Non- invasive Neuromodulation in Neurodegenerative Diseases: from Clinical Follow-up to Eye-tracking as Biomarker

Inaugural-Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

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Düsseldorf, January 2025

Aus dem Institut für Klinische Neurowissenschaften und Medizinischer Psychologie
der Heinrich-Heine-Universität Düsseldorf

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität Düsseldorf

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Tag der mündlichen Prüfung: 11.04.2025

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Acknowledgments

First and foremost, I would like to thank Prof. Wojtecki for believing in me and investing in me. Thank you for sharing your expertise in hour-long meetings, calls, and endless exchange of emails. Thank you for challenging me but supporting me throughout each step of my PhD project. Thank you for the many opportunities and for guiding me in the field of science. Thank you for shaping my career. My success wouldn't have been the same without you.

I would also like to thank my co-supervisor, Prof. Zimmermann. You set the milestone of my academic writing by believing in me when I was just a working student. Thank you for continuing to support me throughout my PhD project.

A very special thank you goes to my coworkers. Thank you for supporting me and always believing in me. Thank you for telling me when to take a break, being proud of me, surprising me with flowers after a successful presentation at a conference, and drinking champagne with me when my paper was published. I couldn't have gone through this without you. Thank you for always having my back; I will forever be grateful that you stood by my side through all of this.

Also, I want to thank the participants and their relatives of the studies published in this thesis. Many of them have been my patients for more than a year now. Thank you for trusting me with your care. It was a challenge but also a great chance to balance my PhD work with clinical practice.

Thank you to my family and friends. To my parents, my grandparents, and my wonderful sister. Thank you for always asking about my work and listening to me while I get lost in the conversation about my research. To my grandpa, who sadly got diagnosed with Parkinson's and dementia and made me choose the path of neuropsychology to understand his disease better: While I could only do little for you, researching about your disease gave me the feeling of fighting against the disease and helping others in the bigger picture.

My amazing husband. There are no words to express my gratitude. We met when I just got my bachelor's degree, and I am not sure I would stand here today writing my PhD thesis if you didn't support me through it all. Thank you for giving me strength and celebrating each little step through the process. Thank you for believing in my dreams and making it possible for me to achieve them.

Abbreviations

A	Amyloid-beta deposition
ADAS	Alzheimer's Disease Assessment Scale
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive Subscale
AD	Alzheimer's Disease
ADE	Adverse Device Effect
AE	Adverse Event
AS	Alzheimer's Syndrome
BDI-II	Beck Depression Inventory-II
BDI-II FS	Beck Depression Inventory-II Fast Screen
CBD	Corticobasal Degeneration
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DBS	Deep Brain Stimulation
drTMS	Deep Repetitive Transcranial Magnetic Stimulation
EEG	Electroencephalography
H-Coil	Hesed Coil
M1	Primary Motor Cortex
MC	Motor Cortex
MCI	Mild Cognitive Impairment
MDS	Movement Disorder Society
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
MSA	Multiple System Atrophy
MSA-C	Multiple System Atrophy, which involves Cerebellar Ataxia

MSA-P	Multiple System Atrophy with Parkinsonian Symptoms
N	Neuronal injury
NIA-AA	National Institute on Aging and Alzheimer's Association
NIBS	Non-invasive Brain Stimulation
NRS	Numeric Rating Scale
PD	Parkinson's Disease
PFC	Prefrontal Cortex
PS	Parkinson Syndrome
PSP	Progressive Supranuclear Palsy
rMT	Resting Motor Threshold
ROC	Receiver Operating Characteristic
ROI	Region of Interest
T	Pathologic tau neurodegeneration or neuronal injury
tACS	Transcranial Alternating Current Stimulation
tDCS	Transcranial Direct Current Stimulation
TPS	Transcranial Pulse Stimulation
TMS	Transcranial Magnetic Stimulation
TUG	Timed Up and Go Test
VR	Virtual Reality

List of papers

Paper I — Cont, C., Stute, N., Galli, A., Schulte, C., Logmin, K., Trenado, C., & Wojtecki, L. (2022). Retrospective real-world pilot data on transcranial pulse stimulation in mild to severe Alzheimer's patients. Frontiers in neurology, 13, 948204.

Paper II — Cont, C., Lehto, A., Stute, N., Galli, A., Schulte, C., Deer, V., Wessler, M., & Wojtecki, L. (2022). Safety of Deep Repetitive Transcranial Magnetic Stimulation (drTMS) against Medical Refractory Symptoms in Parkinson Syndromes: First German Real-World Data with a Specific H5 Coil. Neurology international, 14(4), 1024–1035.

Paper III — Cont, C., Stute, N., Galli, A., Schulte, C. & Wojtecki, L. (2024) Could Eye Tracking Serve as a Sensitive Biomarker in Parkinson's Syndrome? – An Exploratory Pilot Study of Measurements Before and after Deep Transcranial Magnetic Stimulation. Brain Sciences. *In Review*.

Abstract

Background. Neuromodulation is rising as a promising add-on therapy for neurodegenerative diseases, offering the potential to alleviate symptoms, enhance quality of life, and address unmet therapeutic needs. While many patients report a subjective improvement following stimulation, objective assessments using neuropsychological test batteries often yield mixed results. Given the novelty of these techniques, an exploratory approach is essential to evaluate their feasibility, effectiveness, and applicability in real-world scenarios.

Aims. This thesis aims to explore novel neuromodulation techniques in clinical follow-ups, defending an exploratory approach to address the innovative nature of the methods. Furthermore, eye tracking is examined as an innovative and sensitive biomarker in an exploratory pilot study designed to gain objective insights into patients' cognitive and emotional states.

Methods. In Paper I, 11 patients with Alzheimer's Syndrome (AS) underwent Transcranial Pulse Stimulation (TPS), with the stimulation procedures and neuropsychological assessments personally conducted. In Paper II, 21 patients with Parkinson's Syndrome (PS) were treated with Deep Repetitive Transcranial Magnetic Stimulation (drTMS), with both the treatment sessions and subsequent neuropsychological evaluations directly administered. In Paper III, an eye-tracking study was conceptualized, self-designed, and implemented using virtual reality (VR) to collect pre- and post-treatment data from 10 Parkinson's patients undergoing drTMS.

Results. In Paper I, TPS was well tolerated by AS patients. Significant improvements were assessed in some neuropsychological tests, as well as an improvement in depressive symptoms. In Paper II, drTMS for Parkinson's Syndrom was well tolerated, with transient side effects. The treatment reduced the self-reported severity of symptoms and improved depression scores, particularly in older Parkinson's patients, though no significant cognitive benefits were observed. In Paper III, no significant changes in eye-tracking parameters were observed post-treatment; however, there were significant correlations between eye-tracking data and cognitive scores, and depressive symptoms were notably reduced.

Discussions. The results highlight the potential of TPS as a novel treatment for Alzheimer's Syndrome, warranting further research with more extensive, sham-controlled studies. Similarly, drTMS shows promise as an add-on therapy for Parkinson's symptoms but requires validation in larger samples. Furthermore, the pilot eye-tracking study underscores its potential as a sensitive biomarker, though further testing and development are needed. The hands-on approach in administering treatments, conducting assessments, and designing innovative studies reflects the integral role of this work in advancing these methods. In conclusion, while

neuromodulation shows promise in improving patient outcomes, further research is essential to objectively assess its feasibility and ensure its successful integration into real-world clinical settings.

Zusammenfassung

Hintergrund. Die Neuromodulation entwickelt sich zunehmend zu einer vielversprechenden Zusatztherapie für neurodegenerative Erkrankungen, die das Potenzial hat, Symptome zu lindern, die Lebensqualität zu verbessern und ungedeckten therapeutischen Bedarf zu decken. Während viele Patient*innen über eine subjektive Verbesserung nach der Stimulation berichten, liefern objektive Bewertungen mit neuropsychologischen Testbatterien oft gemischte Ergebnisse. Angesichts der Neuartigkeit dieser Techniken ist ein explorativer Ansatz unerlässlich, um ihre Machbarkeit, Wirksamkeit und Anwendbarkeit in real-world Szenarien zu bewerten.

Ziele. Ziel dieser Arbeit ist es, neuartige Neuromodulationstechniken in der klinischen Nachsorge zu erforschen und dabei einen explorativen Ansatz zu verteidigen, um den innovativen Charakter der Methoden zu berücksichtigen. Darüber hinaus wird das Eye-Tracking als innovativer und sensitiver Biomarker in einer explorativen Pilotstudie untersucht, um objektive Einblicke in den kognitiven und emotionalen Zustand von Patient*innen zu gewinnen.

Methoden. In Paper I wurden 11 Patient*innen mit Alzheimer-Syndrom (AS) einer transkraniellen Pulsstimulation (TPS) unterzogen, wobei die Stimulationsverfahren und neuropsychologischen Beurteilungen persönlich durchgeführt wurden. In Paper II wurden 21 Patient*innen mit Parkinson-Syndrom (PS) mit tiefer repetitiver transkranieller Magnetstimulation (drTMS) behandelt. In Paper III wurde eine Eye-Tracking-Studie konzipiert, selbst entworfen und unter Verwendung von Virtual Reality (VR) durchgeführt, um Daten vor und nach der Behandlung von 10 Parkinson-Patient*innen zu sammeln, die sich einer drTMS unterzogen.

Ergebnisse. In Paper I wurde die TPS von den AS-Patient*innen gut vertragen. Es wurden signifikante Verbesserungen in einigen neuropsychologischen Tests sowie eine Verbesserung der depressiven Symptome festgestellt. In Paper II wurde die drTMS bei Parkinson-Syndrom gut vertragen, mit vorübergehenden Nebenwirkungen. Die Behandlung verringerte den selbstberichteten Schweregrad der Symptome und verbesserte die Depressionswerte, insbesondere bei älteren Parkinson-Patient*innen, obwohl keine signifikanten kognitiven Vorteile beobachtet wurden. In Paper III wurden nach der Behandlung keine signifikanten Veränderungen bei den Eye-Tracking-Parametern beobachtet, jedoch gab es signifikante Korrelationen zwischen den Eye-Tracking-Daten und den kognitiven Scores, und die depressiven Symptome wurden deutlich reduziert.

Diskussion. Die Ergebnisse unterstreichen das Potenzial von TPS als neuartige Behandlung des Alzheimer-Syndroms und rechtfertigen weitere Untersuchungen in größeren, Placebo-kontrollierten Studien. Auch die drTMS ist als Zusatztherapie für Parkinson-Symptome vielversprechend, muss aber in größeren Stichproben validiert werden. Darüber hinaus unterstreicht die Pilotstudie zum Eye-Tracking ihr Potenzial als sensitiver Biomarker, auch wenn weitere Tests und Entwicklungen erforderlich sind. Der praktische Ansatz bei der Anwendung von Behandlungen, der Durchführung von neuropsychologischen Bewertungen und der Konzeption innovativer Studien spiegelt die wesentliche Rolle dieser Arbeit bei der Weiterentwicklung dieser Methoden wider. Zusammenfassend lässt sich sagen, dass die Neuromodulation zwar vielversprechend ist, um die Ergebnisse für die Patient*innen zu verbessern, dass aber weitere Forschungsarbeiten erforderlich sind, um ihre Durchführbarkeit objektiv zu bewerten und ihre erfolgreiche Integration in real-world Situationen zu gewährleisten.

1. Introduction

Pharmacological therapies have so far achieved only limited success in treating most major neurological diseases, prompting the development of new approaches aimed at alleviating drug-induced side effects or pharmacoresistant symptoms (Tierney et al., 2013). Recent studies have increasingly investigated the potential of neuromodulation techniques (such as non-invasive brain stimulation techniques, or NIBS) to counteract patients' deterioration. These techniques can stimulate the brain using, for example, transcranial magnetic stimulation (TMS) or by applying direct current (tDCS) and alternating current (tACS) externally (Marson et al., 2021). Due to their ability to modulate brain activity directly, NIBS techniques have been widely applied in treating neurodegenerative diseases.

Transcranial Pulse Stimulation and deep repetitive Transcranial Magnetic Stimulation are advanced neuromodulation techniques that have gained attention for their ability to provide targeted, non-invasive brain stimulation in treating neurological disorders. TPS uses focused ultrasound pulses to stimulate brain regions, potentially enhancing neural plasticity and cognitive function without the need for surgery (Beisteiner et al., 2020). In contrast, drTMS applies magnetic fields that penetrate deeper brain layers than standard TMS, allowing for more extensive modulation of brain circuits implicated in various neuropsychiatric and neurodegenerative conditions (Hanlon et al., 2023). Both methods are increasingly explored as promising alternatives or complementary to conventional pharmacological treatments, particularly in cases where drug therapies are ineffective or poorly tolerated. However, studies show mixed results and no common biomarker has been used to test the efficacy of neuromodulation techniques. Here, eye tracking is tested as a potential biomarker for stimulation effects in Parkinson's Disease.

This thesis focuses on both TPS in Alzheimer's Syndrome (Paper I) and drTMS in Parkinson's Syndrome (Paper II) and adds eye tracking as a potential biomarker (Paper III). This thesis aimed to explore non-invasive neuromodulation in neurodegenerative diseases using clinical follow-up data and proposing eye-tracking as a possible biomarker. Moreover, a general need for an overall biomarker is discussed.

2. Background

2.1. Neurodegenerative Diseases

Neurodegenerative diseases are a broad category of chronic disorders characterized by the progressive degeneration of the structure and function of the central nervous system (CNS). These conditions primarily affect neurons, the building blocks of the nervous system responsible for transmitting signals throughout the body. Unlike many other cells in the body, neurons do not typically regenerate, making the impact of these diseases particularly severe and irreversible (Gao & Hong, 2008). Common neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Each of these diseases have unique characteristics, but they often share standard features such as the aggregation of misfolded proteins, mitochondrial dysfunction, and neuroinflammation (Jellinger, 2010). Neurodegenerative diseases pose a significant global healthcare challenge. Currently, millions of people worldwide are affected by these disorders, and with an aging global population, experts expect the prevalence to rise dramatically in the coming decades. Alzheimer's disease alone is estimated to affect 115.4 million people by 2050, while Parkinson's disease is estimated to impact nearly 7 million individuals by the same year (Bach et al., 2011; Prince et al., 2013). This anticipated increase underscores the urgent need for effective treatments and interventions to manage and potentially cure these debilitating conditions (Jellinger, 2010).

2.1.1. Alzheimer’s Syndrome

Alzheimer's Syndrome is the most common neurodegenerative disorder and is responsible for 60-80% of dementia cases globally (Association, 2016). The characterization of the disease is a progressive decline in cognitive function, leading to memory loss, language difficulties, and behavioral changes. Pathologically, Alzheimer's marks the accumulation of beta-amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein in the brain (Khan et al., 2020). These aggregates disrupt neuronal communication, resulting in neuronal death and brain atrophy. For many years, the definition of Alzheimer’s Disease (AD) was a combination of clinical symptoms and pathological findings. When patients presented with cognitive decline, often involving memory impairment and

functional difficulties in daily life, clinicians would assign a diagnosis of "probable AD" after excluding other causes. However, confirmation of the diagnosis as "definite AD" was only possible through postmortem examination, where the presence of amyloid plaques and tau neurofibrillary tangles in the brain would confirm the pathological basis of the disease (Petersen, 2018). This framework, which tied clinical syndromes to pathological evidence, became the widely accepted standard for over three decades. Recent advancements in biomarker technologies, including neuroimaging and cerebrospinal fluid (CSF) analysis, now allow for detecting AD pathology during a patient's lifetime. Those biomarkers include decreased levels of amyloid-beta 42 and increased levels of total tau and phosphorylated tau, as outlined by the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria (Jack et al., 2011). This has significantly improved diagnostic precision, enabling clinicians to better identify the specific underlying pathology of cognitive syndromes without waiting for postmortem confirmation. Furthermore, the concept of the *Alzheimer's Disease Continuum* has been introduced to describe the progression of the condition from preclinical stages to advanced dementia (Petersen, 2018). While distinct terms like AD or mild cognitive impairment (MCI) describe points along this spectrum, the broader term *Alzheimer's Syndrome* serves as an inclusive framework, encompassing both the clinical manifestations and the individual pathological basis of the disease.

Current treatments primarily focus on symptomatic relief and slowing cognitive decline. Cholinesterase inhibitors, such as donepezil, rivastigmine, and galantamine, are commonly used to enhance cholinergic function and improve cognitive symptoms in mild to moderate stages of the disease (Birks, 2006). Memantine, an NMDA receptor antagonist, is often prescribed for moderate to severe cases to help regulate glutamate activity and reduce neurotoxicity (Reisberg et al., 2003). Non-pharmacological interventions, including cognitive therapy, physical exercise, and social engagement, are also critical for managing symptoms and enhancing the quality of life for patients (Livingston et al., 2020).

2.1.2. Parkinson's Syndrome

Parkinson's syndrome is the second most common neurodegenerative disease, primarily presenting as a movement disorder characterized by tremors, rigidity,

bradykinesia (slowness of movement), and postural instability (Elbaz et al., 2016). This disorder stems from the degeneration of dopaminergic neurons in the substantia nigra, a brain region crucial for movement control. The resultant dopamine deficiency leads to characteristic motor symptoms. Additionally, the presence of Lewy bodies and abnormal aggregates of alpha-synuclein protein is a pathological hallmark. Parkinson's syndrome encompasses various syndromes, including idiopathic Parkinson's disease (PD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD) (Elbaz et al., 2016). While the exact etiology of Parkinson's remains elusive, researchers believe it involves a complex interplay of genetic and environmental factors (Lew, 2007). Treatment for Parkinson's syndrome aims to restore dopamine levels and manage motor symptoms. Levodopa, often combined with carbidopa to prevent premature breakdown, remains the most effective treatment (Fahn, 2003). Other pharmacological approaches may involve adding an MAO-B inhibitor, a COMT inhibitor, a dopamine agonist, or an extended-release formulation of levodopa (Giugni & Okun, 2014). Additionally, treatments like safinamide and dopamine or levodopa pumps are also considered. While pharmacotherapeutic therapies are very effective for motor symptoms, long-term use can lead to disabling side effects. Also, symptoms like freezing of gait, cognitive functions such as speech disturbances, and apathy can resist pharmacotherapy (Hanlon et al., 2023). For advanced Parkinson's cases unresponsive to medication, deep brain stimulation (DBS) offers a surgical option to alleviate symptoms (Liu et al., 2014). However, there are patients with Parkinson's syndrome who are refractory to these treatments, posing a significant challenge (Olanow, 2008). Additionally, therapies such as physical therapy are essential in maintaining mobility and functionality and, thus, quality of life (Radder et al., 2020).

2.2. Neuromodulation

Neuromodulation can be defined as altering neuronal activity using devices, lesions, or electromagnetic energy to modify brain function (Horn & Fox, 2020). It is widely used to treat patients with neurological or psychiatric disorders or answer scientific research questions. However, understanding how specific neuromodulation techniques affect the brain is continuously evolving.

Here, two relatively novel neuromodulation techniques are introduced: transcranial pulse stimulation and deep repetitive transcranial magnetic stimulation.

2.2.1. Transcranial Pulse Stimulation

Transcranial pulsed stimulation (TPS) uses short, repetitive shockwaves and is, therefore, a sound-based technique. Through a neuro-navigated device (Neurolith©, Storz Medical), those mechanical waves are applied highly focal and could possibly stimulate up to 8cm in depth. Shockwaves have been successfully used for over 40 years in orthopaedical or cardiac indications. Similar to ultrasound, shock waves are acoustic waves. However, ultrasound shows continuous waves with frequent oscillations (Figure 1A), while shock waves show a single pressure pulse followed by a tensile wave of lower amplitude (Figure 1B). With a low energy wave (0.2 or 0.25), leading energy into the tissue.

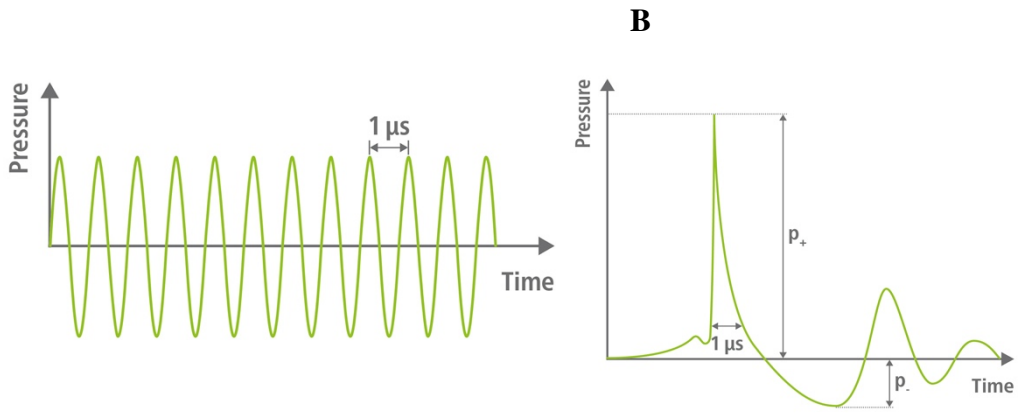

Figure 1. Shockwaves vs. ultrasound. The y-axis represents the pressure, while the x-axis represents time. *A*. ultrasound waves in continuous oscillations. *B*. Representation of shock waves as a single pressure pulse and a tensile wave (TPS Neuro, n.d.). Reproduced with permission from Storz Medical AG.

Studies demonstrated that shockwaves could indorse mechanotransduction (Beisteiner et al., 2020), vascular endothelial growth factors (Hatanaka et al., 2016; Yahata et al., 2016), and release nitric oxide, which then could lead to an improved blood flow (Mariotto et al., 2005). Moreover, a recent EEG study after one single stimulation session confirmed measurable neurophysiological effects in patients with AD. These findings suggest that there might be a direct electroencephalographic effect of TPS, reflected in changes in spectral power, coherence, Tsallis entropy, and cross-frequency coupling across multiple brain

regions, highlighting its potential to modulate brain activity in AD (Wojtecki et al., 2024).

