMS applied over different brain areas using the same stimulation protocol e.g. [41,49]. But the situation is even more complicated since there are many potential confounders involved in clinical studies (see the text above). Of note, results of studies in PD cohorts have demonstrated that targeting different brain areas may lead to similar cognitive behavioral outcomes [47,48,50]. Conversely targeting the same brain area and using the same stimulation protocol may lead to different results in AD patients depending on the stage of the disease [44,45]. Stimulating more than one brain area at the same time might induce additional beneficial after-effects as compared to stimulating just one brain area [66] as well as no additional effect to the single-site rTMS stimulation [67,68]. Therefore, future research employing particularly EEG, functional MRI, PET/SPECT in combination with rTMS is needed to explore the exact mechanisms of different stimulation protocols and brain targets before the best stimulation protocols can be tested in larger double-blind studies. The individualized rTMS treatment tailored for specific diseases, patient groups or even individual subjects may be the right way forward [64].

The study design with respect to the controlling procedure is another important issue. rTMS may produce very high placebo effects [65], and therefore proper verification of the active stimulation is necessary in order to evaluate the real effects induced by specific rTMS. In our review, most of the studies used a tilted coil or the commercially available sham coils that do not produce the same sensory stimulus on the scalp as the active coils. This difference is more problematic when the high stimulation intensities are used. Success of blinding is indeed a very important part of part of double-blind studies. Assessment of a success of blinding was mentioned only in two controlled studies [48,51]. A systematic review [96] dealing with a blinding success of rTMS applied to the DLPFC showed that only 13 out of 96 studies reported the blinding success. Authors also stated that the most frequently used control paradigms such as tilting of the coil or even using a regular sham coil are not suitable for proper mimicking the real rTMS. Taken together, the placebo effect of active stimulation performed in these studies cannot be fully excluded. In this respect, the control site stimulation or the use of novel sham coils that produce the sensory stimulation of the scalp similar to that produced by active coils is recommended.

Different methods were also used for proper localization of the coil. The conventional 5 cm anterior to the "hand motor area hot spot" method and 10–20 EEG system are not considered to be precise in localizing the targeted area due to between-subject brain gray matter variability. For future research, modern frameless stereotaxic systems based on subject's brain MRI are recommended [62,97–99]. Furthermore, small clinical sample sizes, clinical heterogeneity within the specific patients groups and inconsistency of selection the neuropsychological methods that measure cognition and/or specific cognitive domains make the outcome results difficult to generalize and compare. In case that several cognitive tests are used for evaluation of cognitive outcomes the correction for multiple comparisons is needed to prevent false positive results. Of note, the Bonferroni correction was mentioned in only 5 controlled studies [43–45,47,50].

MMSE, as a basic screening tool for dementia and an elementary cognitive measure, and the activities of daily living (ADL) were used to measure global cognitive and functional status in four controlled studies: two in AD and two in PD patient populations [42,43,48,51]. rTMS induced some positive after-effects in people with neurodegenerative diseases in two studies [42,51] while no changes in the MMSE scores were observed in two other studies [43,48]. In studies involving AD patients, the global MMSE or ADL scores did not change after stimulation if these patients were in a severe dementia state [42,43]. Moreover, it has to be pointed out that MMSE is a very basic and unspecific measure that only reflects global cognition. It does not examine executive functions, and the memory evaluation is totally insufficient. Because of low sensitivity it is not recommended for use in PD and MCI patients. Rabey et al. (2012) used ADAS-cog as a more detailed and appropriate global cognitive measure in their rTMS controlled study in AD patients who scored an average 22 points on the MMSE [41]. Some clinically meaningful change was observed in that study (rTMS-induced mean change of 3.5 points on the ADAS-cog from the baseline) but the study lacks methodological clarity and it is performed in very small patient samples (see also the critical points mentioned above).

In addition to global cognitive measures, several studies tested the effects of rTMS on specific cognitive tasks including memory recognition [39], associative memory [40], episodic memory [100] or working memory [49,50,101], psychomotor speed [48,49], and executive functions [46–50,102–104]. These studies were conducted in different patient populations and in healthy volunteers, targeting particularly the left DLPFC. Although variable results were demonstrated, subtle possible effects of rTMS were observed in most of the studies. However, it is difficult to generalize the results of rTMS on specifically chosen cognitive test performance, mainly due to small sample sizes enrolled in these studies. In addition, clinical heterogeneity within the specific patients groups and inconsistency in the selection of the neuropsychological methods that measure cognition and/or specific cognitive domains make the study outcomes difficult to compare. Having said that, the observed results have to be considered as preliminary.

8. Conclusion, future directions

Despite the aforementioned limitations, rTMS is a noninvasive, non-anesthesia required, non-pharmacological tool representing an interesting area for research with possibly therapeutic implications in people with neurodegenerative diseases. For future research, people with mild to moderate stages of the disease should be included. We think that at

the moment there is neither a good rationale nor evidence for stimulating many brain regions simultaneously and bilaterally for prolonged periods of time in large heterogeneous patient samples. Conversely, for future studies we suggest rather a more precise scientific approach. We recommend the use of EEG and/or brain imaging (fMRI for studying brain activity and connectivity patterns and PET for studying metabolic changes) results in order to define the best targets for receiving optimal cognitive after-effect in individual subjects. These cognitive outcomes should be specific depending on the patient group and clinically relevant [47]. There is some evidence showing that the DLPFC stimulation may improve working memory, specific executive functions, memory recall and naming while the IFG stimulation may enhance cognitive speed in patients with AD and/or PD. Other brain regions should also be targeted including e.g. the temporo-parietal cortices. Multimodal brain imaging and EEG/evoked potentials or combination of both techniques (e.g. high density EEG-fMRI) may assist in studying the individual underlying mechanisms of rTMS effects. We recommend individual-based neuronavigation of the coil using frameless stereotaxy and a suitable control procedure. Multiple-session protocols may lead to improved efficacy and prolonged treatment duration. Watching for and studying potential confounders in larger well-characterized groups is another important topic for future research particularly in genetically and clinically heterogeneous cohorts of patients with neurodegenerative brain diseases.

Although some cognitive effects of rTMS in people with neurodegenerative diseases were observed in various studies, based on the current available literature we cannot recommend rTMS for treatment of cognitive deficits in patients with neurodegenerative brain diseases. In future research we should look for specific but clinically meaningful changes induced by specific rTMS that reflect everyday functioning in different groups of cognitively impaired and aged population.

Conflict of interest statement

The authors have no conflict of interest.

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14. Theta Burst Stimulation Enhances Connectivity of the Dorsal Attention Network in Young Healthy Subjects

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Commentary.

This study investigated the neural mechanisms through which TBS can modulate large-scale functional brain networks. Healthy participants were studied in order to characterize stimulation-induced plasticity without the confounding effects of neurodegeneration. We hypothesized that stimulation would produce measurable changes in the rs-FC, demonstrating that focal stimulation can influence distributed brain systems. A single session of intermittent TBS over the left superior parietal lobule improved cognitive processing speed and increased rs-FC within the DAN, whereas stimulation of the right inferior frontal gyrus did not produce comparable effects. The novel contribution was the demonstration of network-level effects of TBS using functional neuroimaging. This work represented a methodological development from earlier clinical studies by focusing on the mechanisms underlying stimulation effects. It also provided an experimental foundation for subsequent studies examining task-related network modulation and the validity of control stimulation sites. My contribution should emphasize involvement in study design and implementation, interpretation of connectivity changes, and manuscript preparation.

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Research Article

Theta Burst Stimulation Enhances Connectivity of the Dorsal Attention Network in Young Healthy Subjects: An Exploratory Study

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We examined effects of theta burst stimulation (TBS) applied over two distinct cortical areas (the right inferior frontal gyrus and the left superior parietal lobule) on the Stroop task performance in 20 young healthy subjects. Neural underpinnings of the behavioral effect were tested using fMRI. A single session of intermittent TBS of the left superior parietal lobule induced certain cognitive speed enhancement and significantly increased resting-state connectivity of the dorsal attention network. This is an exploratory study that prompts further research with multiple-session TBS in subjects with cognitive impairment.

1. Introduction

Transcranial magnetic stimulation (TMS) is a noninvasive method which generates a brief strong magnetic field around a coil that induces electric currents in underlying neuronal tissue. Using this technique in repetitive way—repetitive TMS (rTMS)—we can study and influence brain plasticity in humans in vivo. Noninvasive brain stimulation (NIBS) techniques such as rTMS can be further combined with a variety of neuroimaging and electrophysiological methods which can inform subsequent NIBS, providing information about where, when, and how to stimulate the brain. Moreover, neuroimaging and electrophysiology can provide indices of neuronal activity, which make it possible to assess the changes caused by NIBS and the neural underpinnings of its behavioral aftereffects [1, 2].

The main goal of the current study was to assess immediate effects of a short rTMS session on both cognitive task performance (namely the Stroop task behavioral measures) and the changes in resting-state functional connectivity particularly within the dorsal attentional network (DAN), that is, the major large-scale brain network related to goal-directed behaviors such as visual attention tasks [3]. Stroop task is a cognitive visual task, aimed at measuring cognitive speed and executive function (inhibitory cognitive control over conflicting situation) that in fMRI studies shows involvement of anterior (mainly frontal) and posterior (mainly parietal) brain regions [4, 5]. We specifically focused on the Stroop task performance since it has been altered in early Alzheimer's disease (AD) patients [6, 7] as well as Parkinson's disease (PD) patients [8] and found to be associated with a degree of inhibition of cortical acetylcholinesterase activity

by donepezil [9]. Using repeated rTMS in these patient groups may be of clinical relevance [10, 11], and exploring its potential therapeutic effect has been our major research focus [7, 12–15].

More specifically, we examined effects of rTMS using two different theta burst stimulation (TBS) protocols [16] applied over two stimulation sites in 20 healthy young subjects (HYS). We selected TBS protocols because it has been shown that TBS induces effects on excitability when applied over the motor cortex [16]. Moreover, TBS can modulate cognitive functions [17] and other behavioral functions [18], and it might have an effect on functional connectivity of major cognitive control networks [19]. Compared to classical rTMS protocols, TBS is relatively short with good participants' compliance.

Previously, rTMS studies in healthy controls showed that excitatory repetitive TMS (rTMS) increased dopamine release in the striatum [20, 21], while inhibitory protocols such as continuous TBS (cTBS) decreased striatal dopamine release and impaired performance in the cognitive task [17]. We selected two stimulation sites based on the fMRI Stroop task meta-analysis [5] including the right inferior frontal gyrus (rIFG) and the left superior parietal lobule (lSPL). Moreover, both regions are engaged in the dorsal attention network (DAN) which was our network of interest (see above). Our choice of the rIFG was based on its key role in inhibition [22] and cognitive processes that are important for the Stroop test performance. Moreover, this area seems to be involved in the hyperdirect pathway connecting the subthalamic nucleus (STN) with cortical regions engaged in executive functioning and attention processes [23]. Our group previously studied this area using classical TMS protocols and behavioral Stroop task in patients with neurodegenerative brain diseases [7, 14]. We did not want to interfere with the language area located in the left IFG (this is why we focused on the right hemisphere). The DLPFC has already been a quite heavily studied area while only few studies have targeted the IFG so far. The superior parietal lobule is involved in aspects of attention (spatial attention) and visuospatial perception, including the representation and manipulation of objects, and it is also involved in the Stroop task [5]. In our pilot fMRI data analysis of the currently used Stroop task (unpublished data), we observed more task-induced activation on the left side.

We hypothesized that excitatory (intermittent) TBS [16] will induce measurable behavioral changes that will be accompanied by distinct resting-state fMRI (rs-fMRI) changes. We did not have any strong hypothesis regarding cTBS of the IFG or SPL; however, this protocol exerts inhibitory effects (i.e., opposite effects compared to iTBS) when applied over the primary motor cortex. The work was designed as an exploratory study in order to prompt further research using a multiple-session design in subjects with mild cognitive impairment due to neurodegenerative brain diseases such as AD or PD.

2. Materials and Methods

Twenty young right-handed HYS participated in the study (mean age 25.2 ± 2.7 years, men/women ratio: 7/13). Exclusion criteria were any diagnosed psychiatric or neurological disorder or a cognitive deficit based on the results of a detailed neurocognitive battery performed prior to the study entry [7]. T1 MPRAGE (TR 2300 ms; TE 233 ms; voxel size 1 × 1 × 1 mm; FoV read 224 mm, FoV phase 252 mm; base resolution 256; 240 slices; gap 0.5 mm) and T2 FLAIR MRI sequences (TR 6000 ms; TE 387 ms; voxel size 1 × 1 × 1 mm; FoV 256 mm; base resolution 256; 192 slices) [24] were performed using a 3T Siemens Prisma machine and visually inspected by a clinician to exclude any structural brain pathology. Each participant signed an informed consent form, and the study was approved by the local ethics committee.

Based on literature [5] and as mentioned above, we targeted the right inferior frontal gyrus (rIFG; 46 14 32) and the left superior parietal lobule (lSPL; −24 −68 48) using a frameless stereotaxy neuronavigation with Brainsight 2, and very short excitatory (intermittent) TBS (iTBS; 190 s duration, 600 pulses) and inhibitory (continuous) TBS (cTBS; 40 s duration, 600 pulses) protocols [16] using Deymed DuoMAG XT stimulator with 70BF-Cool coil at 80% of individual AMT intensity. Magnetic stimulation was given in the room right next to the scanner over the abovementioned areas using a hand-held figure of eight coils (70 mm standard coil) placed tangentially to the scalp with the handle pointing ventrally. We used a crossover design, and the order of stimulation protocols and sites was randomised across subjects and sessions. The individual stimulation sessions were separated by at least a one-day interval without stimulation. Each session consisted of the prestimulatory fMRI session, a TBS session, and the poststimulatory fMRI session (identical to the prestimulation one).

For the purpose of this preliminary study, we were interested in behavioral outcomes, that is, response times (RT) and error rates for congruent and incongruent stimuli of the Stroop task [25] which was performed inside the scanner (TR 2050 ms; TE 35 ms; voxel size 3 × 3 × 3.5 mm; FoV 192 mm; base resolution 64; flip angle 70°; 35 slices; 165 scans; iPAT 2) and in the neural correlates of the TBS-induced behavioral changes as measured by rs-fMRI. We acquired 200 rs-fMRI scans using a gradient-echo echo-planar imaging sequence: TR = 2.08 ms, TE = 30 ms, FoV = 192 mm, FA = 90°, matrix size 64 × 64, slice thickness = 3 mm, 39 transversal slices.

The behavioral data were evaluated using paired sample *t*-test. The rs-fMRI data were analysed using SPM12 running under Matlab R2015b and preprocessed using realign and unwarp, slice timing correction, and spatial normalization with resampling to 3 × 3 × 3 mm voxels and spatial smoothing (FWHM 6 mm). We controlled data for spatial abnormalities using the tool mask explorer [26] as well as for excessive movement using framewise displacement (FD) with criterion FD < 0.5 mm in less than 10% of scans (nothing excluded) and FD < 1.5 mm in any scans (two sessions excluded). Data was filtered for effects of motion (24 motion parameters).

Seed-based analysis of resting-state data with a seed located at the stimulation site coordinate (a sphere with

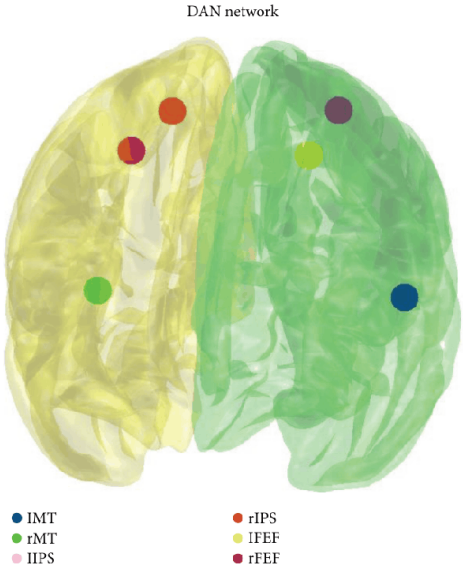

Figure 1: Dorsal attention network: seeds used for the rs-fMRI data analysis. Legend: l/rMT = left/right middle temporal area; l/rIPS = left/right intraparietal sulcus; l/rFEF = left/right frontal eye field.

$r = 6$ mm) was performed using mean as representative signals of the seed [24]. In addition, resting-state functional connectivity of the dorsal attention network (DAN) was analysed. Seeds (a sphere with $r = 6$ mm) were created using 6 coordinates of interest as described in [3], see Figure 1 and Table 1, for the localization of the DAN seeds. Representative mean seed signals were extracted, and correlation matrix was calculated for each subject. Pearson's correlation coefficients were converted using Fisher r-to-z transformation to z values. The average connectivity within DAN was calculated as the mean of z values for each seed pair. Wilcoxon signed-rank test was used to assess the change in DAN connectivity induced by the lSPL iTBS stimulation.

3. Results

In this exploratory study, TBS was generally well tolerated. There were no adverse effects apart from a mild headache reported by one person. Behavioral results of the Stroop task indicate that the RT after the incongruent stimuli were generally longer than the RT after the congruent stimuli resulting from the longer processing of the conflicting stimuli. The overall task accuracy reached 97%, and it was not influenced by any stimulation protocol (data not shown). We observed a trend toward decreased RT due to iTBS of the lSPL (see Table 2).

Table 1: Coordinates of seeds of DAN network, according to Gao and Lin [3].

X	Y	Z	Area	Network
−45	−69	−2	lMT	DAN
50	−69	−3	rMT	
−27	−52	57	lIPS	
24	−56	55	rIPS	
−25	−8	50	lFEF	
27	−8	50	rFEF	

Note: l/rMT = left/right middle temporal area; l/rIPS = left/right intraparietal sulcus; l/rFEF = left/right frontal eye field.

In the next step, we further explored neural underpinnings of the abovementioned behavioral changes (some enhancement of cognitive speed induced by iTBS applied over the lSPL) using rs-fMRI analyses. Seed-based analysis of resting-state data showed a significant increase in connectivity after iTBS of lSPL between lSPL and the left cerebellar nodule (−6 −64 −32, $p = 0.008$) and a nearly significant increase between lSPL and the right anterior insula (33 20 −2, $p = 0.065$). Moreover, we observed a significant increase in connectivity within the DAN network (mean value before versus after stimulation: 0.4685 versus 0.5277, $p = 0.0251$).

4. Discussion

The current work shows for the first time that even a very short single train of rTMS (intermittent TBS (iTBS)) applied over the left posterior parietal cortex (i.e., an area shown to be activated during the Stroop task performance [5]) may enhance cognitive speed in HYS.

Studies using TBS applied over the areas other than DLPFC and involved in cognitive processing such as IFG or SPL are still missing in the literature. Our team showed that rTMS over the right IFG increased the cognitive processing speed (improvement in all subtests of the Stroop task) in nondemented patients with Parkinson's disease [14] or shortened the cognitive event-related potential (ERP of P3) latency in Parkinson's disease patients with implanted DBS electrodes [23]. rTMS applied over the right IFG in patients with mild cognitive impairment and mild Alzheimer's disease showed significant cognitive improvement in attention and psychomotor speed (Stroop test part Words). Differences in the values before and after stimulation were 4.8 times higher with the IFG stimulation than with the vertex stimulation [7]. Studies using TMS protocols applied over the SPL for enhancement of cognitive functions are sparse. Right parietal rTMS improved visual attention as measured by attentional blink paradigm [27]. Luber et al. [28] showed that 5 Hz rTMS applied during the retention period to the midline parietal cortex speeded reaction times in working memory task without decreasing accuracy. Theta (i.e., 5 Hz) and beta (20 Hz) frequency rTMS to the right parietal cortex enhanced global versus local visual processing, respectively [29].

Table 2: Summary of the Stroop task response times (expressed as mean ± SD and tested using paired-sample t-test).

Area	Protocol	Target	Before	After	Difference	p value
rIFG	cTBS	CON	410.6 ± 80.6	406.1 ± 71.1	−4.6 ± 21.3	0.351
		INCON	415.2 ± 75.4	414.0 ± 71.2	−1.2 ± 16.9	0.750
		p value	0.345	0.014	0.422	
rIFG	iTBS	CON	426.3 ± 103.5	426.3 ± 109.6	0.0 ± 32.2	0.999
		INCON	429.7 ± 110.7	420.4 ± 112.2	−9.3 ± 32.5	0.218
		p value	0.326	0.123	0.057	
lSPL	cTBS	CON	418.6 ± 90.2	406.6 ± 79.8	−12.0 ± 30.7	0.096
		INCON	423.0 ± 92.2	412.4 ± 78.2	−10.6 ± 27.2	0.098
		p value	0.200	0.138	0.775	
lSPL	iTBS	CON	412.0 ± 81.7	397.8 ± 64.7	−14.1 ± 31.7	**0.060**
		INCON	418.8 ± 83.8	404.3 ± 68.1	−14.6 ± 32.0	**0.056**
		p value	0.030	0.039	0.888	

Note: rIFG = right inferior frontal gyrus; lSPL = left superior parietal lobule; cTBS = continuous theta burst stimulation; iTBS = intermittent theta burst stimulation; CON = congruent stimuli; INCON = incongruent stimuli. Trends towards enhanced cognitive speed enhanced by the stimulation (although nonsignificant) are depicted in bold.

The lSPL has been the major node of the DAN which is known to be engaged in externally directed conditions and to control processing of visual information while performing a visual cognitive task [3]. Combining rTMS with fMRI showed that iTBS applied over this node enhanced rs-connectivity of the DAN as well as increased connectivity between the stimulated site and other distant brain areas, namely the left cerebellar nodule and the right anterior insula. The cerebellar nodule was shown to be activated by continuous observation of visual stimuli and to have a connection with the frontal eye field, that is, the frontal DAN node [30]. On the other hand, the anterior insula is involved in the frontoparietal control network [3, 31] and heavily interconnected with other prefrontal regions such as the dorsal anterior cingulate cortex, anterior prefrontal regions, and the dorsolateral prefrontal cortex. Activations of the insula together with the abovementioned prefrontal regions are commonly observed with a variety of cognitive control processes such as conflict monitoring, information integration, and response selection [3, 5, 32–35], all of which are involved in the Stroop task. Taken together, our interim data shows that iTBS has an effect particularly on the DAN resting-state functional connectivity and may enhance the cognitive speed of the task performance even in HYS.

We are fully aware of some limitations of this study. We focused solely on the effects of active stimulation applied over specific cortical sites known to be engaged in the task performance. In this exploratory study, we further analysed resting-state functional connectivity changes induced by the TBS protocol and stimulation site with a measurable behavioral aftereffect; that is, we tried to identify neural underpinnings of the iTBS applied over the left SPL. However, the behavioral aftereffect of this specific stimulation was only marginal due to the ceiling effect in cognitively intact healthy young participants, and this is a clear study limitation. Therefore, our results should be interpreted with caution. Despite the fact that the order of the stimulation protocols and stimulation sites was randomised across subjects and sessions and the individual stimulation sessions were separated by at least a one-day interval without stimulation, we cannot exclude the order and carry-over effects completely. This was the exploratory study, and further research controlling for placebo effects is warranted. On the other hand, we for the first time provide evidence for the single-session ultrashort iTBS to induce significant modulatory aftereffects on the rs-fMRI connectivity measures.

5. Conclusions

This exploratory study showed that single-session iTBS applied over the lSPL tended to enhance the speed of the Stroop task performance in the HYS group via increased connectivity of the DAN, brain areas that are known to be engaged in the task performance. To assess possible iTBS "treatment" effects, it will be necessary to include multiple-session TBS and focus on healthy seniors and patients with early AD/PD. However, the current results obtained in HYS are promising, and a future set of data in various patient groups may provide a deeper understanding of brain plasticity mechanisms and outline new possibilities for improving cognitive functions in patients with distinct neurodegenerative brain diseases.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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15. Theta-Burst Transcranial Magnetic Stimulation Induced Cognitive Task-Related Decrease in Activity of the Default Mode Network

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Commentary.

Building on evidence that TBS can alter rs-FC, this study investigated its influence on task-related brain activity. The DMN normally decreases its activity during externally demanding cognitive tasks, and abnormal modulation of this network has been implicated in cognitive dysfunction and aging. The study was performed to test whether TBS could modify this task-related network response. Intermittent TBS over the left superior parietal lobule reduced task-related activity in medial frontal and parietal regions of the DMN, particularly the left anterior cingulate cortex, with effects specific to this stimulation protocol. The findings demonstrated a stimulation-related decrease in task-associated default mode activity, suggesting that NIBS can influence the balance between internally and externally oriented brain systems. The principal contribution was the demonstration that stimulation-induced plasticity can be detected not only during rest but also during active cognition. This publication extended the 2018 connectivity study and strengthened the mechanistic basis for therapeutic stimulation approaches.

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Theta-burst transcranial magnetic stimulation induced cognitive task-related decrease in activity of default mode network: An exploratory study

Dear Editor,

For successful cognitive functioning, engagement of cognitive control networks seems to be crucial. Some of these networks are known to be active during execution of goal-oriented tasks. In contrast, a task-negative network called the default mode network (DMN) is deactivated while the brain is involved in task behaviour. Disruption of both task-positive and task-negative networks is a common finding in many diseases including Alzheimer's disease (AD) [1].