A first pilot study from Vienna showed improvements in cognitive and depressive syndromes in AD patients that lasted up to 3 months (Beisteiner et al., 2020). Since then, several clinical trials have been published. A recent review of six studies of TPS used on AD patients found that TPS significantly improved cognitive performance in multiple neuropsychological test scores and depressive symptoms (Chen et al., 2023). Chen et al. proposed that TPS increased functional connectivity in the brain areas associated with memory, such as the hippocampus, parahippocampal cortex, precuneus, and parietal cortex. Moreover, improving depressive syndromes might be related to decreasing functional connectivity between the ventromedial and salience networks. While these results are promising, it is essential to mention that none of the studies included a sham control group.

2.2.2. Deep Repetitive Transcranial Magnetic Stimulation

Transcranial magnetic stimulation has become a key tool in brain research and is widely used in treating various psychiatric and neurological disorders. Magnetic fields are generated by directing a strong electrical current through an electromagnetic coil on the scalp (Lu & Ueno, 2017). This process induces electric fields and eddy currents in the underlying cortical tissue, leading to localized axonal depolarization. Deep transcranial magnetic stimulation (Deep TMS™ , Brainsway Inc.), however, is used as a development of standard TMS that utilizes a unique, patented Hesed coil (H-Coil) technology to generate those electromagnetic fields that enter deeper into the brain (Figure 2).

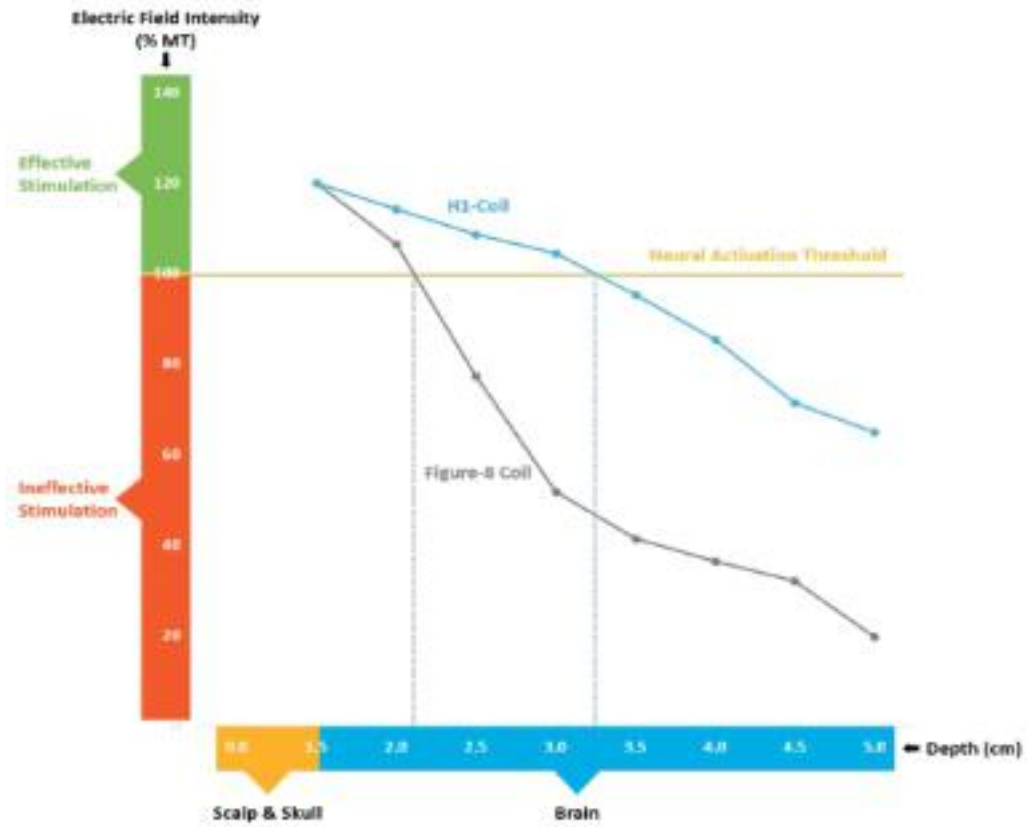

Figure 2. TMS vs. deep TMS. The y-axis represents the electric field intensity with the percentage of the motor threshold. Stimulation over 100% of the motor threshold represents an effective stimulation by reaching the neural activation threshold. The x-axis shows depth measured in cm. 0 – 1.5cm are the scalp and skull, while starting from 1.5 cm reaches the brain. With TMS with the figure-8 coil, the stimulation intensity naturally decreases as it penetrates deeper into the brain. The H-coil design ensures that the strength of stimulation is maintained at effective levels, even in deeper brain regions. Reprinted with permission by BrainsWay Ltd.. Based on data from Rosenberg et al. (2010).

A study compared traditional TMS with the figure-8 coil to drTMS with the H-coil and showed that drTMS uses a slower decay of magnetic fields, which then leads to reaching deeper and broader areas of the brain (Zangen et al., 2005). The study found that drTMS can reach up to 5.5cm in depth while TMS can reach up to 2cm. A recent review has investigated six studies with 220 PD patients in total who have been treated with drTMS using the two-stage protocol targeting the prefrontal cortex and the motor cortex (Hanlon et al., 2023). More than half of the studies showed significant results on motor symptoms, with most studies showing the most prominent effects on individuals with advanced disease. More importantly, a double-blind and sham-controlled study significantly improved a tremor subscale

(Spagnolo et al., 2020). Halon et al. (2023) suggest that the efficiency of drTMS used in PD patients depends on the patient selection, with individuals with advanced PD having the best benefit. Moreover, drTMS also seems to have a promising effect on non-motor symptoms and mood.

2.3. Eye Tracking

Over two centuries, cognitive psychologists tested eye movements to show the mind's inner workings (Eckstein et al., 2017). Even though eye movements are not a direct measurement of brain functions, they can provide details between the brain and behavior and, thus, give relevant information about higher-order processes such as memory and attention (Bueno et al., 2019). One can gather this information by assessing, for example, duration fixations, pupil size, and eye position. Eye movements, combined with theoretical models, provide insights into how attention is distributed and how information within a stimulus processes. For instance, during task performance, eye movement patterns can reveal which areas or details in a text are deemed relevant and, therefore, receive focused attention. This assumption is also called the *eye-mind assumption*, introduced by Just and Carpenter in 1980.

The eye-mind assumption is a foundational principle in cognitive psychology and eye-tracking research, suggesting a close temporal and spatial link between where the eyes are fixated and where cognitive processing occurs (Just & Carpenter, 1980). According to this assumption, the information being processed corresponds to the current fixation's location, and the fixation's duration reflects the time required for cognitive processing of the visual stimulus. Reading and visual perception studies have extensively validated this concept, demonstrating that eye movements provide a reliable window into allocating attention and underlying cognitive processes. The regulation of visual attention is closely linked to situational working memory capacity and prefrontal cortex (PFC) functions.

Numerous research studies derived from eye position data have studied cognitive processes in healthy adults (Eckstein et al., 2017). For instance, fixations measure the duration of attention directed at a specific location, which indicates both attentional engagement and the time required to process stimuli in that area. This metric has been instrumental in exploring various cognitive functions, such as memory (Hannula et al., 2010), reading and information processing (Rayner, 1998), and problem-solving (Grant & Spivey, 2003). Saccades, the rapid eye movements that transition focus between

fixations, indicate attention shifts, whether deliberate or automatically triggered by external stimuli (Luna et al., 2008).

As our technologies progress, eye tracking hardware and software have improved. Like an EEG, eye tracking can range from 25 to 2000 measurements per second, giving sub-millisecond temporal resolution (Eckstein et al., 2017).

Especially in neurodegenerative diseases, neural pathways and brain regions involved in eye movements can indicate the presence of neurodegeneration, including the cerebrum, brainstem, and cerebellum (Anderson & MacAskill, 2013). For example, studies on Parkinson's disease have shown that eye tracking can be an effective tool for early detection of cognitive decline, as noted by Anderson et al. (Anderson & MacAskill, 2013), and for assessing the progression of both cognitive and motor symptoms, as Brien et al. (Brien et al., 2023) demonstrated. Moreover, a review analyzed 11 studies that used eye tracking to test the sensitivity and specificity for detecting cognitive disorders (Liu et al., 2021). Results showed that eye tracking technologies could detect the decline in cognitive impairment in combination with other neuropsychological testing. Integrating eye tracking with neuromodulation techniques opens new possibilities for understanding the neural basis of neurodegenerative conditions.

Various studies have examined eye movements in neurodegenerative diseases, including Parkinson’s (Pretegiani & Optican, 2017). Eye movements, especially fixations and saccades, can indicate basal ganglia functions, which are essential in movement disorders such as PD. While reflexive saccades are primarily preserved in the early stages of PD, voluntary saccades are more severely affected. This is most likely related to the processing of reflexive saccades that bypass the basal ganglia circuit, while voluntary saccades involve this circuit. These voluntary saccades might explain visual search patterns (Pretegiani & Optican, 2017), giving insights into the disease's stage. Therefore, eye tracking could provide neurodegenerative disease severity, progression, or regression information.

2.3.1. Virtual Reality

Virtual Reality (VR) is an emerging technology that creates innovative prospects for research using 3D, 360 real-world environments. Cognitive abilities like memory and attention are usually tested in paper-and-pencil modalities and are still

the gold standard for neuropsychological research and clinical assessment. However, the adequacy of these testing tools is questioned with an emphasis on ecological validity, meaning the relation between cognitive abilities in laboratory settings and actual everyday cognitive abilities (Pieri et al., 2023). The diagnostic of cognitive abilities was permanently restricted to those laboratory situations (e.g., pencil-and-paper tests); however, eye tracking in virtual reality, spatial navigation, and eye movement parameters, and therefore cognitive abilities, can be studied in real situations in real-time with a more in-depth amount of information about the patient (Clay et al., 2019). Moreover, VR supports the illusion of being present in the digital world. A more natural interaction in an artificial world can provide a safe and more controlled testing tool of human behavior while giving the patient freedom of movement. Research has shown that studying the human eye is more effective in seeing real objects than flat images (Jiang et al., 2024). Therefore, a more realistic world should be used to diagnose diseases. VR can provide higher one-dimensional color rendering than flat images, giving more sensatory needs. A review by Pieri et al. suggested that a novel VR diagnostic tool should implement two criteria: adapting established assessment techniques already used in the paper-and-pencil tests and creating virtual modifications of real-life tasks (2023). However, although multiple tests have been developed to test different cognitive domains, some areas seem more suitable and measurable to be tested in VR (see Figure 3, adapted from Pieri et al., 2023).

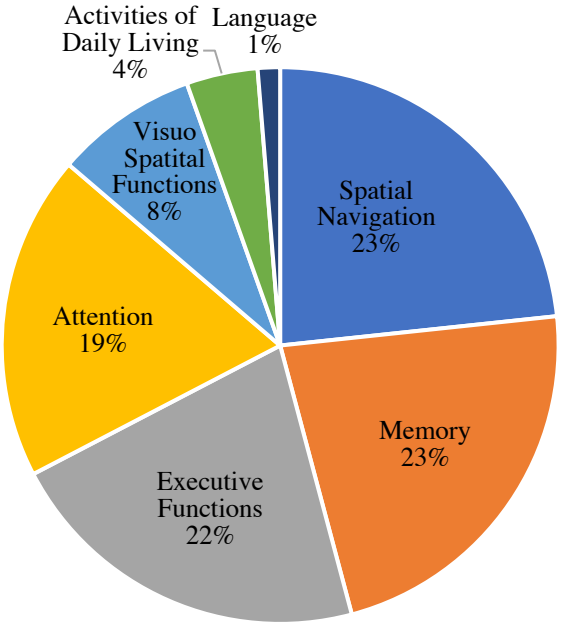

Figure 3. The pie chart shows the percentage of studies conducted in the specific cognitive domains in the years 2000 – 2021, as reviewed in Pieri, Tpso, and Romano (2023). The chart includes research areas such as memory (23%), spatial navigation (23%), executive functions (22%), attention (19%), visuospatial functions (8%), activities of daily living (4%), and language (1%). Some studies addressed multiple cognitive domains. Adapted from Pieri, L., Tosi, G., & Romano, D. (2023). Virtual reality technology in neuropsychological testing: A systematic review. *Journal of neuropsychology, 17(*2), 382–399. .

To assess the diagnostic validity of neuropsychological tests, a test should be able to distinguish between a healthy control group and a clinical group with cognitive impairment. A metanalytic review revealed a moderate to large effect size for the cognitive domains of executive functions, memory, and visuospatial analysis in VR (Neguț et al., 2016), which aligns with the findings by Pieri et al. (2023). These findings highlight the possibility of VR for neuropsychological assessments in either research questions or clinical use and emphasize this new technology as an understudied solution.

3. General Research Questions

The primary objectives of this thesis were to explore the efficacy, safety, and real-world applicability of novel neuromodulation techniques for neurodegenerative diseases. Specifically, Transcranial Pulse Stimulation (TPS) was applied to patients with Alzheimer's Syndrome (AS), and Deep Repetitive Transcranial Magnetic Stimulation (drTMS) was used for patients with Parkinson's Syndrome (PS). Given the innovative nature of these non-invasive brain stimulation methods, an exploratory approach was adopted to evaluate their feasibility and tolerability in a clinical setting. Additionally, eye tracking in a virtual reality (VR) environment was assessed as a potential biomarker to measure treatment effects objectively. This work emphasizes the need to bridge the gap between experimental neuromodulation techniques and their practical integration into real-world scenarios, contributing to the growing knowledge of their potential advantages for individuals suffering from AS or PS.

In Paper I, TPS was studied in AS patients regarding this method's safety and short-term effects. In Paper II, the safety and feasibility of drTMS were examined for various forms of PS. Both indications showed advantages; however, neuropsychological test batteries showed mixed results, indicating improvement in some cognitive abilities, while subjective scores were always superior. In Paper III, eye tracking in VR was added before and after drTMS treatment to investigate eye tracking as a possible biomarker for brain stimulation. Paper I and II combine preliminary results, while Paper III uses an exploratory approach.

The main research questions are as follows:

1) How effective and safe is TPS as a treatment for patients with Alzheimer's Syndrome with different severity levels in terms of cognitive improvement, depressive symptom reduction, and overall tolerability in a clinical setting?
2) What are the effects and safety profiles of drTMS on motor symptoms, depressive symptoms, and cognitive function in patients with Parkinson's Syndrome, and how feasible is its application in real-world clinical settings?
3) Can eye tracking in a VR environment serve as a sensitive biomarker for cognitive and emotional changes in Parkinson's Syndrome patients undergoing drTMS?

4. Methods

4.1. Overview of study design

Papers	Data	Outcome measures
Paper I	Patients with AS (N = 11)	ADAS, ADAS Cog, MMSE, MoCA, NRS
Paper II	Patients with PS (N = 21)	MoCA, BDI-II, BDI-II FS, NRS
Paper III	Patients with PS (N = 10)	MoCA, TUG, BDI-II FS, Fixation duration, longest fixation period, saccade rate, total number of fixations

Table 1. Overview of study design for Paper I, II, and Paper III. AS means Alzheimer's Syndrome, and PS means Parkinson's Syndrome. Outcome measures are Alzheimer Disease Assessment Scale (ADAS), Alzheimer Disease Assessment Scale Cognitive Score (ADAS Cog), Minimental Status Examination (MMSE), Montreal Cognitive Assessment (MoCA), Numeric Rating Scale (NRS), Becks Depression Inventory-II (BDI-II) and its Fast Screen version (BDI-II FS), and Timed Up and Go Test (TUG).

4.2. Participants

Participants were patients from the Hospital Zum Heiligen Geist in Kempen, Germany, from the Department of Neurology and Neurorehabilitation.

For Paper I, patients had to meet the criteria for a least Alzheimer's clinical syndrome, defined as a gradual progressive change in memory function and impairment of the activity of daily living for more than 6 months. Moreover, NIA-AA criteria were used to categorize underlying pathological processes with biomarkers (Jack et al., 2018). Jack et al. (2018) categorize these NIA-AA criteria into three groups: amyloid-beta deposition (A), pathologic tau (T), and neurodegeneration or neuronal injury (N). These biomarkers can be measured using cerebrospinal fluid (CSF) analysis or imaging techniques like PET scans, and they can be used to classify individuals into different categories depending on the presence or absence of these markers. The system uses a combination of these biomarkers to define the stages of Alzheimer's disease:

A+ indicates the presence of amyloid-beta deposition.

T+ indicates the presence of pathologic tau.

N+ indicates evidence of neurodegeneration.

For papers II and III, patients had to meet the criteria for Parkinson's syndrome with a medical refractory main symptom. The 2015 Movement Disorder Society (Postuma et al., 2015) criteria were used for the diagnoses. These included a systematic evaluation of clinical symptoms, supportive features, exclusion criteria, and potential red flags. A diagnosis of PD requires the presence of parkinsonism, characterized by bradykinesia (slowness of movement) in combination with either rest tremor or rigidity. To strengthen the diagnosis, at least two supportive criteria, such as a significant response to Levodopa, Levodopa-induced dyskinesia, rest tremor, hyposmia (reduced sense of smell), or REM sleep behavior disorder, should be present. However, most patients who were treated with drTMS were classified as Parkinson's Syndrome, including MSA, PSP, and CBS. Multiple system atrophy (MSA) is divided into two subtypes: MSA-P, with parkinsonian symptoms, and MSA-C, which involves cerebellar ataxia. MSA often features early autonomic dysfunction, such as urinary incontinence or orthostatic hypotension, and has a poor response to levodopa. Another atypical syndrome, progressive supranuclear palsy (PSP), is marked by early postural instability, frequent falls, and supranuclear gaze palsy, particularly affecting vertical eye movements. PSP also causes axial rigidity, speech difficulties, and swallowing problems. Corticobasal syndrome (CBS) presents with asymmetric motor symptoms, such as rigidity and bradykinesia, combined with cortical features like apraxia, the alien limb phenomenon, or sensory neglect. Unlike PD, CBS often involves dystonia and does not respond to dopaminergic therapy. Imaging studies, including MRI or PET, may show asymmetric cortical atrophy or altered metabolism, helping to distinguish it from other syndromes.

4.3.Transcranial Pulse Stimulation

For the transcranial pulse stimulation, the Neurolith© TPS device from Storz Medical was used, enabling precise neuronavigation through individual 3D T1 isometric voxel MRI scans. The default treatment protocol was set at a frequency of 4 Hz and an energy level of 0.20 mJ/mm^2. The targeted stimulation areas included the bilateral frontal cortex, bilateral lateral parietal cortex, extended precuneus cortex, and bilateral temporal cortex (Figure 4).

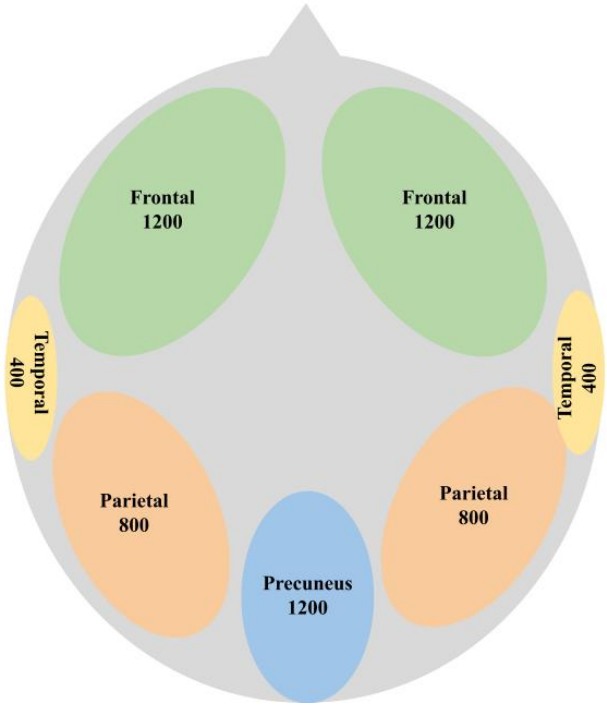

Figure 4. Regions of Interest. A simplified illustration of the human head highlights the regions of interest (ROI) targeted during one stimulation session for Alzheimer's Disease with TPS. In this example, the precuneus received 1200 pulses, the bilateral frontal lobes were stimulated with 1200 pulses each, the bilateral parietal lobes with 800 pulses each, and the bilateral temporal lobes with 400 pulses each. This visualization demonstrates a potential stimulation protocol for key brain areas implicated in Alzheimer's Disease.

The device was calibrated before each treatment session to ensure accurate and effective stimulation. The calibration process began with acquiring MRI scans of each participant's brain. Next, the target areas for stimulation were carefully defined on these MRI images using the device's software. Specific anatomical landmarks were identified to mark the bilateral frontal cortex, bilateral lateral parietal cortex, extended precuneus cortex, and bilateral temporal cortex, ensuring precise targeting during the stimulation sessions. After defining the target areas, the calibration started to match the individual's head orientation and anatomy. The TPS applicator was then placed on the participant's scalp over the predefined target areas, using real-time feedback from the neuronavigation system to ensure accurate alignment.

The treatment protocol varied with either six sessions, each delivering 6,000 pulses over two weeks, with an interval of 48 hours between sessions. In the alternative protocol, 12 sessions were performed with 3,000 pulses administered daily. The applicator was systematically moved across the scalp during each session to ensure that all targeted cortical areas received adequate stimulation.

4.4. Deep Repetitive Transcranial Magnetic Stimulation

The deep repetitive transcranial magnetic stimulation from BrainsWay (Brainsway Inc., Jerusalem, Israel) was used for Papers II and III. The drTMS sessions were conducted with the patient seated in a comfortable position. The BrainsWay helmet, containing the H-coil, was fitted snugly onto the patient's head. Before each session, the motor threshold was determined by locating the region responsible for hand muscle contractions. This is essential to set the intensity of the magnetic pulses relative to the patient's individual neural excitability. The determination of the intensity of the stimulation begins at a low intensity. It gradually increases until a visible muscle twitch (typically in the hand or index finger) is observed. This twitch indicates that the motor cortex has been successfully activated. The lowest intensity producing this consistent motor response is the resting motor threshold. Once identified, the actual treatment is delivered at a percentage of this threshold, for the motor cortex stimulation at 90% of the motor threshold and for the prefrontal cortex stimulation at 100%.

The treatment protocol consisted of sequential stimulation at two different frequencies. First, 1 Hz stimulation was applied to the primary motor cortex (M1), contralateral to the side most affected by motor symptoms (Figure 5A). This low-frequency stimulation lasted for approximately 15 minutes. Immediately after, the coil was moved to target the bilateral prefrontal cortex (PFC), where 10 Hz stimulation was administered for another 15 minutes (Figure 5B). Patients underwent a series of 7-12 drTMS sessions, usually over a 4-week period, with sessions scheduled 3 times per week. Patients were continuously monitored throughout the procedure for any discomfort or adverse effects. Minor side effects, such as transient headaches or facial muscle twitching, were managed by adjusting the coil's position or reducing the stimulation intensity.

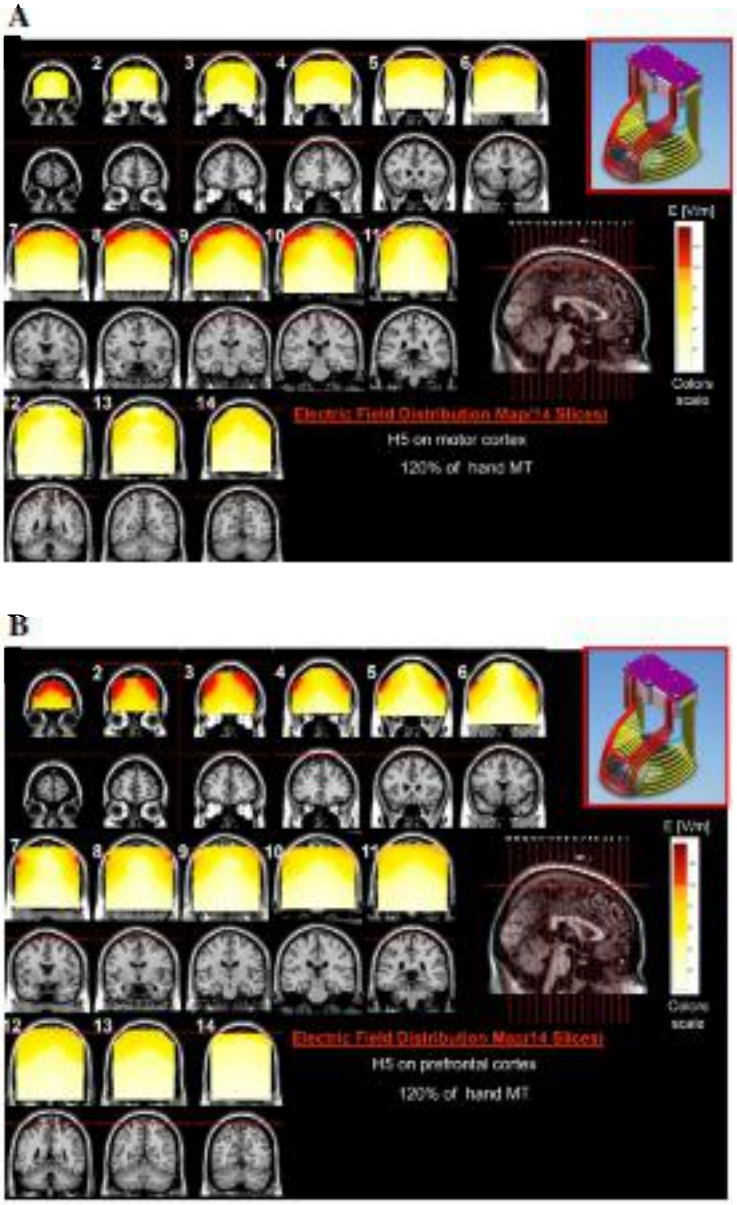

Figure 5. Electric field distribution for the H5 coil used for papers II and III (Hanlon et al., 2023). drTMS was used in a two-step protocol, starting with stimulating the MC and PFC. *A* illustrates the electric field distribution within the brain when positioned over the motor cortex with the H5 coil. These maps illustrate the absolute magnitude of the electric field in each pixel across 14 coronal slices spaced 1 cm apart. The values were adjusted to reflect the average percentage of the maximal stimulator output needed to reach 120% of the hand's resting motor threshold (rMT). Red pixels indicate regions where the electric field magnitude meets or exceeds the neuronal activation threshold, set at 100 V/m. *B* displays the electric field distribution within the brain when positioned over the prefrontal cortex.

4.5. Outcome measurements

4.5.1. Safety

For all papers, the same method was used to measure safety and tolerability. All patients, relatives, and/or caregivers were asked to report any adverse effects they experienced throughout the treatments. A numeric rating scale (NRS) was used, where patients rated the severity of each side effect, including common issues like headaches or discomfort at the stimulation site, from 0 (no effect) to 10 (worst imaginable). This method helped quantify the subjective experience of each patient and allowed for systematic monitoring of side effects over the treatment period.

4.5.2. Neuropsychological Tests

Various neuropsychological tests were used to measure the cognitive and affective effects of the stimulation methods. Trained psychologists conducted all tests.
For Paper I, the Alzheimer's Disease Assessment Scale (ADAS), including the ADAS cognitive score (ADAS Cog) and ADAS affective scores, Mini-Mental Status Examination (MMSE), and Montreal Cognitive Assessment (MoCA) were used. For Paper II, MoCA, Beck Depression Inventory-II (BDI-II), and Beck Depression Inventory—Fast-Screen (BDI-FS) were used. For Paper III, the same tests were used as in Paper II with the addition of the Timed Up and Go Test (TUG). In the following, all neuropsychological tests are presented:

ADAS. The ADAS is a comprehensive tool designed to assess the severity of cognitive dysfunction in individuals with Alzheimer's disease. It is widely used in clinical trials and cognitive assessments for Alzheimer's and related dementias (Verhey et al., 2004). The test has two major components: the ADAS Cog and the non-cognitive portion. The ADAS-Cog subscale evaluates various cognitive domains such as memory, language, attention, and reasoning. The tasks involve word recall, following commands, object and finger naming, and the ability to recognize spoken language. It includes 11 sections with scores ranging from 0 (no impairment) to 70 (severe impairment).
The non-cognitive part evaluates behaviors like mood changes, aggression, or wandering, which are assessed by interview questions asked by the person administering the test.

MMSE. The MMSE is a brief 30-point questionnaire used to screen for cognitive impairment, especially in older adults. It is quick to administer and can provide a

general idea of a person's cognitive status. It's commonly used in dementia evaluations (Ciesielska et al., 2016). It assesses five key areas of cognitive function:

1. Orientation with questions about time and place (e.g., "What is the date today?").
2. Registration: The examiner names three objects, and the patient must repeat them.
3. Attention and Calculation: Tasks like serial sevens (subtracting seven from 100 repeatedly) or spelling "radio" backward.
4. Memory/ Recall: The patient must recall the three words given earlier from the registration task.
5. Language and Executive Functions: Naming two objects, following a three-step command, and copying a simple diagram.

MoCA. The MoCA is a screening tool used to detect mild cognitive impairment and early Alzheimer's disease. It covers many cognitive functions, making it more sensitive than the MMSE (Ciesielska et al., 2016). The test takes about 10-15 minutes. It evaluates several cognitive domains: Visuospatial/executive functions (involves drawing tasks (e.g., clock drawing)), naming (naming three animals in a picture), memory (recalling a list of five words), attention (digit spans and vigilance tasks), language (sentence repetition and verbal fluency), abstraction (identifying similarities between items), orientation (knowing the current date and location).

BDI-II. The BDI-II is a widely used self-report inventory for measuring the severity of depression, which is used in both clinical practice and research (Wang & Gorenstein, 2013). It consists of 21 multiple-choice questions, each rated on a scale from 0 to 3. The questions cover a range of depressive symptoms, including mood, behavior (like sleep disturbances and/or appetite changes), subjective problems in cognition, and physical symptoms (like fatigue and weight changes). The total score determines the severity of depression, ranging from minimal (0-13) to severe (29-63).

BDI-II FS. The BDI-II FS is a shortened version of the BDI-II, designed to assess depressive symptoms quickly (Elben et al., 2021). The fast screen includes seven

items selected from the full BDI-II. These focus on symptoms more related to mood, excluding somatic or physical symptoms that may overlap with medical conditions.

TUG. The TUG test is a simple tool to assess a person's mobility, balance, and fall risk (Ortega-Bastidas et al., 2023). It is often used in older adults or individuals with neurological conditions like Parkinson's disease. For this test, the patient must stand up from a seated position, walk a distance of 3 meters, turn around and walk back, and sit down again while measuring time. Less than 10 seconds is considered normal, while times greater than 12-14 seconds suggest mobility problems and a higher risk of falls.

4.5.3. Eye Tracking

For Paper III, in addition, and for comparison of neuropsychological tests, eye tracking measurements were also assessed before the first stimulation and after the last stimulation (see Figure 6).

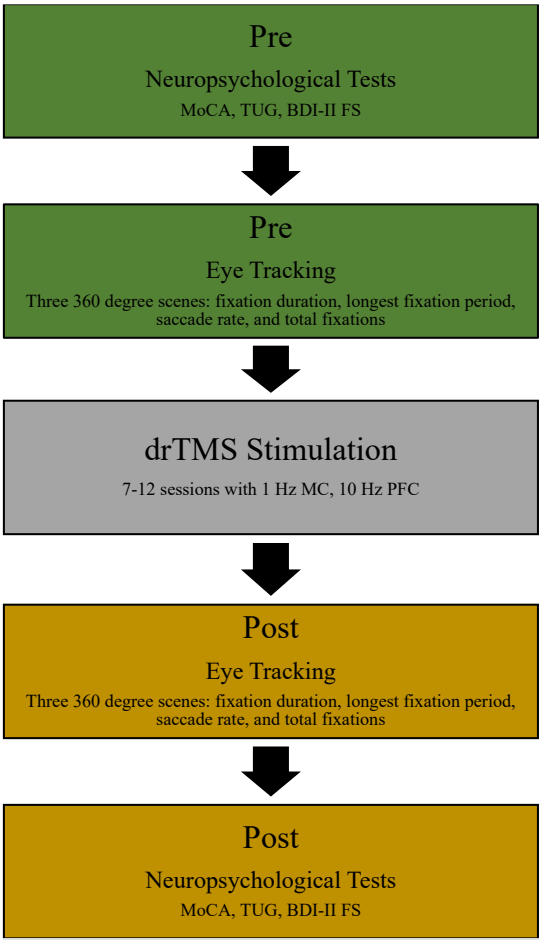

Figure 6. Procedure of Paper III. Before the first stimulation, neuropsychological tests and eye tracking parameters were assessed. The same test battery was used after the last stimulation. Dark green represents procedures before the first stimulation, and dark yellow represents the procedure after the last stimulation (7-12 sessions).

The commercial head-mounted display HTC VIVE Pro Eye VR headset, which has an integrated Tobii eye tracker, was used. Figure 7 shows the HTC Vive with integrated Tobii Eye Tracker, as photographed by the author.

Figure 7. HTC Vive Pro Eye headset with built-in Tobii eye tracker. The eye-tracking cameras and infrared illuminators are positioned around the lenses inside the headset, allowing them to monitor eye movements. *Photo by author*.

The setup required two base stations to track the virtual reality (VR) headset. The VR system operated using Steam VR version 1.14.16. It featured a dual OLED display measuring 3.5 inches diagonally, with a resolution of 1440 x 1600 pixels per eye (resulting in a total resolution of 2880 x 1600 pixels) and a refresh rate of 90 Hz. The field of view was 110°, which is also the range for the integrated eye-tracking system in the HTC VIVE Pro Eye. Tobii Eye Tracking, used within the experiment, had an accuracy ranging from 0.5° to 1.1°. Gaze origin and direction were recorded in a three-dimensional right-handed coordinate system. The experiment was programmed using Unity, and the Tobii XR SDK (Tobii Technology Inc., Sweden) handled the preprocessing of eye-tracking data. This SDK provided a 3D gaze direction vector with normalized gaze coordinates between -1 and 1. Time data and frame sequences were logged via timestamps using HTC's SRanipal SDK. For the scenes shown for the study, an independent developer

created three static 360-degree scenes: a beach, a pier, and a park by a river, chosen for their symmetrical designs and variety of visual stimuli (see Figure 8). Participants sat on a chair; the VR headset was placed on the head, and an eye-tracking calibration was performed. Each scene was shown for one minute, resulting in a total experiment time of three minutes.

Figure 8. Three static scenes used for eye tracking measurements. The first picture shows a park, the second a pier, and the last image a beach (Cont et al., *in review*)

Eye tracking parameters were programmed so that a fixation begins with an eye movement < 30 degrees per second. A "blink" or invalid data interrupts the fixation. At > 30 degrees per second, fixation ends, and a saccade is counted. The direction of movement was not taken into account for the saccade.

4.6. Statistical Analysis

To test whether TPS had an effect in Paper I, neuropsychological measurements were compared before and after the stimulation. For possible side effects, NRS scales were descriptively analyzed. Moreover, a one-sided t-test with an alpha level of 0.05 for significance was used to assess the effect of self-reported symptom intensity and cognitive and affective performance. Furthermore, a one-way Spearman's rank correlation was conducted between the different affected patients (mild, moderate, and severe) using the MMSE scores and changes in cognitive scores after treatment.

For Paper II, the occurrence, frequency, and severity of adverse events (AEs) and adverse device effects (ADEs) were evaluated, and the ADE rate was calculated per patient. Next, correlational analyses were conducted to explore relationships between ADE severity and rate with various personal and stimulation-related factors. Also, the short-term impacts of drTMS on motor and cognitive functions were assessed using one-tailed paired samples t-tests on self-reported symptom intensity and MoCA scores, with significance determined at an alpha level of 0.05.

For Paper III, a Receiver Operating Characteristic (ROC) analysis was conducted to evaluate the discriminative ability of various eye movement parameters. Moreover, Pearson correlations were used to examine the relationship between eye movement parameters and changes in neuropsychological tests. Additionally, paired one-sided t-tests were conducted to assess the difference in eye movements as well as in neuropsychological tests before and after the drTMS treatment.

4.7. Ethics

A registry involving human participants was reviewed and approved by Ärztekammer Nordrhein, Nr. 2021026, as well as collecting eye tracking data (Nr. 2022031). Patients gave written informed consent to the treatment for either TPS or drTMS, for being included in the registry and consenting to eye tracking measurements. Information about the studies was given both verbally and in written form. All data was stored and published anonymously.

5. Summary of Research Papers

5.1. Paper I

Cont, C., Stute, N., Galli, A., Schulte, C., Logmin, K., Trenado, C., & Wojtecki, L. (2022). Retrospective real-world pilot data on transcranial pulse stimulation in mild to severe Alzheimer's patients. Frontiers in neurology, 13, 948204.

This study aimed to evaluate the safety and short-term effects of transcranial pulse stimulation (TPS) as an add-on therapy for patients with mild to severe Alzheimer's syndrome (AS) in a real-world clinical setting. Eleven AS patients underwent six sessions of TPS over two weeks, targeting brain regions implicated in cognitive and emotional functions. Cognitive function was assessed using the ADAS-Cog, ADAS, MMSE, and MoCA scales, along with patient-reported symptom severity, before and after the intervention.

Participants tolerated TPS well, with rare (4% of sessions) adverse effects reported. The results showed significant improvements in ADAS-Cog and ADAS scores, indicating enhanced cognitive performance. These findings suggest a potential benefit of TPS in improving certain cognitive domains in AS patients, though no significant effects were observed on MMSE or MoCA scores. Moreover, depressive symptoms measured with an ADAS subscale also improved. Interestingly, improvements in symptom severity were also noted based on self-reports from patients.

The study concludes that TPS appears to be a promising non-invasive add-on therapy for cognitive and emotional enhancement in AS patients. However, due to the small sample size and lack of a placebo-controlled group, the need for further research with larger cohorts and altered trial designs to confirm these preliminary findings and better understand the therapeutic potential of TPS for Alzheimer's disease is emphasized.

5.2. Paper II

Cont, C., Lehto, A., Stute, N., Galli, A., Schulte, C., Deer, V., Wessler, M., & Wojtecki, L. (2022). Safety of Deep Repetitive Transcranial Magnetic Stimulation (drTMS) against Medical Refractory Symptoms in Parkinson Syndromes: First German Real-World Data with a Specific H5 Coil. Neurology international, 14(4), 1024–1035. This study investigated the safety and feasibility of deep repetitive transcranial magnetic stimulation (drTMS) in patients diagnosed with various Parkinson's Syndromes (PS) and medical refractory symptoms. The study analyzed real-world data from 21 patients treated with drTMS using the H5 coil, which included low-frequency stimulation of the primary motor cortex and high-frequency stimulation of the prefrontal cortex.

The results indicated that drTMS could be safely administered without severe adverse events. The most common side effects included headaches, nausea, and eye discomfort, all of which were transient. The treatment significantly decreased the self-rated severity of main symptoms, especially in older patients with Parkinson's disease (PD). It showed an improvement in depression scores using the Beck Depression Inventory-II (BDI-II). However, no significant effects were observed on cognitive performance as measured by the Montreal Cognitive Assessment (MoCA). The study suggests that while drTMS is a promising therapy for reducing subjective symptom severity and depressive symptoms in PS patients, there remains considerable variability in its effectiveness across individuals.

These findings support the potential of drTMS as a non-invasive add-on treatment for refractory symptoms in Parkinson's. Still, the study also emphasizes the need for further research to understand its effects better, optimize stimulation protocols, and explore long-term benefits. Future studies should involve larger sample sizes and randomized control groups, as well as exploring different stimulation parameters to determine the most effective protocol for various types of PS.

5.3. Paper III

Cont, C., Stute, N., Galli, A., Schulte, C. & Wojtecki, L. (2024) Could Eye Tracking Serve as a Sensitive Biomarker in Parkinson's Syndrome? – An Exploratory Pilot Study of Measurements Before and after Deep Transcranial Magnetic Stimulation. Brain Sciences. *In Review*.

The exploratory pilot study aims to evaluate the potential of eye-tracking parameters and deep repetitive transcranial magnetic stimulation in assessing and managing Parkinson's syndrome. It investigates whether these methods can serve as sensitive diagnostic tools and help improve symptoms such as depression and cognitive impairment in PS patients.

Ten patients with Parkinson's Syndrome underwent eye movement measurements using the HTC Vive Pro Eye VR headset before and after drTMS sessions. drTMS targeted the motor and prefrontal cortex, and neuropsychological assessments (MoCA, TUG, BDI-II FS) were conducted to measure cognitive and motor function and depressive symptoms. Eye movement data were collected on fixation duration, longest fixation period, saccade rate, and total fixations.

ROC analysis showed a moderate ability to differentiate between patient states using eye-tracking data, with small to moderate effect sizes across different parameters. Significant correlations were found between changes in the longest fixation period and cognitive scores, as well as between fixation durations and depressive symptoms. Although no significant differences were found in eye movement parameters post-drTMS, a significant reduction in depression scores was observed. These results suggest that drTMS may help alleviate depressive symptoms in PD, and eye movement parameters could serve as biomarkers for cognitive changes. However, further research with larger samples is necessary.

6. Discussion

This thesis aimed to explore non-invasive neuromodulation in neurodegenerative diseases using clinical follow-up data and proposing eye-tracking as a possible biomarker. More specially, measurements before and after noninvasive neuromodulation (TPS and drTMS) were analyzed in patients with Alzheimer's and Parkinson's Syndrome.

6.1. Summary of main findings

Paper I) Transcranial Pulse Stimulation for Alzheimer patients. First, reported side effects were rare in three out of 75 total sessions (4%), supporting TPS's safety and general tolerability. However, one side effect was ranked as a 10/10 on a self-reported numeric rating scale, highlighting the need for closely monitoring the patient shortly after the stimulation and the need for medical assessments that should be readily available. Stimulation procedures should be conducted under careful medical oversight to ensure patients receive comprehensive care. Second, patients reported noticeable improvements in their primary symptoms following the treatment. Additionally, there was a significant reduction in depressive symptoms, as measured by a self-reported subscale of the ADAS. The results also showed significant cognitive improvements in the total ADAS score and its cognitive subscale, ADAS-Cog, following TPS treatment (see Figure 9).

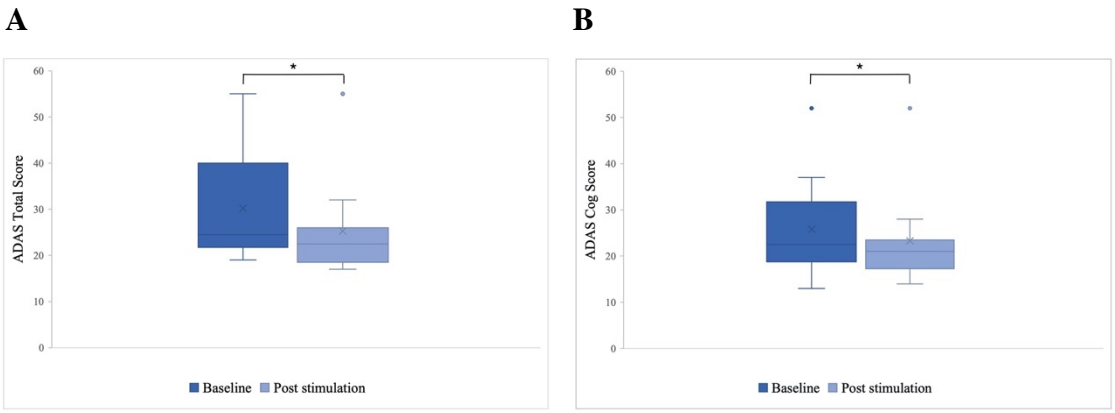

Figure 9. The patient's group score on the Alzheimer Disease Assessment Scale before the first stimulation, highlighted in dark blue, and after the last, highlighted in light blue. *A* shows the total ADAS score, including the cognitive and affective scores, and *B* shows the subscale ADAS Cog, which represents only the cognitive parameters. Both scales show a significant improvement after the stimulation measured by a one-sided t-test. Bars represent the median values for each time point, and the

* indicates statistically significant differences between baseline and post-stimulation scores with $p < 0.05$ (Cont et al., 2022). Reprinted with Permission.