To this date, little is known about how we can noninvasively influence task-related activity of these networks. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive tool that was shown to influence the underlying brain region and also distant brain areas. Moreover, it was shown to have positive effects on altered cognitive functions in AD [2]. The main goal of this study was to assess the effect of rTMS on the activity of task-related brain networks.

We examined twenty healthy young right-handed subjects (mean age 25.2 ± 2.7 years, men/women ratio: 7/13) that underwent fMRI measurement using 3T Siemens Prisma machine with 64 channel head coil before and immediately after rTMS. The fMRI measurement consisted of an fMRI Stroop task [3] and resting state measurement (results of the resting state part of the study are described elsewhere [4]). The goal of the fMRI Stroop task was to match the colour of a cue stimulus to the colour of a Stroop target stimulus that appeared after an interstimulus interval. Cue and target colours were either red, green or blue. The colour cue either matched or did not match the colour of the Stroop target, which was either congruent (e.g. word blue written in blue ink) or incongruent (e.g. word blue written in red ink). All subjects practiced the task before entering the scanner. Subjects used index and middle fingers of their dominant hand to press a YES-key for cue-target colour matches and a NO-key for nonmatches. We measured response times (RT) and error rates for congruent and incongruent stimuli of the fMRI Stroop task (TR 2050 ms; TE 35 ms; voxel size 3 × 3 × 3.5 mm; FoV 192 mm; base resolution 64; flip angle 70°; 35 slices; 165 scans; iPAT 2).

As TMS targets, we selected the right inferior frontal gyrus (rIFG, 46 14 32) and the left superior parietal lobule (lSPL, −24 −68 48), which are both involved in the top-down control of complex visual tasks [3,5]. TMS sessions were performed using a frameless stereotaxy neuronavigation with Brainsight2, either intermittent theta burst stimulation (iTBS; 190 s duration, 600 pulses) or continuous TBS (cTBS; 40 s duration, 600 pulses) protocols [4] using Deymed DuoMAG XT stimulator with 70BF-Cool coil at 80% of individual active motor threshold intensity. Crossover design was used, and the order of stimulation protocols and sites (4 rTMS sessions: iTBS rIFG, cTBS rIFG, iTBS lSPL, cTBS lSPL) was randomised across subjects and sessions to make them counterbalanced across the group. The individual stimulation sessions were separated by at least a one-day interval without stimulation. Each participant gave their written informed consent, and the study was approved by the Ethics Committee of St. Anne's University Hospital in Brno (3G/2016).

The fMRI data were analysed using SPM12 running under Matlab. Standard preprocessing [4] and general linear modeling (using task time course convoluted with canonical hemodynamic response function) with contrasting incongruent vs. congruent stimuli (i.e. our main fMRI outcome) were used. Quality of the data was checked using the tool mask explorer [6]. Group results were assessed using cluster level inference at p (FWE) < 0.05 with initial cut-off $p < 0.005$.

TMS was very well tolerated with no major adverse effects. The behavioural data were evaluated using a paired sample t-test and the results were published previously [4]. They showed a trend toward decreased RT due to iTBS of the lSPL ($p = 0.060$ for congruent stimuli, $p = 0.056$ for incongruent stimuli). The overall task accuracy reached 97% (ceiling effect), and it was not influenced by any stimulation protocol.

Based on the behavioural results, we specifically aimed at identifying neural correlates of the effects of iTBS applied over the lSPL using task fMRI analysis contrasting incongruent vs. congruent stimuli (post vs. pre-stimulation). The stimulation induced the significant decrease of activity in medial frontal and parietal regions of DMN with the peak in the left anterior cingulate cortex (lACC, -3 56 1) $p = 0.00013$ (344 voxels in cluster), see Fig. 1. Moreover, we found a significant difference when directly comparing effects of the excitatory (iTBS) and inhibitory (cTBS) stimulations over the lSPL with regard to BOLD signal changes within this cluster (paired sample t-test, $p = 0.005$), indicating that our result is stimulation protocol specific (see Table S1 in Supplementary materials). Neither iTBS over the rIFG nor cTBS rIFG produced any significant changes in task-induced brain activations. We did not observe with any significant effect of the sessions order on either RT changes or BOLD signal changes in the ACC.

Our exploratory study showed that even a single session of brief TMS may have an influence on a cognitive task and activity of

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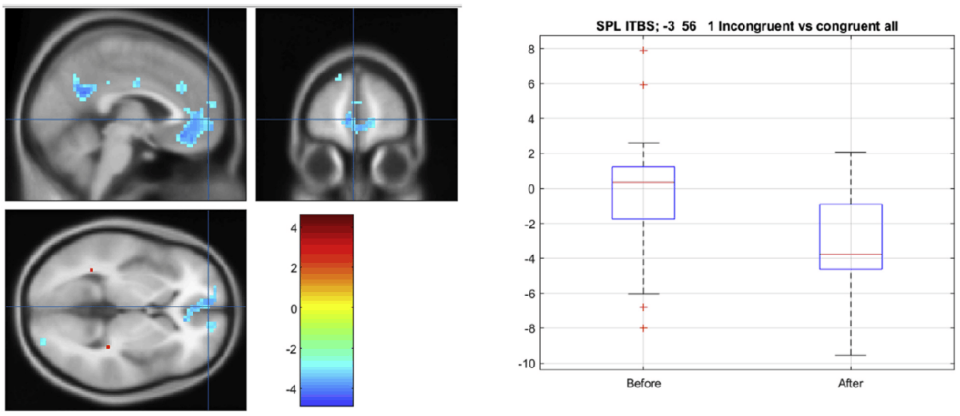

Fig. 1. Significant decrease of activity in medial frontal and parietal regions of default mode network after intermittent theta burst stimulation over the left superior parietal lobule.

related cognitive brain networks. The pattern of decreased activation was stimulation protocol specific and it was observed in regions known to be part of the DMN with the peak in ACC, which is a major anterior hub of the network.

Using resting state fMRI, other authors [7] reported that iTBS to the left inferior parietal lobule increases resting state functional connectivity (rs-FC) to the anterior part of DMN in young adults and in part of the older adults with superior cognitive performance. Using cTBS to the left dorsolateral prefrontal cortex (DLPFC), the rs-FC was reduced between the targeted region and posterior part of the DMN (posterior cingulate and precuneus) in young subjects [8].

In patients with amnestic mild cognitive impairment, repeated sessions of high frequency rTMS of the right DLPFC led to reductions of rs-FC between the posterior node of DMN and other regions including the ACC which was associated with clinical cognitive improvements [9]. The authors hypothesized that rTMS might have "normalized" pathological increases of rs-FC within the anterior part of DMN in this patient population, however, healthy subjects were not included. Finally, a study combining high frequency rTMS to precuneus with EEG in early AD patients also showed that memory improvement after rTMS was accompanied by modulation of activity and connectivity within DMN [10]. However, results of rs-FC and task-related activations cannot be directly compared.

Limitations of our study are its exploratory nature and the lack of placebo control. In summary, this study demonstrates iTBS-induced task-related BOLD signal decreases of the DMN. The future therapeutic strategies using TMS in patients with cognitive dysfunctions such as AD may be beneficial through normalizing disrupted activity and connectivity of large-scale brain networks and the DMN in particular. We want to highlight a network-based approach for studying neuromodulation for therapeutic purposes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.01.015.

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16. Is the Vertex a Good Control Stimulation Site? Theta Burst Stimulation in Healthy Controls

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Commentary.

This methodological study addressed an important issue in the design of NIBS experiments: whether stimulation of the vertex can be considered an appropriate control condition. The vertex is/was frequently used as an active control site, but the assumption that it is neurophysiologically inert has not been sufficiently examined. We hypothesized that TBS of the vertex might itself produce measurable effects on brain function, thereby complicating its interpretation as a neutral control. The study provided evidence relevant to this methodological question and highlighted the importance of carefully selecting control conditions in stimulation research. Its novel contribution lies in addressing a fundamental source of potential bias in experimental neuromodulation studies. In relation to previous work, it reflects the maturation of our research from demonstrating stimulation effects to critically evaluating the methodological tools used to establish those effects. The study has implications for the design, interpretation, and reproducibility of future clinical and experimental stimulation trials.

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NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - ORIGINAL ARTICLE

Is the vertex a good control stimulation site? Theta burst stimulation in healthy controls

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Abstract
The vertex has been used as a suitable control stimulation site in repetitive transcranial magnetic stimulation studies. The objectives of this study are (1) to assess cognitive performance (CP) after theta burst stimulation (TBS); (2) to evaluate whether clinically relevant cortical areas might be reached by vertex stimulation and how that might influence CP. Twenty young healthy subjects performed a cognitive task prior to and immediately after intermittent TBS (iTBS) and continuous TBS (cTBS) of two active cortical stimulation sites and the vertex. We used the Wilcoxon signed-rank test to compare the pre- and post-stimulation reaction times (RTs) and a mixed ANOVA analysis to evaluate the effect of the stimulation on changes in RTs. A three-dimensional finite-element model (FEM) was used to calculate the vertex TBS-induced electrical field (E-field) in the adjacent regions of interest (ROIs). Correlation analyses were performed between E-fields in the ROIs and cognitive outcomes. We found a significant effect only of the stimulation time factor (F (1,12) = 65.37, $p < 0.001$) on RT shortening, with no superiority of the active site stimulation compared to the vertex stimulation. In 73.5% of vertex TBS sessions, a significant E-field was induced in at least one ROI. We found a negative association between the magnitude of the iTBS-induced E-fields and RT changes ($R = -0.54$, $p = 0.04$). TBS protocols may lead to changes in CP when applied over the craniometrically targeted vertex. We therefore suggest not using a conventional approach as a vertex targeting method.

Keywords Transcranial magnetic stimulation · rTMS · SimNIBS · Vertex stimulation · Cognitive · Control condition

Introduction

In the field of neuroscientific research, there is always a need for a good control condition. The placebo effects of repetitive transcranial magnetic stimulation (rTMS) are relatively high compared to the effects of pharmacotherapy (Meister et al. 2017; Razza et al. 2018; Jiang et al. 2019); it is therefore crucial to choose a relevant control condition. The choices include a designated placebo coil (Arana et al. 2008; Duecker and Sack 2015), a placebo coil with additional electrical scalp stimulation (Arana et al. 2008; Rossi et al. 2007), the diversion of the pulse direction (Loo et al. 2000), and the active stimulation of a control stimulation site that is not engaged in the studied outcome measures, such as the vertex (Jung et al. 2016).

The vertex has been used by researchers as a control site particularly in cross-over study designs in which maintaining an authentic scalp sensation is necessary (Collins and Jacquet 2018; Tse et al. 2018; Tik et al. 2017). The vertex is described as a point in the midline between the hemispheres that can be found as the intersection of an abscissa between the nasion and the inion and an abscissa between the left and right tragus. The conventional craniometric approach described above is used almost exclusively (Ferrari et al. 2018, 2019; Gatti et al. 2020, 2021; Donato et al. 2020; Fu et al. 2021). Alternatively, specific vertex MNI coordinates can be inserted into neuronavigation software and adjusted to the individual subject's MRI (Okamoto et al. 2004; Okamoto and Dan 2005).

Our main goal was to shed light on the eligibility of conventional vertex targeting in terms of its suitability as a control stimulation site.

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We used a cognitive task to assess the immediate effects of one session of distinct theta burst stimulation (TBS) protocols (Huang et al. 2005) applied over two active cortical stimulation sites engaged in the frontoparietal control network and over the vertex as a control stimulation site, employing a randomized controlled cross-over study design in healthy volunteers (HC). We further evaluated whether any clinically relevant cortical brain areas might be reached by the vertex stimulation with a particular interest in the supplementary motor area (SMA) and paracentral lobule (PCL), i.e., our regions of interest (ROIs). We hypothesized that vertex TBS-induced E-fields in our ROIs would be associated with changes in the performance of a visual cognitive task and would thus affect the overall study results.

As our active stimulation sites, we chose the right inferior frontal gyrus (rIFG) and the right superior parietal lobule (rSPL) as they both are involved in top–down visual processing control and the modulation of attention (Cooper et al. 2004; Chikazoe et al. 2007; Hampshire et al. 2010; Romei et al. 2011; Srovnalova et al. 2011; Jacobson et al. 2011; Anderkova et al. 2015). In our previous research, both sites showed activation in a visual object-matching task; the activation differed between the healthy controls and the patients with mild cognitive impairment (Nemcova Elfmarkova et al. 2017). We thus hypothesized that theta burst TMS over these areas would influence cognitive performance in our task.

In the past 16 years, a form of patterned, high-frequency rTMS called theta burst stimulation (TBS) has shown an ability to induce corticospinal excitability changes that outlast the period of stimulation through the synaptic mechanisms of long-term potentiation (LTP)- or long-term depression (LTD)-like plasticity (Diamond et al. 1988; Huang et al. 2005; Suppa et al. 2016; Morris et al. 2019). In recent TMS-EEG studies, theta burst TMS demonstrated similar effects on cortical excitability, influencing not only the stimulated brain region but also its connections influencing the brain function on a network level (Casula et al. 2016; Chung et al. 2017; Koch et al. 2020; Di Lorenzo et al. 2020). At the same time, TBS uses shorter protocols with lower intensity, which makes it more tolerable for subjects (Lefaucheur et al. 2020; Di Lazzaro et al. 2021).

Materials and methods

Twenty young right-handed healthy controls (HC) participated in the study (mean age 23.6 ± 2.3 years, men/women ratio: 5/15). Recruitment for the study took place in the form of leaflet advertising and through our institute's social media pages. All subjects were enrolled and examined at the Central European Institute of Technology (CEITEC, Masaryk University). Each participant signed an informed consent form, and the study was approved by the local ethics committee.

Based on the results of our previous research (Nemcova Elfmarkova et al. 2017), we chose a complex visual object-matching task (VOMT) and two active stimulation sites that were involved in the top–down control of the VOMT processing: the right inferior frontal gyrus (rIFG; MNI coordinate: 46 14 32) and the right superior parietal lobule (rSPL; MNI coordinate: 21 −58 61). In addition, the vertex served as a control stimulation site. The VOMT task (see description below) was performed prior to and immediately after each stimulation session. A reaction time (RT) change was our cognitive outcome measure. T1 MPRAGE scan (TR 2300 ms; TE 2.33 ms; voxel size 1 × 1 × 1 mm; FoV read 224 mm, FoV phase 252 mm; base resolution 256; 240 slices) was performed using a 3 T Siemens Prisma machine as a basis for frameless stereotactic neuronavigation, which was used for targeting the active stimulation sites with Brainsight 2 software. The vertex was localized by a conventional craniometric method, i.e., by localizing the intersection of an abscissa between the nasion and the inion and an abscissa between the left and right tragus. After placing the neuronavigation pointer tool on this point on the scalp, it was converted into a marker in the Brainsight 2 software, which was subsequently used for the individual subject's coil navigation. (Note that this point is represented by Cz in the international 10–20 system, Jurcak et al. 2007.)

Both intermittent TBS (iTBS; 600 pulses over 192 s) and continuous TBS (cTBS; 600 pulses over 40 s) protocols (Huang et al. 2005; Anderkova et al. 2018) were used. Stimuli were delivered using the Deymed DuoMAG XT stimulator with 70BF-COOL coil at an intensity of 80% of the individual active motor threshold (AMT). The coil was placed tangentially on the scalp with the handle pointing ventrally. Each participant underwent altogether six stimulation sessions over the three stimulation sites (rIFG, rSPL, and vertex). The individual stimulation sessions were separated by at least a 1-day interval without intervention. The order of stimulation sites and protocols was randomized across subjects and sessions. The distribution of the order of visits and particular stimulation variants is presented in Figure S1 in the supplementary materials.

The VOMT task was described in our previous work (Nemcova Elfmarkova et al. 2017). In brief, it consisted of pairs of common objects (30 pairs of conventional views of objects and 30 pairs of objects presented in unconventional orientations—spatially rotated). Each event consisted of the following sequence: a mask stimulus (1 s), a picture of the first object (1 s), a mask (1 s), a picture of the second object (1 s), a mask (1 s), and a fixation cross (5 s) (see Fig. 1). Participants had to respond as quickly as possible by pressing the YES button if the second object of the paired image was the same as the first object (regardless of spatial

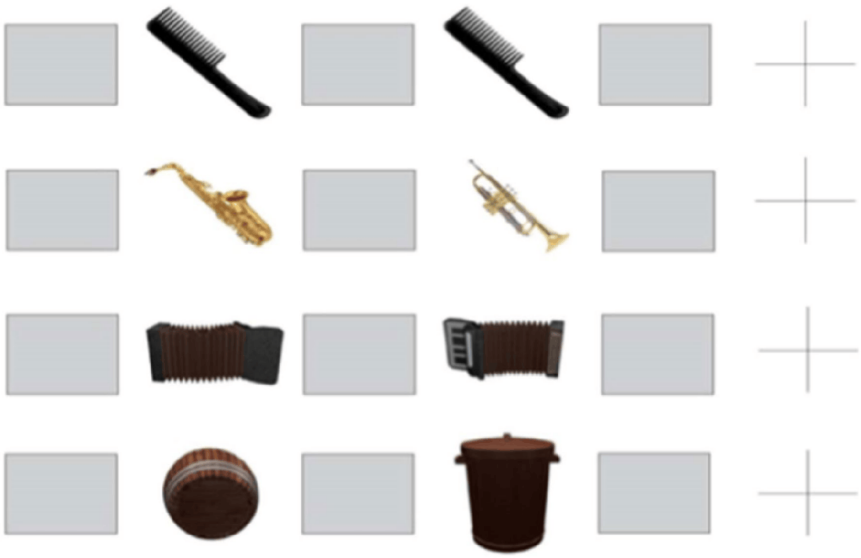

Fig. 1 Visual object-matching task using conventional and unconventional views of object pairs. Line 1: conventional view task condition (non-rotated); the correct answer is YES (left button), Line 2: conventional view task condition; the correct answer is NO (right button), Line 3: unconventional view task condition (rotated); the correct answer is YES (left button), Line 4: unconventional view task condition; the correct answer is NO (right button)

orientation) or by pressing the NO button if they were different (Nemcova Elfmarkova et al. 2017). Conditions were randomly intermixed; the percentage of correct responses and RTs were calculated as the mean of both conventional and unconventional views of objects (see Table 1).

Before conducting an analysis of the RTs, we used a general linear model to filter out the effect of order of visit to eliminate this effect from the observed results. For dependence of reaction time (RT) on order of visit see Fig. S2 in the supplementary materials. For data analyses, we applied a nonparametric test (Wilcoxon signed-rank test) to compare the pre- and post-stimulation RTs in the VOMT for each stimulated region and protocol. We used $p < 0.0083$ (Bonferroni correction of $p < 0.05$ due to six tests used) as a significance threshold for paired tests. We calculated effect size r (see Table 1) based on effect size estimates for Wilcoxon nonparametric test (Fritz et al. 2012). Mixed ANOVA analysis was used to evaluate the effect of the stimulated site (rSPL, rIFG, and VTX), stimulation protocol (iTBS, cTBS), and time (before and after stimulation) on RTs for our cognitive task of interest (VOMT). We used $p = 0.05$ as the level of statistical significance for the analyzed effects and their interactions. We tested assumptions for mixed ANOVA with Mauchly's sphericity test. Statistical analysis was performed in STATISTICA 12 (Stat Soft, Inc.) and MATLAB (The MathWorks Inc., Natick, MA, US, R2019a version).

Table 1 Reaction times in VOMT in relation to different types and sites of stimulation

Site	Protocol	RT [ms] Before	RT [ms] After	RT [ms] Difference	Wilcoxon signed-rank test p value	Wilcoxon signed-rank test z value	Effect size r
IFG	CTBS	396.9 ± 116.5	314.9 ± 76.4	−82.0 ± 64.4	< 0.001	−3.68	−0.87
IFG	ITBS	417.5 ± 106.9	324.3 ± 75.5	−93.2 ± 50.8	< 0.001	−3.72	−0.88
SPL	CTBS	413.7 ± 108.9	344.3 ± 87.9	−69.4 ± 59.1	< 0.001	−3.18	−0.82
SPL	ITBS	402.0 ± 90.4	320.0 ± 68.2	−82.0 ± 66.0	< 0.001	−3.38	−0.82
VTX	CTBS	394.3 ± 106.9	326.4 ± 85.2	−67.9 ± 67.4	< 0.001	−3.33	−0.79
VTX	ITBS	415.1 ± 141.2	313.2 ± 75.3	−101.9 ± 89.4	< 0.001	−3.70	−0.85

Evaluation of vertex stimulation outcomes using E-field modelling in SimNIBS

We hypothesized that the vertex stimulation might partially reach behaviorally relevant adjacent prefrontal cortical regions such as the supplementary motor area (SMA) (Arai et al. 2012; Narayana et al. 2012) and the paracentral lobule (PCL) (Verwey et al. 2002; Laakso et al. 2018; Lee et al. 2018; Shirota et al. 2019), i.e., our ROIs.

To test our hypothesis, we exported neuronavigational data from the Brainsight 2 software, including the localization of the coil, pulse trajectory axis orientation, distance of the coil to the target, and the target error (Thielscher and Wichmann 2009). Using a 3D finite-element model (FEM) based pipeline called SimNIBS (www.simnibs.org), we modelled in MATLAB the peak and the overall induced norm E-field in our ROIs, individually for each subject and each stimulation session (Thielscher et al. 2015). The norm E-field value represents the magnitude, i.e., the vector length of the E-field, and the vector is not further decomposed into normal (orthogonal) and tangential components (Saturnino et al. 2019). As the coil model, we chose the built-in model of the Magstim 70 mm figure-of-8 coil, since in this setting, its parameters are equivalent to our Deymed 70BF-Cool coil. The E-field input in the form of dI/dt was calculated as the maximum output of the stimulator (Deymed DuoMAG XT-100; dI/dt max = 108 A/μs), multiplied by the individual intensity of stimulation used in each stimulation session (80% of AMT) (Konakanchi 2020). To account for outlier values, we used the 99.999 percentile as a peak E-field for the given ROI. To be able to perform a correlational analysis between the accuracy of the stimulation and any change in performance in the cognitive task, we first needed to prove that a relevant E-field magnitude was induced in one of the adjacent active areas as a function of stimulation inaccuracy. Thielscher and Kammer (2002) estimated that the E-field values required for activating the hand motor area lie in the range of 70–100 V/m; Huang et al. proved that TBS protocols at an 80% intensity of the individual AMT are able to produce significant changes in motor-evoked potentials (MEPs) (Huang et al. 2005). According to these findings, we accepted 56 V/m as a reasonable minimum threshold for peak E-field values in one of the ROIs to produce the neural aftereffects of TBS (Eggers et al. 2015; Casula et al. 2016; Koch et al. 2020). Recent TMS-EEG studies reported the induced E-field value of 50 V/m as a threshold value to effectively cause neuronal activation (Komssi et al. 2007; Rosanova et al. 2009; Casali et al. 2010; Gosseries et al. 2015; Conde et al. 2019). Spearman's correlation coefficients were used to study the relation between the vertex TBS-induced E-fields in the ROIs and improved performance in the VOMT.

Results

Behavioral results

Since the task accuracy was very high already prior to the stimulation (97.7% of correct responses for objects in the conventional view and 96.6% for objects presented in the unconventional view), we concentrated on the RT results only. There was a shortening of RTs after the stimulation of the active sites (rIFG, rSPL) and after the simulation of our control site (vertex) (see Table 1; for normalized values of RTs, see Table S1 in the supplementary materials).

Distributions of RTs used in the analyses are shown in Fig. 2. Because Mauchly's sphericity test of compound symmetry of response variables showed statistically significant p values ($p = 0.021$; for details, see Table S3 in the supplementary materials), we reported a resulting p value of p_{LB} (p value with lower bound adjustment) of a mixed ANOVA, corrected for sphericity. Mixed ANOVA analysis showed a statistically significant mean effect of the time factor, $F(1,12) = 65.37$, $p_{LB} < 0.001$ on the shortening of RT; there was no clearly significant effect for the stimulation protocol factor (iTBS, cTBS), $F(1,12) = 0.83$, $p_{LB} = 0.381$). In other words, TBS applied over our control vertex stimulation site produced equal cognitive aftereffects or even surpassed the positive effects of TBS applied over our active stimulation sites. Other factors and interactions did not exceed the threshold for significance ($p_{LB} < 0.05$). For details, see Table 2.

Induced E-field modelling results in our ROIs

Out of the initial 40 stimulation sessions, 34 were eligible for the E-field analysis. In six sessions, the SimNIBS pipeline was unable to produce E-field maps properly, and therefore, we excluded them from further analysis.

In 73.53% of the stimulation sessions, there was a suprathreshold induced norm E-field in at least one of the selected ROIs (see Table 3 for average stimulation intensities and Table S2 in the supplementary materials for individual intensities).

Spearman's correlation analysis (see Fig. 3) shows a strong negative correlation between the overall induced norm E-field and RT change ($R = -0.54$, $p = 0.04$) after the iTBS stimulation. On the other hand, while using the cTBS protocol, we found a weak positive correlation between the overall induced norm E-field and RT change ($R = 0.23$, $p = 0.41$). In other words, iTBS correlated with a positive behavioral outcome depending on the magnitude of the induced E-fields in the ROIs, while cTBS correlated with a weak negative cognitive outcome depending on E-field values.

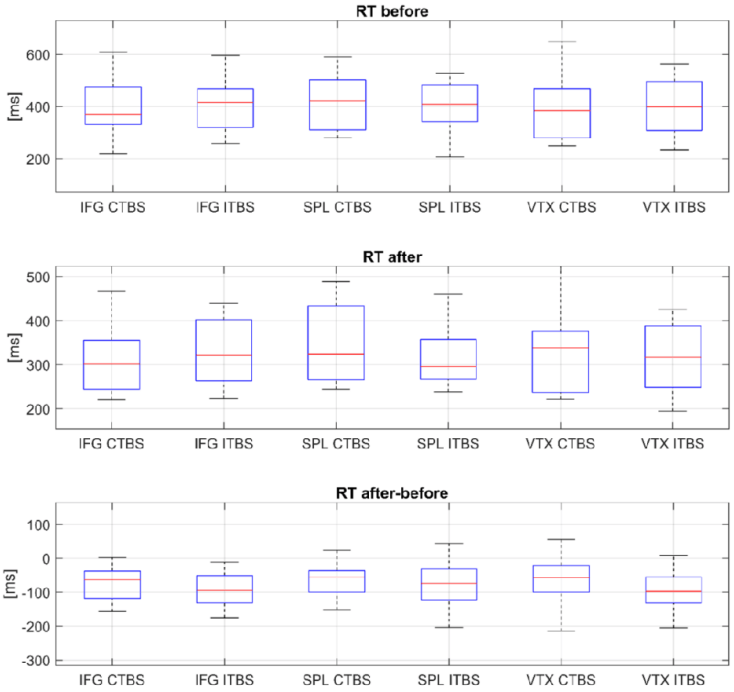

Fig. 2 Boxplots of RT used in analyses. The upper graph represents values before stimulation; the middle graph is for values after stimulation; and the bottom graph represents differences (after–before RT) in RT values. The red line represents the median; boxes represent the upper and lower quartiles

Table 2 Results of mixed ANOVA, performed on reaction times

	SumSq	MeanSq	F	p	p_{LB}
Intercept	20,374,955.0	20,374,955.0	287.06	< 0.001	< 0.001
Main effect: site	233.7	116.9	0.03	0.967	0.857
Main effect: protocol	1249.2	1249.2	0.83	0.381	0.381
Main effect: time	249,450.0	249,450.0	65.37	< 0.001	< 0.001
Interaction: area × protocol	12,817.2	6408.6	2.11	0.144	0.172
Interaction: area × time	1495.0	747.5	0.33	0.722	0.576
Interaction: protocol × time	3555.7	3555.7	2.58	0.134	0.134
Interaction: area × protocol × time	1786.4	893.2	0.75	0.484	0.404

Variable p_{LB} is the p value, corrected for sphericity

Table 3 Simulated induced peak norm E-fields for the respective ROIs after the vertex stimulation using mean stimulation intensities

Peak induced norm E-field in ROIs (V/m)				
	SMA L	SMA R	Paracentral lobule L	Paracentral lobule R
iTBS	82.0 ± 52.9 (98.7)	80.9 ± 54.1 (91.2)	61.9 ± 41.1 (65.1)	58.5 ± 47.4 (52.9)
cTBS	102.6 ± 47.5 (110.6)	102.9 ± 44.9 (116.2)	73.5 ± 41.0 (76.2)	67.7 ± 42.8 (62.9)

Values in the table are for mean ± standard deviation; median is in bracket

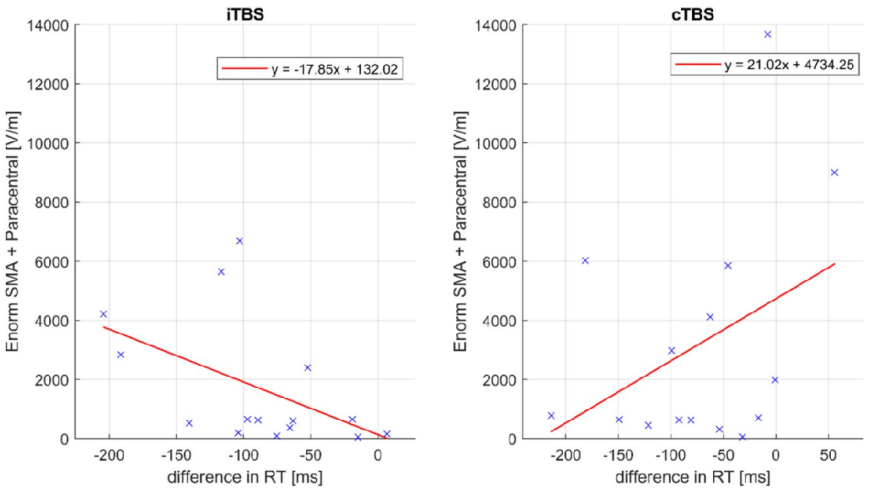

Fig. 3 Graphs of correlations for two different stimulation protocols. The *Y*-axis represents the sum of induced norm E-field inside the ROI masks. The *X*-axis represents the difference between post-stimulation RTs and pre-stimulation RTs in VOMT (i.e., shortening of RTs). We excluded three outliers from the iTBS graph, based on inner fence criterion (Ross 1983) The Spearman correlation for the iTBS graph is $r=-0.54$, $p=0.04$ with 95% confidence interval $[-0.79, -0.13]$. The Spearman correlation for the cTBS graph is $r=0.23$, $p=0.41$ with 95% confidence interval $[-0.24, 0.61]$. The red line is for approximation with linear trend

Discussion

The cognitive results of our TBS study were negative. In fact, TBS applied over all stimulation sites produced significant RT shortening, and we did not identify any superiority of the active site stimulation compared to the vertex stimulation.

To clarify the electrophysiological underpinnings of the behavioral changes induced by the vertex TBS, we applied modern E-field modelling methods with the SimNIBS software. In the past year, there has been a growing focus on increasingly realistic head models that are able to account for various properties of different intracranial tissues and substances (Thielscher et al. 2011, 2015; Laakso et al. 2018; Lee et al. 2018).

We uncovered an induction of significant norm E-fields in adjacent active cortical areas of interest, namely in the SMA and PCL. Using conventional craniometric vertex targeting, this was true in almost three out of four subjects, probably due to the interaction of the passing pulse with other tissues (Lee et al. 2018), individual anatomical factors (Laakso et al. 2018), or possible deviations in targeting. The SMA is a key structure during the execution of visually paced hand movements (Thickbroom et al. 2000; Grefkes et al. 2008). This is substantiated by the state-dependent effective SMA-primary motor cortex (M1) connectivity and their intra- and interhemispheric coupling (Nachev et al. 2008; Arai et al. 2012). The active role of the SMA has also been described in mental rotation visual tasks similar to the one used in our study (Cona et al. 2017). This suggests that stimulation of the SMA may have contributed to the shortening of RTs (Carlsen et al. 2015) both by its effect on cognition and by modulating the motor response. It has been shown that damage to the SMA may lead to deficits in executive control of motor functions (Salo et al. 2018; Sjöberg et al. 2019).

The PCL is considered to be an important motor region as well, related to the mental representation of actions (Péran et al. 2010) and to spatial attention (Hirjak et al. 2014). The PCL is further involved in motor impulsiveness control (Ai et al. 2019). Our correlation analyses illustrated the clinical relevance of our results, clearly showing that iTBS leads to RT shortening with increasing norm E-field values in our ROIs, while the opposite (though less strong) direction of correlation was observed with vertex cTBS. It has been repeatedly shown in the literature that iTBS leads to a persisting increase in the amplitude of MEPs when applied over the primary motor cortex, while cTBS produces a consistent inhibitory effect of MEPs that serve as a measure of corticospinal excitability (Huang et al. 2005; Quartarone et al. 2005; Di Lazzaro et al. 2010; Iezzi et al. 2011; Gamboa et al. 2011; Suppa et al. 2016; Morris et al. 2019). Previous studies showed that high-frequency rTMS over the SMA is able to relieve motor symptoms in Parkinson's disease (Hamada et al. 2009) and increase the evoked MEPs in M1

(Matsunaga et al. 2005; Raux et al. 2010), while cTBS over the SMA induced inhibition in the motor network (Wu et al. 2014; Ji et al. 2017).

When studying the effect of TMS on cognitive performance, it is necessary to always consider not only the spread of induced E-fields in the active areas in close proximity, but also the effect of TMS on cortical excitability in their distant cortico-subcortical and cortico-cortical projections. TMS-EEG has proven to be a superior method for studying cortical excitability as well as effective connectivity (Ilmoniemi et al. 1997; Bergmann et al. 2016; Casula et al. 2020), oscillatory activity of the brain (Thut and Miniussi 2009; Rosanova et al. 2009), and the neurohumoral relationships in the brain (Du et al. 2018) with a considerable clinical diagnostic potential (Pellicciari et al. 2018; Koch et al. 2019; Tremblay et al. 2019; Koch 2020). However, it is necessary to prove which proximal active areas are activated by vertex stimulation to be able to further study its influence on their distal connections. We believe that our results can serve as a basis for future TMS-EEG research.

A suitable placebo condition for a cross-over rTMS study must meet four parameters: (1) authentic placement of the device on the scalp and visual similarity, (2) specific scalp sensation arising from superficial nerve and muscle stimulation, (3) authentic auditory stimulation and vibration, and (4) not producing the effects arising from active cortical stimulation. Commercially available sham coils with incorporated magnetic field attenuating material and tilted coil techniques with only the edge of the coil touching the scalp generally lack authentic scalp sensation. Modern state-of-the-art sham systems employ a multimodal approach utilizing cutaneous electric stimulation (Rossi et al. 2007; Gordon et al. 2018) in conjunction with a TMS coil in a configuration that is not able to induce a relevant E-field in the underlying cortex. This is usually done by holders made from a rigid material such as plastic that allows the transmission of the vibrations made by the TMS coil and also for the auditory stimulation through the bone conduction while ensuring the appropriate distance between the coil and the cortex. Recent TMS-EEG studies showed that such realistic sham-TMS stimulation, when individually adjusted to correspond with the somatosensory sensation of the real TMS stimulation, are in fact able to evoke EEG responses similar to those evoked by real TMS stimulation (Conde et al. 2019). In the field of cognitive neuroscience, these techniques are suitable preferably for parallel group studies with subjects naive to the real TMS (Duecker and Sack 2015).

To further dissociate the effects of real TMS from sham, there is a need to reduce its multisensory contamination. This can be done by implementing specific noise-masking measures, such as white noise or noise adjusted to the time-varying frequency of the TMS "click," substantially reducing its auditory co-stimulation. Furthermore, a thin layer of foam can be incorporated under the TMS coil to reduce the vibrations and the low-frequency auditory stimulation via bone conduction and to suppress the inductive electric stimulation of myelinated peripheral nerve axons (Conde et al. 2019; Rocchi et al. 2021). However, a complete suppression is often not achievable even when all the currently advised measures to attenuate multisensory co-stimulation are implemented (ter Braack et al. 2015; Biabani et al. 2019). A new option for the auditory stimulus masking may be a recently described adaptable tool (Russo et al. 2021) that seems to perform better than standard noise-masking.

It has been shown (Jung et al. 2016) that well-navigated vertex stimulation, even in suprathreshold intensities, does not evoke BOLD signal increases within regions of the motor network that were activated after a single session of rTMS of the primary motor area (M1). The fMRI effects of 1 Hz rTMS applied over the vertex induced only deactivations of regions engaged in the default mode network. The authors speculated that this might be attributed to a by-product of TMS (sensory responses evoked by TMS may arouse the participant's attention and lead to an interruption of self-related thinking). A functional connectivity analysis revealed increased connectivity within the motor network after M1 stimulation; no connectivity changes were observed after the vertex stimulation in either the motor network or the default mode network. These findings support the use of vertex stimulation as a control condition.

We tried to avoid the effect of learning on our observed results. We regressed the order of visit effect out of the RTs with a general linear model. Moreover, to mitigate the effect of learning, participants properly practiced the VOMT before they performed the task in the actual experimental setting. The learning effect was also partially disrupted by the randomization of the visits. Although we regressed out the effect of learning, the remaining variability was still sufficient to show a significant decrease in RT due to the stimulation. We also examined the effect of individual ability to perform the task on differences in RTs. We normalized the RT values, but the normalization did not influence the observed differences in RT. We present normalized RT and differences in normalized RTs in Table S1 in the supplementary materials.

In our study, we clearly show that with less-precise conventional vertex targeting, the vertex stimulation may reach the adjacent SMA/PCL, i.e., regions that may be engaged in cognitive performance and may lead to RT shortening during a visual working memory task (Chung et al. 2005; Macar et al. 2006; Ai et al. 2019; Respino et al. 2019; Busan et al. 2019; Sjöberg et al. 2019).

To ensure sufficient accuracy in vertex targeting, we suggest using neuronavigation software tools to verify the trajectory of the pulse even after it passes the target point on the scalp to achieve a higher probability of preventing other

active areas from being stimulated. For future studies, we propose using a common coordinate from the MNI atlas, lying exactly at half of the width of the longitudinal fissure at the level of the most dorsal points of the adjacent gyral crowns, and then leading a line from the scalp surface intersecting this vertex MNI coordinate and continuing further ventrally exactly in the middle of the longitudinal fissure until it reaches the trunk of the corpus callosum. This line would be saved as a neuronavigation target in the form of a trajectory as opposed to a target in the form of a point on the scalp. We used this in our study to transfer the craniometrically located vertex to the neuronavigational environment. This trajectory-type target would control for angular errors when placing the stimulation coil on the scalp, in contrast to the target as a point on the scalp, which does not set the direction of the pulse once it enters the cranium. In this study, we show that although we are not able to omit the nonzero width of the stimulation pulse itself or the dispersion of the pulse when passing through various tissues and environments, the TMS pulses were, in some subjects, able to proceed through the midline without inducing relevant E-field amounts in the adjacent cortical areas.

Conclusion

We show for the first time that conventional vertex targeting may induce significant unwanted changes in cognitive performance that are substantiated by induced E-fields in adjacent active cortical regions including the SMA and/ or PCL. To prevent this, we suggest using appropriate neuronavigation tools for vertex targeting. Setting the target as a trajectory might be necessary; this warrants further research.

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Data availability Data are stored at the CEITEC, Masaryk University repository for a minimum of 10 years. Data will be disclosed on request and after approval of the proposed use of the data by the study committee.

Code availability The SimNIBS pipeline is available at https://simnibs.github.io/simnibs/build/html/index.html.

Declarations

Conflict of interest The authors declare no conflicts of interest related to this study.

Ethical approval Approval was obtained from the ethics committee of Masaryk University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate Informed consent was obtained from all individual participants included in the study.

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17. Cognitive Effects of Dance-Movement Intervention in a Mixed Group of Seniors Are Not Dependent on Hippocampal Atrophy

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Commentary.

This study broadened the concept of brain plasticity beyond direct neuromodulation by examining a behavioral intervention based on dance and movement. Physical, cognitive, and social stimulation may support cognitive functioning in older adults, but it remains unclear whether structural brain pathology limits the potential for intervention-induced improvement. The 6-month dance-movement intervention improved executive function, as reflected by better figural fluency performance, in the intervention group comprising individuals with MCI preceding AD and HC, compared with the control group, independently of baseline brain atrophy. An important contribution of this study was to demonstrate that the potential for cognitive improvement was not determined by the degree of baseline structural brain atrophy, supporting the persistence of plastic potential in older adults. This work complemented my research on NIBS by investigating another route to cognitive and neural modulation and established the basis for subsequent multimodal MRI studies of fitness-related brain plasticity.

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NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - ORIGINAL ARTICLE

Cognitive effects of dance-movement intervention in a mixed group of seniors are not dependent on hippocampal atrophy

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Abstract

Dance-movement intervention (DMI) offers multi-component stimulation of cognitive functions, and it may ameliorate cognitive deficits in the elderly. We investigated the effects of intensive DMI on the cognitive performances of healthy seniors (HS) and patients with mild cognitive impairment (MCI). In addition, we evaluated whether the baseline MRI hippocampus-to-cortex volume (HV:CTV) ratio (i.e., a marker of a typical AD-specific brain atrophy and of distribution of neurofibrillary tangles in the brain) has any impact on the DMI-induced cognitive changes. The research cohort consisted of 99 subjects who were randomly assigned (in a 1:1 ratio) to a DMI group or to a control (life-as-usual) group. The DMI group consisted of 49 subjects with an average age of 69.16 years (SD = 5.36), of which 34 were HS (69.4%) and 15 had MCI (30.6%). The control group consisted of 50 subjects aged 68.37 years (SD = 6.10), of which 31 were HC (62%) and 19 (38%) had MCI. The DMI group underwent a 6-month intervention, which consisted of 60 lessons supervised by a qualified instructor. Statistical analysis yielded a significant improvement of the figural fluency task as measured by the five-point test in the DMI group as compared to the control group [t (97) = 2.72; p = 0.008]. The baseline HV:CTV ratio was not associated with cognitive changes on that task or with changes in any cognitive domain's Z scores. We observed DMI-induced effect on the test evaluating executive functions across the spectrum of HS and MCI, which was not dependent on the magnitude of AD-related brain pathology.

Keywords Dance-movement intervention · Aging · Mild cognitive impairment · Executive function · MRI · Hippocampus–to-cortex volume ratio

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Introduction

The population is getting older and the number of persons dealing with cognitive impairment is increasing. The most common cause of dementia is Alzheimer's disease (AD). Environmental components may significantly modulate healthy and pathological aging. One preventative and possibly protective factor is physical activity (PA) (Colcombe et al. 2006), which modulates the brain through neuroplasticity (Colcombe et al. 2006) and also transfers this effect to cognitive enhancement (Colcombe and Kramer 2003). The mechanisms by which PA affects the brain are not yet fully understood; however, they probably involve vascular (e.g., blood perfusion), neurochemical (e.g., endorphins, serotonin increase), cognitive reserve (e.g., neuroplasticity), anti-stress, and functional (e.g., cognitive stimulation) factors (Bowes et al. 2013). The activities with the most potential benefit are the multi-component ones (Ngandu et al. 2015, Liang et al. 2018), especially dance-movement (DM) activity, which combines physical, cognitive, and social stimulations (Burzynska et al. 2017). The cognitive stimulation in dance-movement intervention (DMI) particularly stems from learning new complex skills and choreography through observational learning, working memory, psychomotor coordination, procedural memory, and kinesthetic empathy (Reynolds and Reason 2012). Another source of stimulation is provided by effects of music itself (Liang et al. 2018) which has been shown to have additional positive effect on cognitive performance (Sung and Chang 2005; Särkämö et al. 2014). The social component of the DMI cannot be neglected either (Fratiglioni et al. 2000; Seeman et al. 2001). Studies showed that the social support is a significant health-protective factor (Fratiglioni et al. 2004) reducing the risk of mortality (Ceria et al. 2001; Eng et al. 2002; Iwasaki et al. 2002; Steptoe et al. 2013; Tanskanen and Anttila 2016), and preventing cognitive deficits (Seeman et al. 2001; Zunzunegui et al. 2003; James et al. 2011) and dementia conversion (Fratiglioni et al. 2000).

Preliminary results suggest that DMI may modulate cognitive performance in older healthy subjects (HS) and people with mild cognitive impairment (MCI), although the results vary and remain inconclusive so far (Hokkanen et al. 2008; Coubard et al. 2011; Kim et al. 2011; Kimura and Hozumi 2012; Lazarou et al. 2017; Zhu et al. 2018). Research shows that DM activity stimulates those brain areas that are mostly affected by negative changes during aging and Alzheimer's dementia pathology (Karpati et al. 2015; Burzynska et al. 2017). Decreased volumes of hippocampi are referred to as the main structural imaging biomarker of AD reflecting brain neurodegeneration (McKhann et al. 2011). Whitwell et al. (2012) demonstrated close correlation between the antemortem MRI hippocampus-to-cortex (HV:CTV) ratio and the postmortem distribution of neurofibrillary tangles in a clinico-pathological study in distinct Murray–Dickson AD subtypes. The cut-off ratios were published for differentiating typical AD variants from a less typical hippocampus-sparing AD variant (Risacher et al. 2017). Of note, the tau pathology with neurofibrillary tangles can also be found in the "primary age-related tauopathy" (PART), i.e., an age-related tangle pathology of the neurofibrillary tangle type encompassing a wide range from cognitively normal condition to senile dementia (which is quite rare) (Crary et al. 2014). This pathology is also commonly observed in the medial temporal lobe (Crary et al. 2014). Taken together, the HV:CTV ratio may serve as an MRI marker of typical AD and a marker of neurofibrillary tangle distribution in the brain even in the absence of amyloid plaques.

Since there is no available causal pharmacological treatment for AD (Hardiman et al. 2016) and other degenerative dementias, DMI might serve as an alternative or supportive non-pharmacological therapy for cognitive malfunction. To our knowledge, this is the first randomized controlled study employing DMI conducted on such a large sample of a mixed group of HS and MCI using a very complex clinical evaluation and baseline structural MRI.

Aims of the study

Using a complex neuropsychological battery, our primary study objective was to evaluate whether the 6-month intensive (three times per week) DMI has any symptomatic effect on cognitive performance (Thomas et al. 2016) in a large mixed group of HS and people with MCI (primary study objective 1). In addition, we used MRI to identify the DMI-induced aftereffects on neural brain plasticity (i.e., the primary study objective 2; results will be published elsewhere).

Our secondary objective was to assess whether the magnitude of expression of hippocampal changes as measured by the baseline HV:CTV ratio has any impact on the DMI-induced cognitive changes in a continuum of cognitively healthy and MCI aged subjects. If positive, this result might be of clinical relevance since it would help to identify good candidates for the DMI in future studies.

MCI is a heterogeneous entity that may be caused by various brain pathologies. Structural MRI has potential to reveal brain neurodegeneration prior to any cognitive decline and serve as a biomarker of AD-related brain pathology (McKhann et al. 2011; Albert et al. 2011). Since we did not use other AD-specific biomarkers of the AD brain pathology than MRI (Albert et al. 2011), we also used the baseline HV:CTV ratio cut-off score as a novel MRI biomarker of neurodegeneration due to a typical or limbic variant of AD (Risacher et al. 2017).

Method

Study sample

The study sample consisted of healthy senior volunteers potentially capable to participate in our intensive intervention. Public media, such as local newspapers, radio, and TV news reports and leaflets, were used for subject recruitment. The MCI participants were longitudinally followed at the First Department of Neurology, Faculty of Medicine, Masaryk University in Brno, Czech Republic and they contacted us based on the information provided by their neurologist.

The inclusion criteria were: age > 60, no major neurological or psychiatric diseases, no major medical conditions that would contradict the DMI, non-smoking, and MR compatibility. Individuals included in the study should not engage in a higher intensity or frequency of PA than our planned intervention based on a semi-structured interview. Participants were classified as having MCI if they scored below − 1.5 SD in at least two tests in one or more cognitive domains (Anderkova et al. 2017). In detail, we recruited MCI subjects if they met one of the following criteria: in case of MoCA ≥ 26 points, the subject had to score below 1.5 SD in two tests in at least one cognitive domain as assessed by our cognitive test battery. In case that the MoCA score was < 26 points, the subjects had to score below 1.5 SD in any two tests from our cognitive test battery. In case of an objective memory deficit on the MoCA, the subject had to score below 1.5 SD in at least one test from the memory domain as assessed by our cognitive test battery. Informed consent, in accordance with the ethics committee of Masaryk University, was obtained from each subject. The study was approved by the local ethics committee.

Study design

The final sample size was calculated as follows: when considering the pilot data, we observed a mean change of 0.5 points with SD = 1 in the five-point test score, i.e., a test evaluating executive functions. When using the type I error probability $\alpha = 0.05$, and expecting the power of the test $\beta = 0.8$, we should be able to detect a similar or greater change in the distinct cognitive test score with at least 33 subjects in an intervention group. Based on the power analysis and an estimated study drop out (25%), we aimed at enrolling 42 subjects in each study group (DMI group/control group).

The study was processed in three intervention episodes (September 2015–June 2018). Each episode consisted of five phases: (1) subject enrolment; (2) baseline assessment; (3) randomization; (4) intervention/life-as-usual; and (5) outcome assessment. Phases 1, 2, and 3 took 2–3 months, the intervention/life-as-usual period lasted 6 months, and the outcome assessment took approximately 1 month. The baseline and outcome assessments included neuropsychological assessment and MRI scanning using the 3 T Siemens Prisma MR scanner (Siemens Corp., Erlangen, Germany). For the purpose of this study, we used magnetization-prepared rapid gradient-echo (MPRAGE) high-resolution T1-weighted image (240 sagittal slices, slice thickness = 1 mm, TR = 2300 ms, TE = 2.34 ms, FA = 8°, FOV = 224 mm, matrix size 224 × 224) sequence. The neuropsychological assessment consisted of tests evaluating four cognitive domains: memory, attention, executive functions, and visuospatial functions (Table 1). Depressive symptoms [BDI-II (Beck et al. 1996) and clinician's interview with a subject and self-sufficiency (BADLS (Bucks et al. 1996)] were assessed to prevent inclusion of depressed or demented individuals.