While no significant differences were found in treatment outcomes based on the initial severity of symptoms, there was a slight indication that patients with moderate to severe cognitive impairment experienced more substantial improvements than those with mild impairment. This suggests that TPS may be equally, if not more, beneficial for patients with more advanced stages of AD, though a potential ceiling effect might limit the gains in patients with milder symptoms. In conclusion, TPS might be a promising and safe add-on therapy.

Paper II) Deep repetitive Transcranial Magnetic Stimulation for Parkinson's patients. The findings indicated that drTMS is safe, with AEs occurring in 12.56% of sessions, primarily headaches, which aligns with previous studies. The frequency and severity of AEs varied widely between patients, with no apparent relation to stimulation parameters, suggesting individual factors influence AE experiences. Importantly, no severe or long-lasting adverse effects were observed, further supporting the safety of drTMS.

Analysis of short-term effects showed significant reductions in the severity of the primary symptoms reported by patients. However, the intensity of motor cortex or prefrontal cortex stimulation was not directly associated with this improvement. Interestingly, older patients with Parkinson's disease (PD) reported greater reductions in symptom severity, which may reflect prior findings that drTMS is more effective in patients with more advanced disease stages (see Figure 10).

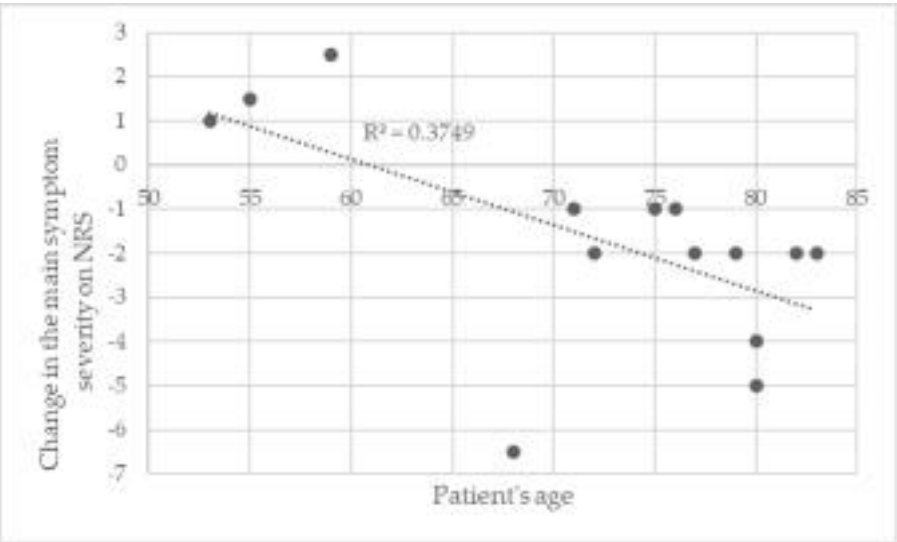

Figure 10. The figure illustrates the correlation between the change in main symptom severity and patients' age after stimulation, as described in Cont, Lehto, et al. (2022). The y-axis shows the change in main symptom severity, measured on a Numerical Rating Scale. Positive values indicate an increase in symptom severity, while negative values indicate improvement. The X-axis represents the patient's age, calculated in years. A correlation was found between the change in main symptom severity and patients' age, illustrated by the dashed line. The older the patient, the more the main symptom was improved after the stimulation (Cont, Lehto, et al., 2022). Reprinted with Permission.

Motor symptom improvements were observed, particularly in gait. Additionally, there was a significant decrease in depression scores, mainly when measured with the BDI-II, likely due to the high-frequency stimulation of the PFC, which has been shown to have antidepressant effects. However, no significant effects on cognition in PD patients were noted. In conclusion, drTMS is generally well-tolerated and safe and could lead to a decrease in subjective symptom severity and depressive symptoms.

Paper III) Eye tracking before and after drTMS in Parkinson's. The ROC analysis indicated that eye movement parameters could moderately differentiate between states. Specifically, measures such as the longest fixation period and saccade rate showed moderate discriminative power. Small to moderate effect sizes were observed across different parameters (mean fixation duration and total number of fixations), suggesting that these metrics reflect some state change. Correlations revealed that changes in the longest fixation period were significantly associated with cognitive improvements measured by the MoCA, while fixation durations were linked to reductions in depressive symptoms. Though no significant differences were found in eye movement parameters before and after drTMS treatment, a significant decrease in depressive symptoms was observed, indicating a potential benefit of drTMS on mood in PD patients. These findings highlight the potential of eye movement metrics, particularly the longest fixation period, as possible biomarkers for cognitive changes. However, further research is needed with larger samples to confirm these results.

6.2. Limitations

6.2.1. Limitations of sample size

A major limitation noted in all three papers is the relatively small sample size, given the clinical nature of the study. Small sample sizes reduce the findings' statistical power, leading to difficulty in detecting meaningful effects or differences in

treatment outcomes. Additionally, small samples can lead to reduced generalizability, limiting the extent to which the results can be applied to the broader population. This can result in increased variability and a higher likelihood that the results show individual differences rather than reflecting consistent treatment effects across diverse participants. Especially for Paper III, which introduced a possible biomarker for neuromodulation, the study design should be tested with a larger sample size to draw a full conclusion. Moreover, small samples often make it challenging to conduct robust subgroup analyses, which is especially relevant for Paper I and the different stages of the patients with Alzheimer's. Therefore, more extensive and comprehensive studies are necessary to confirm the findings for all papers and ensure the reliability of these two interventions.

6.2.2. Limitations of outcome measures

Neuropsychological tests used as outcome measures for these papers, such as the Alzheimer's Disease Assessment Scale (ADAS), the Mini-Mental State Examination (MMSE), and the Montreal Cognitive Assessment (MoCA), also pose limitations. These tests are widely used to assess cognitive function. Still, they often lack the sensitivity required to detect subtle changes in cognitive performance, particularly in the early stages of neurodegenerative diseases. Furthermore, each test focuses on different cognitive domains, which might not fully capture the broad spectrum of cognitive impairments seen in patients with Alzheimer's or Parkinson's disease. Additionally, variability in test administration and patient cooperation, as well as the inherent subjectivity in interpreting results, can further affect the reliability of the data. For example, a study found substantial differences in the ADAS translations used in different countries (Verhey et al., 2004). The difference was most prominent in the verbal memory subtest recall and recognition, which is among the most sensitive to change in the early stages of Alzheimer's Disease.
Though commonly used, these assessments may not always accurately reflect functional improvements following neuromodulation due to issues related to their construct validity and responsiveness.

Eye-tracking technology, such as the Tobii eye tracker integrated into the HTC Vive VR headset that was used for Paper III, presents its own set of limitations in experimental settings. While it provides valuable insights into gaze behavior, its

reliability can be influenced by both technical and user-related factors (Sipatchin et al., 2021). Calibration issues, variable lighting conditions, and individual differences in eye physiology can lead to inconsistent data capture, reducing the precision of measurements. Additionally, eye-tracking data obtained in VR environments may not always translate effectively to real-world settings, potentially limiting the ecological validity of the findings. Furthermore, the relatively short duration of the VR tasks (3 minutes) might not be sufficient to assess long-term cognitive effects or changes in attentional patterns, which are crucial for understanding the impact of neuromodulation on neurodegenerative diseases like Parkinson's.

6.3. Future directions

For the clinical follow-up studies, TPS and drTMS were found to be safe add-on therapies for indicated neurodegenerative diseases. However, up to this date, no sham-controlled study on TPS has been published, which is needed to confirm these results and to rule out a placebo effect. Moreover, most of the studies of TPS used for AD patients used the protocol proposed by the first pilot study (Beisteiner et al., 2020). Therefore, the optimal frequency, number of pulses, stimulation areas, and sessions are unknown (Chen et al., 2023). Moreover, the study is essential since most AD treatments focus on early AD stages, such as the relatively new monoclonal antibodies treatment (Cheng et al., 2023). However, up to this date, there is no effective treatment for AD, especially for moderate to severely impaired patients. In this study, we found that TPS may be equally or more beneficial for patients with more advanced stages of AD (Cont et al., 2022). Future studies need to address the optimal parameters and protocol and include control groups. Moreover, side effects were rare and diminished after one day; however, one patient reported experiencing a high severity, highlighting the need for more systematic monitoring of side effects. Given the limited number of studies on TPS as a novel approach, we plan to establish a multicenter registry focused on the safety of this treatment. The registry will include detailed medical data, such as comorbidities and medication histories, and document all adverse device effects (ADEs) and adverse events (AEs). With this registry, we aim to provide a comprehensive and robust assessment of the safety profile of TPS.

For drTMS, more studies are needed, and the findings in the literature are still mixed. Most TMS research thus far has concentrated on stimulating the motor or premotor

cortex, often employing low-frequency protocols. However, the outcomes have been inconsistent, potentially due to insufficient cumulative TMS dosage or stimulation parameters that may not adequately target the necessary depth and breadth of neural networks in this patient population (Hanlon et al., 2023). Another discussion is using low or high frequency during motor cortex stimulation. While several studies with PD patients showed that high-frequency rTMS improves dyskinesia for several weeks after the treatment (González-García et al., 2011), other studies showed that cortical inhibition is compromised in PD patients. Therefore, low-frequency stimulation should be considered (Cheng et al., 2023). Another proposal was that high-frequency rTMS may induce endogenous dopamine release in the ipsilateral dorsal striatum and may promote the production of dopaminergic neurons. Therefore, the high-frequency protocol of the PFC and the MC should be explored and tested in further studies.

Moreover, the potential use of eye tracking as a biomarker needs to be discussed. In general, no common biomarker is used for neuromodulation in neurodegenerative diseases. A recently published study explored the neurophysiological effects of a single session of TPS in Alzheimer's disease patients using EEG markers like spectral power, coherence, Tsallis entropy, and cross-frequency coupling (Wojtecki et al., 2024). The results showed significant changes in brain activity across frontal, occipital, and temporal regions, suggesting that EEG could serve as a potential biomarker for TPS effects. These findings highlight the importance of objective neurophysiological measures in monitoring and understanding neuromodulation therapies like TPS. While the EEG was recorded directly after stimulation in this study, it is also possible to use closed-loop brain stimulation.

The effect of stimulation on the brain depends not only on the stimulation itself but also on the current state when it is received. Closed-loop brain stimulation involves a two-way interaction between the stimulation device and the brain: the system continuously monitors brain activity to tailor the stimulus in real time (Zrenner & Ziemann, 2024). Specifically, the system analyzes neurophysiological feedback from the brain to adapt the parameters of the next stimulus, ensuring that each adjustment aligns with the brain’s ongoing dynamics. This responsive approach allows the stimulation to be personalized and more effective by directly modulating the therapy based on immediate feedback from the brain.

A review by Hoang et al. (Hoang et al., 2017) stated that while closed-loop brain stimulation holds great promise, its success depends on the availability of an effective biomarker that closely reflects clinical symptoms. Ideally, a biomarker should align with symptom severity, allowing devices to respond directly to it as a stand-in for the symptoms, such as beta band oscillations linked to Parkinson 's-related bradykinesia (Kühn et al., 2006). Such a biomarker would need to reliably signal symptom changes and dynamically mirror the effects of neural stimulation on the brain's circuits (Hoang et al., 2017). However, biomarker signals present challenges, including variability in signal quality depending on sampling location and interference from surrounding brain signals.

Eye Tracking, however, can provide insight into biological processes with higher temporal resolution (Tao et al., 2020). It can be used nonverbally and is a less cognitively demanding method, which is especially crucial for AD patients. Eye tracking provides data about brain function and neural mechanisms and can be used simultaneously with the stimulation. Diagnostic eye tracking can overcome the difficulties of testing in neurodegenerative diseases. Often, patients, for example, patients with Alzheimer's Disease, suffer from significant verbal impairments, which makes it impossible to test cognitive abilities. Furthermore, patients with Parkinson's Disease who suffer from motor impairments and sit in wheelchairs are unable to do a walking test. Eye tracking overcomes those challenges and allows testing for cognitive as well as motor abilities.

For future directions, more evidence-based research is crucial for implementing eye tracking as a possible biomarker for non-invasive neuromodulation in neurodegenerative diseases.

7. Overall conclusion

TPS as an add-on therapy for patients with Alzheimer's Syndrome was well tolerated, with minor side effects. Significant improvements were observed in cognitive function, particularly in ADAS-Cog and ADAS scores, but no notable effects were found on MMSE or MoCA scores. The study suggests TPS may improve cognitive and emotional functions in Alzheimer's patients, but further research with larger samples is necessary to confirm these findings.

DrTMS for Parkinson's Syndrom was well tolerated, with transient side effects such as headaches and nausea. The treatment reduced the self-reported severity of symptoms and improved depression scores, particularly in older Parkinson's patients, though no significant cognitive benefits were observed. The study highlights drTMS as a potential therapy for Parkinson's symptoms but calls for further research with larger samples and varied stimulation protocols.

Eye movement data, including fixation and saccade metrics, were collected before and after drTMS sessions targeting motor and prefrontal regions in patients with Parkinson's syndrome. While no significant changes in eye-tracking parameters were observed post-treatment, there were significant correlations between eye-tracking data and cognitive scores, and depressive symptoms were notably reduced. The findings suggest eye-tracking could be a valuable biomarker for cognitive changes in Parkinson's, but further research is required.

Given that TPS and drTMS are still not standard-of-the-art treatments, placebo-controlled studies with larger sample sizes are needed. However, a big concern is the comparability between studies, consistency, and objectivity in findings. To compare studies, we need a reliable and accurate biomarker to assess the effect of both neuromodulation techniques in neurodegenerative diseases.

This dissertation provides critical insights into the potential of novel neuromodulation techniques as possible add-on treatments for neurodegenerative diseases. By focusing on both the feasibility and safety of these methods in clinical settings, this work highlights the importance of systematically addressing the challenges of integrating innovative technologies into real-world practice. The exploration of eye tracking as a potential biomarker further emphasizes the commitment to advancing objective measures for

evaluating treatment effects, paving the way for more precise and personalized approaches in neuromodulation.

A key contribution of this dissertation is the establishment of a framework for future research, including the development of a multicenter registry to document safety profiles systematically. Additionally, it advocates for dedicated studies exploring eye tracking as a robust tool for measuring cognitive and emotional changes, intending to validate its use as an objective biomarker in neuromodulation. By addressing gaps in current knowledge and proposing strategies for systematic data collection, such as longitudinal placebo-controlled studies and comprehensive registries, this work offers a foundation for translating these promising approaches into robust clinical applications. Ultimately, it underscores the role of innovative, evidence-driven methodologies in shaping the future of therapies for neurodegenerative disorders.

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Paper I-III

Paper I

Retrospective real-world pilot data on transcranial pulse stimulation in mild to severe Alzheimer's patients

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Frontiers in neurology, 2022

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 14 September 2022
DOI 10.3389/fneur.2022.948204

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Dementia and Neurodegenerative Diseases,
a section of the journal Frontiers in Neurology

RECEIVED 19 May 2022
ACCEPTED 15 August 2022
PUBLISHED 14 September 2022

CITATION
Cont C, Stute N, Galli A, Schulte C, Logmin K, Trenado C and Wojtecki L (2022) Retrospective real-world pilot data on transcranial pulse stimulation in mild to severe Alzheimer's patients. *Front. Neurol.* 13:948204. doi: 10.3389/fneur.2022.948204

Retrospective real-world pilot data on transcranial pulse stimulation in mild to severe Alzheimer's patients

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Introduction: Transcranial pulse stimulation (TPS) is a non-invasive neuromodulation therapy that uses short, repetitive shockwaves through a neuro-navigated device. Current research suggests that these pulses lead to a wide range of vascular, metabolic, and neurotrophic changes. This relatively new CE-marked treatment provided first promising results in a clinical pilot study for improving cognition in mild-to-moderate Alzheimer's. Data from other centers is lacking, so here we analyzed safety and pilot real-world short-term results of TPS from the first center in Germany. To gain information about effects in different stages, patients with not only mild but also moderate-to-severe Alzheimer's were analyzed.

Methods: A total of 11 patients were retrospectively examined for cognitive and emotional function before and after the first stimulation series. The effect was assessed using several neuropsychological tests [Alzheimer's Disease Assessment Scale (ADAS), including the ADAS cognitive score (ADAS Cog) and ADAS affective scores, Mini-Mental Status Examination (MMSE), and Montreal Cognitive Assessment (MoCA)] including in comparison between the groups of mild-to-severe patients. Moreover, subjective improvement of symptom severity, potential effects on depressive symptoms, and side effects were analyzed using Numeric Rating Scales (NRS).

Results: Side effects were rare (in 4% of sessions) with moderate subjective severity and only transient. Patients significantly improved in the ADAS and ADAS Cog, while there was no significant effect in MMSE and MoCA. Patients' self-reported symptom severity improved significantly. The depressive symptoms measured in an ADAS subscale also improved significantly. Statistical data analyses revealed no significant correlation of clinical improvement with baseline symptom severity.

Conclusion: TPS might be a safe and promising add-on therapy for Alzheimer's, even for moderate-to-severe patients. More research on long-term effects in patients as well as studies with sham control groups is needed. Moreover, translational research on the mechanisms of action and effects

on cerebral network physiology will be needed to understand this new neuromodulation technique.

KEYWORDS

neuromodulation, transcranial pulse stimulation, Alzheimer's disease (AD), dementia, real-world data

Introduction

The most common type of dementia is Alzheimer's disease (AD), which is defined as a progressive neurodegenerative disease characterized by plaques and neurofibrillary tangles (1). Symptoms of this dementia are characterized mainly by a decline in memory and independence in personal daily activities. Around 50 million patients suffer from this disease, and no treatment is available to prevent or cure AD. Two types of symptomatic drugs are used, including cholinesterase inhibitors and antagonists to N-methyl-d-aspartate (1). One newly FDA-approved drug, called Aducanumab, is one of the first approved medications to target the possible cause of AD. This monoclonal antibody clears out the plaque of amyloid-ß (2). However, more research is needed. As a non-pharmacological treatment, non-invasive brain stimulation (NiBS) has already shown encouraging preliminary results as it integrates the multilevel biological and neurophysiological complexity of AD (3–5). For AD, different brain stimulation techniques are already used: transcranial magnetic stimulation (TMS) as well as electrical stimulation using transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS), the latter with a possible amyloid-clearance effect using gamma frequencies (6). Recent reviews suggest the use of NiBS in AD as promising, yet it should be used in addition to multidisciplinary therapies (5).

One relatively newly CE-marked therapy for AD is transcranial pulse stimulation (TPS) (7). TPS might have some advantages compared to other neuromodulation devices: it is applied highly focal and is possibly not restricted to superficial layers of the brain and, therefore, stimulates up to 8 cm in depth. This non-invasive neuromodulation therapy uses short, single pulses of mechanical waves called shockwaves. The characteristics of shock repetitive waves are pulses that each last about 1 μs. In contrast to ultrasound, the pulse is followed by a tensile wave with a relieving effect of lower amplitude, which lasts for about 4–5 μs. Subsequently, the result is a reciprocal effect with high pressure and low tension, emerging due to the asymmetrical pulse validating both momentums, which do not compensate for each other. The focal energy deposition was tested for its practicability with rats, human skulls, and brain specimens (7). The results of the stimulation of mechanosensitive ion channels manifest themselves in increased metabolism, angiogenesis, and anti-inflammatory effects caused by the release of nitric oxide in the treated areas (7). The stimulation affects vascular growth factors (VEGF), neurogenesis (eNGF and GF-2), and brain-derived neurotrophic factors (BDNF) (8). The first evidence for beneficial clinical effects after a series of six TPS sessions in an uncontrolled pilot study with 35 AD patients discovered an effect on cognitive performance after TPS treatment (7). The cognitive effect was measured using the CERAD test, which significantly improved after treatment, with an increase of total points of about 10.5%. This effect lasted up to a 3-month follow-up period. Additionally, a significant improvement of depressive symptoms after 2–4 weeks of TPS treatment was reported, which suggests TPS as an add-on therapy for depression in AD (10).

However, besides in healthy subjects (9), no AD placebo-controlled trial has been published for TPS. Furthermore, there is a lack of information about the real-world applicability, safety, and effects of other centers outside the pioneer center in Vienna. Therefore, in this paper, we provide a pilot retrospective analysis of the feasibility, safety, and short-term effect of TPS on the cognitive and emotional performance of 11 patients with AD as the first center in Germany. To date, TPS is recommended for mild-to-moderate AD. Furthermore, we investigated patients with severe forms. Specific hypotheses were as follows:

I) TPS is safe and generally well tolerated.
II) TPS improves subjective symptom severity in cognition and mood.
III) TPS shows positive short-term effects on cognitive functions assessed in the objectives test.
IV) TPS is effective in patients with mild, moderate, and severe AD.

Methods

Patients

A consecutive number of 11 TPS-treated patients with Alzheimer's disease from the Department of Neurology and Neurorehabilitation at Hospital zum Heiligen Geist in Kempen, Germany, were examined (nine men, two women, age range 59–77 years, $M = 69.82$). Inclusion and exclusion criteria

for TPS treatment were based on clinical evaluations, MRI, CSF, and EEG. The inclusion criteria was at least Alzheimer's clinical syndrome, which was defined in a gradual progressive change in memory function (using the MMSE as screening tool for severity score) and impairment of activity of daily living for more than 6 months. *In vivo* evidence from CSF and/or MRI scans and/or PET was used for the NIA-AA criteria, which categorizes the underlying pathological processes using biomarkers (11). These biomarkers are grouped into ß amyloid deposition, pathological tau, and neurodegeneration [AT(N)], which can be detected in imaging and biofluids. The biomarker category is shown in Table 1. A total of eight patients were defined as Alzheimer's continuum, seven of them with Alzheimer's disease (AD) and one with Alzheimer's disease and concomitant suspected non-Alzheimer's pathological change. Two patients were simply defined as having Alzheimer's clinical syndrome due to a lack of biomarker and one as having Alzheimer's clinical syndrome with non-Alzheimer's pathological change.

The exclusion criteria for TPS treatment were relevant intracerebral pathologies (including vascular lesions Fazekas $>$ 2) unrelated to Alzheimer's disease, non-compliance with the protocol, blood clotting disorders, oral anticoagulation, corticosteroid treatment in the last 6 weeks, pregnancy, breastfeeding, or epilepsy. Patients signed informed consent to receive the stimulation treatment. The retrospective analysis of all patients treated with TPS was part of the local registry approved by the Ethics Committee of the regional Medical Chamber (Ärztekammer Nordrhein, Nr. 2021026). Patients varied in the severity of cognitive symptoms: four patients with mild, five with moderate, and two with severe impairment (see Table 1).

Materials

Numerous cognitive and affective scores were assessed as part of the standard assessment before the first stimulation and on the last day of the stimulation protocol.

Mini-mental status examination

The MMSE tests orientation, word recall, attention, language abilities, calculations, and visuospatial ability. This test was conducted before the first treatment and after the last treatment, and it was used as a screening tool for the classification of symptom severity. A heterogeneous group that was defined in its symptom severity using the Mini-Mental Status Examination (MMSE) with a range from 2 to 27 (M = 17.64, SD = 7.74) was treated and thus included in the database average of 17.64 (SD = 7.74).

Alzheimer's disease assessment scale

The ADAS total score, including the ADAS cognitive score (ADAS Cog), were used as a parameter for follow-up on the cognition of the patients. The noncognitive subscale includes interviews with the patient on the mood and behavior changes. The cognitive subscale includes 11 tasks with subject-completed tests and observations from the neuropsychologist. The ADAS test takes about 45 min, and the patient's performance is ranked with a score from 0 to 150 by summing the number of errors made on each task. Therefore, the lower the score, the better the performance of the patient. After the last TPS treatment, a parallel version was administered.

Montreal cognitive assessment

The MoCA assesses the same areas as the MMSE but provides more depth and includes additional cognitive functions measured with a clock-drawing test and a trail-making test. A parallel version of this test was administered after the TPS treatment.

Numeric rating scale

The severity of the symptoms as well as side effects were assessed using the NRS. This scale ranges from 0 to 10, with higher numbers indicating higher intensity. Before each treatment, the patient was asked if they experienced any side effects and to subjectively evaluate the severity of their main symptom during the last 24 h. If the patient was not able to answer the question due to the severity of the disease, caregivers were questioned.

Stimulation

For the stimulation, the Neurolith© TPS device from Storz Medical was used, which allows neuronavigation using individual 3D T1 isometric voxel MRI scans (Figure 1). The treatment protocol was 4 Hz, 0.20 mJ/mm^2 by default. The stimulated areas were similar to Beisteiner et al. (7), including the bilateral frontal cortex, bilateral lateral parietal cortex, and extended precuneus cortex (Figure 2A). Yet, the bilateral temporal cortex was also stimulated (Figure 2B). Treatment protocol was either in six sessions with 6,000 pulses over 2 weeks with a $\geq$48 h break between sessions or in 12 sessions with 3,000 pulses every day.

Statistical analysis

Several hypotheses were addressed: For hypothesis I, NRS scales were descriptively analyzed. For hypothesis II, NRS scales were also analyzed using a one sided t-test with an

TABLE 1 Demographics of the patients.

ID	Age	Sex	Cognitive impairment	Biomarker category / diagnosis
1	76	M	Mild	A+T+(N)+ / AD
2	74	M	Severe	A+T+(N)+ / AD
3	77	M	Moderate	Alzheimer's clinical syndrome without biomarkers tested
4	59	M	Moderate	A+T-(N)+[a]
5	60	M	Moderate	A+T+(N)+/ AD
6	65	M	Moderate	A+T+(N)+ / AD
7	61	F	Mild	A+T+(N)+ /AD
8	74	M	Severe	Alzheimer's clinical syndrome without biomarkers tested
9	74	F	Moderate	A+T+(N)+ /AD
10	76	M	Mild	A-T-(N)+[b]
11	72	M	Mild	A+T+(N)+ / AD

Cognitive impairment was defined using the Mini-Mental Status Examination (MMSE): 30–27, no impairment, 26–20, mild impairment, 19–10, moderate impairment, and <10, severe impairment. Diagnostic criteria were assessed according to the NIA-AA criteria. "A" labels biomarker of Aß plaques, "T" labels biomarkers of fibrillar tau, and "N" labels biomarkers of neurodegeneration or neuronal injury (10). Two patients were included with no biomarkers tested. [a]Alzheimer's and concomitant suspected non-Alzheimer's pathological change. [b]Alzheimer's clinical syndrome with non-Alzheimer's pathological change.

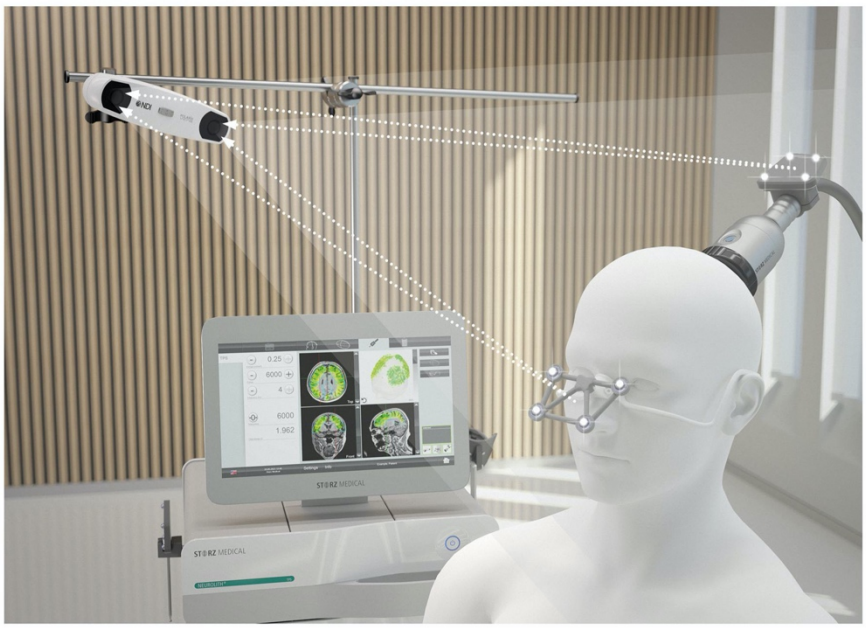

FIGURE 1
TPS system. 3D camera, navigation tracker and headpiece, and TPS handpiece with naviagation tracker. Image Source: Storz Medical.

alpha = 0.05 for significance. Hypothesis III was tested using the changes in ADAS total score, ADAS-Cog, MMSE, and MoCA from the baseline to the follow-up assessment and by computing a one-sided t-test with alpha = 0.05 for significance. For testing hypothesis IV, patients were divided into three groups, namely, mild cognitive impairment ($N = 4$), moderate

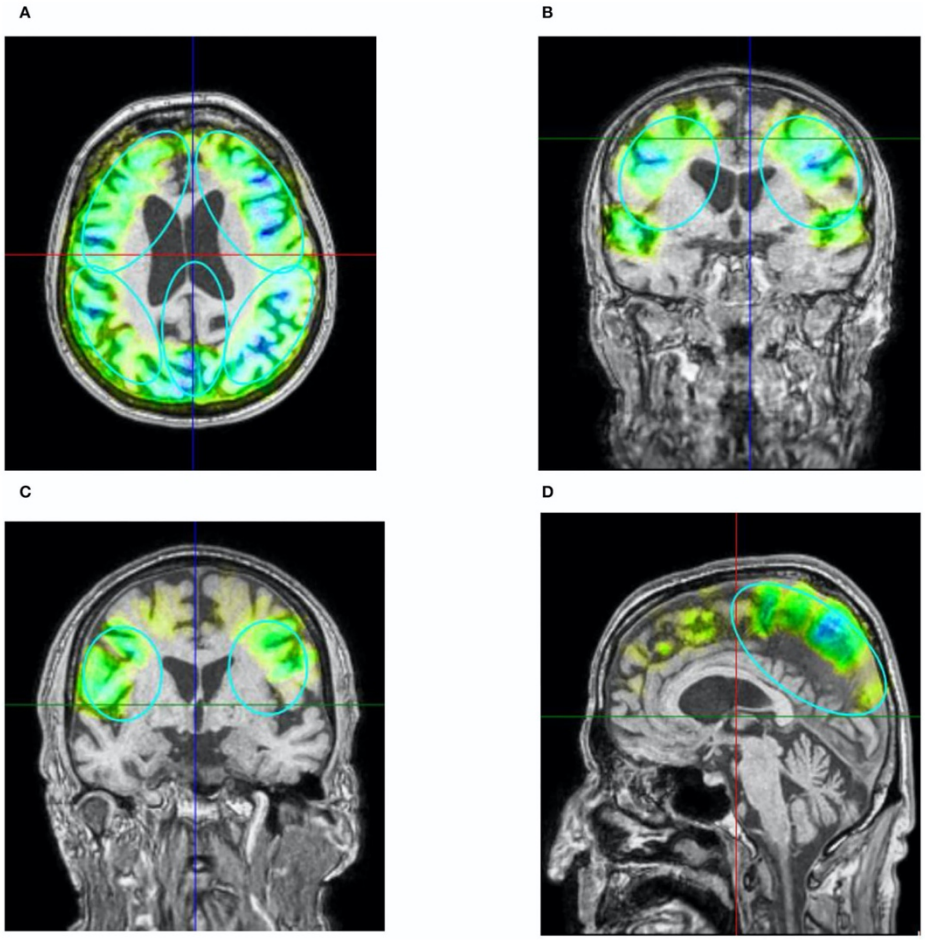

FIGURE 2
(A) Example of axial T1 image with navigated visualization of applied pulse energy. Color code shows quantity of pulses and energy applied in predefined ROIs (turquoise ellipses) frontal, parietal, and precuneus with green indicating low, yellow indicating medium, and blue indicating high energy applied. Image source: Storz Medical. **(B)** Coronar T1 image of patient 10. Besides predefined ROI, pulses were also applied to the temporal cortex perinsular. **(C)** The parietal treatment ROI on coronar T1 from patient 2. **(D)** The treatment of precuneus with 600 pulses visualized on ROI on sagittal T1 image of patient 3. Please note that 3D ROIs are partly superimposed from other 2D plane sections.

cognitive impairment ($N = 5$), and severe cognitive impairment ($N = 2$) using the MMSE cutoff criteria and compared descriptively. Furthermore, a one-way spearman's rank correlation between MMSE scores from the baseline testing and the improvement in each test after stimulation was calculated. For all analyses, SPSS Version 27.0.1.0 and Microsoft Excel were used.

Results

Side effects

Notably, three out of 11 patients (27%) reported side effects in three out of 75 total sessions (4%). These included pain in the jaw (NRS 4/10), feeling of nausea (NRS 7/10), and drowsiness

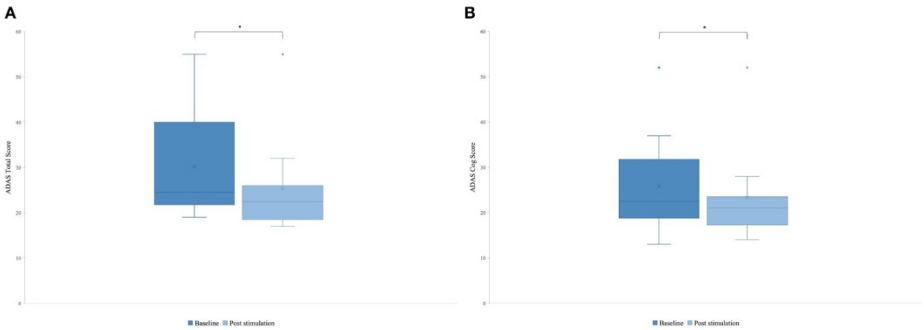

FIGURE 3
Mean of the patient group's score of the Alzheimer's Disease Assessment Scale (ADAS) before the first stimulation (dark blue) and after the last stimulation (light blue). A lower score indicates a better performance. Box plot show destribution of the patients' data. **(A)** ADAS total score. The line represents the median of the group (*baseline* = 24.5, *post-stimulation* = 22.5), and the cross represents the mean scores [*M baseline* = 30.2 (*SD* 11.55), *M post-stimulation* = 25.8 (*SD* 10.71), *$p = 0.01$]. **(B)** ADAS cog score. The line represents the median of the group (*baseline* =22.5, *post-stimulation* = 21), and the cross represents the mean scores [*M baseline* = 25.8 (*SD* 10.77), *M post-stimulation* = 23.3 (*SD* 10.27), *$p = 0.04$].

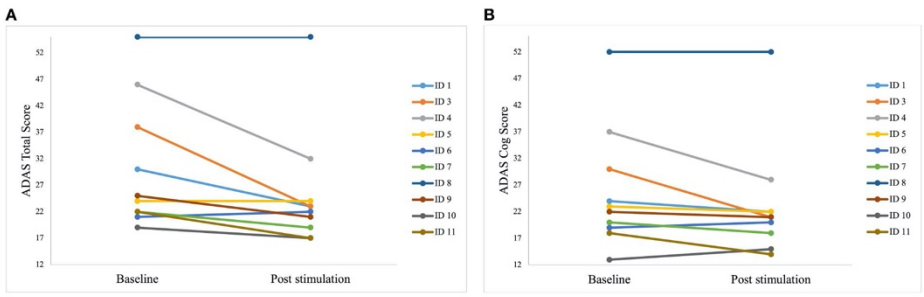

FIGURE 4
Individual test results of the patients in Alzheimer's Disease Assessment Scale (ADAS) before the first stimulation (*baseline*) and after the last stimulation (*post-stimulation*). A lower score indicates a better performance. Each line represents one patient. **(A)** Individual scores of each patient in the ADAS total score. Best improvement was 15 points (*ID 3*). **(B)** Individual scores of each patient in the sub scale ADAS cog score. Best improvement was 14 points (*ID 3* and *ID 4*).

(NRS 10/10). Medical assessments (blood count, blood sugar, and blood pressure) could not reveal the cause of the drowsiness of one patient, and external reasons could not be ruled out. None of the side effects lasted longer than one day, and, therefore, no permanent side effects were observed.

Subjective improvement of symptom severity

Descriptive analysis showed a large individual variation in estimating the improvement. Four of the patients were not able to evaluate the severity of their symptoms. Of the seven patients, six reported an improvement. The mean subjective improvement of the symptom severity ($N = 7$) in NRS was from 5.7 ($SD = 2.3$) to 3.4 ($SD = 3$) [$t_{(5)} = 2.65, p = 0.023$]. Moreover, a one-tailed *t*-test reveal a significant difference in the depressive symptoms in a self-reported subscale of the ADAS test before ($M = 0.7$, $SD = 1.1$) and after stimulation ($M = 0.2$, $SD = 0.4$) [$t_{(8)} = 1.859, p < 0.01$].

Short-term effects on cognitive functions

There was a significant difference in the post-stimulation compared to the baseline in the ADAS total score (Figure 3A)

and in the ADAS Cog score (Figure 3B). Means were for ADAS total score of 30.2 (SD = 11.55) and 25.8 (SD = 10.71) with $t_{(8)} = 2.87$ and $p = 0.01$ and for ADAS Cog 25.8 (SD = 10.77) and 23.3 (SD = 10.27) with $t_{(8)} = 2$, and $p = 0.04$, giving a total improvement in the ADAS total score of 15.76% and in the ADAS Cog score of 8.65%.

Some patients only showed minor improvements, but the best improvement in a patient was 40% (Figure 4).

However, no significant difference was found neither in the MMSE with means of 17.64 (SD = 7.74) and 18 (SD = 7.12) with $t_{(9)} = -0.80$, $p = 0.22$, nor in the MoCA with means of 11.73 (SD = 6.2) and 12.09 [SD = 6.68 with $t_{(9)} = -0.13$, $p = 0.45$].

Effectiveness between groups

The descriptive analysis of the different groups revealed a large improvement in the severe group (MMSE <10) and the moderate group (MMSE 19–10) in the MMSE. When looking at the patients with severe AD (MMSE <10), the mean score of the MMSE improved by 20% (*M improvement* = 0.55). Patients with mild symptoms (MMSE >20) worsened slightly (Table 2). In all tests, the moderate group improved more than the mild cognitive impairment group. In MoCA, the severe group (MMSE<10) worsened, while the moderate group and the mild group improved.

The statistical test of hypothesis IV–if TPS effects differed between mildly, moderate, or severely patients–however, showed no significant correlation between baseline MMSE and changes of cognitive scores after treatment.

Yet the MMSE showed a negative and moderate correlation with $\rho = -0.436$, and the ADAS total score showed a moderate and positive correlation with $\rho = 0.396$. Weak correlations were found in the ADAS Cog with $\rho = 0.12$ and MoCA with $\rho = 0.063$.

The three non-diagnosed AD patients (two patients without biomarkers tested and one with Alzheimer's clinical syndrome with non-diagnosed Alzheimer's pathological change) did show cognitive changes after stimulation in a comparable range as did the AD group ($N = 8$) in most tests. Due to the small sample size, we did not apply statistics. In detail, the mean numbers were as follows:

ADAS total score (non-diagnosed AD group: M improvement = 5.67; AD group: M improvement = 4) and ADAS Cog (non-diagnosed AD group: M improvement = 2.33; AD group: M improvement = 2.2).

MMSE (non-diagnosed AD group: M improvement = 1.67; AD group: worsened slightly, M improvement = −0.13), MoCA (non-diagnosed AD group: M improvement = 1; AD group: M improvement = 0.125).

TABLE 2 Normalized absolute and relative mean change of the scores for the different groups: Mild cognitive impairment, moderate cognitive impairment, and severe cognitive impairment.

	Mild ($N = 4$)	Moderate ($N = 5$)	Severe ($N = 2$)
MMSE	−0.75 (−2.91%)	+1.4 (8.64%)	+0.55 (20%)
ADAS total	+4.25 (18.28%)	+6.4 (20.78%)	-*
ADAS Cog	+1.5 (8%)	+3.8 (14.5%)	-*
MoCA	+0.25 (3.83%)	+1.6 (15.69%)	−1.5 (−60%)

Positive values indicate improvement and negatives values indicate worsening. *For ADAS, the severe cognitive impairment group was N = 1.

Discussion

This research implied a retrospective investigation of TPS in a heterogeneous sample of patients with AD and tested several hypotheses: (I) side effects occurred rarely, which indicates that TPS is a safe and in general well tolerated; (II) the subjective reports of the improvement of the main symptom exposed a significant effect after the treatment. Also, a significant difference was found in the depressive symptoms measured by a self-reported ADAS subscale; (III) these preliminary results display a significant cognitive improvement in patients after TPS treatment in the ADAS total score and the ADAS Cog; and (IV) no significant difference in improvement according to baseline symptom severity was proven. However, between the groups' mild cognitive impairment, moderate cognitive impairment, and severe cognitive impairment, a slight difference was suggested by the data: descriptive analysis of the data indicates a larger improvement in severe and moderately affected patient compared to mildly affected patients in most tests. Our findings suggest that severe and moderately affected patients at least benefit equally from TPS as mildly affected patients, but a ceiling effect in mildly affected patients must be considered. Moreover, two of the patients were identified as having Alzheimer's clinical syndrome without having biomarkers tested, and one showed an Alzheimer's clinical syndrome with non-diagnosed Alzheimer's pathological change. This subgroup's mean scores also improved cognitively after stimulation, indicating that no pathological AD diagnosis is needed to find an improvement after stimulation. Yet, it must be considered that the sample size is not representative enough and the difference in scores between subgroups might be due to the small sample size ($N = 3$) and the fact that the non-diagnosed AD group included two moderate/severe cognitive impairment patients as possible bias. Furthermore, the improvements in cognition varied between the different neuropsychological tests, which could be explained by the different sensitivities of the assessments. The Alzheimer's Disease Assessment Scale (ADAS), which is used in this study, has been conducted to assess the effects of anti-dementia treatments since 1980 (12). It has been developed to evaluate the severity of cognitive and non-cognitive deficits from mild

to severe AD. However, the ADAS test has been criticized for not being able to detect changes at milder stages of Alzheimer's disease (12). The MoCA was developed to detect earlier stages of dementia and is commonly used for mild cognitive impairment (MCI) since it is more challenging than other dementia tests (13). The MMSE was also developed to detect MCI, yet it is less sensitive due to its lack of complexity and absence of executive function items (14). These differences in the sensitivity of the tests between the stages of AD could also explain the different results of this study within the tests. The total patient group showed a significant cognitive improvement in ADAS total and ADAS Cog, but not in the MMSE or MoCA. This is underlined by the fact that the group sample used for this study was more advanced in the symtopms, which the ADAS test is more sensitive to than the other tests.

Additionally, the patients reported a significant improvement in subjective symptom severity. However, the scores showed a large individual variation regarding the change, which might be caused by a placebo effect. This is the first demonstration of improving cognition in patients with severe Alzheimer's disease using TPS; however, there are limitations to be considered. First, there was no sham stimulation as a control condition. Second, the sample size is small. Due to the limitations of a retrospective analysis, data were entered in a clinical database and not collected for research, which caused some missing data from patients in some tests. In conclusion, TPS can be included as an effective and safe add-on treatment for Alzheimer's disease. Even though the first studies (7, 9, 10), as well as our findings, show promising results, more research is needed, including long-term results in patients. Besides larger sham controlled trials, translational research on the mechanisms of action and effects on cerebral network physiology will be needed. Vascular, metabolic, neurotrophic, and (meta-)plasticity effects will need to be investigated (15).