Professional instructors from the Faculty of Sport Studies at Masaryk University conducted the 6-month intervention. Each lesson lasted 60 min and took place three times a week. The priority was to create a program with a maximum stimulation intensity. Dances such as Irish country, African dance, Greek dance, and tango were performed. The choreography was divided into several short learning segments, which were continually developed and combined into a final choreographed dance during ongoing lessons. Based on previous reports, a 3-month aerobic exercise performed three times per week has already shown measurable cognitive as well as brain plasticity aftereffects (Maass et al. 2016). Three months of DMI induced significant cognitive effects in patients with MCI (Zhu et al. 2018). Of note, significant cognitive effects were described already after nine DMI sessions in patients with dementia (Hokkanen et al. 2008). Therefore, we decided to analyze data from all subjects whose attendance was at least 60%. Of note, the real compliance with the study protocol was much higher in the vast majority of participants (mean 78.1%).

Data acquisition and processing

The baseline and outcome assessments included neuropsychological testing and MRI data scanning, altogether lasting approximately 3 h. The cognitive domain Z scores were computed as the average Z scores of the tests included in the particular domain (Anderkova et al. 2017). Subjects with dementia and depression, based on the BADLS-CZ, BDI-II, and clinician's interview were not included in this study. Equivalency in between-group baseline data was checked by Chi-square tests and two-sample t tests. Repeated measures MANOVA and following post hoc tests were applied for examination of DMI-induced cognitive effect.

Table 1 Neuropsychological assessment: tests and cognitive domains

Domain	Abbreviation	Test (subtest)
Overall cognitive level	MoCA	Montreal cognitive Assessment (Nasreddine et al. 2005)
Memory	TCF 1	Taylor figure test recall 3 min after copy (Taylor 1969)
	TCF 2	Taylor figure test recall 30 min after copy (Taylor 1969)
	WMS III: LogPam 1	Logical memory subtest from WMS III immediate recall (Wechsler 1997)
	WMS III: LogPam 2	Logical memory delayed recall after 30 min (Wechsler 1997)
Attention	WAIS III: symbols	Symbol search subtest from WAIS III (Wechsler 1997)
	WAIS III: DigitSpan	Digit span subtest from WAIS III (Wechsler 1997)
Executive function	ToH 3	Tower of Hanoi (3 disks) (Humes et al. 1997)
	ToH 4	Tower of Hanoi (4 disks) (Humes et al. 1997)
	FPT	Five-point test (Tucha et al. 2012)
Visuospatial function	JLO	Judgment of line orientation test (Benton et al. 1994)
	TCF copy	Taylor figure test copy (Taylor 1969)
Self-dependency	BADLS-CZ	Bristol Activities of Daily Living Scale (Bucks et al. 1996)
Mood	BDI-II	Beck depression inventory II (Beck et al. 1996)

T1-weighted images were processed using Freesurfer 6.0 (http://surfer.nmr.mgh.harvard.edu). The longitudinal pipeline (Reuter et al. 2012) was used for preprocessing, which included: (1) processing each timepoint independently with recon-all pipeline (CROSS); (2) the creation of within-subject template, unbiased to either timepoint, and using a cross-sectional stream to process the template (BASE); and (3) re-processing every timepoint using the unbiased template (LONG). Upon completing each step, automatic subcortical segmentation labels (Fischl et al. 2002), gray and white matter boundaries were visually inspected and later manually corrected for each subject. Thirteen subjects (7 DMI and 6 in the control group) were excluded due to segmentation imprecisions. For the purpose of this study, we calculated baseline hippocampal-to-cortical volume ratio (HV:CTV ratio), see below in the Calculation section. Spearman correlation was used to assess the relationship between the baseline HV:CTV ratio and changes in cognitive domain's *Z* scores or changes in cognitive tests of interest, i.e., tests that revealed significant DMI-induced changes.

Hippocampal-to-cortical volume ratio (HV:CTV ratio) was calculated according to Risacher et al.(2017) as the sum of left and right hippocampus volumes divided by the sum of left and right lateral frontal cortices (caudal middle frontal gyrus, pars opercularis, pars triangularis, rostral middle frontal gyrus), lateral parietal cortices (superior parietal gyrus, inferior parietal gyrus, supramarginal gyrus), and superior temporal gyri

$$\text{HV:CTV} = \frac{\text{Hippocampi}}{\text{Lateral frontal cortices} + \text{lateral parietal cortices} + \text{superior temporal cortices}}.$$

Freesurfer cortical structures were defined using Desikan–Killiany et al. (2006) atlas. Spearman partial correlations of the baseline HV:CTV ratio with the change in five-point test (timepoint 0–timepoint 1) in the DMI group were calculated. Age, gender, and total intracranial volume (TIV) were included as covariates of no interest.

For the purpose of our study, we further used the cut-off for the baseline HV:CTV ratio (≤ 0.0501) to identify MCI and HS subjects with a distinct pattern of MRI atrophy suggestive of typical AD variants with hippocampal involvement (Risacher et al. 2017). To eliminate the effects of age, gender, and TIV, HV:CTV ratio values were adjusted using the following formula:

$$\begin{aligned}\text{Adjusted HV : CTV} = \text{Baseline HV : CTV} - \big(&\beta_{\text{age}} \\ &\times\left(\text{age} - \bar{x}_{\text{age}}\right) + \beta_{\text{sex}} \times \left(\text{sex} - \bar{x}_{\text{sex}}\right) \\ &+\beta_{\text{TIV}} \times \left(\text{baseline ICV} - \bar{x}_{\text{TIV}}\right)\big)\end{aligned}$$

Results

Study participants

We recruited 147 subjects for the study. After a careful application of the inclusion and exclusion criteria (see above), a total of 120 participants were enrolled and randomized. During the study, 11 dancers did not complete the required number of DMI sessions. The most common reason was an unexpected health problem of the participating subject or his/her partner, and problems with keeping up with the time schedule of the intervention. If the attendance in the DMI program was

lower than 10%, the subject was offered to be reallocated to the control group (6 out of 11 participants). Altogether, 11 subjects were lost to the follow-up visit in the control group. All subjects who completed the DMI also underwent the post-treatment follow-up assessment. For a detailed flowchart, see Fig. 1.

Demographics

Our final research sample ($n=99$) consisted of 65 HS and 34 patients with MCI from which 49 individuals were randomly (in 1:1 ratio using opaque envelopes method) assigned to the DMI group (for more details, see Table 1) and 50 participants were assigned to the control group. The intervention and control groups did not differ in sociodemographic data or in the baseline neuropsychological test results, see Table 2 and Supplementary material (Table S1). The differences between HC and MCI, and between MCI in the experimental group and MCI in the control group in cognitive tests at baseline are displayed in the Supplementary material (Tables S2 and S3).

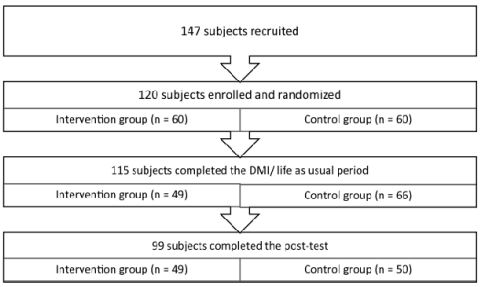

Fig. 1 Flowchart of this study

Cognitive effects of DMI

For raw cognitive test results in the DMI and control groups, see Supplementary Table S1. Statistical analysis yielded no statistically significant change (see Table 3) in any cognitive domain (memory, attention, executive function, visuospatial function) between pre-test and post-test outcome measures in the DMI group as compared to the control group ($V=0.031$; $F(3, 93)=0{,}98$; $p=0.408$). However, significant changes between the baseline testing and the cognitive assessment after 6 months (see Table 4) indicated improvement of the TCF 1 ($t(48)=-2.68$; $p=0.010$), TCF 2 ($t(48)=-3.48$; $p=0.001$), ToH 4 time ($t(35)=2.74$; $p=0.010$), and FPT ($t(48)=-4.04$; $p<0.001$) in the DMI group. Regarding multiple comparisons (13 tests in the assessment), the α correction according to Sidak was applied ($\alpha_{SID}=0.004$). Only TCF 2 and FPT survived this correction. The between-group (intervention/control) comparison for FPT indicates a significant change in the DMI group as compared to the control group ($t(97)=2.72$; $p=0.008$). The between-group comparison was not significant for TCF 2 ($t(97)=0.35$; $p=0.727$).

The typical AD-related pattern of brain neurodegeneration based on the adjusted HV:CTV ratio was found in 10 out of 43 subjects in the DMI group (23.3%), and in 12 out of 45 subjects (26.7%) in the control group. The mean HV:CTV ratio in the DMI group was 0.0543 ± 0.0050 and it was 0.0547 ± 0.0057 in the control group.

Table 2 Descriptive characteristics of the study sample

	Intervention group ($n=49$)		Control group ($n=50$)		p^1
	n	%	*n*	%	
Sex					
Male	8	16.3	15	30	0.107
Female	41	83.7	35	70	
Cognitive health					
HS	34	69.4	31	62	0.439
MCI	15	30.6	19	38	
	M	Sd	*M*	Sd	p^2
Age	69.16	5.36	68.37	6.10	0.495
Length of education	14.51	2.56	14.80	3.10	0.613
MoCA	29.96	2.88	26.06	2.71	0.113

MoCA Montreal Cognitive Assessment

[1]Based on Chi-square Test

[2]Based on two-sample *t* test

Table 3 Effect of intervention on cognitive domains (controlled for age)

Effect	*V*	*F*	*df*	Error *df*	Effect size	Power	*p*
Domains	0.031	0.98	3	93	0.031	0.26	0.408
Domains × age	0.046	1.49	3	93	0.046	0.38	0.223
Domains × group	0.028	0.89	3	93	0.028	0.24	0.451
Time	0.048	4.80	1	95	0.048	0.58	0.031*
Time × age	0.038	3.76	1	95	0.038	0.48	0.055
Time × group	0.015	1.46	1	95	0.015	0.22	0.231
Domains × time	0.056	1.85	3	93	0.056	0.47	0.143
Domains × time × age	0.051	1.67	3	93	0.051	0.43	0.179
Domains × time × group	0.036	1.17	3	93	0.036	0.31	0.324

Covariates: age, cognitive health (HS/MCI); *V* Pillai's trace, time = baseline/outcome assessment; group = intervention/control

Table 4 Change between baseline testing and cognitive assessment after 6 months

	Intervention group ($n=49$)					Control group ($n=50$)					Between groups
	M	Sd	*T*	*df*	*p*	*M*	Sd	*T*	*Df*	*p*	*p*
TCF 1	−0.42	1.1	−2.68	48	0.010	−3.68	0.98	−2.67	49	0.010	0.802
TCF 2	−0.57	1.1	−3.48	48	0.001	−0.49	1.05	−3.31	49	0.002	0.727
WMS III: LogPam1	−0.09	0.9	−0.70	48	0.488	−0.25	0.99	−1.76	49	0.085	0.418
WMS III: LogPam2	−0.07	0.7	−0.64	48	0.526	−0.24	0.94	−1.78	49	0.081	0.323
WAIS III: Symbols	−0.14	0.82	−1.21	48	0.234	0.08	0.80	0.67	49	0.506	0.252
WMS III: DigitSpan	−0.12	0.75	−1.12	48	0.268	0.05	0.66	0.58	49	0.562	0.22
ToH 3 time	5.04	47.8	0.73	47	0.469	19.6	59.4	2.34	49	0.024	0.185
ToH 3 movements	0.12	1.00	0.81	47	0.425	−0.13	1.2	−0.7	49	0.469	0.286
ToH 4 time	37.8	83.0	2.74	35	0.010	16.5	76.6	1.38	40	0.176	0.244
ToH 4 movements	0.09	1.61	0.35	38	0.732	0.19	1.7	0.74	43	0.462	0.466
FPT	−0.53	0.91	−4.4	48	0.000	0.02	1.1	0.16	49	0.872	0.008
JLO	0.01	0.78	0.10	48	0.920	−0.13	0.81	−1.17	49	0.248	0.366
TCF: copy	−0.01	0.61	−0.03	48	0.974	0.06	0.48	0.95	49	0.348	0.542

There was no correlation between the baseline HV:CTV ratio and the change in FPT due to the DMI ($r=0.02$, $p=0.92$). The same negative results were found in individual cognitive domain's *Z* scores (memory: $r=0.16$, $p=0.31$; attention: $R=-0.05$, $p=0.76$; executive functions: $r=0.08$, $p=0.64$; visuospatial functions: $r=-0.01$, $p=0.97$; language: $r=0.09$, $p=0.57$).

Separate analysis for the MCI subgroup showed some significant DMI-induced effects on memory, namely in TCF 1, TCF 2, and WMS III: LogPam2 (see Supplementary Tables S3 and S4). Between-group (intervention/control) analysis yielded a significant difference only in the TCF 1 performance ($p=0.036$). However, this result did not survive the alpha correction ($\alpha_{SID}=0.01$).

Discussion

Our results indicate that there is no striking effect of a 6-month DMI on cognitive performance in a mixed group of healthy seniors and MCI patients. This finding is consistent with the literature exploring effects of a 6-month DMI in older adults (Barnes et al. 2015; Burzynska et al. 2017; Müller et al. 2017; Rehfeld et al. 2018). However, in our study, the DMI yielded significant improvements in the five-point test (Tucha et al. 2012) examining nonverbal (figural) fluency. Figural fluency reflects divergent thinking, attention division, planning, and mental flexibility; i.e., it evaluates executive functions (Johanidesová et al. 2014). Executive functions and specifically figural fluency are known to decline in Alzheimer's disease, and even during healthy aging (Fama et al. 1998). Our results

accord with preliminary results of Coubard et al. (2011) who reported the DMI-induced improvement in switching attention of older adults ($n = 16$) as compared to two other motor training programs. Nine sessions of DM intervention compared to no intervention also improved executive and visuospatial functions as measured by the clock-drawing test in patients with already fully blown dementia ($n = 19$ and 10, respectively)(Hokkanen et al. 2008). Two 40-min aerobic dance programs applied on 34 seniors, showed that the complex choreography program is efficacious to improve the executive functions rather than simply learning separate dance steps (Kimura and Hozumi 2012). Ten sessions of DMI led to improved executive functions and short-term memory in a sample of 12 healthy seniors compared to control group with non-dance activity (Colcombe et al. 2006).

The change in the TCF 2 task evaluating non-verbal delayed memory recall performance in the DMI group survived correction for multiple comparisons; however, no between-group differences were detected. This result may suggest a possible practice effect, which led to improved performance even in the control group. Separate analysis of the MCI subgroup showed a significant between-group (intervention/control) effect on the TCF 1, i.e., a memory test evaluating immediate visual memory recall. This result is consistent with results of (Zhu et al. 2018) who found improved logical memory in 29 MCI patients after only 3-month DM intervention compared to 31 MCI subjects with no intervention. These results suggest that cognitively impaired subjects may profit from the DMI even more than HS. Similar results demonstrating memory improvement were shown in another study including only amnestic MCI patients (Lazarou et al. 2017) or patients with a metabolic syndrome (Kim et al. 2011).

Our secondary objective was to identify whether the magnitude of expression of the HV:CTV ratio (i.e., a marker of both typical AD-related pattern of atrophy and neurofibrillary tangle distribution in the brain) has any impact on the DMI-induced cognitive aftereffects. Despite the fact that typical AD-related pattern of atrophy as indicated by the published cut-off score (Whitwell et al. 2012; Risacher et al. 2017) was present in about one quarter of all subjects in both DMI and control groups, we were not able to show any association between the baseline MRI changes and the DMI-induced cognitive aftereffects.

Conclusion

In conclusion, the results of this study indicate a subtle favorable effect of a relatively short-term DMI on executive functions in a mixed group of elderly participants with variable baseline cognitive performance without dementia. The magnitude of hippocampal atrophy as a biomarker of typical AD and of neurofibrillary tangle distribution in the brain had no impact on cognitive changes due to DMI. More studies are warranted to assess effects of longer interventions, and explore underlying mechanisms of DMI-induced cognitive aftereffects.

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18. Multishell Diffusion MRI Reflects Improved Physical Fitness Induced by Dance Intervention

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Commentary.

This study investigated whether improvements in physical fitness induced by a dance intervention are reflected in brain microstructure. It was motivated by growing evidence that lifestyle interventions may influence brain health, together with the need for sensitive neuroimaging markers capable of detecting these changes. We hypothesized that advanced multishell dMRI would identify microstructural correlates of intervention-related fitness improvement. The dance intervention improved physical fitness and attention, with fitness gains associated with changes in white matter microstructure, particularly in the corticospinal tract and superior longitudinal fasciculus, despite no significant overall intervention-related differences in white matter changes between groups. The findings demonstrated associations between changes in physical fitness and diffusion-based measures, suggesting that brain microstructure may be responsive to behavioral intervention. The novel contribution was the application of advanced dMRI, previously used in our research to characterize neurodegeneration, to the investigation of positive brain plasticity. This methodological transfer is an important feature of my research trajectory: imaging biomarkers were used not only to detect disease-related damage but also to assess intervention-induced change.

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Research Article

Multishell Diffusion MRI Reflects Improved Physical Fitness Induced by Dance Intervention

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Using multishell diffusion MRI and both tract-based spatial statistics (TBSS) and probabilistic tracking of specific tracts of interest, we evaluated the neural underpinnings of the impact of a six-month dance intervention (DI) on physical fitness and cognitive outcomes in nondemented seniors. The final cohort had 76 nondemented seniors, randomized into DI and control (life as usual) groups. Significant effects were observed between the DI and control groups in physical fitness measures and in attention. We detected associations between improved physical fitness and changes in diffusion tensor imagining (DTI) measures in the whole white matter (WM) skeleton and in the corticospinal tract and the superior longitudinal fascicle despite the fact that no significant differences in changes to the WM microstructure were found between the two groups.

1. Introduction

Dance intervention (DI), regardless of the type of dance, was shown to have a positive impact on the overall physical health of older adults [1]. Dancing is an activity that comprises a wide range of skills, including motor learning, action observation, sensorimotor coordination, and synchronization with a group; it engages physical endurance, balance control, motor learning, and cognitive functions [1, 2]. Keogh et al. [3] in their review concluded that dancing can significantly improve the aerobic power, lower body muscle endurance, strength, flexibility, balance, and agility of older adults. Another more recent review [4] proved the beneficial effect of dance on physical fitness (mostly reported as balance and motor skills in general) in patients with different kinds of pathology.

The influence of the different types of dance activity on cognition in healthy seniors has been tested behaviorally [5, 6]. Coubard et al. [5] reported that contemporary dance training (one lesson/week, 5.7 months) leads to improvement in switching attention in older adults. Kattenstroth et al. [6] reported positive effects of regular dancing (one lesson/week, six months) on attention in particular. Neither of these studies examined any possible neuroimaging correlates of the positive effect of DI.

Diffusion tensor imaging (DTI) is the most widely used model of diffusion MRI, which allows the evaluation of changes in the brain microstructure, especially in the white

matter (WM) [7]. Voss et al. [8] used conventional single-shell DTI and examined the effect of aerobic fitness training (three lessons/week, one year). The authors observed a relationship between increased aerobic fitness and increased fractional anisotropy (FA) in prefrontal, parietal, and temporal areas. On the other hand, they found no difference in cognitive performance between groups. The only study evaluating the effect of the DI (three lessons/week, six months) in healthy seniors with conventional DTI was performed by Burzynska et al. [9]. The authors used tract-based spatial statistics (TBSS) and selected regions of interest on the TBSS skeleton using the DTI WM atlas. They found that FA in the fornix increased in the DI group as compared to control groups. However, the change in fornix integrity did not correlate with the change in cognitive outcomes. In fact, there were no significant cognitive changes during DI surpassing changes in the control groups.

In our previous paper [10], we demonstrated subtle effects of an optimized, structured six-month dance intervention on executive functions in aged people without dementia, particularly in the Five-Point Test (FPT) which evaluates attention and executive functions [11]. In the current DTI substudy, we aimed to explore the neural correlates of the DI-induced changes in physical fitness and cognition using a multishell diffusion MRI protocol. The use of multiple shells improves the modeling of crossing fibers within each voxel [12]. Moreover, by quantifying the non-Gaussian diffusion, the multishell DTI provides more precise information about the microstructural properties of WM tissue heterogeneity [12, 13]. However, the changes in microstructural properties of the tissue derived from the multishell DTI might be caused by variety of factors, such as axonal density, axonal ordering, degree of myelination, accumulation of pathological proteins, brain atrophy, or microglial activation, and the method does not inform about distinct pathological underlying mechanisms of the observed DTI changes [14].

2. Materials and Methods

The cohort consisted of healthy senior volunteers and seniors with mild cognitive impairment (MCI), all potentially capable of participating in the intensive dance intervention. Healthy seniors were recruited using the public media such as local newspapers and radio and TV news. MCI participants were recruited from patients longitudinally followed at the First Department of Neurology, Faculty of Medicine, Masaryk University, Brno, Czech Republic. In brief, we included subjects aged over 60 years, nonsmokers with no alcohol and/or drug abuse, and patients without serious brain injury, dementia, or major depressive disorder. For detailed information about the enrolment and randomization process, see Kropacova et al. [10]. Briefly, 120 participants were randomized to a dance intervention (DI) group and a life as usual (LAU, control) group, 60 participants in each group, using the opaque envelope method.

All subjects underwent the neuropsychological, physical fitness, and MRI examination at the baseline and after 6 months. Informed consent, in accordance with the ethics committee of Masaryk University, was obtained from each subject. The study was approved by the local ethics committee.

2.1. Dance Intervention. The dance intervention was organized by specialists from the Faculty of Sports Studies, Masaryk University, Brno, Czech Republic. The intervention took six months and included three training units (each 60 minutes) per week. The whole study lasted for three years with the DI taking place between November and April each year in small groups of up to 20 subjects. The DI program was performed at a medium physical load intensity, and subjects were supervised by an experienced tutor. The load intensity was monitored by the Borg Rating of Perceived Exertion (RPE) scale during each supervised session. The RPE is a user-friendly numerical scale that evaluates an individual's self-reported level of effort, physical exertion, and fatigue during exercise using a 15-point scale ranging from 6 (no exertion) to 20 (maximum exertion) [15]. The physical load was adjusted to the current health condition and physical fitness levels of the individual seniors, and it was kept between 11 and14 points on the RPE. The DI units included folk, country, African, Greek, and tango dancing. The choreographies were divided into smaller blocks that were gradually taught in individual lessons and modified and developed over time into the final choreography. Only subjects who completed at least 60% of the DI program were included in the final cohort [10]. The real average completion of the DI program is 78.1%.

2.2. Physical Fitness Examination. The effect of the DI was evaluated by two tests from the functional fitness assessment [16]. The 8-Foot Up-and-Go Test evaluates the agility and dynamic balance. It measures time (in seconds) required to get up from a seated position, walk 8-foot distance, return to the chair, and sit down. The lower values indicate better performance. The 30-Second Chair Stand Test evaluates lower body strength and physical endurance by measuring the number of repetitions of full stands from a chair in 30 seconds. The higher values indicate better performance.

2.3. Neuropsychological Examination. Global cognition, five cognitive domains, and activities of daily living were evaluated by complex neuropsychological testing [10]. The examination included the MoCA score [17] and individual tests from the memory domain (Taylor Figure Test [18], Wechsler Memory Scale III: Logical Memory [19]), attention domain (Wechsler Adult Intelligence Scale III: Digit Span, symbol search [20]), executive domain (Five-Point Test [11], Tower of Hanoi [21]), visuospatial domain (Taylor Figure Test [18], Judgement of Line Orientation [22]), language domain (Mississippi Aphasia Screening Test [23]), and activities of daily living (Bristol Activities of Daily Living Scale [24]). The cognitive domain Z-scores were calculated as the average Z-scores of the tests included in the particular domain [25].

Participants were classified as having MCI if they scored below -1.5 SD in at least two tests in one or more cognitive domains [25]. More specifically, we used the following criteria: MoCA $\geq$ 26 points and the score below 1.5 SD in two tests in at least one cognitive domain, MoCA $<$ 26 points

and the score below 1.5 SD in any two tests, and objective memory deficit on the MoCA and the score below 1.5 SD in at least one test from the memory domain.

2.4. DTI-MRI Examination. All subjects were scanned using the 3T Siemens Prisma MR scanner (Siemens Corp., Erlangen, Germany) in CEITEC Masaryk University, Brno, Czech Republic, employing the following sequences: magnetization-prepared rapid gradient-echo (MPRAGE) high-resolution T1-weighted images (240 sagittal slices, slice thickness = 1 mm, TR = 2300 ms, TE = 2.34 ms, FA = 8°, FOV = 224 mm, and matrix size 224×224) and diffusion-weighted images (114 sagittal slices, slice thickness = 2 mm, TR = 9300 ms, TE = 97 ms, and FOV = 228 mm) and thirty noncollinear diffusion directions with b-values 500, 1000, and 2000 s/mm^2, ten T2-weighted acquisitions with b-value 0 s/mm^2, and three acquisitions with b-value 0 s/mm^2 with opposite polarity of phase encoding. FA and mean diffusivity (MD) were the parameters of interest.

2.5. Processing of the MRI Data. The structural connectivity of the WM was evaluated using the FSL software [26] and TBSS method [27]. Each subject's raw data was first corrected for susceptibility-induced distortions, eddy current distortions, and movements using the topup [28] and eddy [29] tools. Nonbrain voxels were excluded using the Brain Extraction Toolbox (BET) [30], and the brain extracted masks were checked one by one. Diffusion tensor at each voxel was modeled by DTIFIT function. Maps of FA and MD were calculated. The bedpostx tool was used for modeling with recommended settings [12, 31].