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 registry involving human participants was reviewed and approved by Ärztekammer Nordrhein, Nr. 2021026. Patients gave written informed consent to the treatment and consent to be included in the registry. Registry data and images have been anonymized.

Author contributions

CC, NS, AG, CS, KL, CT, and LW contributed to conception and design of the study. CC, NS, AG, CS, KL, and LW did the data acquisition. NS, AG, CS, and CC organized the database. CC performed the statistical analysis and wrote the first draft of the manuscript. LW wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

This study received funding from the Storz Medical for payment of publication fees and conference costs for data presentation. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Conflict of interest

Author CC received travel fees from Storz Medical. LW has previously received funding grants and institutional support from the German Research Foundation, Hilde-Ulrichs-Stiftung für Parkinsonforschung, and the ParkinsonFonds Germany, BMBF/ERA-NETNEURON, DFG Forschergruppe (FOR1328), Deutsche Parkinson Vereinigung (DPV), Forschungskommission, Medizinische Fakultät, HHU Düsseldorf, UCB; Medtronic, UCB, Teva, Allergan, Merz, Abbvie, Roche, Bial, Merck, Novartis, Desitin, Spectrum. LW owned stock in company BioNTech SE. He is consultant to the following companies or received travel honarium from: TEVA, UCB Schwarz, Desitin, Medtronic, Abbott/Abbvie, MEDA, Boehringer I, Storz Medical, Kyowa Kirin, Guidepoint, Merck, Merz, Synergia, BIAL, Zambon, Sapio Life, STADA, Inomed, Vertanical.

The remaining 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.

Publisher's note

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Paper II

Safety of Deep Repetitive Transcranial Magnetic Stimulation (drTMS) against Medical Refractory Symptoms in Parkinson Syndromes: First German Real-World Data with a Specific H5 Coil

Cont, C., Lehto, A., Stute, N., Galli, A., Schulte, C., Deer, V., Wessler, M., & Wojtecki, L

Neurology international, 2022

neurology international

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Article

Safety of Deep Repetitive Transcranial Magnetic Stimulation (drTMS) against Medical Refractory Symptoms in Parkinson Syndromes: First German Real-World Data with a Specific H5 Coil

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Citation: Cont, C.; Lehto, A.; Stute, N.; Galli, A.; Schulte, C.; Deer, V.; Wessler, M.; Wojtecki, L. Safety of Deep Repetitive Transcranial Magnetic Stimulation (drTMS) against Medical Refractory Symptoms in Parkinson Syndromes: First German Real-World Data with a Specific H5 Coil. *Neurol. Int.* **2022**, *14*, 1024–1035. https://doi.org/10.3390/neurolint14040082

Academic Editor: Diego Santos-García

Received: 25 October 2022
Accepted: 7 December 2022
Published: 12 December 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: So far, deep repetitive transcranial magnetic stimulation (drTMS) has shown promising results as an add-on treatment for Parkinson's disease (PD) but not for non-idiopathic Parkinson Syndromes (PS). We aimed to investigate the safety and feasibility of drTMS application in patients with different Parkinson Syndromes and medical refractory symptoms. Multifaceted real-world data (n = 21) were retrospectively analyzed regarding adverse effects as well as short-term effects of the drTMS treatment on patients' self-rated symptom severity and motor, cognitive, and emotional functions. The drTMS treatment with H5 coil included a sequential 1 Hz primary motor cortex stimulation contralateral to the more-affected body side and a bilateral 10 Hz stimulation of the prefrontal cortex. Overall, drTMS could be safely administered to patients with different PSs and medical refractory symptoms, but large variation was apparent in the rate and severity ratings of the reported adverse event/adverse device effect. The treatment significantly decreased the subjective main symptom severity. This effect was more pronounced in older patients with PD. Furthermore, analysis showed an improvement in depression, but no effect could be established in terms of cognitive performance. drTMS can be safely administered to patients with PS and medical refractory symptoms and can decrease the subjective motor symptom severity and depression.

Keywords: deep transcranial magnetic stimulation; Parkinson Syndrome; adverse events; adverse device effect; real-world data; patients

1. Introduction

Current treatment options of levodopa-refractory symptoms in the later stages of Parkinson's disease (PD) and especially non-idiopathic Parkinson Syndromes (PS) remain suboptimal for several patients. In these patients, the initial beneficial effect of medication is difficult to maintain, and over the years patients often develop debilitating refractory symptoms such as freezing of gait [1,2]. In advanced PD, dopaminergics are a common therapeutic option, while levodopa optimization is critical [2]. Another pharmacological treatment includes the addition of an MAO-B inhibitor; a COMT inhibitor; a DA; or an extended-release levodopa formulation, safinamide, and DA- or Levodopa pumps. Moreover, invasive deep brain stimulation has a high level of evidence in advanced treatment

options [2]. While novel non-dopaminergic disease-modifying pharmacotherapies and cellular therapies are under development [3], non-invasive neurostimulation approaches, such as repetitive transcranial magnetic stimulation (rTMS), have surfaced as an add-on treatment option to pharmacotherapy [4,5].

Deep TMS (drTMS) is a type of rTMS applied with an H-coil that generates less focal magnetic fields than the commonly used figure-of-eight coil [6]. One advantage of the H-coil compared to the figure-of-eight coil is the slower decay of magnetic fields, which therefore reach deeper and stimulate a larger proportion of the brain [6–8]. Furthermore, the H5 coil in particular, designed for PD (Brainsway Inc., Jerusalem, Israel), can be used to bilaterally target motor cortex regions and/or the prefrontal cortex [9–11]. This is in accordance with findings from rTMS studies, which suggest that bilateral stimulation has the highest efficacy on motor symptoms in PD [5]. As an add on therapy for PD, drTMS has been reported to improve motor functions, autonomic and depressive symptoms, and activities of daily living, e.g., [9,10,12]. However, the application of drTMS in the treatment of other Parkinson Syndromes (PS) or patients with medical refractory symptoms has not yet been investigated.

Adverse device effects (ADEs) associated with drTMS have been studied in the context of various indications. The most common potential ADEs of drTMS include headaches, discomfort at the stimulation site, and facial discomfort during stimulation [13]. Various studies assessing the tolerability and effectiveness of drTMS in PD [10–12] have reported the forementioned ADEs and added dizziness, nausea, and sleepiness to the list. All these effects are generally reported as transient and mild. A further risk of rTMS is to trigger a seizure, although this occurs rarely and often in combination with other risk factors such as preexisting epilepsy, changes to the medication dosage, or alcohol consumption [13]. Therefore, drTMS seems to offer a safe and tolerable add-on therapy for PD patients, which encourages further investigation of its utility in treating refractory symptoms in the broader group of PS patients. The stimulation protocol that is most effective for PD is still unclear. While some prefer stimulating with high frequency of the prefrontal cortex (PFC) and low frequency of the motor cortex [12,14], some stimulate with high frequency of both [9,10]. Additionally, an evidence-based guideline on the therapeutic use of rTMS suggests targeting the bilateral motor cortex with high frequency [5]. However, several studies reported an impaired intra-cortical inhabitation in PD patients, which highlights the importance of low-frequency stimulation of the primary motor cortex (for a summary, see the introduction in [12]). Moreover, high-frequency stimulation of the prefrontal cortex has been shown to increase striatal dopamine release, which causes an improvement of motor symptoms (for a summary, see the introduction in [12]). The stimulation protocol for this study used low-frequency stimulation of the motor cortex as well as high-frequency stimulation of the prefrontal cortex.

The current research entails a retrospective analysis of real-world data regarding drTMS application in a clinical context. Namely, existing multifaceted information gathered as part of standard clinical practice in the neurology department of the Hospital zum Heiligen Geist was analyzed according to specific hypotheses. The data came from a heterogeneous sample of consecutive patients with different neurodegenerative forms of PS and medical refractory symptoms, who differed from each other in terms of diagnosis, age, treatment goal, and levels of symptom severity. Taking place in the first center using the H5 coil in Europe, this investigation aimed to examine the safety and feasibility of drTMS treatment with this coil in PS. The hypotheses for this analysis included the following: (i) drTMS application is safe and not associated with severe adverse events or device effects; (ii) drTMS can be successfully applied to patients regardless of their diagnosis, age, impairment profile, and symptom severity; (iii) drTMS application improves motor and cognitive functions as well as patients' subjective symptom severity.

2. Materials and Methods

2.1. Patients

Patient data (n = 21, 13 males, 8 females) from the Hospital zum Heiligen Geist in Kempen, Germany were analyzed. The exclusion criteria for receiving the drTMS treatment included diagnosed epilepsy, pregnancy, presence of an implanted pacemaker or other metal implants, and alcohol and/or drug use on the day before or on the day of the stimulation. The inclusion criteria for the stimulation treatment included (i) a diagnosis of a PS and (ii) refractory hypokinetic or tremor symptoms from levodopa medication or the need for reduction in levodopa dose due to side effects from the medication. The diagnostic criteria for PS were bradykinesia and at least one of the following features: rest tremor, muscular rigidity, or disturbances of posture and gait. The main treatment goal or symptom for each patient was defined before stimulation.

Before the start of the treatment, patients consented to receiving the stimulation treatment according to the CE-mark of the system in a real-world setting. Patient also consented to various data being recorded in an anonymous registry (Ethic Commission Number 2021026 Ärztekammer Nordrhein, 22 February 2021). The criteria for inclusion in the current analysis consisted of the patient's assignment to drTMS treatment with H5 coil according to the manufacturer's (Brainsway Inc., Jerusalem, Israel) treatment protocol for Parkinson's. The majority of the patients had a diagnosed PD (n = 16); others had atypical neurodegenerative forms of PS (e.g., progressive supranuclear palsy, multiple system atrophy, and combined motor neuron disease) or mixed forms of PD with symptomatic PS. Although multiple patients received more than one drTMS treatment cycle, only the data regarding their first treatment cycle were included in the current analysis.

Some characteristics of the sample are summarized in Table 1. The estimated severity of motor impairments for the patients in this sample is indicated by the average score of the motor evaluation of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS III) when off dopaminergic medication (n = 15, M = 37.3, SD = 10.9) and on dopaminergic medication (n = 15, M = 25.9, SD = 16.6). Although some patients formally responded to levodopa in the MDS-UPDRS III, all patients had a main refractory symptom or side effect by medication according to the above-named inclusion criteria for stimulation. Due to the retrospective creation of the data registry, not all patient data are complete.

2.2. Materials

The neuropsychological tests and questionnaires, which were obtained as part of the standard assessment, are outlined below. Before the start of each stimulation session, patients evaluated the severity of their main symptom during the last 24 h on a numeric rating scale (NRS) ranging from 0 to 10, with higher numbers indicating higher intensity. Furthermore, they were asked to report and rate any side effects they had experienced since the last stimulation session on an identical NRS.

Movement Disorder Society (MDS)–sponsored Unified Parkinson's Disease Rating Scale (UPRDS). This instrument is a widely used clinical rating scale for PD [15]. The third subscale, the motor examination (MDS-UPDRS III), is administered as part of the standard assessment to indicate patients' motor abilities and levodopa response before drTMS. For some patients, the scores were available also from the day after completing the treatment.

Montreal Cognitive Assessment (MoCA). This widely used screening test assesses a variety of cognitive functions and has been rated as a recommended cognitive scale for PD [16]. MoCA is administered on the starting day of the drTMS treatment before the first stimulation and a parallel version of this test is administered immediately after the last stimulation.

Beck Depression Inventory-II (BDI-II). This questionnaire is a widely used measure in both research and clinical practice for assessing depression. BDI-II is administered in the same assessment with MoCA.

Beck Depression Inventory—Fast-Screen (BDI-FS). This brief self-report inventory comprising seven items is used to evaluate depression in patients whose behavioral and

somatic symptoms attributable to medical problems may confound diagnosis. This instrument has been found to have good psychometric properties and its use in screening PD patients has been encouraged [17]. The BDI-FS replaced the BDI-II as the depression instrument halfway through the data collection.

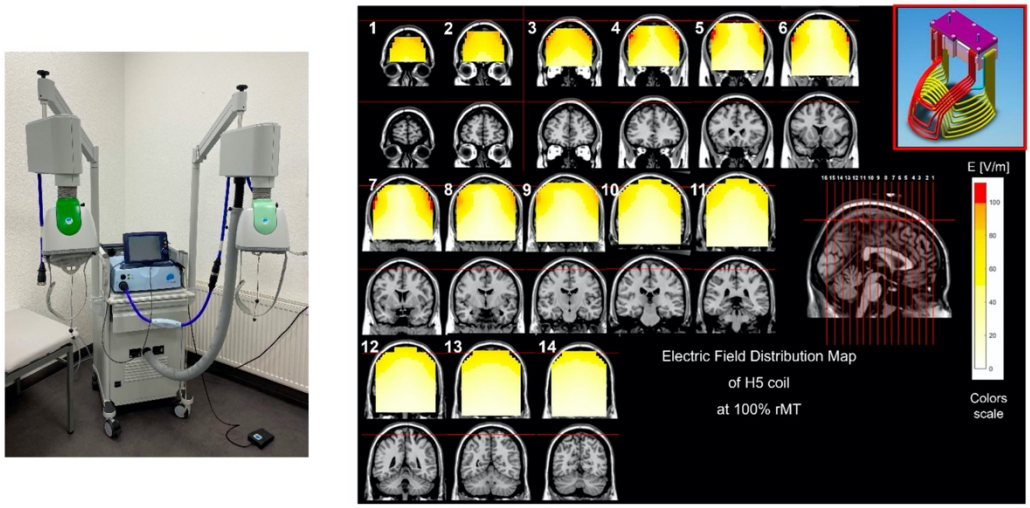
Table 1. This table summarizes various baseline characteristics for all consecutive patients with Parkinson Syndromes that received the stimulation treatment.

Patient	Age	Diagnosis	Sex	Main Symptom	MDS-UPDRS III off [1]	MDS-UPDRS III on [2]
1	79	PD-AR [3]	Male	Rigidity		25
2	82	aPS [4]	Male	Hypokinetic gait	25	
3	41	PD-AR	Male	Rigidity	11	1
4	59	PD	Male	Hypokinetic gait, Freezing	29	9
5	83	PD-AR	Female	Hypokinetic gait	38	18
6	68	PD-E [5]	Male	Tremor	41	18
7	53	PD	Male	Hypokinetic gait	48	8
8	71	PD-D [6]	Male	Tremor		68
9	72	aPS: PSP [7]	Female	Hypokinetic gait	50	40
10	76	PD & sPS [8]	Male	Hypokinetic gait		
11	80	PD-AR	Female	Finger/Hand Hypokinesia	39	
12	55	PD-E	Male	Tremor	31	19
13	80	aPS: CMND [9]	Female	Hypokinetic gait		
14	72	PD & sPS	Male	Hypokinetic gait, Postural instability	40	20
15	77	aPS: probable MSA [10]	Female	Upper extremity Hypokinesia	51	43
16	60	PD-AR	Female	Speech	48	38
17	82	PD-AR	Male	Hypokinetic gait		
18	77	PD-AR	Male	Cognition		
19	75	PD-AR	Male	Hypokinetic gait	29	27
20	76	aPS: possible PSP	Female	Finger/Hand Hypokinesia	44	29
21	80	PD-AR	Female	Hypokinetic gait	36	26

[1] MDS-UPDRS III off = the score of the motor evaluation of the Unified Parkinson's Disease Rating Scale when off medication; [2] MDS-UPDRS III on = the score of the motor evaluation of the Unified Parkinson's Disease Rating Scale when on medication [15]; [3] PD-AR = Parkinson's Disease with predominant symptoms of akinesia and rigidity; [4] aPS = atypical Parkinson's syndrome; [5] PD-E = Parkinson's Disease subtype of the equivalent type; [6] PD-D Parkinson's Disease with Dementia; [7] PSP = Progressive Supranuclear Palsy; [8] sPS = symptomatic Parkinson's syndrome; [9] CMND = Combined Motor Neuron Disease; [10] MSA = multiple system atrophy.

2.3. Stimulation

After screening for contraindications for drTMS and completion of the neuropsychological assessment, the patients received between 5 and 11 treatments on consecutive workdays. The standard protocol of the manufacturer suggests 12 treatments over 4 weeks on an outpatient basis. Due to inpatient treatment, this protocol was adjusted and compressed to a shorter timeframe. Number of sessions was determined by the treating physician after considering individual clinical factors such as effect, side effects, and patients' preferences. A dTMS H5 coil (Brainsway Inc., Jerusalem, Israel) was used with a MagStim stimulator (MagStim Company, Ltd., Whitland, UK, see Figure 1a,b).



(**a**) (**b**)

Figure 1. (**a**) Deep TMS system at the Department of Neurology in Kempen with Brainsway H5 Coil CE-marked for Parkinson's (light green, right) and H2 Coil CE-marked for Alzheimer's (dark green, left); Magstim Stimulator and Air-cooling. (**b**). Distribution of electric field induced by the H5 coil. The electric field distribution was measured in a model of the human head (15 × 13 × 18 cm) filled with physiologic saline solution. The colored field maps indicate the electric field absolute magnitude in each pixel, for 14 coronal slices, 1 cm apart, along with the appropriate MRI coronal images. The H5 coil was placed over the theoretical frontal cortex of the head model and the field in each pixel was measured using a 'pick-up' dipole probe attached to an oscilloscope. The red pixels indicate field magnitude above the threshold for neuronal activation, which was set to 100 V/m based on the average threshold for motor activation of the hand. The field maps are adjusted to obtain 100% of the threshold at a depth of 1.5 cm (Image provided by Brainsway).

The helmet with the H5 coil was positioned above the hemisphere contralateral to the symptom-dominant side. The hotspot of stimulation was determined by moving the helmet on the anterior–posterior and lateral–medial planes and monitoring the muscle activity of the appropriate index finger by using an EMG (resting motor threshold, RMT). When the hotspot with the highest muscle response was located, the position of the helmet was recorded according to the two measurement tapes fastened to the cap from anterior to posterior and from the left side to the right side and the position was used for the M1 stimulation. For the subsequent PFC stimulation, the helmet was centered and moved 6 cm anterior. The stimulation intensity was calculated according to the manufacturer's guidelines (Brainsway Inc., Jerusalem, Israel), and the manufacturer's stimulation protocol was followed. This consisted of a 1 Hz M1 stimulation centered on the more-affected hemisphere and a consequent bilateral 10 Hz PFC stimulation as also used by other studies [12,14]. The M1 stimulation was standard at 90% intensity from the resting motor threshold (RMT) intensity and consisted of 900 pulses applied at 1 s intervals. The PFC stimulation was standard at 100% (RMT) intensity and consisted of 40 trains of 20 pulses applied with 20 s intervals between the trains and amounting to 800 pulses. The calculated stimulation intensity was too uncomfortable for 15 out of 21 patients; so, the intensity was lowered until the patients could tolerate the treatment (see Table 2). Intensity values are stated below in Section 3.1.

Table 2. This table shows the number of sessions as well as the intensity parameters (percent of stimulator output) of M1 and PFC stimulation. Please note that the stimulation intensity was calculated according to the manufacturer's guidelines based on resting motor threshold but was adjusted if it was too uncomfortable (15 out of 21 patients).

Patient	Number of Sessions	Intensity M1	Intensity PFC
1	5	40	45
2	7	35	45
3	1	45	-
4	6	45	50
5	5	54	40
6	5	32	35
7	10	45	40
8	7	45	35
9	6	58	60
10	6	59	65
11	11	49	55
12	11	50	50
13	8	54	40
14	9	45	40
15	10	41	45
16	11	59	50
17	7	45	45
18	10	36	40
19	10	59	50
20	8	-	45
21	9	54	50

2.4. Data Processing and Analysis

All the data were processed using Excel software and several hypotheses were addressed. Firstly, the data were screened for any treatment cancellations. Additionally, the presence, frequency, and severity of AE—respectively, ADEs—were assessed, and the rate of ADE was calculated per patient. Secondly, correlational analyses were carried out between the severity and rate of ADE and various personal and stimulation characteristics. Lastly, the short-term effects of drTMS on motor and cognitive functions were examined through one-tailed paired samples t-tests on the self-rated main symptom intensity and MoCA scores with alpha = 0.05 for significance.

3. Results

3.1. Safety of drTMS and AE/ADEs

The drTMS treatment was stopped in one out of twenty-one patients. The stimulation was stopped shortly after starting the first session due to patient's discomfort. During the stimulation application, the patient's face turned pale and he experienced nausea and stimulus-locked facial muscle contractions, prompting the cancellation of the treatment. No seizure was prompted. This reaction may have been associated with the levodopa test completed by the patient earlier that day. Thus, it is unclear whether it should be accounted as ADE or rather as AE. Besides this exception, other patients tolerated the stimulation treatment well.

A total of 199 stimulation sessions were carried out and an AE was recorded in 25 of them (12.56%). A total of eight different ADEs (thus possibly related to the stimulation) were reported by the patients. The most common ADE was headache, which was reported 15 times in total across the whole treatment by seven patients, followed by nausea and discomfort of the eye region or tearing of the eyes during stimulation, both reported in total four times by three patients. The application site discomfort was reported once by two patients and the rest of the ADEs (shoulder pain, increased rest tremor, tiredness, sleeplessness, intensified drifting to the left when walking) were mentioned once across the treatment by one patient each.

The intensity of AEs was rated subjectively on an NRS ranging from 0 to 10. A total of 10 from 20 patients (50%) reported at least 1 AE throughout the treatment and the severity of reported AEs varied greatly (M = 5.4, SD = 2.6). None of the AEs lasted beyond the day of stimulation. The ratio between the number of sessions where AEs were reported, and the total number of stimulation sessions was calculated per patient. The values of the ratio ranged from 0 to 0.75 (M = 0.2, SD = 0.2), as portrayed in Figure 2, displaying a differing level of tolerability between individuals.