After preprocessing and quality control, the data underwent TBSS [27]. FA images of all subjects were nonlinearly registered to FMRIB58_FA_1mm target image and then affine transformed to the $1 \times 1 \times 1$ mm MNI152 standard space. The mean FA map was calculated, and the skeleton representing the centres of all tracts was created at the threshold 0.2. All individual FA maps were projected onto the skeleton. MD maps were processed using the information from the FA procedure. Individual maps were nonlinearly registered to the common space and projected onto the original mean FA skeleton. Mean values of DTI parameters were extracted from the final WM skeleton.

Paired differences within subjects were calculated, and a two-sample t-test design was set in a general linear model (GLM), as suggested by the FSL GLM User Guide. A randomization tool [32] with 5000 permutations was used to calculate the differences between the DI and control groups, controlled for the effect of gender, age, years of education, and the baseline MoCA score.

In addition to the whole-brain WM skeleton exploratory analysis, mean FA and MD were also computed for the corticospinal tract (CST) and the superior longitudinal fascicle (SLF) which are known to be related to motor planning and execution as well as spatial attention and speech comprehension [8, 9, 33, 34]. We used a bidirectional iterative parcellation (BIP) [35] which applies the FSL option of "probabilistic tracking with classification targets" in a bidirectional and iterative manner [35]. The method requires specific gray matter endpoint definition. Initial seed regions and inverse masks were downloaded from BIP's creator Bitbucket depository (https://bitbucket.org/dpat/). Gray matter endpoints for CST were defined as motor-sensory and brainstem. The motor-sensory endpoint mask was created using MARINA [36] by merging masks of the precentral gyrus, postcentral gyrus, and supplementary motor area. The brainstem endpoint originated in the Harvard-Oxford subcortical structural atlas and was truncated at $z = -20$ mm. The gray matter endpoints for SLF were derived from the AAL atlas [37], with the first endpoint being the angular gyrus and the second endpoint being the frontal middle gyrus. We chose specifically the angular gyrus since it is a crossmodal hub where converging multisensory information is combined and integrated to comprehend commands, manipulate mental representations, solve problems, and reorient attention to relevant information [38]. For the probabilistic tractography, the GPU version of Probtrackx was used [39]. The final tracts were thresholded at 5% probability, binarized, and masked with whole-brain WM segmentation. In order to be comparable with the methods and results of a study by Burzynska et al. [9], we additionally segmented the fornix using T1-weighted anatomical images and the FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu) [40] and the mri_cc function.

The longitudinal pipeline was used for preprocessing [41]. All segmentations were visually inspected, and 9 subjects (3 DI and 6 LAU) were excluded due to segmentation imprecisions. Binary masks of the fornix were created for each subject and registered to native space DWI b0 images using SPM (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). For segmentation of all tracts, see Figure 1.

2.6. Evaluation of Changes in Cognitive/Physical Fitness Measures of Interest and Changes in DTI Parameters. Equivalency in between-group baseline data was checked by chi-square tests and the Mann-Whitney test.

Mixed ANOVA and the following post-hoc tests were applied to examine DI-induced behavioral, cognitive, and DTI changes, both in the whole brain and in tracts of interest.

Paired t-tests were additionally used to test the DI-induced changes in the DI group only.

Partial correlations (Spearman correlation coefficient; MATLAB 2018) were calculated between changes in clinical measures of interest (i.e., those that revealed significant time * group effects) and changes in FA and MD parameters in the whole-brain WM and above-mentioned tracts of interest in the DI group.

3. Results

Altogether, 99 (49 in the DI group and 50 in the LAU group) completed successfully the DI/LAU period. The most common reason to withdraw from the study during the DI period was an unexpected health problem of the participant or his/her partner and problems with keeping up with the time schedule of the intervention. The final research sample with good-quality clinical, cognitive, and diffusion MRI data both at the baseline and at the follow-up examination after the DI/LAU consisted of 76 participants (51 HC and 25 MCI

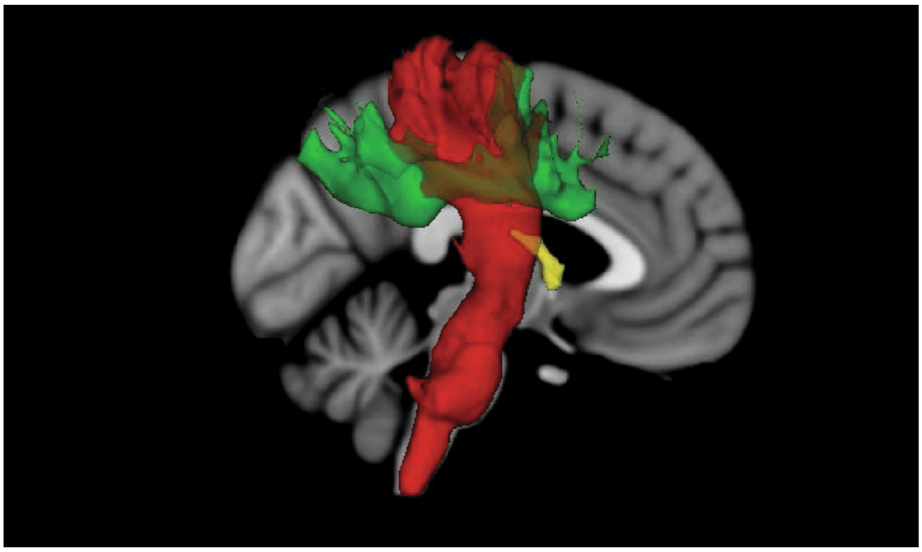

Figure 1: Visualisation of tracts of interest: red—corticospinal tract, green—superior longitudinal fasciculus, and yellow—fornix.

subjects). At the baseline, groups in the final cohort were not significantly different in age, years of education, and relative proportion of MCI participants. We found between-group differences at the baseline visit in gender and in the global cognitive MoCA score. Gender differences were due to the dropout rate, which was clearly gender-related in the DI group: fewer men than women completed the DI program. Therefore, we controlled all results for the effect of gender, age, years of education, and the baseline MoCA score. All cognitive, physical fitness, and DTI data at the baseline visit and at the follow-up visit after six months in the DI and control groups are depicted in Table 1. All cognitive, physical fitness, and DTI data at the baseline visit and at the follow-up visit after six months in both groups, divided into HC and MCI subgroups, are depicted in Supplementary Material Tables S1 and S2.

3.1. Behavioral Results. Mixed ANOVA revealed a significant time $*$ group effect (see Figure 2), in the 8-Foot Up-and-Go Test ($p = 0.006$) and in the 30-Second Chair Stand Test ($p = 0.021$). There was an effect in the attention domain ($p = 0.015$), but it did not survive the FDR correction for five cognitive domains. Complete results can be found in Supplementary Material Table S3.

Paired t-tests in the DI group revealed significant DI-induced changes (improvement) in the 30-Second Chair Stand Test ($p = 0.002$) and in the 8-Foot Up-and-Go Test ($p = 0.014$). The DI also led to improved attention ($p = 0.033$) and executive domain Z-score ($p = 0.007$) in the DI group, but the results did not survive the FDR correction for five cognitive domains. For complete results, see Supplementary Material Table S4.

3.2. DTI Results in the Whole-Brain WM and in the WM Tracts of Interest and Relation to Physical Fitness. TBSS showed no significant differences between the DI and control groups. As for the DTI measures in the subanalyses of the tracts of interest, no significant changes in FA and MD were observed between the two groups (for details, see Supplementary Material Table S3).

Partial correlation analysis showed a significant relationship between the performance in the 30-Second Chair Stand Test and global WM FA ($p = 0.016$, $R = 0.41$) in the DI group (see Figure 3).

Additional analyses in the tracts of interest in the DI group showed positive medium strength correlations between FA in the left SLF and performance in the 30-Second Chair Stand Test ($p = 0.006$, $R = 0.47$) and between MD and FA in the right CST and time to perform the 8-Foot Up-and-Go Test ($p = 0.006$, $R = 0.46$ and $p = 0.023$, $R = -0.39$, respectively). For complete results, see Supplementary Material Table S5.

4. Discussion

Our study focused on WM microstructure correlates of behavioral and cognitive effects of intensive six-month dance exercise training in mixed nondemented seniors including cognitively intact individual as well as a small proportion of MCI subjects.

We observed DI-induced effects in the physical fitness measures, namely, the 8-Foot Up-and-Go Test and 30-Second Chair Stand Test, in the DI group as compared to the control group. A better performance in these tests means improvements in the dynamic balance, agility, lower body

Table 1: (a) Descriptive characteristics. (b) Physical fitness, cognitive, and DTI data at the baseline. (c) Physical fitness, cognitive, and DTI data after 6 months. Mean ± standard deviation. *Significant difference.

	DI group	Control group	p value
(a) Descriptive characteristics			
N	37	39	—
Gender	32 F/5 M	26 F/13 M	0.042*
Age	69.3 ± 5.3	68.9 ± 6.3	0.607
Years of education	15.0 ± 2.2	15.0 ± 3.0	0.084
HC/MCI	28/9	23/16	0.121
(b) Baseline—physical fitness, cognitive, and DTI data			
8-Foot Up-and-Go Test (seconds)	5.1 ± 1.5	5.3 ± 1.4	0.890
30-Second Chair Stand Test (number of repetitions)	14.9 ± 4.0	16.8 ± 4.8	0.191
MoCA	27.4 ± 2.7	25.8 ± 2.7	0.004*
Memory (Z-score)	1.11 ± 1.01	0.93 ± 0.89	0.374
Attention (Z-score)	0.08 ± 0.58	0.02 ± 0.71	0.559
Executive (Z-score)	−0.35 ± 0.63	−0.30 ± 0.74	0.831
Visuospatial (Z-score)	0.29 ± 0.55	0.38 ± 0.56	0.417
Language (Z-score)	0.39 ± 0.46	0.40 ± 0.42	0.872
Global WM FA	0.43 ± 0.02	0.42 ± 0.02	0.979
Global WM MD (mm^2s^{-1})	0.0007 ± 0.00002	0.0007 ± 0.00005	0.230
(c) After 6 months—physical fitness, cognitive, and DTI data			
8-Foot Up-and-Go Test (seconds)	4.8 ± 1.2	5.6 ± 2.3	0.537
30-Second Chair Stand Test (number of repetitions)	16.4 ± 4.1	16.2 ± 6.3	1.000
MoCA	27.0 ± 2.7	26.6 ± 2.5	0.367
Memory (Z-score)	1.30 ± 0.77	1.15 ± 0.92	0.318
Attention (Z-score)	0.26 ± 0.71	−0.06 ± 0.75	0.046*
Executive (Z-score)	−0.02 ± 0.82	−0.22 ± 0.65	0.278
Visuospatial (Z-score)	0.30 ± 0.59	0.48 ± 0.55	0.166
Language (Z-score)	0.46 ± 0.43	0.42 ± 0.51	0.826
Global WM FA	0.43 ± 0.02	0.43 ± 0.02	0.751
Global WM MD (mm^2s^{-1})	0.0007 ± 0.00002	0.0007 ± 0.00005	0.238

strength, and physical endurance. These parameters are key factors in preserving mobility and independence in older age [16]. A positive effect of DI on physical fitness was consistently reported by others and was summarized in a review by Hwang and Braun [1]. On the other hand, the effects of DI on cognition are more heterogeneous and have remained inconsistent. Some authors reported a positive DI-induced effect on attention [5, 6, 42, 43] and memory [42, 43] functions, but positive changes were reported also in groups who underwent conventional fitness training [42, 43]. In this DTI substudy, we observed a DI-induced effect solely on the attention domain, but this result did not survive the FDR correction and therefore should be interpreted with caution. We did not observe any DI-induced effects between both groups in the microstructure in the WM skeleton or in tracts of interest.

Our major study aim was to identify whether WM microstructural changes, as assessed by FA and MD measures derived from a multishell diffusion protocol, may underlie dance-induced behavioral improvements. FA is a directionally dependent sensitive marker of microstructural changes (e.g., myelination, fiber orientation, and axonal diameter) [14]. Increase in FA can be explained by, e.g., higher packing density or increased directional organization of axons and/or stabilization or increase in myelin integrity [14, 44]. MD is a directionally independent measure that describes overall diffusion within the voxel [45]; increased MD is characteristic for regions where neural microstructures (e.g., axonal cell membranes, myelin sheaths, and neurofilaments) are displaced by intra- and extracellular water [46]. Studies showed that the increase in MD and decrease in FA are the common process in healthy aging [47]. The WM integrity also reflects age-related variability in cognitive outcomes in healthy aged individuals [48] such that increased FA/decreased MD relates to increased cognitive performance [46].

A positive relationship between improved physical fitness and increased WM integrity in this study was detected in the

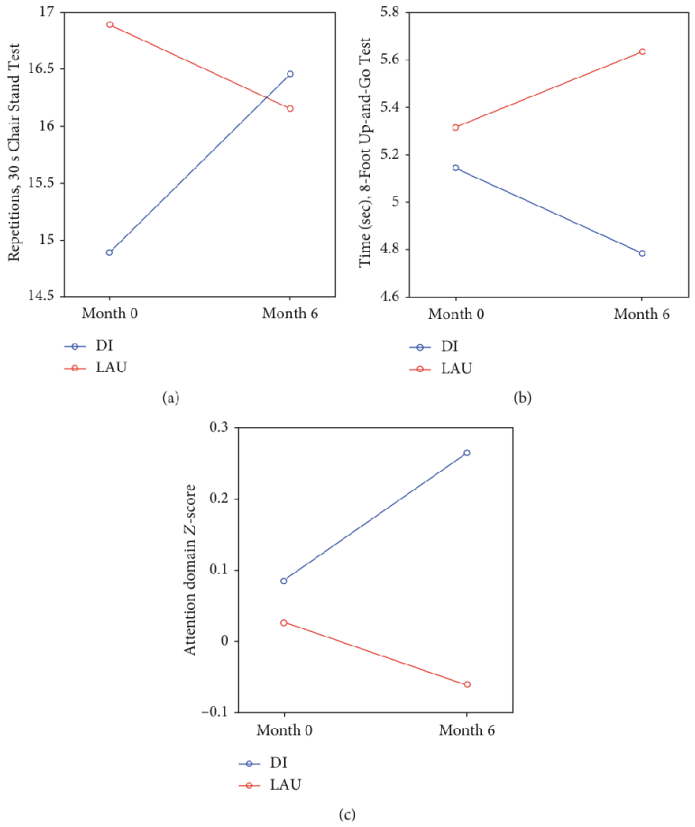

FIGURE 2: Mixed ANOVA results: significant time * group changes. DI: dance intervention; LAU: life as usual. (a) Number of repetitions, 30-Second Chair Stand Test (increase means improvement); (b) time, 8-Foot Up-and-Go Test (decrease means improvement); and (c) attention domain Z-score.

whole brain as well as in the WM tracts of interest related to motor learning and movement execution, as well as to spatial attention, manipulation of mental representations, and speech comprehension. The CST projects from the motor cortices to the spinal cord and plays a key role in the control of voluntary movement [34]. The SLF is considered to be the major cortical association fiber pathway. As for the particular results of correlation with increased physical endurance, the SLF plays a role in regulating motor learning and higher aspects of motor behavior [33, 34]. Based on the results of Burzynska et al. [9], we also focused on the fornix, the major efferent tract of the hippocampus with a key function of memory formation and consolidation [49], although engagement in motor functions in normal aging has also been described [50]. Unlike Burzynska et al. [9], we did not observe any relationship between changes in DTI measures within this tract and cognitive functions or physical fitness.

Our results were similar to those of Voss et al. [8] who did not find significant DTI changes on a group level even after a whole year of the aerobic intervention (walking performed three times per week). The authors observed that increased FA in the WM of prefrontal, parietal, and temporal areas (no tracts were specified in this work) was related to changes in cardiorespiratory fitness measures in the walking intervention group. However, significant associations were not supported by the TBSS analysis. No specific tracts were analysed.

Some novel methodological aspects of the current study should be highlighted: we used the multishell diffusion MRI protocol which is thought to surpass conventional single-shell DTI in terms of its ability to accurately evaluate microstructural properties with varied restrictions to diffusion [13]. In addition to TBSS, tracts of interest were delineated including the probabilistic tracking method applicable for evaluating long association/projection pathways [35]. However, despite

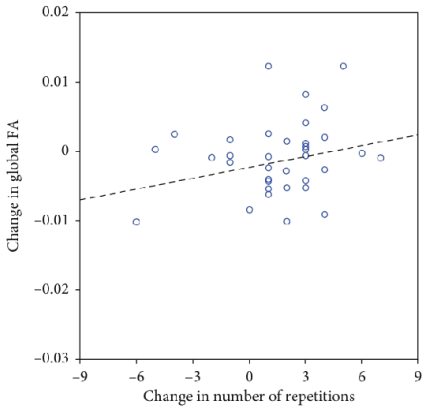

FIGURE 3: Relationship between change in FA and change in performance in the 30-Second Chair Stand Test.

using these methods, we were not able to identify changes in WM integrity due to the DI.

It would have been interesting to examine the HC and MCI subjects separately. However, this was not possible due to a small proportion of MCI subjects in DI and LAU cohorts.

5. Study Limitations

The distribution of demographic and cognitive characteristics and the number of MCI subjects in both groups were comparable in the whole cohort of 99 participants [10]. However, some subjects had to be discarded because of incomplete or low-quality diffusion MRI data. This led to rather disproportional distribution of MCI subjects in the groups of this DTI substudy although the number of MCI subjects in the DI and LAU groups was not significantly different.

The number of MCI patients in DI and LAU groups was too low to perform separate analysis for MCI.

The MoCA score was lower in the LAU group as compared to the DI group. Despite the fact that we controlled for the effect of the baseline MoCA score in our further analyses, we cannot fully exclude a possible effect of unequally distributed MCI participants in both groups. Another limitation of the study is a rather low number of applied diffusion directions for probabilistic tractography analysis.

6. Conclusion

In conclusion, we showed that 6 months of intensive DI can increase physical fitness measures evaluating lower body muscle endurance, agility, and balance in aged nondemented individuals and is associated with the enhancement in structural integrity, particularly in specific tracts that are engaged in motor behavior, regulation of motor learning, and coordination and control of voluntary movement. Future studies should focus on possible differences in the behavioral effects of DI and related DTI changes separately in the groups of heathy seniors and MCI patients.

Data Availability

Anonymised imaging data of this study will be available on reasonable request from any qualified researcher, following the EU General Data Protection Regulation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

We thank Anne Johnson for English language editing.

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Supplementary Materials

Table S1: DI group and HC and MCI subgroups; mean ± standard deviation. Table S2: LAU group and HC and MCI subgroups; mean ± standard deviation. Table S3: mixed ANOVA—behavioral, cognitive (FDR-corrected), and DTI (FDR-corrected) results. *Significant. WM: white matter; CST: corticospinal tract; SLF: superior longitudinal fasciculus; FA: fractional anisotropy; MD: mean diffusivity. Table S4: paired *t*-tests: DI-induced changes in the DI group—behavioral, cognitive (FDR-corrected), and DTI (FDR-corrected) results; mean ± standard deviation. *Significant. WM: white matter; CST: corticospinal tract; SLF: superior longitudinal fasciculus; FA: fractional anisotropy; MD: mean diffusivity. MD unit (10^{-3} mm^2 s^{-1}). Table S5: partial correlations (Spearman correlation coefficient; MATLAB 2018) between changes in clinical measures of interest (i.e., those that revealed significant time * group effects) and changes in FA and MD parameters in the WM in the DI group; *p* value/correlation coefficient. *Significant. WM: white matter; CST: corticospinal tract; SLF: superior longitudinal fasciculus; FA: fractional anisotropy; MD: mean diffusivity. *(Supplementary Materials)*

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19. Dance Intervention Impact on Brain Plasticity: A Randomized 6-Month fMRI Study in Non-expert Older Adults

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Commentary.

This randomized study provided a direct examination of functional brain plasticity induced by a sustained dance intervention. The study was performed to determine whether six months of structured dance training could produce measurable changes in brain rs-FC in older adults without professional dance experience. We hypothesized that the intervention would modify functional brain organization in parallel with behavioral or cognitive changes. The results showed improved attention and increased intra- and inter-network rs-FC, particularly involving the DMN, insulo-opercular, FPN, VN, language, and cerebellar networks, with some connectivity changes associated with attention and executive function. The findings provided evidence that sustained multimodal behavioral activity can influence functional brain organization in later life. The principal contribution was the integration of a randomized intervention design with fMRI, thereby strengthening the translational relevance of the findings. In relation to the preceding studies, this work combined the themes of cognitive intervention, network neuroscience, and neuroimaging biomarkers. It demonstrates that the same brain networks affected by neurodegeneration may retain substantial capacity for experience-dependent modification.

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BRIEF RESEARCH REPORT
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Dance Intervention Impact on Brain Plasticity: A Randomized 6-Month fMRI Study in Non-expert Older Adults

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Background: Dance is a complex activity combining physical exercise with cognitive, social, and artistic stimulation.

Objectives: We aimed to assess the effects of dance intervention (DI) on intra and inter-network resting-state functional connectivity (rs-FC) and its association to cognitive changes in a group of non-demented elderly participants.

Methods: Participants were randomly assigned into two groups: DI and life as usual (LAU). Six-month-long DI consisted of supervised 60 min lessons three times per week. Resting-state fMRI data were processed using independent component analysis to evaluate the intra and inter-network connectivity of large-scale brain networks. Interaction between group (DI, LAU) and visit (baseline, follow-up) was assessed using ANOVA, and DI-induced changes in rs-FC were correlated with cognitive outcomes.

Results: Data were analyzed in 68 participants (DI; $n = 36$ and LAU; $n = 32$). A significant behavioral effect was found in the attention domain, with Z scores increasing in the DI group and decreasing in the LAU group ($p = 0.017$). The DI as compared to LAU led to a significant rs-FC increase of the default mode network (DMN) and specific inter-network pairings, including insulo-opercular and right frontoparietal/frontoparietal control networks ($p = 0.019$ and $p = 0.023$), visual and language/DMN networks ($p = 0.012$ and $p = 0.015$), and cerebellar and visual/language networks ($p = 0.015$ and $p = 0.003$). The crosstalk of the insulo-opercular and right frontoparietal networks were associated with attention/executive domain Z-scores ($R = 0.401$, $p = 0.015$, and $R = 0.412$, $p = 0.012$).

Conclusion: The DI led to intervention-specific complex brain plasticity changes that were of cognitive relevance.

Keywords: dance intervention, resting state fMRI, independent component analysis, intra-network connectivity, attention, cognitive

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INTRODUCTION

Dance is a complex activity combining physical exercise with cognitive, social, and artistic stimulation (Burzynska et al., 2017). While the beneficial effect of dance intervention (DI) programs on physical fitness have been repeatedly proven, papers assessing the effects of DI on cognition have mixed results (Alpert et al., 2009; Coubard, 2011; Kim et al., 2011; Kattenstroth et al., 2013; Müller et al., 2017; Rehfeld et al., 2017, 2018; Brustio et al., 2018; Qi et al., 2018b). The effects of dance intervention on brain plasticity include various structural changes, such as an increase in gray matter volume (left hippocampus, left dentate gyrus, left precentral gyrus, etc.) as well as an increase in white matter integrity (fornix, corpus callosum), for a full review please see Teixeira-Machado et al. (2019). These findings are in concordance with our recent works, where 6-month-long DI as compared to life activities as usual (LAU) and resulted in significant improvement in a Five Point Test (FPT) which evaluated attention and executive functions (Kropacova et al., 2019), and in increases of cortical thickness of the right lateral occipitotemporal cortex (Rektorova et al., 2020) implicated in learning new skilled movements (Gatti et al., 2017). Physical fitness also significantly improved due to the 6-month DI, which was linked to changes in diffusion tensor imagining measures in the whole white matter skeleton and in the corticospinal tract and the superior longitudinal fascicle, which are engaged in motor learning and movement execution, as well as in spatial attention, manipulation of mental representations, and speech comprehension (Sejnoha Minsterova et al., 2020).

Brain connectivity changes following aerobic exercise of various types and intensities were studied by several authors. Increase of resting-state functional connectivity (rsFC) following aerobic exercise was often reported in the default mode network (DMN) and hippocampus and parahippocampal gyrus regions (Burdette et al., 2010; Rosano et al., 2010; Voss et al., 2010; Leavitt et al., 2014; Boraxbekk et al., 2016; Tozzi et al., 2016; Chirles et al., 2017; McGregor et al., 2018; Ikuta et al., 2019). A 6-month-long aerobic exercise intervention in MCI patients led to increased rsFC of the prefrontal cortex (Hugenschmidt et al., 2017), similarly, the aerobic exercise intervention of the same duration led to increased rsFC in the prefrontal cortex, as well as superior parietal gyrus/precuneus in older overweight adults compared to the control group (Prehn et al., 2019). Not only regular but also a single session of moderate aerobic exercise resulted in increased FC in the sensorimotor areas (pre/post central gyri, thalamus and secondary somatosensory area) of healthy young adults (Rajab et al., 2014). Similarly, a 30-min long exercise bout was reported to increase rsFC in the right fronto parietal network (rFPN), sensorimotor network (SMN), and right affect and reward and network (ARN) in male athletes (Schmitt et al., 2019). However, some studies reported limited or no effect of aerobic exercise on rsFC (Flodin et al., 2017; Cui et al., 2019).