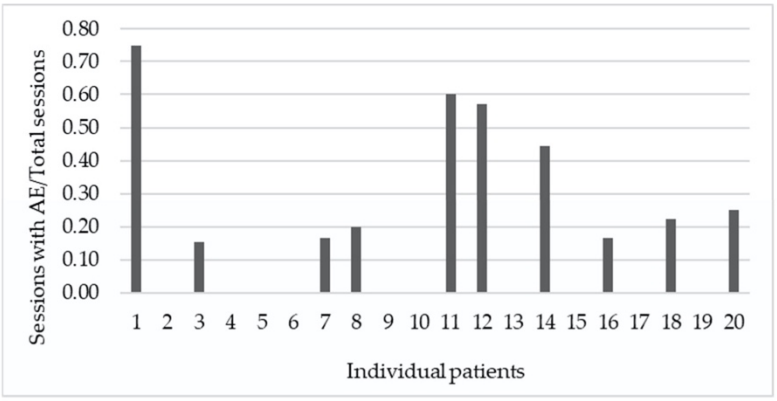


Figure 2. The y-axis represents the ratio between the number of sessions with AEs reported and the total number of stimulation sessions. The x-axis shows the individual patients displaying different levels of tolerability. The patients differed greatly in their reporting of AEs. Whereas some patients had a high ratio signifying frequent AE/ADEs, other patients did not report any AEs.

Numerous correlational analyses were carried out to examine the relationships between the average AE severity and the rate of AEs on one hand and various personal and stimulation characteristics on the other hand. No relationship was found between the severity or rate of AEs and patients' age, their self-reported main symptom severity before treatment, their cognitive level as indicated by their MoCA score before treatment, and their baseline main symptom severity according to the MDS-UPDRS III while on or off medication. In terms of stimulation parameters, the intensity of M1 and PFC stimulation were recorded (M = 47.6, SD = 8.5 and M = 46.3, SD = 7.8, respectively). The severity and rate of AEs were not associated with the M1-stimulation-intensity ($r(36) = 0.06$, $p = 0.792$; $r(36) = 0.04$, $p = 0.885$) nor the PFC-stimulation-intensity ($r(38) = -0.16$, $p = 0.509$; $r(38) = 0.01$, $p = 0.981$).

3.2. The Short-Term Effects on Main Symptom Severity and Cognitive Functions

In general, a large individual variation was apparent in the change in main symptom severity, as evaluated subjectively on the NRS. The decrease in the severity of the patients' main symptom, as evaluated subjectively on the NRS before (M = 7.2, SD = 1.8) and after the drTMS treatment (M = 6.1, SD = 2.2), was significant as evidenced by a one-tailed paired samples t-test ($t(18) = -2.06$, $p = 0.027$), shown in Figure 3. The number of sessions or the intensity of M1- or PFC-stimulation were not related to the change in main symptom severity in our sample ($r(36) = 0.04$, $p = 0.866$; $r(34) = 0.03$, $p = 0.897$; and $r(36) = 0.15$, $p = 0.544$, respectively).

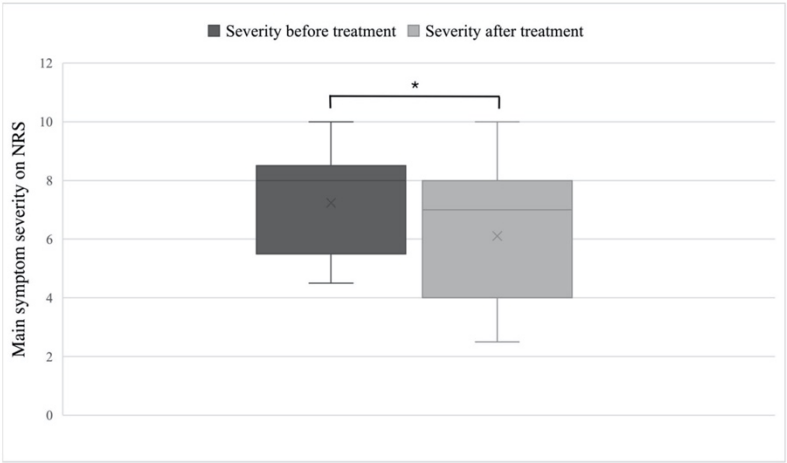


Figure 3. Mean of the patient group's score of the main symptom severity measured on NRS before the treatment (dark grey) and after the treatment (light grey). The line represents the median of the group and the cross the mean scores. Despite the large amount of individual variation, the ratings on the NRS revealed a significant decrease in the severity of the main symptom from before to after the treatment (* $p \leq 0.05$).

The most common main symptom was hypokinetic gait, reported by 11 patients. The descriptive analysis of this subgroup revealed a large individual variation regarding change in the main symptom severity from before (M = 7.2, SD = 2.1) to after the drTMS treatment (M = 6.7, SD = 2.2).

The post-treatment on-medication MDS-UPDRS III scores were available for six PS patients and varied greatly (M = 34.5, SD = 19.2). Change from the baseline score could be calculated for four patients (Pre M = 31.8, SD = 9.4; Post M = 28.3, SD = 11). The descriptive analysis of depression symptoms as measured by BDI-II or BDI-FS before treatment (M = 10.6, SD = 7.0 and M = 2.3, SD = 2.3) and afterwards (M = 3.6, SD = 3.8 and M = 1.5, SD = 1.2) revealed a decrease or a maintenance in the reported symptoms in 10 from 11 PS patients. A one-tailed paired samples t-test revealed a significance difference before and after treatment in BDI-II ($t(3) = -3.8$, $p = 0.015$) but no significant difference in BDI-FS ($t(4) = 1.2$, $p = 0.145$). An overview is shown in Table 3.

Table 3. This table shows the mean scores, standard deviations, and significance level (* $p \leq 0.05$) of the assessments that were used. A significant effect was shown in the depressive score using the BDI-II questionnaire and in the subjective main symptom using the NRS. A trend for improvement can be seen in the UPDRS ON and BDI-FS.

Scale	Pre Mean (SD)	Post Mean (SD)	*p*
MDS-UPDRS III (ON)	31.8 (9.4)	28.3 (11)	0.115
Main Symptom Severity NRS	7.2 (1.8)	6.1 (2.2)	0.027 *
MoCA	24.7 (6.2)	24 (5.6)	0.274
BDI-II	10.6 (7)	3.6 (3.8)	0.015 *
BDI-FS	2.3 (2.3)	1.5 (1.2)	0.145

Some further analyses were conducted with available data from PD patients. No effect of drTMS on cognition as tested with MoCA before (M = 24.7, SD = 6.2) and after (M = 24.0, SD = 5.6) the treatment was found ($t(13) = -0.62$, $p = 0.274$). Moreover, changes in MoCA scores were not related to age, number of stimulation sessions, nor to changes in

main symptom severity. Lastly, a correlation was found between the change in the main symptom severity and patients' age ($r(26) = -0.61$, $p = 0.020$), depicted in Figure 4.

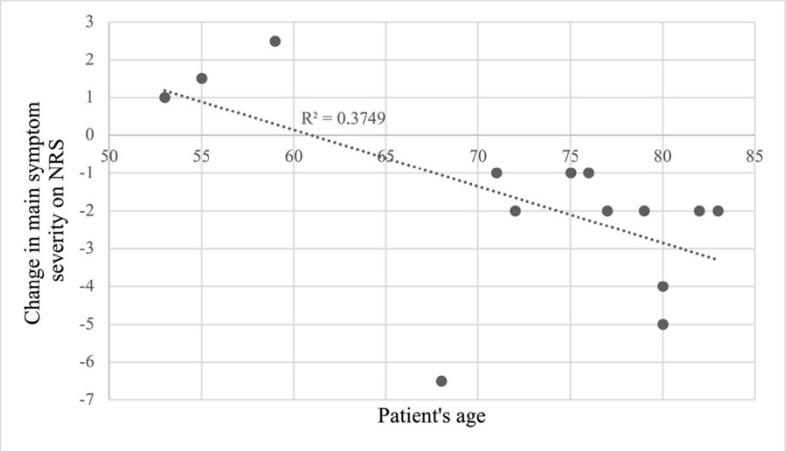


Figure 4. A correlation between the change in main symptom severity and patients' age. The decrease in the main symptom severity was greater for older patients, whereas the younger patients did not seem to benefit from the treatment to the same degree and some even reported a worsening of symptom severity.

4. Discussion

The current research entailed the first retrospective real-world data analysis of the application of drTMS in a heterogeneous sample of patients with different forms of PS and medical refractory symptoms in a hospital setting. The study aimed to evaluate the safety and feasibility of this add-on therapy using the H5 coil for this patient population as well as to explore the short-term effects regarding subjective refractory symptom relief, motor and cognitive functions, and depression. We found that drTMS could be safely administered. AEs were recorded in 12.56% of sessions, with the most common AE reported as a headache, which has to be accounted as ADE. This shows that the profile of recorded AEs was congruent with previous literature, e.g., [11–13,18]. Both the frequency and the severity ratings of AEs varied largely from patient to patient and were not related to the examined personal and stimulation parameters. Therefore, the experience of AEs seems to be related to still uncovered personal factors. In summary, the number of AE is not low, but no SAE, long-lasting, or severe events occurred. Thus, drTMS is considered as safe.

The analysis of short-term treatment effects revealed a large individual variation regarding its benefits. The decrease in subjective main symptom severity was significant; however, the intensities of M1- or PFC-stimulation were not related to this improvement. Interestingly, PD patients' age was correlated with the change in main symptom severity, indicating that older patients with PD reported larger decreases in symptom severity. This finding may reflect the previously reported results of drTMS being more beneficial for patients with higher MDS-UPDRS III scores and longer disease durations [11] since these variables could not be included in the current analysis. Moreover, a trend for improvement in motor symptoms could be demonstrated by an improvement in the mean score of the UPDRS-III ON scale. As the main symptom was gait hypokinesia, this symptom could have been underrepresented in the UPDRS-III and, thus, not sensitive enough to pick up treatment effect. A more walking-related score could have been more helpful. Still, the effect on subjective rating in walking abilities can be due to activating stimulation of the PFC in a sense of improvement in the executive control of walking.

Lastly, the analysis illustrated a significant decrease in depression scores for most PS patients using the BDI-II questionnaire but no effect of drTMS was found on cognition in PD patients. No significant improvement, but a trend of improvement, of the depressive symptom was found when using the BDI-FS scale, which could be explained by the shortness of the scale compared to the BDI-II questionnaire. This improvement of depressive symptoms could be due to the high-frequency stimulation of the prefrontal cortex, which has shown to achieve antidepressive effects [14].

This research offers numerous new insights. Firstly, it supports the safety and feasibility of utilizing drTMS treatment in a hospital setting for patients with different forms of PS and refractory symptoms. Secondly, it indicates a benefit of drTMS on refractory symptoms (especially hypokinesia/freezing of gait), particularly in older PD patients. Thirdly, it offers validation for the intense treatment schedule of stimulating on consecutive workdays, which is better suited for a hospital stay than previously reported schedules (e.g., [9–11]). However, the number of sessions needed are hard to conclude from our data. We suggest to start with five consecutive workdays when an inpatient protocol is chosen and then to perform an interim clinical examination. Another remaining question is the duration of the treatment effect. In our study, we found an immediate improvement in motor and depressive symptoms as well as in the subjective refractory symptom relief. However, the duration of that effect is still unclear. A study with a follow-up session 30 days after drTMS treatment showed a remaining significant improvement, suggesting that the treatment effect of the stimulation could last for numerous weeks [14]. Multiple further points of interest, however, remain to be explored. The most effective drTMS protocol, for instance, is still undetermined. Despite the successful use of low-frequency M1 stimulation in this and some other drTMS studies (e.g., [12]), various rTMS studies [5] and a recent drTMS study [9] have demonstrated a beneficial effect of high-frequency M1 stimulation. Moreover, more extensive investigation is needed to determine which types of PS besides PD benefit from drTMS the most.

The current findings must be considered while keeping in mind the retrospective nature of this research and the limitations associated with that. Firstly, due to the open label nature of the study and the lack of a control group comparison, the extent of a placebo effect on the patient cannot be determined. As the raters of the study were not blinded, a placebo effect on the raters also cannot be ruled out. Secondly, the small sample size and partly incomplete data limited the nature and strength of possible conclusions. Thirdly, the analyzed sample of patients was heterogenous in some respects while similar in other aspects such as high cognitive performance and low depression symptoms, which may have led to a ceiling effect. The reliance on self-reported measures and the restricted availability of an objective measure for motor functions must be taken into consideration. Future studies should investigate the number of sessions needed for an effect. This study showed that the subjective self-reported symptom severity improved, yet neuropsychological tests failed to assess this improvement. Future studies should reevaluate the assessments and add a more sensitive screening tool.

5. Conclusions

This retrospective analysis found the drTMS treatment to be generally well-tolerated by patients with different forms of PS and medical refractory symptoms. The treatment led to a decrease in self-rated symptom severity, especially in older PD patients, and in depressive symptoms.

Author Contributions: Conceptualization, A.L. and L.W.; methodology, A.L. and L.W.; formal analysis, A.L. and C.C.; investigation, C.C., A.L., N.S., A.G., C.S., V.D. and M.W.; resources, L.W.; writing—original draft preparation, A.L.; writing—review and editing, C.C., N.S. and L.W.; visualization, A.L., L.W. and C.C.; supervision, L.W.; project administration, L.W.; funding acquisition, L.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Hilde-Ulrichs-Stiftung für Parkinsonforschung to L.W. from 14 November 2018.

Institutional Review Board Statement: The registry involving human participants was reviewed and approved by Ärztekammer Nordrhein, Nr. 2021026.

Informed Consent Statement: Patients gave written informed consent to the treatment and consent to be included in the registry. Registry data and images have been anonymized.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The data are not publicly available due to privacy and ethical restrictions.

Conflicts of Interest: Author L.W. has previously received funding grants from the German Research Foundation (DFG/BMBF), the Parkinson Fonds Germany and Deutsche Parkinson Vereinigung. Author L.W. is consultant to or received honoraria for lecture or boards or travel payments from the following companies: Kyowa Kirin, Bial Gmbh, STADA Arzneimittel AG, Zambon GmbH, Storz Medical AG, Sapio Life Gmbh, Synergia, Dialectica, Merck KGaA, Medtronic Inc. AbbVie Deutschland GmbH and Co KG, Abbott, Meda AB, Teva Pharmaceuticals Ltd., Boehringer Ingelheim Pharma GmbH and Co KG, Cephalon, Pfizer, Solvay Pharma, Inomed Medizintechnik GmbH, Mag & More GmbH, Alcimed, Esteve Pharma, Vertanical Phama, Angilini Pharma.

Abbreviations

PD	Parkinson's disease
PS	Parkinson Syndromes
AE	Adverse event
ADE	Adverse device effect
drTMS	deep repetitive transcranial magnetic stimulation
rTMS	repetitive transcranial magnetic stimulation
PFC	Prefrontal cortex
PD-AR	Parkinson's Disease with predominant symptoms of akinesia and rigidity
aPS	Atypical Parkinson's syndrome
PD-E	Parkinson's Disease subtype of the equivalent type
PD-D	Parkinson's Disease with Dementia
PSP	Progressive Supranuclear Palsy
sPS	symptomatic Parkinson's syndrome
CMND	Combined Motor Neuron Disease
MSA	multiple system atrophy
MDS-UPDRS	Movement Disorder Society sponsored Unified Parkinson's Disease Rating Scale
NRS	Numeric rating scale
MoCA	Montreal Cognitive Assessment
BDI-II	Beck Depression Inventory-II
BDI-FS	Beck Depression Inventory—Fast-Screen

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Paper III

Could Eye Tracking Serve as a Sensitive Biomarker in Parkinson's Syndrome? – An Exploratory Pilot Study of Measurements Before and after Deep Transcranial Magnetic Stimulation.

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Brain Sciences. In Review.

Article

Could Eye Tracking Serve as a Sensitive Biomarker in Parkinson's Syndrome? – An Exploratory Pilot Study of Measurements Before and after Deep Transcranial Magnetic Stimulation

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Abstract: Background/Objectives Neurodegenerative diseases such as Parkinson's disease (PD) are becoming increasingly prevalent, necessitating diverse treatment options to manage symptoms. The effectiveness of these treatments depends on accurate and sensitive diagnostic methods. This exploratory pilot study explores the use of eye tracking and deep transcranial magnetic stimulation (dTMS) to enhance PD assessment. **Methods**: We used the HTC Vive Pro Eye VR headset with Tobii eye tracker to measure eye movements in 10 Parkinson syndrome patients while viewing three 360-dergree scenes. Eye movements were recorded pre- and post-dTMS, focusing on fixation duration, longest fixation period, saccade rate, and total fixations. Neuropsychological assessments (MoCA, TUG, BDI) were conducted before and after stimulation. dTMS was performed using the Brainsway device with the H5 helmet, targeting the motor cortex (1 Hz) and the prefrontal cortex (10 Hz) for 7-12 sessions. **Results:** ROC analysis indicated a moderate ability to differentiate between states using eye movement parameters. Significant correlations were found between changes in the longest fixation period and MoCA scores ($r = 0.65$, $p = .025$), and between fixation durations and BDI scores ($r = -0.55$, $p = .043$). Paired t-tests showed no significant differences in eye movement parameters, but BDI scores significantly reduced post-dTMS ($t(5) = 2.57$, $p = .049$). **Conclusions**: dTMS may positively influence depressive symptoms in PD, and eye movement parameters, particularly the longest fixation period, could serve as biomarkers for cognitive changes. Further research with larger samples is needed to validate these findings and clarify the diagnostic utility of eye movements in PD.

Keywords: Parkinson's Disease, Eye Tracking, Deep Transcranial Magnetic Stimulation, Biomarker, Neuromodulation

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

1. Introduction

Neurodegenerative diseases such as Parkinson's disease are becoming increasingly prevalent, necessitating a range of treatment options to manage their symptoms [1]. However, the effectiveness of these treatments hinges on the availability of accurate and sensitive diagnostic methods to measure their impact. This study aims to explore the use of

advanced diagnostic tools, such as eye tracking for the measuring the effect of neuromodulation techniques such as deep transcranial magnetic stimulation (dTMS). Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a range of motor symptoms, including bradykinesia, rigidity, tremor, and postural instability, as well as non-motor symptoms such as cognitive impairment, mood disorders, and autonomic dysfunction [2]. Traditional therapeutic approaches include pharmacological treatments, primarily using dopaminergic agents, and surgical interventions such as deep brain stimulation [DBS; 3, 4]. However, these treatments often have limitations and side effects, prompting the exploration of non-invasive neuromodulation techniques such as transcranial magnetic stimulation [TMS; 5, 6].

One specific type of TMS is deep transcranial magnet stimulation (dTMS), which involves using magnetic fields to stimulate deeper brain structures, has shown promise in various neurological and psychiatric disorders, including PD. A study by Shirota et al. [7] demonstrated that dTMS could modulate cortical excitability and improve motor symptoms in PD patients. Another study by Fregni et al. [6] indicated that dTMS targeting the motor cortex could lead to significant improvements in motor performance and quality of life. Recently, we published the first real-world German data and demonstrated that drTMS is a safe intervention for managing resistant Parkinson symptoms [8]. Moreover, we found that drTMS had a positive impact on depressive symptoms, indicating a potential benefit for mood improvement in patients undergoing this treatment. Low-frequency transcranial magnetic stimulation (TMS) at 1 Hz targeting the motor cortex has been studied for its potential to improve motor symptoms in Parkinson's disease (PD). For instance, Lefaucheur et al. [9]. found that low-frequency rTMS of the motor cortex could improve motor symptoms in PD patients by reducing cortical excitability. This was further supported by the study from Pal et al. [10] which showed significant improvements in motor performance following low-frequency stimulation. High-frequency TMS at 10 Hz targeting the prefrontal cortex has shown potential benefits in treating non-motor symptoms of PD, such as depression and cognitive impairment. For example, Benninger et al. [11] conducted a randomized controlled trial demonstrating that high-frequency rTMS over the prefrontal cortex significantly improved mood symptoms in PD patients. Similarly, studies [7, 12] have shown that high-frequency TMS can enhance cognitive functions and executive control in PD patients, providing evidence of its efficacy in treating non-motor symptoms.

Eye tracking provides objective and quantitative measurements of eye movements, making it a sensitive tool for detecting subtle oculomotor abnormalities in neurodegenerative diseases, such as PD. Studies have shown that eye tracking can reveal early signs of cognitive and motor impairments, which might not be detected through traditional clinical assessments. Multiple brain regions, including the fronto-insular cortex, anterior cingulate cortex, supplementary motor area, superior colliculi, and thalamus, have been shown to be activated during fixation tasks [13]. Additionally, the bilateral dorsolateral prefrontal cortex is associated with fixation durations [14]. Executive function engages various cortical and subcortical areas, which are involved in tasks such as saccades, visual searching, and social cognition tasks. Therefore, eye tracking can be used to measure the bridge between behavior, brain function, and neural

mechanisms. Ansons et al. [15] demonstrated that eye tracking could detect early cognitive decline in PD patients, while Brien et al. [16] showed that it could identify stating and classification of cognitive and motor dysfunction in PD. Eye tracking has emerged as a valuable tool for assessing oculomotor function in PD. Studies have shown that PD patients exhibit various eye movement abnormalities, including increased fixation duration, reduced saccade frequency, and impaired smooth pursuit. Uc et al. [17] reported that PD patients had longer fixation durations and fewer saccades compared to healthy controls. Similarly, Mosimann et al. [18] found that PD patients demonstrated impaired smooth pursuit and increased latency in saccades.

Combining TMS and eye tracking offers a novel approach to understanding the neural mechanisms underlying oculomotor dysfunction in PD. A study by Mano et al [19] indicated that rTMS could modulate eye movement control and improve oculomotor performance in PD patients. These findings suggest that TMS, particularly when combined with eye tracking, could serve as an effective therapeutic and diagnostic tool in PD. Especially free exploration in eye tracking, where participants are allowed to view visual stimuli in a natural and unrestricted manner, is crucial as it mimics real-world visual behavior. Unlike controlled tasks or paper-pencil tests, free exploration captures spontaneous eye movements and provides insights into how patients interact with their environment. This approach is particularly relevant for PD patients, whose real-world visual and cognitive challenges might not be fully captured in structured test settings. Tatler et al. [20] have emphasized the importance of naturalistic eye movement recordings in understanding visual attention and cognitive processes.

The present study aimed to evaluate the effectiveness of deep transcranial magnetic stimulation (dTMS) in influencing eye movement parameters and neuropsychological test scores among Parkinson's Syndrome (PS) patients. Additionally, we sought to determine whether eye movement parameters could serve as sensitive biomarkers for cognitive and emotional changes in this population. The following hypotheses were tested:

I) Eye tracking in Virtual Reality serves as a sensitive measurement for Parkinson’s Syndrome

II) Changes in Neuropsychological Tests correlate with Changes in Eye Tracking Measurements

III) There is a significant Difference in Eye Movements as Well as Neuropsychological Tests Before and After Stimulation

2. Materials and Methods

Participants

We analyzed data from 10 patients (8 males, 2 females) with various forms of Parkinson’s syndrome at the Hospital zum Heiligen Geist in Kempen, Germany (see Table 1). Exclusion criteria for receiving deep repetitive transcranial magnetic stimulation (drTMS) included diagnosed epilepsy, pregnancy, presence of an implanted pacemaker or other metal implants, and alcohol and/or drug use on the day before or on the day of

stimulation. Inclusion criteria comprised (i) a diagnosis of Parkinson's syndrome (PS) and (ii) refractory hypokinetic or tremor symptoms despite levodopa medication or the necessity to reduce levodopa dose due to side effects. The diagnostic criteria for PS included bradykinesia and at least one of the following: rest tremor, muscular rigidity, or disturbances of posture and gait. The primary treatment goal or symptom for each patient was established before stimulation. Patients were on various medication regimens, primarily involving Levodopa and its combinations. Details are provided in Table 1.

Table 1.

Patient	Diagnosis	Medication
1	PS1	Levodopa
2	MSA2	Levodopa
3	PSP3	Levodopa
4	MSA2	Levodopa
5	MSA2	None
6	PD4	Levodopa
7	Overlap Syndrome(CBD5, PSP3)	Levodopa
8	PD4	Levodopa
9	PS1	Levodopa
10	PS1	Levodopa

Patient Diagnoses and Medication. 1PS = atypical Parkinson Syndrome, not further classified. 2MSA = Multiple System Atrophy, 3PSP = Progressive Supranuclear Palsy, 4PD = Parkinson's Disease, 5CBD = Corticobasal Degeneration.

Eye Tracking Measurements in Virtual Reality

For this study, we used the HTC VIVE Pro Eye VR headset, which features an integrated Tobii eye tracker to measure participants' eye movements. SR_Runtime was utilized to enable eye tracking. Two base stations were required to track the VR headset. The VR hardware operated with Steam VR version 1.14.16. The display was a dual OLED 3.5" diagonal screen with a resolution of 1440 x 1600 pixels per eye (2880 x 1600 pixels combined) and a refresh rate of 90 Hz. The field of view was 110°, which also represents the trackable field for the eye tracking integrated into the HTC VIVE Pro Eye. Tobii Eye Tracking accuracy within FOX 20 ranged from 0.5° to 1.1°. Gaze origin and direction were recorded in a three-dimensional, right-handed coordinate system. The experiment was developed in Unity. Eye tracking data was preprocessed by the Tobii XR SDK (Tobii Technology Inc., Sweden), which provides a three-dimensional gaze direction vector in the right-handed coordinate system with normalized gaze data between -1 and 1. Time data was recorded with a timestamp in the SRanipal SDK, which also logged the frame sequence provided by HTC.

Procedure

To create the experimental stimuli, an independent programmer developed three static 360-degree scenes, each representing a symmetrical environment: a beach, a pier, and a park scene with a river (see Figure 1).

These scenes were selected to ensure a variety of visual stimuli while maintaining symmetrical properties. Participants viewed each scene for one minute, resulting in a total experiment duration of three minutes. Before viewing each scene, an eye tracking calibration was conducted.

Eye movements were recorded before and right after the final dTMS session, focusing on the following parameters: fixation duration (ms), longest fixation period (ms), saccade rate, total number of fixations. Fixations were defined as any eye movements where the velocity was less than 40 degrees per second. Eye movements exceeding this threshold were classified as saccades.

Figure 1.

To prevent the possibility that changes in eye movements were an effect of the medication, care was taken to ensure that the pre- and post-testing occurred at approximately the same time of day (see Table 2). The Parkinson's patients were admitted as inpatients, which ensured that they took their medication at the same time each day. On average, the time difference between the pre- and post-tests was -0.65 hours (M = -.65, excluding patient 5 since that patient doesn't take levodopa).

Table 2.

Patient	Pre Test Time	Post Test Time	Time difference (Hours)
1	12:42	10:33	-2.15
2	13:09	11:35	-1.57
3	12:52	10:48	-2.07
4	13:58	11:51	-2.12
5	14:54	11:20	-3.57
6	12:43	12:05	-0.63
7	10:19	11:42	1.38
8	10:21	10:57	0.60
9	14:07	13:01	-1.10
10	11:26	13:17	1.85

Table 2. Pre and post test time of eye tracking parameters.

Neuropsychological Assessments

Moreover, neuropsychological tests were assessed before the first stimulation and after the last stimulation. To include cognitive, mood and motor functions, the following tests were used:

The Montreal Cognitive Assessment (MoCA) was used to evaluate cognitive performance across multiple domains, including memory, attention, language, visuospatial abilities, and executive functions. The test consists of tasks such as word recall, clock drawing, and serial subtraction, with a total score ranging from 0 to 30 [21]. Scores below 26 were considered indicative of cognitive impairment. The MoCA is a validated tool widely used for detecting mild cognitive impairment and monitoring cognitive changes over time.

The Timed Up and Go (TUG) test was performed to assess functional mobility and balance. Participants began seated in a standard chair, stood up, walked 3 meters at a comfortable pace, turned around, returned to the chair, and sat down. The time taken to complete the task was recorded in seconds. Higher times indicate reduced mobility and an increased risk of falls. The TUG is a simple and reliable measure frequently used in clinical and research settings to evaluate motor function [22].

The Beck Depression Inventory Fast Screen (BDI-II FS) is a brief, 7-item version of the full Beck Depression Inventory-II (BDI-II) designed to assess depressive symptoms, particularly in populations with medical or neurological conditions [23]. Unlike the full BDI-II, this version excludes somatic symptoms (e.g., fatigue, sleep disturbance, and appetite changes) that could overlap with physical symptoms of conditions like Parkinson's disease. Each item evaluates cognitive and affective aspects of depression, such as sadness, pessimism, and loss of interest, using a 4-point Likert scale ranging from 0 (not at all) to 3 (severe). The total score ranges from 0 to 21, with higher scores indicating greater severity of depressive symptoms.

Deep Transcranial Magnetic Stimulation

For the deep transcranial magnetic stimulation (dTMS), we utilized the Brainsway dTMS device equipped with the H5 helmet, which is specifically designed for Parkinson's disease indications. The Brainsway dTMS system features a stimulator, a touchscreen interface, and an efficient cooling system. The dTMS stimulation was conducted in two separate sessions: one targeting the motor cortex and the other targeting the prefrontal cortex. The patient wore a helmet containing the H5 coil and a measuring tape to precisely determine the motor threshold before the first session. The motor threshold was identified by gradually increasing the stimulation intensity until a visible muscle contraction was observed in the hand or thumb. During the first session, the motor cortex was stimulated with low-frequency dTMS at 1 Hz. In the second session, the prefrontal cortex was stimulated with high-frequency dTMS at 10 Hz. To minimize discomfort from the noise generated during the stimulation, patients were provided with earplugs. Each session was conducted under controlled conditions to ensure accurate targeting and patient safety.

Patients were stimulated between 7 and 12 sessions in total every second day. Thus, most patients were stimulated for 4 weeks, 3 times a week.

2. Results

I) Eye tracking in Virtual Reality serves as a sensitive measurement for Parkinson's Syndrome

Receiver Operating Characteristic (ROC) analysis was conducted to evaluate the discriminative ability of various eye movement parameters. The results indicated a moderate ability to differentiate between states using these parameters. The area under the curve (AUC) for Mean Fixation Duration was 0.60 with a 95% confidence interval (CI) of [0.31, 0.89]. The AUC for Longest Fixation Period was 0.66, with a 95% CI of [0.37, 0.94]. For the Total Number of Fixations, the AUC was 0.54 with a 95% CI of [0.24, 0.84], and for the Saccade Rate, the AUC was 0.72 with a 95% CI of [0.46, 0.99]. Therefore, hypothesis I can be confirmed by showing a moderate ability to differentiate between states.

Effect sizes (Cohen's d) were calculated to determine the magnitude of changes in eye movement parameters and neuropsychological test scores. The Cohen's d for Mean Fixation Duration was 0.10, with a 95% CI of [-0.57, 0.76]; for Longest Fixation Period, it was 0.35, with a 95% CI of [-0.33, 1.01]; for the Total Number of Fixations, it was 0.20, with a 95% CI of [-0.46, 0.86]; and for the Saccade Rate, it was 0.45, with a 95% CI of [-0.23, 1.12].

II) Changes in Neuropsychological Tests correlate with Changes in Eye Tracking Measurements

Pearson correlations were conducted to examine the relationships between changes in eye movement parameters and changes in neuropsychological test scores (see Table 3). A positive correlation was observed between changes in the Longest Fixation Period and improvements in MoCA scores, $r(8) = 0.65$, $p = .025$. Saccade Rate changes were negatively correlated with TUG test times, $r(4) = -0.45$, $p = .082$, though this was not statistically significant. Changes in Fixation Durations correlated negatively with BDI-II FS scores, $r(7) = -0.55$, $p = .043$. Additionally, a positive, non-significant correlation was observed between Mean Fixation Duration and MoCA scores, $r(8) = 0.45$, $p = .187$. A negative, non-significant correlation was found between Longest Fixation Period and TUG test times, $r(4) = -0.40$, $p = .221$, and between Total Fixations and BDI scores, $r(7) = -0.39$, $p = .221$. A positive, non-significant correlation was observed between Saccade Rate and MoCA scores, $r(8) = 0.30$, $p = .421$. A negative, non-significant correlation was found between Total Fixations and TUG test times, $r(4) = -0.35$, $p = .312$, and a very low, non-significant correlation was observed between Saccade Rate and BDI scores, $r(7) = 0.10$, $p = .781$. Therefore, hypothesis II can be confirmed for some parameters (see table 3).

Table 3.

Parameter	MoCA Change (r, p)	TUG Change (r, p)	BDI-II FS Change (r, p)
Longest Fixation Period Change	0.65, .025*	-0.40, .221	-0.39, .221
Mean Fixation Duration Change	0.45, .187	-	-0.55, .043*
Total Fixations Change	-	-0.35, .312	-0.39, .221
Saccade Rate Change	0.30, .421	-0.45, .082	0.10, .781

Table 3. Pearson correlation coefficients between changes in eye movement parameters and changes in neuropsychological test scores. *Indicating a significant ($p < 0.05$) correlation.

III) There is a significant Difference in Eye Movements as Well as Neuropsychological Tests Before and After Stimulation

Paired t-tests were conducted to compare pre- and post-dTMS measurements of eye movement parameters (see Table 5). For Mean Fixation Duration, there was no significant difference, $t(9) = 0.053$, $p = .959$. For Longest Fixation Period, there was no significant difference, $t(9) = 1.019$, $p = .335$. For the Total Number of Fixations, there was no significant difference, $t(9) = 0.353$, $p = .732$. For the Saccade Rate, there was no significant difference, $t(9) = 0.960$, $p = .361$.

Paired t-tests were also conducted to compare pre- and post-dTMS measurements of neuropsychological test scores (see Table 4 for mean scores and Table 5 for paired t-test). For MoCA scores, there was no significant difference, $t(9) = -1.27$, $p = .238$. For TUG test times, there was no significant difference, $t(4) = 0.37$, $p = .728$. For BDI scores, there was a significant difference, $t(5) = 2.57$, $p = .049$, indicating a reduction in depressive symptoms. In sum, the hypothesis III could not be confirmed based on the data collected.

Table 4.

	M*pre*	**M***post*
Neuropsychological Tests		
MoCA Scores	23.3	24.3
TUG Test Times	28.18	27.28
BDI Scores	3.67	1.67

Table 4. Mean scores in neuropsychological test batteries administered before dTMS stimulation and after stimulation.

Table 5.

Parameter	t(df)	p-value
Eye Movement Parameters		
Mean Fixation Duration	0.053 (9)	.959
Longest Fixation Period	1.019 (9)	.335
Total Number of Fixations	0.353 (9)	.732

Saccade Rate	0.960 (9)	.361
Neuropsychological Tests		
MoCA Scores	-1.27 (9)	.238
TUG Test Times	0.37 (4)	.728
BDI Scores	2.57 (5)	.049*

Table 5. Paired t-test results for eye movement parameters and neuropsychological test scores pre- and post-dTMS. *indicating a significant ($p < 0.05$) value.

4. Discussion

For hypothesis I, if eye tracking serves as a sensitive measurement, analysis indicated a moderate ability to differentiate between states using eye movement parameters. Specifically, the area under the curve (AUC) values were 0.60 for Mean Fixation Duration, 0.66 for Longest Fixation Period, 0.54 for Total Number of Fixations, and 0.72 for Saccade Rate. Effect sizes (Cohen's d) for these parameters were 0.10, 0.35, 0.20, and 0.45, respectively, indicating small to moderate changes. Therefore, hypothesis I can be confirmed.

For hypothesis II, if changes in neuropsychological tests correlate with changes in eye tracking measurements, significant correlations were found between changes in the Longest Fixation Period and MoCA scores ($r = 0.65$, $p = .025$), and between changes in Fixation Durations and BDI scores ($r = -0.55$, $p = .043$). Although the correlation between Saccade Rate changes and TUG test times was not statistically significant ($r = -0.45$, $p = .082$), it suggested a trend where higher saccade rates might be associated with better motor performance. Therefore, hypothesis II can be confirmed for some parameters.

For hypothesis III, if there is a significant difference in eye movement as well in neuropsychological test scores, paired t-tests comparing pre- and post-dTMS measurements showed no significant differences in Mean Fixation Duration ($t(9) = 0.053$, $p = .959$), Longest Fixation Period ($t(9) = 1.019$, $p = .335$), Total Number of Fixations ($t(9) = 0.353$, $p = .732$), and Saccade Rate ($t(9) = 0.960$, $p = .361$). However, the BDI scores showed a significant reduction post-dTMS ($t(5) = 2.57$, $p = .049$), suggesting that dTMS may positively impact depressive symptoms in PD patients. In sum, the hypothesis III could not be confirmed based on the data collected.

The findings from this study contribute to evidence supporting the use of dTMS in PS treatment. The current study extends these findings by exploring the effects of dTMS on eye movement parameters and neuropsychological outcomes.

The significant correlation between changes in the Longest Fixation Period and MoCA scores aligns with earlier studies that have highlighted the relationship between eye movements and cognitive function in [24, 25]. These findings suggest that eye movement parameters, particularly the Longest Fixation Period, could serve as reliable biomarkers for cognitive changes in PD. The moderate effect size observed for the Saccade Rate further underscores its potential utility as a diagnostic tool.

Eye tracking offers several advantages as a sensitive diagnostic tool. It provides objective, quantifiable data that can detect subtle changes in cognitive and emotional states, often before these are apparent in traditional neuropsychological tests [15]. Eye tracking can be conducted in a naturalistic setting, which may better reflect real-world functioning compared to paper-pencil tests. This is particularly important in PD, where cognitive and motor symptoms can fluctuate and be context-dependent.

The reduction in BDI-II FS scores post-dTMS is consistent with prior research indicating the antidepressant effects of TMS [8,12]. This is particularly relevant for PD patients, who often experience comorbid depression [26]. The observed correlation between changes in BDI-II FS scores and Fixation Durations suggests that eye tracking parameters may reflect not only emotional states but also cognitive and attentional mechanisms relevant to social functioning. Future research could build on these findings by incorporating measures of social cognition and evaluating how changes in eye movement patterns relate to improvements in real-world social interactions. This could provide deeper insights into the broader implications of dTMS and eye tracking for enhancing quality of life in PD.

Although the paired t-tests did not show significant changes in most eye movement parameters, the observed trends and effect sizes highlight the need for further investigation with larger sample sizes. The non-significant negative correlation between Saccade Rate changes and TUG test times suggests that eye movements might be linked to motor performance, albeit weakly in this study. Future research should explore this relationship more deeply, considering the small sample size and variability in TUG test times. Neuropsychological tests like the MoCA, TUG, and BDI provide valuable insights into cognitive, motor, and emotional functions. The MoCA assesses various cognitive domains, while the TUG test measures mobility and balance, and the BDI evaluates depressive symptoms. However, these tests can be subjective and influenced by patient effort and external factors. In contrast, eye tracking offers quantitative and objective measurements that can detect subtle changes in oculomotor function. Eye tracking can be used to detect cognitive function. Cognitive psychology and eye tracking research highlighted this by suggesting the *eye-mind assumption* [27]. This assumption suggests that there is a close temporal and spatial link between eye fixating and cognitive processing. The assumption demonstrates that eye movements provide information about cognitive processes. Therefore, eye tracking measurements should be a key tool for future studies to investigate a new approach of detecting cognitive functions.

A significant limitation of this study is the small sample size, which may have reduced the statistical power to detect significant changes and correlations. The limited number of participants also increases the risk of type II errors, where meaningful differences or relationships could remain undetected. Furthermore, the lack of diversity within the sample, particularly in terms of age, gender, disease severity, and comorbidities, restricts the generalizability of the findings to broader populations. Parkinson's disease is a heterogeneous condition, and the observed effects might not be representative of the wider population or specific subgroups,

such as those with advanced disease stages or different treatment histories. Additional limitations include variability in TUG test performance, potentially influenced by differences in participant motivation or fatigue during testing, which could introduce noise into the data. Moreover, missing data for some neuropsychological test scores further complicates the interpretation of results, as these gaps may have biased the analysis. Another important limitation of this study is the absence of a control group. Without a control group of participants without Parkinson's syndrome or those who did not receive the intervention, it is challenging to determine whether the observed changes and correlations are truly attributable to the intervention or represent natural variability. A control group would provide a baseline for comparison, allowing researchers to isolate the specific effects of the intervention from other confounding factors, such as placebo effects, fluctuations in symptom severity, or environmental influences.

To address these issues, future studies should prioritize larger, more diverse cohorts that capture the demographic and clinical variability of Parkinson's disease populations. Stratified sampling methods could be employed to ensure representation across disease stages, treatment modalities, and comorbid conditions.

Moreover, while this study focused on the effects of dTMS on eye movement parameters and neuropsychological outcomes, it did not explore the underlying neural mechanisms. Neuroimaging studies could provide valuable insights into how dTMS modulates brain activity related to eye movements and cognitive/emotional functions in PD.

5. Conclusions

In conclusion, this study provides preliminary evidence that dTMS may positively influence depressive symptoms in PD patients and that eye movement parameters, particularly the Longest Fixation Period and Saccade Rate, hold potential as biomarkers for cognitive changes. The moderate effect sizes and significant correlations observed warrant further research with larger samples to validate these findings and fully elucidate the diagnostic utility of eye movements in PD. This exploratory pilot study suggests that eye tracking, due to its objective and sensitive nature, may offer a superior alternative to traditional neuropsychological tests in detecting subtle cognitive and emotional changes in PD.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: Conceptualization, C.C. and L.W.; methodology, C.C., L.W..; software, C.C.; validation, C.C., L.W., and N.S.; formal analysis, C.C.; investigation, C.C.; resources, N.S., A.G., and C.S.,C.C:; data curation, N.S., A.G., and C.S., C.C.; writing—original draft preparation, C.C.; writing—review and editing, C.C. and L.W.; visualization, C.C.; supervision, L.W.; project administration, L.W., C.C.

Funding: This research received no external funding

Informed Consent Statement: Prior to the treatment, patients provided consent to undergo stimulation according to the CE-mark of the dTMS device. Moreover, a registry involving human participants was reviewed and approved by Ärztekammer Nordrhein (Nr. 2021026) as well as collecting eye tracking data, (Nr. 2022031). Patients gave written informed consent to the treatment, for being

included in the registry as well as consenting to eye tracking measurements. Also, we confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines

Acknowledgments: We sincerely thank all the patients who participated in this study for their valuable time and effort, which made this research possible. We also extend our gratitude to our MTAs, Veronika Hirsch and Michaela Wessler, for their technical and administrative support throughout the project. Their contributions were invaluable in ensuring the smooth execution of the study.

Conflicts of Interest: Author Lars Wojtecki has previously received funding grants and institutional support from the German Research Foundation, Hilde-Ulrichs-Stiftung für Parkinsonforschung, and the ParkinsonFonds Germany, BMBF/ERA-NETNEURON, DFG Forschergruppe (FOR1328), Deutsche Parkinson Vereinigung (DPV), Forschungskommission, Medizinische Fakultät, HHU Düsseldorf, UCB; Medtronic, UCB, Teva, Allergan, Merz, Abbvie, Roche, Bial, Merck, Novartis, Desitin, Spectrum. Author Lars Wojtecki owned stock in company BioNTech SE. Author Lars Wojtecki is consultant to the following companies: TEVA, UCB Schwarz, Desitin, Medtronic, Abbott/Abbvie, MEDA, Boehringer I, Storz Medical, Kyowa Kirin, Guidepoint, Merck, Merz, Synergia, BIAL, Zambon, Sapio Life, STADA, Inomed, Vertanical. Author Celine Cont is consultant to Storz Medical.

The remaining 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. Moreover, the authors declare that there are no additional disclosures to report.

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9. Erklärung

Ich versichere an Eides Statt, dass die Dissertation von mir selbstständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

Die Dissertation wurde in der vorliegenden oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

Düsseldorf, den

Celine Cont