Although dance, unlike simple aerobic activity, involves learning and attention, emotions, coordination and balance, acoustic stimulation and social interaction (Kshtriya et al., 2015) only one study to date (Qi et al., 2018b) used rs-fMRI to examine the effect of the intervention on FC. The results of this pilot 3-month-long aerobic dance intervention in older adults with MCI showed an increase in brain spontaneous activity (analysis of the amplitude of low-frequency fluctuations) in bilateral fronto temporal, hippocampal, entorhinal and anterior cingulate cortices, compared to baseline (Qi et al., 2018b).

Based on this promising preliminary finding, we decided to use fMRI and a data-driven independent componential analysis (ICA) approaches with a primary objective to assess the effect of DI on rsFC in a mixed group of healthy elderly with or without MCI but no dementia. We hypothesized that DI-induced task-specific changes in both within-network and between-network rsFC in the DI group compared to the LAU group. Our secondary objective was to evaluate the association between the DI-induced changes in rsFC and cognitive performance. Based on our previous results (Kropacova et al., 2019; Rektorova et al., 2020), we particularly focused on DI-induced changes in attention and executive function domains. We hypothesized that DI-induced brain plasticity changes will be linked with our cognitive outcomes of interest.

METHODS

Study Participants and Intervention

Volunteers older than 60 were recruited using several sources, such as free advertising in community centers (senior centers, libraries), University of the Third Age courses, and in public media such as local newspapers and leaflets, etc. The exclusion criteria included serious brain injury, major psychiatric disorder or central nervous system disease, other serious neurological, orthopedic, oncological or internal disease disabling participation in the dance-exercise intervention, as well as any contraindications of MRI scans as followed in clinical practice. The abuse of alcohol, other addictive substances and smoking were other exclusion criteria. Volunteers practizing sport activities more frequently or at a more demanding level than the dance exercise intervention were not included in the study.

Participants were randomly assigned into two groups in a 1:1 ratio: DI (dance intervention) and LAU (life as usual) using the opaque envelope method. The study sample was estimated based on pilot data analysis, when a mean change of 0.5 points was observed in the five-point test score (Kropacova et al., 2019). Considering the type 1 error probability α of 0.05 and expecting the test power β of 0.8, and also considering a dropout rate of 25%, the minimum number of participants in one group was calculated to be 42. It should be noted that in the presented paper, the five point test is not included in the analysis. Participants who were eventually included in the analysis were those who completed the study protocol (at least 66.6% attendance of dance-exercise intervention in DI group, 2 fMRI scans, 2 detailed neuropsychological examinations before and after the intervention and physical fitness assessment).

Participants included in the DI group followed a 6-month-long dance exercise course taking place three times a week, the duration of a single lesson was 60 min and it took place at the Faculty of Sports Studies, Masaryk University in Brno. The dance exercise training was prepared and led by professionals from the faculty. The intervention included various dance types, e.g., Irish country, African, and Greek, etc., which were combined into a final choreography and performed at medium physical load intensity three times per week (Kropacova et al., 2019; Rektorova et al., 2020). Participants in the LAU group led normal lives.

Neuropsychological Examination

The complex neuropsychological testing evaluated global cognitive functions (Montreal Cognitive Assessment—MOCA) and five domains: memory, attention, executive functions, visuospatial functions, and language. Subjects who scored below −1.5 SD in two tests in at least one cognitive domain compared to normative data were categorized as having MCI. Activities of daily living and depressive symptoms were also evaluated to exclude subjects with dementia or depression (Kropacova et al., 2019). For further details, see the **Supplementary Material**.

MRI Data Acquisition and Preprocessing

All subjects were scanned in 3T Siemens Prisma MR scanner (Siemens Corp., Erlangen, Germany) at the Central European Institute of Technology (CEITEC), Masaryk University in Brno, using the following sequences: magnetization-prepared rapid gradient-echo (MPRAGE) high-resolution T1-weighted images (240 sagittal slices, slice thickness = 1 mm, TR = 2,300 ms, TE = 2.34 ms, FA = 8°, FOV = 224 mm, matrix size 224 × 224) and gradient-echo echo-planar imaging sequence for resting state fMRI (200 images, 34 transversal slices, slice thickness = 3.5 mm, TR = 1,990 ms, TE = 35 ms, FA = 70°, FOV = 192 mm, matrix size 64 × 64).

The resting state fMRI data were preprocessed using the SPM 12 toolbox and Matlab 2014b. Preprocessing included realignment and unwarping, normalization into standard anatomical space (MNI) and spatial smoothing with 5 mm FWHM. The level of motion was thoroughly checked in terms of frame-wise displacement (FD) (Power et al., 2012). No FD was higher than 3 mm and scans that displayed FD > 0.75 mm were scrubbed (Power et al., 2012). No more than 2.5% of subject scans were removed. Moreover, the six movement regressors (obtained during realignment and unwarping), FD and extracted signals from white matter and cerebrospinal fluid were regressed out of the data in subsequent analysis. Assessment of functional connectivity changes using independent component analysis (ICA).

Regarding the rs-fMRI data analyses, we utilized the data-driven approach using independent component analysis (ICA) methods. The intra-network and inter-network connectivity (intra-NC and inter-NC) of major large-scale brain networks were also evaluated, see the details below.

The data from all subjects (n = 68, 36 DI and 32 LAU) and both sessions (a total of 136 fMRI datasets) were entered into single group spatial Independent Component Analysis (gsICA) (Calhoun et al., 2001) implemented in the GIFT toolbox running under MATLAB. At first, each dataset was further preprocessed to remove the mean per each voxel time series and reduced by Principal Component Analysis (PCA) with the number of components set to the maximum Minimum Description Length (MDL) estimate for all datasets (Li et al., 2007). Then, a second PCA reduction was applied to all PCA components from all datasets with the number of components set to median over MDL estimates. Finally, the Infomax algorithm and ICASSO framework (Himberg et al., 2004) was used to derive maximally spatially group-specific independent components and the GICA algorithm was used to render dataset-specific spatial maps and time-series (20 runs with random initialization, minimal cluster size 16, maximal cluster size 24). We visually inspected spatial maps of the resulting components and those that represent functional networks were used for ensuing intra-NC analysis and the respective back-reconstructed time-series were used to evaluate inter-NC connectivity.

Experimental Design and Statistical Analysis

The study was completed between September 2015 and June 2018 and was divided into three parts. In September and October, the participants underwent neuropsychological and physical assessment and fMRI data were acquired, the data acquisition took place at CEITEC, Masaryk University, and at Faculty of Sports Studies, Masaryk University in Brno.

The intervention itself took place from November to April. In May and June, the re-assessments were performed in both groups.

Two sample t-test and Fischer Exact tests were used to compare the demographic data and cognitive performance between the two groups before the intervention. The effect of the intervention on cognitive performance was tested using ANOVA with three factors ("subject," "group"—DI or LAU, and "visit"—before and after intervention), with age, sex and education as covariates.

Intra-Network Connectivity Analysis

For intra-network connectivity analysis, the groups at baseline were first compared using ANOVA, with a group (DI or LAU) as a factor. Then, the group differences between "before" and "after" intervention were compared using ANOVA, with three factors: group (DI or LAU), subject and visit (before, after), and an interaction group x visit. Both models included three nuisance covariates (age, gender and years of education). Both models were analyzed for each selected component and the analysis included only voxels significantly active within the given network. Altogether, 20% of voxels with the highest absolute value were included in the analysis. The significance level was set to $p < 0.05$ FWE corrected (cluster level, initial cut $p = 0.001$ uncorrected) corrected for multiple comparisons using Random Field Theory (Nichols and Hayasaka, 2003).

Inter-Network Connectivity Analysis

For inter-network connectivity analysis, subject-specific static functional connectivity between all pairs of selected ICA

components (correlation coefficient) was calculated based on the components' time series. The resulting correlation coefficients were transformed into Z-scores using Fisher Z-transform. Again, the groups at baseline were first compared using ANOVA with a single factor—group (DI or LAU). The group differences between "before" and "after" intervention were compared using ANOVA, with three factors: group (DI or LAU), subject and visit (before, after) and an interaction group x visit. The main effect of all three factors and interaction of group (DI or LAU) and "visit" (before, after) was modeled. For both models, three covariates were included (age, gender and years of education). Both models were calculated for each connection (altogether 36 connections, 9 components between each other). The significance level was set to $p < 0.05$ with Bonferroni corrections on two contrasts ($p < 0.025$, 2 × main effect of a factor "group" (DI or LAU) or 2 × interaction "group" X "visit").

All ANOVAs with three factors included the "subject" factor, which modeled an effect of repeated measures. In case of any significant group vs. visit results, we also performed a correlation between changes in the rs-FC and cognitive domain z-scores in the DI group. The connectivity changes were corrected for age, sex and education.

RESULTS

Participant Characteristics

Altogether, 147 subjects were recruited, 120 were included in the study after reviewing the inclusion and exclusion criteria. Eleven participants included in the DI group did not meet the criterion of the minimum attendance (66.6%), those whose attendance was lower than 10% were offered to be moved to the LAU group (6 participants). Eleven participants of the LAU group were lost to follow-up.

In the present study, we analyzed data from 68 subjects from the final cohort who had good quality fMRI data, 36 DI participants and 32 LAU participants. There were no differences in age, education and MOCA scores (two sample *t*-test, $p < 0.05$) and men/women ratio (Fischer Exact test, chi square = 1.24, $p = 0.265$) between the two groups. A significant difference between the groups was found for gender distribution (Fischer Exact test, chi square = 3.95, $p = 0.047$). Both DI and LAU were heterogeneous groups consisting of both healthy controls (HC) and subjects with mild cognitive impairment (MCI). There was no difference between the DI and LAU groups in healthy seniors (HS) and MCI ratio.

Cognitive Outcomes

The results of cognitive examinations are summarized in **Supplementary Table 1**. There were no differences in cognitive domain Z scores between the groups before the intervention (two sample *t*-test, $p < 0.05$) (see **Table 1**).

The interaction of factors "group" and "visit" was significant for the attention domain, with Z scores increasing in the DI group and decreasing in the LAU group ($F = 6.00$, $p = 0.017$) (see **Supplementary Figure 2**).

TABLE 1 | Baseline demographic and cognitive examination data.

	DI (*N* = 36)	LAU (*N* = 32)	*p*-value
Age (years)	69.2/5.47	69.0/6.08	0.878
Gender (M/F)	5/31	11/21	0.047
Control/MCI	27/9	20/12	0.265
Education (years)	14.8/2.31	15.0/3.02	0.697
MOCA	27.2/2.81	25.9/2.93	0.069
Memory (Z score)	1.12/1.03	1.12/0.64	0.997
Attention (Z score)	0.11/0.57	0.05/0.76	0.738
Executive (Z score)	−0.36/0.64	−0.32/0.65	0.795
Visuospatial (Z score)	0.30/0.56	0.40/0.53	0.727
Language (Z score)	0.39/0.47	0.40/0.45	0.938

Independent Componential Analysis Resting-State fMRI Results

Altogether 20 components were identified using the Independent Component Analysis. We visually inspected the spatial maps of the components and for further analysis of inter and intra-network connectivity, we selected those representing functional networks—cerebellum, DMN, visual network, right and left frontoparietal network, language network, salience (insulo-opercular) network, frontoparietal control network and sensory-motor network (see **Supplementary Figure 1**).

Dance Intervention-Induced Changes in Intra-Network Connectivity

There were no significant differences in intra-network connectivities between DI and LAU groups at baseline. We observed significant interaction "group" x "visit" for the DMN. Based on extracted average values, the connectivity increases in the DI group and decreases in the LAU group (ANOVA, $p = 0.048$ FWE). The rs-FC in the precuneus (MNI coordinate −9, −58, 25) increased in the DI group and it decreased in the LAU group ($p = 0.048$) (see **Supplementary Figure 3**).

Dance Intervention -Induced Changes in Inter-Network Connectivity

There were three significant differences in inter-NC between the DI and LAU groups before the intervention, in all three the connectivity was higher in the LAU group compared to the DI group (cerebellum –visual network—$p = 0.0088$, cerebellum—language—$p = 0.01$, language—sensory-motor network—$p = 0.0218$).

We found six significant results for the group vs. visit interaction. Based on the extracted average values, the inter-NC increased in the DI group (five results) and decreased in the LAU group (one result). The inter-NC between the networks decreased significantly more in LAU than in the DI group (see **Table 2** and **Figure 1**). Briefly, DI, when compared to LAU, induced increases in the crosstalk between the insulo-opercular and right frontoparietal/frontoparietal control networks ($p = 0.019$ and $p = 0.023$), visual and language/DMN networks ($p = 0.012$ and $p = 0.015$), and cerebellar and visual/language networks ($p = 0.015$ and $p = 0.003$).

TABLE 2 | The significant results in inter-NC connectivity between the pairs of networks.

Networks	*P*-value
Cerebellum vs. visual network	0.0147
Default mode network vs. visual network	0.0149
Cerebellum vs. language network	0.003
Visual vs. language network	0.0119
Right frontoparietal vs. salience network	0.0186
Salience network vs. frontoparietal control network	0.023

Association Between Resting-State Functional Connectivity Changes and Cognitive Outcomes of Interest

The DI-induced changes in inter-NC between the right frontoparietal network and salience network significantly correlated with changes in attention domain z-scores ($R = 0.402$, $p = 0.015$) and executive function domains ($R = 0.412$, $p = 0.012$) in the DI group (see **Figures 2A–C**).

DISCUSSION

The significant behavioral effect of DI was found in the attention domain, which is in concordance with previous research (Diamond, 2015; Xiong et al., 2021) and our other research, which was performed in the same final cohort with a larger sample size (Kropacova et al., 2019). The current cohort remains smaller due to discarding data from participants with motion artifacts during fMRI scanning.

Our main goal was to assess brain rsFC changes that reflect DI-induced ability to enhance the recruitment of relevant cognitive large-scale brain networks and/or communication between them. For this purpose, we analyzed rsFC changes within specific networks as well as those of individual network pairings. We observed group vs. visit interaction for the DMN intra-network connectivity which increased in the DI group and decreased in the LAU group. DMN is a main resting state cognitive network with high degrees of functional connectivity (Greicius et al., 2003). Reduced rsFC within DMN has been described in both normal and pathological aging (such as in MCI or Alzheimer's disease), with DMN having a strong correlation with cognitive processes (Agosta et al., 2012; Krajcovicova et al., 2014; Vidal-PiÃ±eiro et al., 2014; La et al., 2015; Qi et al., 2018a; Zonneveld et al., 2019). The positive effect of exercise, both short and long-term, on the plasticity of DMN, has already been described by several studies (Boraxbekk et al., 2016; Li et al., 2017; McGregor et al., 2018).

We found more significant results for the DI-induced rs-FC enhancement of the inter-network crosstalk than of the intra-network connectivity. This finding may relate to the fact that aging is associated with the network de-differentiation and enhanced inter-network pairing, which is one of the

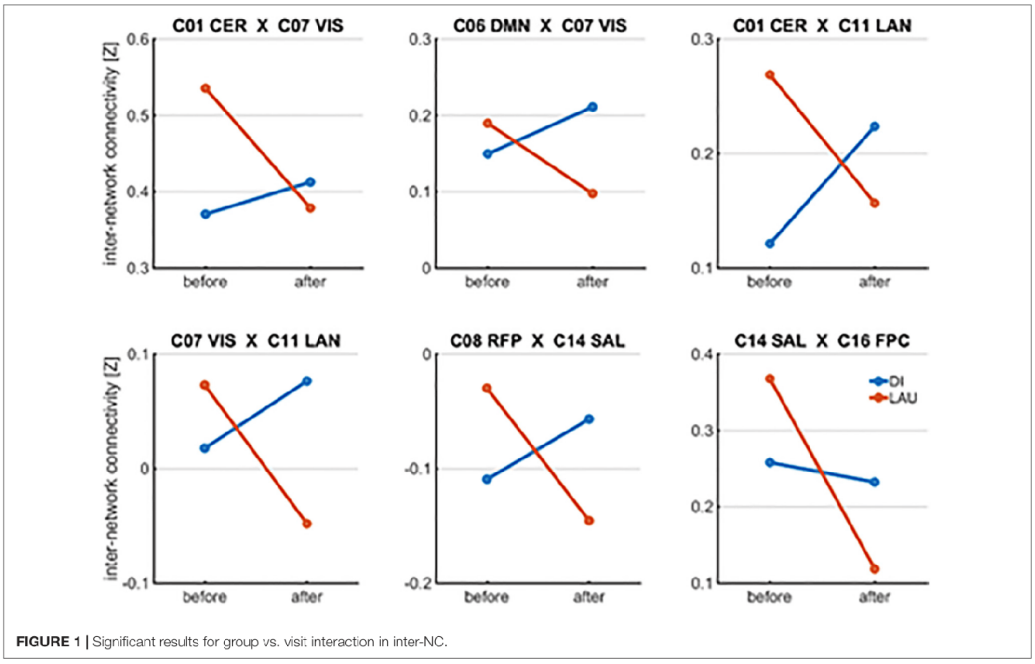

FIGURE 1 | Significant results for group vs. visit interaction in inter-NC.

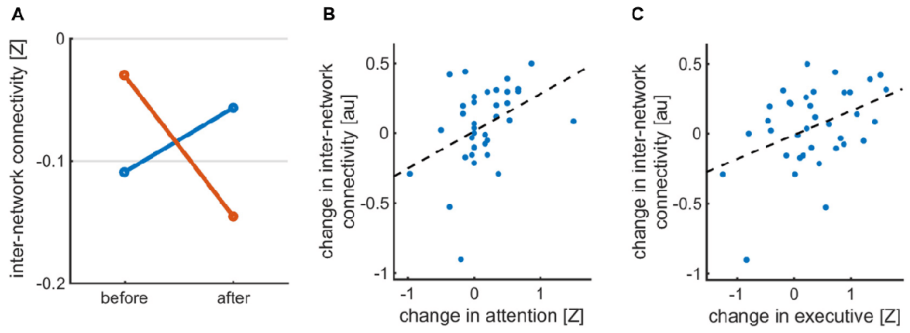

FIGURE 2 | (A) Inter-network connectivity between RFPN and SAL networks in DI and LAU groups before and after the intervention **(B)** correlation between inter-NC between FRP and SAL in DI group and z-scores in attention domain **(C)** correlation between inter-NC between FRP and SAL in DI group and z-scores in executive domain.

well-described neural compensatory mechanisms for decreased modularity and network segregation with age (Geerligs et al., 2015). While several significant results have been found for the group vs. visit interaction, only the inter-network rsFC increases between right frontoparietal network and salience (insulo-opecular) network in the DI group were associated with our cognitive outcome measures, i.e., with z-scores in attention and executive function domains. The salience network comprises anterior cingulate and ventral anterior insular cortices, bilateral Rolandic opercula, with nodes in the amygdala, thalamus, hypothalamus, ventral striatum and specific brainstem nuclei (Menon, 2015; Blefari et al., 2017; Seeley, 2019). The right FP network is located within the right frontal and parietal cortices. Both salience/fronto-opercular and right FP networks (the latter is also referred to as the ventral attention network (Farrant and Uddin, 2015) are engaged in the control of salient (i.e., behaviorally relevant) stimuli processing from internal (interoceptive awareness and bodily self-consciousness) as well as external (outside world) environment (Blefari et al., 2017; Seeley, 2019). They also play important roles in the control of working memory (Wallis et al., 2015). ACC is repeatedly associated with executive functions (Carter et al., 1999), the anterior insula is also functionally connected with frontal regions implicated in executive functions (Eckert et al., 2009). The finding that the DI enhanced this specific inter-network crosstalk related to attention to behaviorally relevant stimuli and to executive functions is novel and future studies should assess the long-term cognitive and behavioral sequelae of these changes.

We also identified increased rsFC between the cerebellum and visual/language networks, and between DMN and visual/language networks. Some of these results have to be taken with caution because of the significant differences in the inter-network connectivity strength between DI and LAU groups already at baseline. On the whole, the abovementioned networks are known to be engaged in movement coordination, and visual and speech processing (Solé et al., 2010; van den Heuvel and Hulshoff Pol, 2010; Manto et al., 2012), thus supporting the notion that DI is an enjoyable multimodal cognitive, movement and social activity that modulates brain plasticity in a specific behaviorally relevant manner.

Study Limitations

One of the study limitations of this study is that there was no active control for the DI group, who would perform, e.g., aerobic activity, such as jogging (Müller et al., 2017). Moreover, due to the low number of MCI subjects in both DI and LAU groups, we could not perform analyses for these groups separately.

In conclusion, dance is a multimodal activity that led to complex intervention-specific brain plasticity changes that were of cognitive relevance.

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 author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Masaryk University Ethical Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IR and ZB contributed to conception, design of the study, and wrote the first draft of the manuscript. ZB, LN, NN-E, SK, LB, RG, PV, and LS participated in data acquisition. RG, PV, and LS were responsible for DI program preparation and evaluation. RM performed statistical analysis of data. LN, RM, and IR wrote

sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2021.724064/full#supplementary-material

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20. Digital Speech Biomarkers for Assessing Cognitive Decline Across Neurodegenerative Conditions

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Commentary.

This publication represents a recent expansion of our research toward digital and clinically scalable biomarkers. Speech is a complex behavior that integrates language, executive function, memory, motor control, and other cognitive processes, making it a promising source of information about neurodegenerative decline. The study was performed to investigate whether quantitative digital speech measures can support the assessment of cognitive impairment across different neurodegenerative conditions (MCI in AD/PD and MCI with Lewy bodies). Speech biomarkers showed distinct linguistic and acoustic profiles across the studied groups, were associated with structural brain changes, and improved machine-learning classification and prediction of cognitive status beyond clinical measures alone. The findings supported the potential utility of digital speech analysis as an accessible and sensitive approach to disease assessment. The novel contribution lies in extending the biomarker framework from MRI-based measures of brain structure and function to scalable behavioral data that may be collected more easily in clinical and remote settings. In relation to my previous work on language networks and sentence comprehension, this study represents a logical translational extension from laboratory-based fMRI paradigms to digital assessment of language and cognition. Its potential impact lies in multimodal biomarker development, combining speech, cognition, neuroimaging, and clinical information to support earlier and more individualized detection of neurodegenerative disease.

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Digital speech biomarkers for assessing cognitive decline across neurodegenerative conditions

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ABSTRACT

This study investigates speech impairments in individuals with mild cognitive impairment due to Alzheimer's disease (MCI-AD), mild cognitive impairment with Lewy bodies (MCI-LB), and Parkinson's disease with mild cognitive impairment (PD-MCI), compared to healthy controls (HC), aiming to identify linguistic and acoustic digital biomarkers that differentiate these groups. Monologue recordings were collected from 68 HC, 42 MCI-AD, 50 MCI-LB, and 47 PD-MCI participants (ON state). Participants were instructed to speak spontaneously for one and a half minutes. Speech was automatically transcribed, manually corrected, and analyzed using natural language processing to extract eight linguistic (lexical/syntactic) and four acoustic (prosodic) biomarkers. Group differences were assessed using the Mann–Whitney U test, with Spearman's correlation used to examine associations with clinical and MRI measures (FDR-corrected). Machine learning models (XGBoost) were applied to evaluate the classificatory and predictive potential of speech features. Distinct speech patterns were observed across groups: MCI-AD participants exhibited reduced use of function words, resulting in increased content density, PD-MCI participants used shorter sentences and fewer coordinating conjunctions with longer pauses, and MCI-LB participants exhibited greater lexical repetition than MCI-AD. Altered speech features correlated with structural brain changes but not with global cognition (MoCA) or depressive symptoms (GDS). Sentence structure and pausing features showed strong interrelationships. Machine learning models showed that adding speech biomarkers improved classification performance compared to using clinical scores alone. In regression analyses, the models predicted MoCA with a normalized error of 10%, performing similarly on automatic and manually corrected transcripts. These findings suggest that speech biomarkers and traditional clinical assessments may offer complementary information about cognitive status and brain health, supporting their use in scalable, non-invasive cognitive monitoring.

1. Introduction

Neurodegenerative diseases pose an escalating threat to global health, demanding enhanced diagnostic and therapeutic strategies [1]. Conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD) are becoming increasingly prevalent, primarily due to the aging global population and the consequent rise in life expectancy [1]. The Global Burden of Disease (GBD) 2021 studies underscore the significant impact of these conditions, with dementia, predominantly AD, emerging as a leading cause of disability and dependence among older adults [1]. Similarly, PD's prevalence is on the rise, contributing substantially to the global burden of disease and presenting considerable health, social, and economic challenges [1]. This growing burden necessitates the development of more refined diagnostic tools and effective interventions to manage these debilitating conditions. Furthermore, the symptomatic and mechanistic overlap observed across various neurodegenerative diseases complicates the diagnostic process, highlighting the need for more precise methods to differentiate between them [2,3].

Mild cognitive impairment (MCI) is recognized as a transitional phase between the cognitive changes associated with normal aging

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and the more severe decline indicative of dementia [3,4]. This stage is characterized by objective cognitive decline that is noticeable but does not yet significantly impair an individual's ability to perform daily activities [3]. MCI is not merely an age-related phenomenon but rather a significant risk factor for the development of dementia, including AD and PD dementia (PDD) [2,5]. Studies indicate that a substantial proportion of individuals with newly diagnosed PD exhibit MCI, and this prevalence increases over the course of the disease [2]. MCI with Lewy Bodies (MCI-LB), MCI due to AD (MCI-AD), and PD-related MCI (PD-MCI) are distinct clinical forms of MCI, each with overlapping but specific features. MCI-LB was formally defined in 2020, establishing standardized diagnostic criteria [6]. Its core clinical features include cognitive fluctuations, recurrent visual hallucinations, REM sleep behavior disorder, and parkinsonism [3,6]. Neuropsychological assessments often reveal prominent visuospatial and executive dysfunction, and language impairments such as reduced verbal fluency, pragmatic discourse difficulties, and word-finding problems are commonly secondary to these deficits [7,8]. MCI-AD, commonly considered a prodromal stage of AD, is primarily characterized by episodic memory impairment [9,10]. However, emerging research indicates that language difficulties, particularly at the lexical-semantic level, are also prevalent. Individuals with MCI-AD may experience impairments in verbal fluency, confrontation naming, and the processing of complex syntactic structures such as anaphora, reflecting challenges in integrating form and meaning beyond the sentence level [10]. Although PD is primarily recognized as a motor disorder, cognitive impairments, including PD-MCI and dementia, occur frequently [2,11]. Linguistic tasks are commonly affected in PD-MCI, with difficulties observed in verbal fluency, word retrieval, morpho-syntactic processing, and the comprehension of complex syntax [11–14]. Additionally, a high proportion of PD patients experience hypokinetic dysarthria, affecting respiration, phonation, articulation, and prosody [15–17]. While language deficits are present across MCI-LB, MCI-AD, and PD-MCI, their manifestations may differ depending on the underlying cognitive and neuropsychological profiles, affecting syntactic complexity, lexical retrieval, discourse organization, and speech production [9,11,14,18–20]. Depressive symptoms are clinically relevant across the MCI spectrum and differ by subtype, with higher prevalence reported in MCI-LB than MCI-AD and frequent reports in PD-MCI compared with MCI-AD [9,21,22]. Greater severity has also been described in MCI-LB relative to PD-MCI [23]. Affective status can influence connected speech, including prosody, tempo, fluency, and lexical choice, and may therefore interact with language measures in these populations.

Recent advances in Natural Language Processing (NLP) have enabled non-invasive, cost-effective, and scalable tools to assess cognitive functioning in individuals with neurodegenerative diseases. Digital linguistic biomarkers, quantifiable features extracted from spontaneous or written language, capture diverse dimensions of language production including syntactic complexity, lexical diversity, and semantic coherence. For example, features such as type-token ratios, syntactic subordination, and referential cohesion have been linked to early signs of cognitive decline [24,25]. These features are often extracted via NLP pipelines utilizing part-of-speech tagging and dependency parsing to enable objective, reproducible analyses across large populations [26,27]. The utility of these methods in detecting MCI subtypes has been confirmed by recent studies. Kim et al. demonstrated that written discourse elicited via picture description tasks can distinguish between MCI-AD and non-amnestic MCI with up to 90% accuracy using a BERT-based classifier, surpassing traditional neuropsychological tests in diagnostic sensitivity [28]. Their findings highlight the clinical value of linguistic patterns, including pronoun frequency and subordinating conjunction usage, in subtype differentiation. Similarly, Lust et al. found that individuals with MCI-AD exhibit selective impairments in complex anaphoric constructions, indicating disruptions at the syntax-semantics interface [29]. In parallel, studies on PD-MCI consistently show impairments in morphosyntactic and lexical-semantic domains. For instance, Aresta et al. (2025) found that features like action verb ratios, sentence structure variability, and utterance-level errors reliably distinguish PD-MCI from both healthy controls (HC) and PD-nonMCI, linking these patterns to frontostriatal and executive dysfunction [30]. Similarly, García et al. (2022) demonstrated that automated analysis of action-related language in naturalistic storytelling tasks can differentiate PD-MCI from HC and PD-nonMCI using a novel Proximity-to-Reference-Semantic-Field metric, which outperformed general-purpose language models and provided a scalable, theory-driven tool for cognitive phenotyping in PD [31]. Several additional studies further emphasize the diagnostic and predictive value of speech-based linguistic features. Orimaye et al. showed that machine learning models trained on syntactic and lexical features from spontaneous speech could accurately distinguish probable AD from HC, achieving strong classification performance and identifying meaningful linguistic deficits [32]. Similarly, Beltrami et al. demonstrated that multidimensional linguistic analysis, encompassing acoustic, syntactic, and lexical features, could reliably differentiate healthy individuals from those with MCI and early dementia, supporting the value of spontaneous speech as a sensitive marker of preclinical decline [33]. In the context of PD, Smith et al. identified linguistic markers of word-finding difficulty that aligned with neuropsychological scores, particularly in participants with MCI-AD and multiple-domain MCI, further validating the clinical relevance of these features [34]. Subert et al. found that individuals with isolated REM sleep behavior disorder exhibited reduced content density and lexical diversity, especially in MCI-positive subgroups. Their results suggest that these markers may help predict progression to PD or Lewy body dementia DLB [35]. Further advancing this field, Subert et al. recently demonstrated that dopaminergic therapy in de-novo PD patients improves syntactic language features, such as sentence length and sentence development, over a 12-month period, with no changes observed in untreated patients or healthy controls. Improvements were strongest in those treated with dopamine agonists rather than levodopa. These findings highlight the role of dopamine in cognitive-linguistic processing and support the use of automated speech analysis to track language changes over time [36]. Importantly, the integration of NLP-derived features with machine learning models enables objective and scalable assessment of cognitive-linguistic impairments. Yeung et al. demonstrated that linguistic and acoustic variables extracted from speech significantly correlate with clinician-rated markers of language pathology in individuals with MCI and Alzheimer's dementia, offering a promising complement to traditional assessments [37]. While their study was cross-sectional, it supports the use of automated analysis for consistent evaluation of speech-language characteristics. Furthermore, the application of NLP to spontaneous language tasks, such as picture description or narrative recall, facilitates deployment in remote and telemedicine settings, expanding their potential role in routine cognitive screening [38,39]. Taken together, these studies affirm the role of NLP-driven linguistic analysis as a powerful complement to traditional cognitive assessment. By enabling fine-grained and objective measurement of language disturbances, digital linguistic biomarkers may improve our ability to detect and differentiate MCI subtypes in early stages, paving the way for more targeted interventions and better clinical outcomes.

Connected speech engages lexical–syntactic organization together with temporal–prosodic control, so linguistic markers and acoustic timing measures may offer complementary views on the cognitive and motor systems involved across MCI-AD, MCI-LB, and PD-MCI. When both domains are of interest, a brief spontaneous monologue is well suited. In contrast to picture description or story retell, where visual search or memory demands can inflate pauses and constrain sentence planning, an unconstrained monologue minimizes external task demands so that pausing and tempo more directly reflect internal planning and speech motor control while still permitting naturalistic discourse and syntax. This choice is also compatible with low-burden recording suitable for remote monitoring.

Table 1
Distribution of participants by gender across diagnostic groups.

	HC	MCI-AD	MCI-LB	PD-MCI	Total
Women	38	23	28	22	111
Men	30	19	22	25	96
Total	68	42	50	47	207

Building on this rationale, our primary objective is to characterize subtype-specific linguistic and acoustic biomarkers in MCI-AD, MCI-LB, and PD-MCI using spontaneous monologue speech. Secondary objectives are to: (1) test whether altered speech features associate with structural magnetic resonance imaging (MRI) within each subtype, (2) examine whether these features relate to clinical scores that index cognition and depressive symptoms after adjustment for confounders, (3) evaluate whether speech features improve clinical group classification beyond routine clinical scales, and (4) assess robustness across automatic and manually corrected transcripts.

Relative to prior work that commonly targets a single condition and elicits English language output in picture description or narrative tasks, with limited integration of MRI, this study analyzes three MCI syndromes within one center and protocol using spontaneous Czech monologue, integrates digital linguistic and acoustic analysis with structural MRI to relate candidate markers to neuroanatomy, evaluates how speech information may complement routine clinical scales that index cognition and depressive symptoms, and addresses deployability by comparing an automatic transcription route with a manually corrected route while releasing a language-specific, reproducible extraction pipeline. This design responds to reviewed needs for standardized connected-speech methods and broader language coverage.

2. Materials and methods

2.1. Database

The study cohort comprised 207 participants: 68 healthy controls (HC), 42 individuals with MCI-AD, 50 with MCI-LB, and 47 with PD-MCI. The sample included 111 women and 96 men, with the gender distribution across diagnostic groups summarized in Table 1. The cohort was racially and ethnically homogeneous; all participants were native Czech speakers of white ethnicity. Recruitment was via social media and local newspaper advertisements, followed by a brief telephone pre-screening with a standardized questionnaire to identify risk of prodromal DLB and verify basic eligibility. The assessment took place at the Central European Institute of Technology, Masaryk University, Brno, Czech Republic, between 2017 and 2024 under a standardized speech recording protocol. Screening and enrollment used the same exclusion criteria: comprised alcohol or drug abuse, the presence of ferromagnetic metals in the body, left-handedness, inability to communicate in the Czech language, and a history of psychiatric disorders. Individuals with PD-MCI were examined in the ON state. Group differences in age, medication, and education are presented in Fig. 1. Statistical comparisons were performed using the Mann–Whitney U test with p-values corrected for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) procedure. All diagnostic groups were defined on clinical grounds at a single center. Participants were assigned to clinical groups based on established diagnostic criteria [40–42] without pathological confirmation or blood-based biomarkers.

2.2. Clinical assessment

We collected a comprehensive set of neuropsychological and clinical measures from all participants. These were transferred to standardized z-scores across key cognitive domains: memory, visuo-spatial ability, attention, and executive function. These scores served as a central tool in quantifying cognitive performance and contributed to classifying participants into clinically defined groups. Global cognitive functioning was assessed using the Montreal Cognitive Assessment (MoCA) [43], a widely used screening tool for detecting MCI and early dementia. To evaluate depressive symptoms, we administered the Geriatric Depression Scale (GDS) [44], which is particularly suited for older adult populations. Participants were assigned to clinical subgroups based on established diagnostic criteria. MCI subjects had subjective cognitive complaints and scored with at least two neuropsychological tests with a cutoff score under -1 standard deviation (SD) below the age-appropriate norms or with one test with a cutoff score under -1 SD below the age-appropriate norms plus a MoCA cutoff score of 26. MCI-AD subjects have to have at least one of impaired test in memory domain. PD-MCI patients had MCI and clinically established PD, were longitudinally followed by a neurologist, and were on a stable dopaminergic medication (mean Levodopa Equivalent Dose = 930 ± 459 mg/day) at least 4 weeks prior to the baseline assessment and during the whole study and were tested in the ON medication state without dyskinesias. MCI-LB subjects were diagnosed with MCI and had at least two core clinical features of MCI-LB (fluctuating cognition, visual hallucinations, or REM sleep behavior disorder, and one or more spontaneous cardinal features of parkinsonism).

2.3. Structural MRI data

High-resolution T1-weighted MRI scans were analyzed using FreeSurfer 7.1.[1] Data was first processed with the recon-all pipeline, resulting in segmented images and cortical thickness maps. Upon completing each step, automatic subcortical segmentation labels [45], gray and white matter boundaries were visually inspected for each subject. Volumes of segmented subcortical structures (i.e. volumes of bilateral hippocampus, amygdala, basal ganglia and thalamus) and volumes of segmented cortical regions defined by Desikan–Killiany atlas [46] were calculated. These volumes were divided by estimated total intracranial volume (eTIV) to yield relative volumes of subcortical structures for every subject. Moreover, cortical thickness values were extracted for all Desikan–Killiany [46] cortical regions. Together, these measures provided a detailed anatomical profile of both cortical and subcortical brain regions.

2.4. Digital speech biomarkers

All speech recordings were collected in Czech language and were obtained in a sound-controlled environment to ensure high audio fidelity. Participants were seated in a quiet room and recorded using the same microphone and setup across sessions. The examiner delivered a standardized prompt: "*Please speak continuously for approximately one and a half minutes about anything you like, for example your day, a recent activity, your hobbies, or your family. Please do not read from notes or recite a memorized text*". The examiner started a timer, remained silent during the recording, and provided no follow-up questions or topic suggestions. Speech was stopped at 90 s or at the end of the current utterance if it slightly exceeded the time. Participants were free to choose any topic. Across groups, topics were typically everyday and low in cognitive demand, such as hobbies, family matters, and descriptions of daily routines. Retakes were not permitted. All participants completed the spontaneous monologue as instructed; no recordings were terminated early. To standardize recording conditions across sessions, a studio-grade condenser microphone was mounted on a fixed stand at approximately 30 cm from the participant's mouth. Prior to subsequent speech processing, all recordings were resampled to a standardized sampling frequency of 16 kHz with a bit depth of 16 bits to ensure compatibility with downstream analytical tools and maintain consistency across the dataset.

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

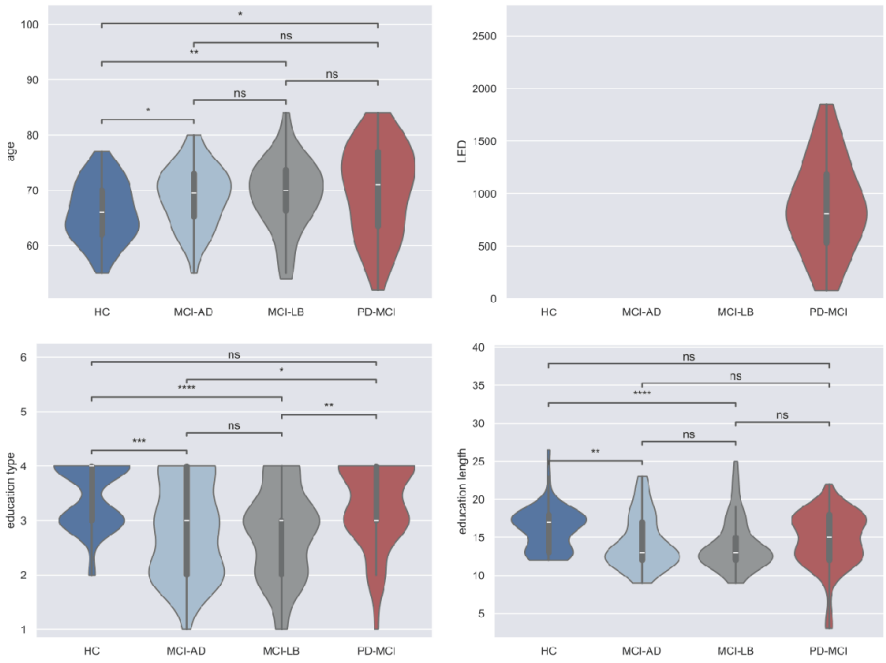

Fig. 1. Demographic and treatment data (*p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant); LED – daily levodopa equivalent dose in mg; educational type: 1 – primary education, 2 – secondary school, 3 – secondary school with diploma, 4 – university degree.

2.4.1. Linguistic biomarkers extraction

Linguistic biomarkers were derived through a multi-step pipeline to ensure accurate and language-specific text analysis. First, speech recordings were transcribed into text using Whisper, an open-source automatic speech recognition (ASR) system developed by OpenAI [47]. We employed the "large-v2" model with the Czech language setting, which provides robust transcription capabilities based on a transformer architecture trained on diverse speech data collection. A certified speech-language therapist subsequently reviewed and corrected all automatically generated transcripts to improve transcription accuracy and enable comparative analyses. For linguistic analysis, we utilized Stanza [48], a Python-based NLP toolkit developed by the Stanford NLP Group, which supports a wide range of human languages. Specifically, we applied part-of-speech (POS) tagging using the Czech-PDT Universal Dependencies (UD) treebank, a resource grounded in the Prague Dependency Treebank – Consolidated 1.0 (PDT-C) [49], developed at Charles University in Prague. We extracted a set of linguistic biomarkers from the resulting POS-tagged texts, detailed in Table 2, capturing lexical and syntactic characteristics relevant to cognitive and neurodegenerative conditions. The Python code for biomarker extraction is publicly available in the newly established GitHub repository.[2]

2.4.2. Acoustic biomarkers extraction

In addition to the primary set of linguistic biomarkers, we extracted several digital acoustic biomarkers to capture prosodic features that may further reflect speech disturbances. Given that linguistic and acoustic characteristics often interact in spontaneous speech, these acoustic measures serve as a complementary layer of analysis. Specifically, we focused on disprosodic features such as reduced pitch and loudness variations and altered pausing patterns (see Table 3). Analyses were performed with the utilization of Praat, a widely used tool for phonetic and acoustic analyses [50].

Table 2
Linguistic biomarkers and their classifications.

Biomarker	Category	Description
NW	Syntactic	Mean number of words per sentence.
NS	Syntactic	Number of sentences in text relative to the total number of words.
PSR-CCONJ	Syntactic	Number of coordinating conjunctions (e.g., and, but, or) relative to the total number of words.
PSR-SCONJ	Syntactic	Number of subordinating conjunctions (e.g., because, although, while) relative to the total number of words.
SC	Syntactic	Syntactic complexity calculated as (2*SCONJ + 2*WH-PRON + NOUNS + VERBS)/total number of words.
CD	Lexical	Content density. Ratio of open-class words, also known as content words (e.g., nouns, verbs, adjectives, adverbs), to closed-class words, also known as function words (e.g., pronouns, prepositions, conjunctions, determiners).
NG	Lexical	The sum of repeated n-grams relative to the total number of words in the text. In this study, we consider 2-grams (bigrams), 3-grams (trigrams), and 4-grams.
MATTR	Lexical	Moving-average type-token ratio. Number of unique words (types) divided by the total number of words (tokens) within each sliding window of 58 words.

CCONJ – coordinating conjunctions; SCONJ – subordinating conjunctions; WH-PRON – who, whose, whom, or which.

[2] https://github.com/BDALab/linguistic-digital-biomarkers-lexicon-toolkit.

Table 3
Acoustic biomarkers quantifying disprosody.

Biomarker	Description
DurMED	Median duration of silences longer than 250 ms.
DurMAD	Median absolute deviation of silence duration (silences longer than 250 ms).
relF0SD	Pitch variation defined as a standard deviation of F0 contour relative to its mean.
relSEOSD	Speech loudness variation, defined as a standard deviation of intensity contour relative to its mean after removing silences exceeding 50 ms.

2.5. Confounding factors

To assess and account for potential confounding effects, we evaluated the influence of age, gender, education type, and years of education on all measured variables. Spearman's rank correlation tests were conducted across the entire cohort (including HC, MCI-AD, MCI-LB, and PD-MCI), and the resulting p-values were corrected for multiple comparisons using the FDR technique. The analysis revealed that all four variables significantly influenced clinical scores (MoCA, GDS, and cognitive domain z-scores), as well as acoustic and linguistic features. In contrast, only age and gender were found to affect the neuroimaging biomarkers significantly. Additionally, within the PD-MCI group, no significant effect of medication status was observed on any analyzed features. Multiple linear regression models were trained on the full dataset to correct for these confounding effects and used to adjust the clinical, acoustic, linguistic, and neuroimaging data before further analyses [51].

2.6. Statistical analysis

Statistical analyses were conducted in two stages:

1. Group differences were assessed using the Mann–Whitney U test for cognitive domain z-scores, MoCA, GDS, and linguistic and acoustic biomarkers. Pairwise comparisons were performed between each clinical group and the HC group, as well as among the clinical groups themselves.
2. We then focused on the linguistic and acoustic biomarkers. When a statistically significant difference was observed between any two groups, we performed Spearman's rank correlation analyses within the group that showed worsening in the respective biomarker compared to HC. These correlations were used to examine associations between the affected speech features and all available clinical measures.

Additionally, we computed pairwise Spearman correlations among all linguistic and acoustic biomarkers to explore interrelationships within the speech-derived features. Obtained p-values were corrected for multiple comparisons using the FDR method.

2.7. Machine learning

We utilized the computed digital speech biomarkers in the machine learning modeling to investigate their potential applicability in decision-support systems. Specifically, the aim was to evaluate whether these features could support both the prediction of clinical scores through regression and the classification of clinical groups. Two transcription scenarios were considered to reflect real-world variability and assess model robustness: one using ASR outputs and the other based on manually corrected transcripts. We employed the Extreme Gradient Boosting algorithm (XGBoost)[3] for both regression and classification tasks. Hyperparameter tuning was conducted using Bayesian optimization via the Optuna framework,[4] which builds a surrogate model of the objective function and efficiently explores the hyperparameter space. A total of 500 optimization trials were performed for each model to ensure robust tuning. This approach is particularly effective for high-dimensional search problems and is more sample-efficient than traditional grid or random search methods. To ensure reproducibility, we report the exact XGBoost hyperparamcters tuned with Optuna and the corresponding search space in Table 4. All other XGBoost parameters were left at their library defaults. The optimization objectives were to minimize the mean absolute error (MAE) for regression tasks and to maximize the area under the receiver operating characteristic curve (AUC) for classification tasks. Leave-One-Out Cross-Validation (LOOCV) was used during both hyperparameter tuning and final model evaluation to maximize the use of available data. The final performance of the regression models was reported using an estimation error rate (EER), which is defined as the MAE divided by the range of the corresponding clinical score in the training set. Classification models were evaluated based on AUC.

3. Results

In the fixed 90 s monologue, the mean percentage of speech time within the full recording was 42.36% in HC, 43.53% in MCI-AD, 47.24% in MCI-LB, and 46.13% in PD-MCI; the remaining portion corresponds to silence and pauses. The mean total number of words was 180.56 in HC, 156.36 in MCI-AD, 170.88 in MCI-LB, and 172.47 in PD-MCI. The results of the Mann–Whitney U test for clinical scores are shown in Fig. 2. Significant differences in speech biomarkers between groups are illustrated in Fig. 3. Individuals with MCI-AD exhibited higher content density (CD) compared to HC. The MCI-LB group showed higher n-gram repetition (NG) relative to the MCI-AD group. Participants with PD-MCI demonstrated a lower mean number of words per sentence (NW) and proportion of coordinating conjunctions (PSR-CCONJ), alongside a higher number of sentences relative to word count (NS) and median pause duration (DurMED) compared to HC. Several other biomarkers, including moving-average type–token ratio (MATTR) and syntactic complexity (SC), did not show significant group differences in this cohort and task. Spearman's rank analysis revealed no correlations between the speech biomarkers that showed deterioration and clinical scores within the affected group. However, several strong associations were found between these speech biomarkers and MRI measures (see Table 5). Significant correlations between linguistic and acoustic biomarkers are illustrated in Fig. 4. In MCI-AD, higher content density (CD) was associated with reduced cortical thickness in the left frontal pole, a region implicated in executive control and language regulation. In MCI-LB, increased n-gram repetition (NG) correlated negatively with volumes in the right postcentral gyrus, bilateral hippocampi, and left pars opercularis. In PD-MCI, alterations in sentence structure (NW, NS, PSR-CCONJ) and pausing (DurMED) correlated with structural measures in regions including the right medial orbitofrontal cortex, left insula, posterior and isthmus cingulate cortex, and motor related regions such as paracentral, precentral, caudal middle frontal, and transverse temporal cortex (Table 5). At the level of cross feature relationships, shorter sentences and a higher number of sentences relative to word count were associated with longer and more variable pauses across the cohort, as shown in Fig. 4. Using XGBoost models, speech biomarkers demonstrated predictive capability for cognitive domain z-scores, MoCA, and GDS, with estimation error rates ranging from approximately 10% to 17% (Table 6). Although these error rates indicate that models are not precise enough to replace standard clinical assessments, they support the potential for tracking broader cognitive or affective status over time. For group classification, models trained on

[3] https://github.com/dmlc/xgboost.

[4] https://github.com/optuna/optuna.

Table 4
XGBoost hyperparameters tuned with Optuna and the search space used in our experiments.

Parameter	Type	Range	Purpose
n_estimators	Integer	[100, 2500]	Number of boosting trees (iterations)
learning_rate	Float	[0.01, 0.40]	Shrinkage per tree
max_depth	Integer	[3, 15]	Max tree depth (interaction complexity)
subsample	Float	[0.60, 1.00]	Row subsampling per tree
colsample_bytree	Float	[0.60, 1.00]	Feature subsampling per tree
gamma	Float	[0, 5]	Min loss reduction to split
min_child_weight	Integer	[1, 20]	Min child Hessian sum
reg_alpha	Float	[0, 5]	L1 regularization (lasso regression)
reg_lambda	Float	[0, 5]	L2 regularization (ridge regression)

Table 5
Significant correlations between significant speech biomarkers and clinical measures based on Spearman's rank analysis.

Clinical group	Speech biomarker	Neuroimaging measure	Brain region	Coefficient	P-value
MCI-AD	CD	CT	Left frontal pole	−0.474	0.037
MCI-LB	NG	CSV	Right postcentral gyrus	−0.453	0.043
		SSV	Left hippocampus	−0.527	0.008
		SSV	Right hippocampus	−0.459	0.038
		CSV	Left inferior frontal gyrus – pars opercularis	−0.491	0.019
		CSV	Left postcentral gyrus	−0.480	0.024
PD-MCI	NW	CT	Right medial orbitofrontal cortex	−0.469	0.015
	NS	CT	Right medial orbitofrontal cortex	0.452	0.015
	PSR-CCONJ	CSV	Left insula	−0.451	0.044
		CT	Right medial orbitofrontal cortex	−0.465	0.015
		CSV	Right medial orbitofrontal cortex	−0.548	0.005
		CSV	Right posterior cingulate cortex	−0.597	0.001
	DurMED	CT	Left isthmus of the cingulate gyrus	−0.450	0.046
		CSV	Left paracentral lobule	−0.487	0.021
		CSV	Left precentral gyrus	−0.469	0.031
		CSV	Right caudal middle frontal gyrus	−0.473	0.028
		CSV	Right transverse temporal gyrus	−0.486	0.022

CD – content density; NG – n-gram repetition; NW – mean words per sentence; NS – number of sentences; PSR-CCONJ – proportion of coordinating conjunctions; DurMED – median pause duration; CT – cortical thickness; CSV – cortical structure volume; SSV – subcortical structure volume.

Table 6
Estimation error rates [%] for clinical score prediction using XGBoost models with digital speech biomarkers as independent variables.

Speech biomarkers	Memory	Visuo-spatial	Attention	Executive	MOCA	GDS
Linguistic	13.67 | 13.81	17.53 | 17.15	15.70 | 15.71	15.09 | 15.28	10.28 | 9.75	15.35 | 15.21
Acoustic	14.07	17.36	15.82	15.14	10.16	14.78
Combination	13.69 | 13.55	17.54 | 17.19	15.67 | 15.68	15.10 | 15.28	10.30 | 9.82	15.40 | 15.07

Automatic transcription | manual correction.

clinical scores achieved moderate AUCs between 0.57 and 0.78 depending on the contrast. Adding speech biomarkers improved discrimination in most scenarios. For example, when differentiating HC from PD-MCI, the AUC increased from 0.78 to 0.82 using automatic transcription, with a similar pattern across other pairs (Fig. 5). Results were comparable when models used automatic transcripts and when they used manually corrected transcripts, for both clinical score prediction and classification, which supports feasibility of an automatic pipeline in large scale applications. An exception was the PD-MCI versus MCI-LB contrast, where manual correction improved discrimination. PD-MCI is primarily characterized by prosodic timing changes that are captured by acoustic features and are relatively insensitive to transcription route, whereas MCI-LB in our cohort showed a higher number of repeated n-grams. Because n-gram repetition depends on accurate tokenization, ASR errors can attenuate this signal and manual correction likely restores it.

4. Discussion

The clinical assessments confirmed expected cognitive profiles across the groups (Fig. 2), generally aligning with known characteristics: predominant memory impairment in MCI-AD [9], broader deficits potentially encompassing visuospatial and executive domains in MCI-LB [3,6], and variable cognitive patterns in PD-MCI, often involving executive function [2,11]. These observations provide a foundation for interpreting the speech biomarker differences.

The elevated ratio of content to function words in MCI-AD relative to HC is intriguing and partly contrasts with reports of reduced lexical richness or information content in MCI-AD and AD [18,24,33]. A higher content density (CD) may arise from a compensatory focus on core information or from use of simpler grammatical constructions. This interpretation is consistent with neurobiological evidence that abstract grammatical function words rely more on perisylvian cortices, whereas content words engage broader bilateral networks [52]. Early perisylvian abnormalities in MCI-AD, including cortical thinning, hypometabolism, and white matter disruption [53,54], could selectively affect function word processing while relatively sparing content word access, yielding increased CD. The absence of a difference in moving-average type–token ratio (MATTR) suggests preserved vocabulary range with altered usage strategy. Related work in Japanese cohorts has emphasized that language deviations are more prominent in AD than in DLB, whereas prosodic and acoustic changes dominate in Lewy body disorders [55]. Our finding of higher CD in MCI-AD is compatible with this emphasis on language change in the Alzheimer spectrum.

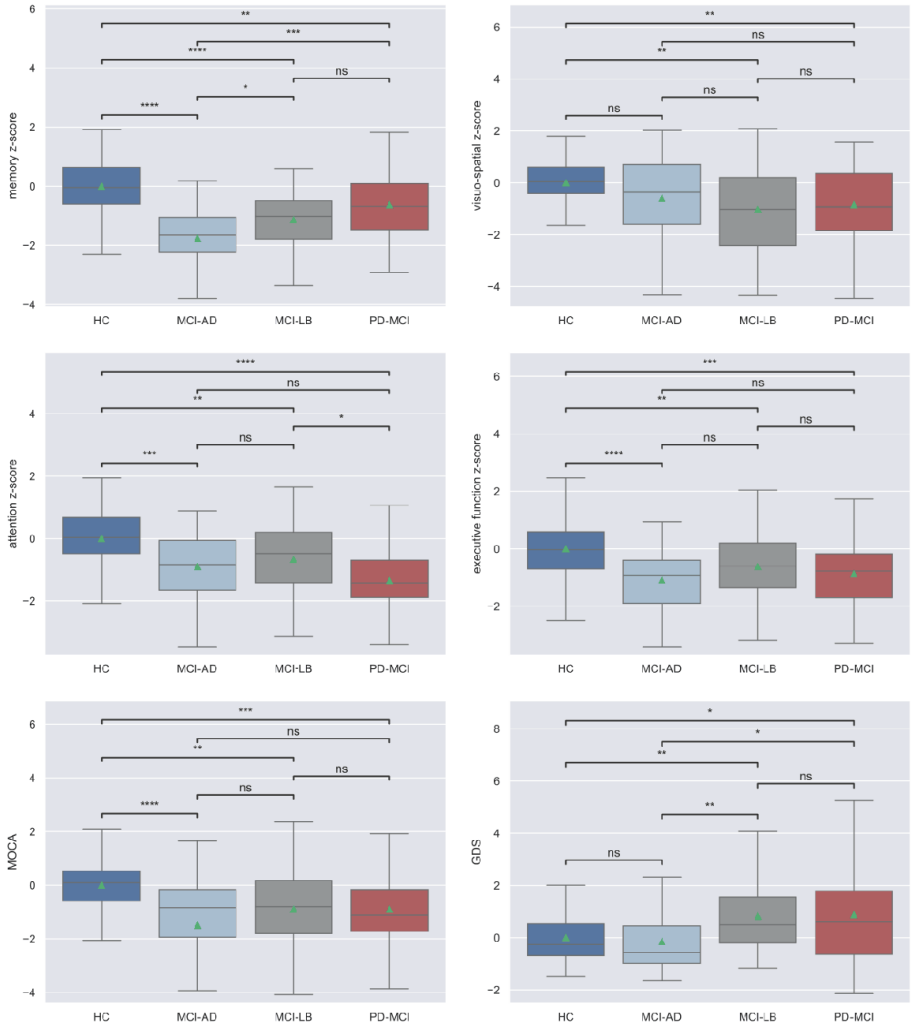

Fig. 2. Group differences in standardized clinical scores (*p < 0.05, **p < 0.01, ***p < 0.001, ***p < 0.0001, ns = not significant).

In MCI-LB, higher n-gram repetition (NG) relative to MCI-AD is compatible with cognitive inflexibility, perseveration, or word finding difficulty that increases reliance on familiar phrases. Executive dysfunction and attentional fluctuations are common in MCI-LB [6,8], and discourse level impairments such as incoherence or perseveration have been described in Lewy body disorders [8]. The contrast with reduced content density and diversity reported in iRBD [35] suggests that NG may index cognitive control processes rather than broad lexical variety in this spectrum. Complementing our repetition based linguistic signal, prior studies have highlighted prosodic and affective speech changes as characteristic of the Lewy body spectrum, including reduced vocal emotional expressiveness that discriminates DLB from Alzheimer's disease and controls [56]. Together these findings indicate that both discourse level repetition and prosodic alterations can mark Lewy body pathology, with relative prominence likely shaped by disease stage and task demands.

The PD-MCI pattern of shorter sentences (NW), fewer coordinating conjunctions (PSR-CCONJ), a higher number of sentences relative to total words (NS), and longer median pauses (DurMED) points to difficulty formulating and executing complex syntax together with disrupted fluency. Such patterns are consistent with prior reports in PD-MCI [14,16,57]. The linguistic impairments likely reflect the combined effects of executive dysfunction on planning and sequencing [11,13] and motor speech deficits characteristic of hypokinetic dysarthria that affect fluency during the ON medication state [15,17]. Subert et al. reported that long term dopaminergic therapy improved syntactic aspects of speech only in patients treated with dopamine agonists, with no significant language amelioration in patients treated with levodopa [36]. This

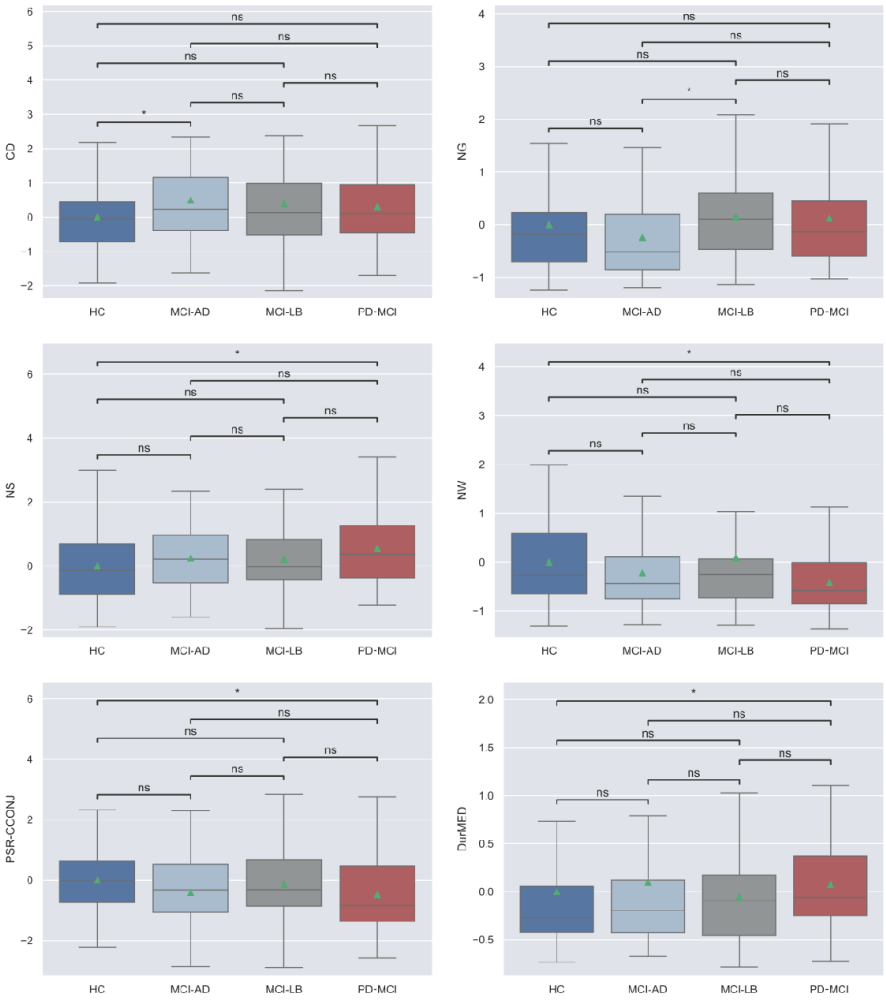

Fig. 3. Significant group differences in standardized linguistic and acoustic speech biomarkers (*p < 0.05, ns = not significant, CD = content density, NG = n-gram repetition, NS = number of sentences, NW = mean words per sentence, PSR-CCONJ = proportion of coordinating conjunctions, DurMED = median pause duration).

pattern is consistent with persistent syntactic impairments in our PD-MCI cohort despite being assessed in the ON state. Taken together, the contrasts observed in PD-MCI relative to healthy controls likely reflect a mixture of cognitive linguistic change and Parkinson related motor speech characteristics rather than cognitive decline alone. Consistent with reports that prosodic and temporal features carry diagnostic signal in Lewy body conditions [55,56], our PD-MCI results underscore the contribution of timing and fluency alongside syntactic simplification in Parkinsonian cognitive impairment.

The neuroanatomical associations observed in Table 5 provide face validity for the speech markers by linking them to regions involved in executive control, language processing, articulatory planning, attention, and motor control [2,11,16,57]. These links support the view that connected speech integrates cognitive and motor systems and that disease related change in these systems can be detected in spontaneous monologue. Related DLB work using a brief reading task reported atrophy in fusiform, insula, and accumbens, with insular thickness the only region showing corrected associations with vocal emotional expressiveness and valence [56]. This aligns with our finding that connected-speech markers correlate with insular measures in PD-MCI, suggesting links to salience and affective networks alongside language and motor systems. Differences in task and disease spectrum likely shape regional emphasis, as Kobayashi et al. targeted affective prosody in DLB, whereas we analyzed spontaneous monologue across prodromal subtypes.

The dissociation between robust speech–MRI correlations and limited speech–clinical score correlations suggests that connected speech may index structural brain change more directly than global clinical

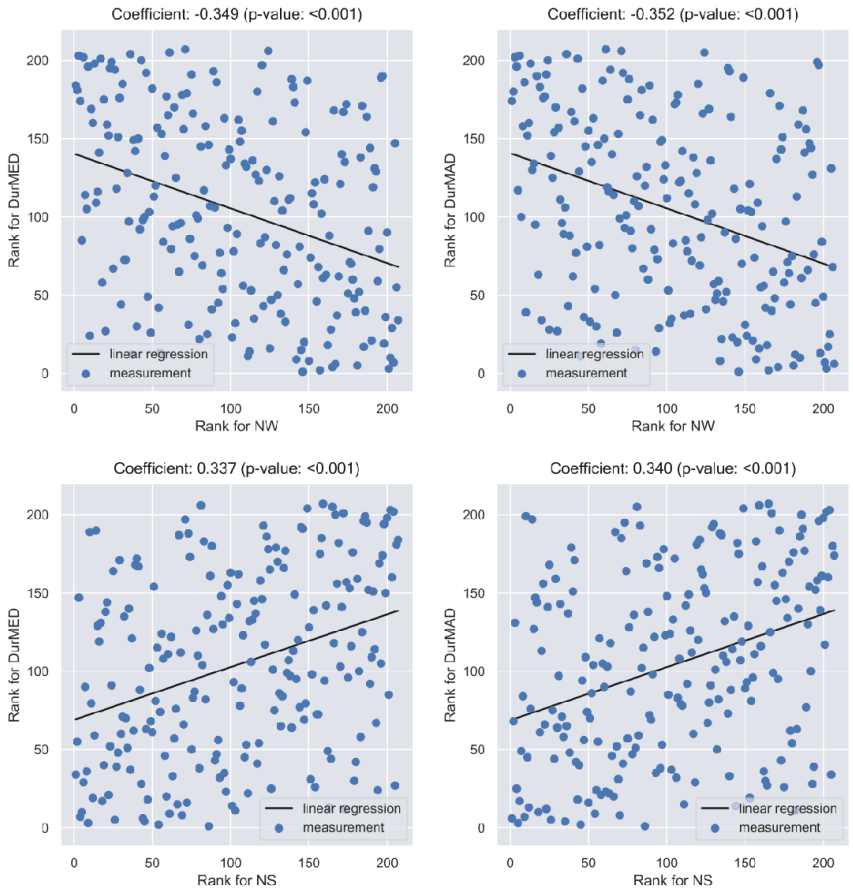

Fig. 4. Significant Spearman's rank correlations between linguistic and acoustic biomarkers NW = mean words per sentence, NS = number of sentences, DurMED = median pause duration, DurMAD = median absolute deviation of pause.

assessments. Standard neuropsychological tests evaluate isolated functions under structured conditions, while spontaneous speech draws on lexical retrieval, syntactic planning, semantic coherence, motor execution, and executive control in real time [25]. This difference in construct coverage may explain why subtle speech deficits do not always align with composite clinical scores in mild impairment stages, implying that speech analysis and clinical assessment provide complementary information about cognitive status and brain health.

Consistent with an integrated view of production, the observed relationships between sentence structure and pausing indicate that increased structural complexity can introduce additional cognitive or motor load that manifests as longer or more variable pauses [58]. This interplay argues for combined analysis of linguistic and acoustic features to characterize deficits in neurodegenerative conditions [16,33].

Integrating semantic rich representations from models like BERT may be valuable for improving classification in remote monitoring while maintaining interpretability. Prior work, focused only on classification and not on predicting continuous clinical scores, has shown that linguistic markers from automatic transcription can match or exceed those from manual transcripts. In conversational German interviews, features from automatic transcripts performed on par with or slightly better than manual features (unweighted average recall of 0.623) [59]. In a Japanese self-administered mobile application, automatically and manually derived linguistic features were strongly correlated with an $r = 0.92$, and multi-class classification of AD, MCI, and HC reached 78.6% in nested cross-validation [60]. In contrast, the present study evaluates both classification and clinical score prediction and relates speech features to structural MRI in Czech spontaneous monologue, reporting ROC AUC for pairwise classification and a normalized estimation error rate for score prediction, with similar performance across automatic and manually corrected transcripts. Overall, while predictive accuracy remains moderate, robustness to automatic transcription supports feasibility for remote cognitive monitoring and early screening, and the primary value may lie in longitudinal tracking rather than direct cross-sectional diagnosis.

Limitations of this study should be acknowledged. Sample sizes may have limited the detection of weaker effects or finer subgroup differences, and recruitment from a single center may affect generalizability. Diagnoses were clinical rather than pathologically confirmed. Standard

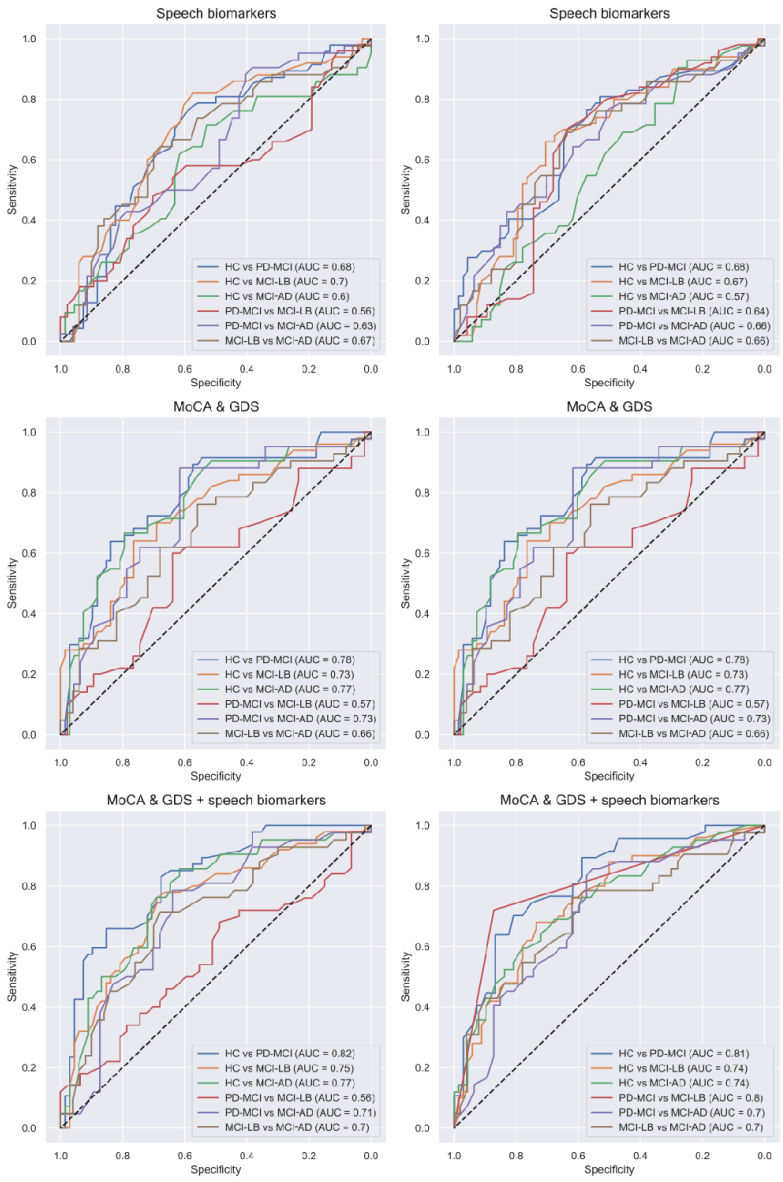

Fig. 5. Classification results (automatic transcription on the left, manual correction on the right).

biomarkers were not obtained, including cerebrospinal fluid assays (for example, Aβ42/40 and phosphorylated tau), blood-based biomarkers, and molecular imaging. There were no amyloid positron emission tomography studies for AD, no tau positron emission tomography studies, no dopamine transporter single-photon emission computed tomography studies, and no cardiac metaiodobenzylguanidine scintigraphy for Lewy body disease. Neuroimaging analyses were limited to structural MRI; functional or connectivity measures could add explanatory value. Although we regressed out confounding factors, a dataset balanced for gender and with groups matched on age and education would provide a cleaner design and reduce residual confounding risk. PD-MCI participants were assessed only in the ON medication state, which may not fully capture their linguistic or motor speech capabilities. The single, relatively unstructured monologue task may not assess all

language functions [28,31]. A free topic by design can introduce topic-related variability. Although instructions were identical across groups and participants tended to choose everyday themes, differences in topic selection could, in principle, influence content-dependent markers. The analysis focused on a limited set of biomarkers; broader linguistic and acoustic features could yield additional insights [17,61]. Classification and regression models' performance was estimated without a nested cross-validation loop due to cohort size, which may yield optimistic estimates.

Several future directions follow from these limitations. Longitudinal designs are needed to track speech biomarkers alongside clinical change and neuroimaging in MCI-AD, MCI-LB, and PD-MCI. A broader range of speech elicitation tasks could provide a more comprehensive profile of language impairment. Assessing PD-MCI in both ON and OFF states would clarify medication effects on speech. Expanding features to include semantic representations from deep learning models, such as BERT embeddings, may improve both classification and score prediction by capturing richer language characteristics [27,28]. Integrating speech with functional neuroimaging or electrophysiology could reveal underlying network dynamics. Given the observed speech–MRI associations, predicting neuroanatomical metrics such as cortical thickness directly from speech is a promising direction. Larger, more diverse cohorts with longitudinal follow-up will be important for establishing clinical utility, particularly for remote monitoring [39]. Generalizability to other languages, including English and Spanish, should be assessed in future work, ideally with parallel ASR-manual comparisons.

5. Conclusion

This study provides evidence that specific linguistic and acoustic digital speech biomarkers, extracted from monologue speech, are tied to particular clinical groups (reduced function word use in MCI-AD, increased lexical repetition in MCI-LB, and shorter sentences with longer pauses in PD-MCI) and correlate meaningfully with structural brain measures in disease-relevant networks. The dissociation between speech-MRI and speech-clinical score correlations suggests that these biomarkers may capture early neurobiological changes not fully reflected in standard clinical scales. Although prediction of concurrent clinical scores achieved only moderate accuracy, classification between clinical groups improved with the inclusion of speech features. The robustness of biomarker extraction using ASR further supports the feasibility of scalable, low-cost, and non-invasive remote monitoring. Future work may enhance performance by integrating semantic features from deep learning models, while maintaining a balance between predictive power and clinical interpretability. This research advances the field of digital biomarkers, highlighting both the promise and challenges of speech-based assessment for early detection and longitudinal tracking of cognitive decline in neurodegenerative diseases.

CRediT authorship contribution statement

Daniel Kovac: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lubomira Novakova:** Writing – original draft, Project administration, Funding acquisition, Data curation, Conceptualization. **Jiri Mekyska:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Krystof Novotny:** Writing – original draft, Software, Methodology. **Lubos Brabenec:** Validation, Data curation, Conceptualization. **Patricia Klobusiakova:** Writing – original draft, Data curation. **Irena Rektorova:** Validation, Supervision, Methodology, Conceptualization.

Ethics statement

All subjects signed an informed consent form that was approved by the respective local ethics committees. The projects under which the datasets were collected were approved by the Masaryk University Research Ethics Committee under Ref. No. EKV-2019-061 (13 March 2020) and EKV-2022-094 (10 November 2022), and by the Ethics Committee of St. Anne's University Hospital in Brno under Ref. No. 66V/2022 (9 November 2022).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are protected by privacy and security laws.

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Conclusion

This habilitation thesis brings together more than a decade of research focused on the relationship between brain connectivity, neurodegeneration, cognitive dysfunction, and neuroplasticity. Although the individual studies address different diseases, brain systems, and methodological approaches, they converge on the view that cognitive impairment is closely related to alterations in the organization and interaction of distributed neural networks.

A central perspective emerging from this work is that structural brain integrity, functional connectivity, and neuroplasticity should be considered as interconnected rather than independent features of neurodegenerative disease. Understanding their interaction may provide a more comprehensive characterization of individual patterns of cognitive decline than the assessment of regional pathology alone.

The integration of multimodal neuroimaging with neuromodulation and behavioral interventions further extends this perspective from describing disease-related brain changes toward investigating their potential modification. This approach provides a basis for linking biomarkers of neurodegeneration with mechanisms of brain plasticity and, ultimately, with clinically meaningful outcomes.

Taken together, the work presented here establishes brain connectivity and plasticity as complementary frameworks for investigating cognitive dysfunction in neurodegenerative diseases. The findings provide a foundation for future research aimed at identifying sensitive and clinically relevant biomarkers, understanding individual differences in disease and recovery trajectories, and developing more targeted approaches to preserving cognitive function.