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„Effects of Transcranial Ultrasound Pulse Stimulation on Motor and Mood Symptoms in Parkinson's Disease"

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Abstract (en)

Transcranial pulse stimulation (TPS) is a newly developed non-invasive brain stimulation tool and recently has been shown to be beneficial in the treatment of Alzheimer's disease (AD). The aim of the present study is to investigate whether ultrasound-based brain stimulation benefits motor and mood symptoms in Parkinson's disease (PD) patients.

PD is the second most common neurodegenerative disorder after AD. It is characterized by motor features (i.e. tremor, bradykinesia, rigidity), and non-motor symptoms including depression and cognitive impairments.

In this uncontrolled pilot study 29 PD patients were treated with 10 sessions of TPS. No serious adverse events were reported. The efficacy of TPS over motor area and supplementary motor area was assessed using motor and depression scales to evaluate motor and non-motor symptoms in 17 and 12 patients respectively. The results of the present study demonstrate significant changes in the Unified Parkinson's Disease Rating Scale part III scores after TPS treatment, indicating an improvement of motor signs. The Becks Depression Inventory-II scores also show a trend towards alleviation of depressive symptoms after TPS intervention, but below significance. In conclusion, the application of TPS appears to be a safe and possibly beneficial add-on treatment in patients with PD.

Abstract (de)

Morbus Parkinson ist eine neurodegenerative Erkrankung, bei der die Betroffenen an fortschreitenden Störungen des Bewegungsapparates leiden. Nicht-motorische Symptome, unter anderem Depressionen und kognitive Einschränkungen, gehören ebenso zu dem Krankheitsbild. Die Parkinson-Krankheit ist nach Morbus Alzheimer die zweithäufigste neurodegenerative Erkrankung. Bislang ist keine Heilung der Krankheit möglich. Symptome können durch Medikamente gelindert werden, allerdings sind diese mit hohen Nebenwirkungen verbunden. Daher herrscht ein großer Bedarf an neuen und wirksamen Behandlungsoptionen.

Das Ziel der Forschung der vorliegenden Masterarbeit ist es, den Effekt von Transkranieller Pulsstimulation (TPS) in Parkinson-Patient:innen zu untersuchen. TPS ist eine neu entwickelte und sichere Hirnstimulationsmethode, bei der Neuronen mittels Ultraschallwellen aktiviert werden. TPS ist für die Behandlung von Alzheimer-Demenz bereits zugelassen und hat das Potential auch bei anderen neurologischen und psychiatrischen Erkrankungen positive Effekte zu erzielen.

Um den Effekt von TPS auf die Symptome von Parkinson zu untersuchen wurde eine Pilotstudie mit 29 Parkinson-Betroffenen durchgeführt. Während des zweiwöchigen TPS-Behandlungszyklus wurden keine schwerwiegenden Nebenwirkungen berichtet. Vor und nach der TPS Behandlung wurden motorische und psychologische Tests bei 17 beziehungsweise 12 Patient:innen durchgeführt und in weiterer Folge statistisch ausgewertet. Die Ergebnisse zeigen eine Verbesserung der motorischen Symptome sowie einen Trend zur Verbesserung von Depressionen. Daraus lässt sich schließen, dass TPS eine neue Chance in der Behandlung von Morbus Parkinson und anderen neurologischen Erkrankungen darstellen könnte.

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1. Introduction

Parkinson's disease (PD) is a complex and heterogeneous progressive neurodegenerative disorder. It is characterized by both the classic motor features including bradykinesia, tremor, rigidity, and gait dysfunction (Dauer & Przedborski, 2003; Davie, 2008) as well as non-motor manifestations such as depression, cognitive changes, and sleep disorder (Obeso et al., 2010; Stennis Watson et al., 2010). It remains unclear which exact mechanism causes PD. Pathobiological hallmarks are the intracytoplasmic inclusions of α-synuclein, known as Lewy bodies (Goedert et al., 2013), and the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The latter results in perturbations affecting the whole basal ganglia (BG) network and is associated with the cardinal motor symptoms of PD, particularly rigidity and bradykinesia (Braak et al., 2003; Dauer & Przedborski, 2003).

The BG encompass a group of subcortical nuclei involved in voluntary movement and can be categorized in input nuclei, output nuclei, and intrinsic nuclei. Caudate nucleus, putamen, and accumbens nucleus are considered input nuclei and receive cortical, thalamic, and nigral information (Lanciego et al., 2012). The output nuclei are the internal segment of the globus pallidus and the substantia nigra pars reticulata. Those output nuclei send information from the BG to the thalamus, which in turn project back to the cerebral cortex (Hoover & Strick, 1993). The intrinsic nuclei consist of the external segment of the globus pallidus, the subthalamic nucleus, as well as the SNpc and relay information between the input and the output nuclei (Albin et al., 1989). Dopamine plays a critical role in controlling the flow of information in the BG (Alexander, 2004). The pigmented dopaminergic neurons of the SNpc project and transmit dopamine to the striatum, including the motor territory within the putamen (the nigrostriatal pathway). The classical BG model claims that two distinct pathways with opposite effects facilitate movement: The direct pathway to promote and the indirect pathway to inhibit movement (Lanciego et al., 2012). The progressive deterioration of the SNpc dopaminergic neurons in PD patients results in the depletion of dopamine in the striatum and malfunction of the whole BG network (Wu et al., 2012). Consequences are a decreased activation of the direct pathway and increased

inhibition of the indirect pathway which lead to a diminished excitation of the motor cortex and the motor signs of PD (Delong, 1990).

Current pharmacological treatment options are anchored on substitution of striatal dopamine. The most widely used drug is the dopamine precursor levodopa. However, those treatment options are limited by arising dopa resistance and drug related side effects (Kalia & Lang, 2015; Martínez-Fernández et al., 2016). Moreover, they focus on symptomatic management and are still not able to substantially alter the course of disease progression (Lang & Espay, 2018; J. Obeso et al., 2017).

In our rapidly aging society, neurodegenerative diseases are a central public health issue of increasing relevance. PD is the second most common neurodegenerative disorder and has undergone the fastest growth in prevalence among neurological diseases in recent years (de Lau & Breteler, 2006). The progressive character of PD and the degeneration effects on mobility and muscle control strongly impact the life of patients, their families, and caregivers. Therefore, strategies to slow disease progression are of major importance to maintain or even improve the patient's quality of life as well as ameliorate the burden on relatives and health care systems (Dorsey et al., 2013). Consequently, there is a great need for novel approaches in the treatment of neurodegenerative diseases including PD.

Non-invasive brain stimulation (NIBS) technologies encompass different modalities of brain therapy for neurological and psychiatric disorders. They use energy to modify neuronal activity and cortical excitability (Bhattacharya et al., 2021). In PD repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) are two of the most studied NIBS (Cosentino et al., 2022). In tDCS neuronal membrane potentials are modulated by a weak electrical current which is directly applied to the scalp. In rTMS high intensity, transient rapid changing magnetic fields generate electrical currents of brief duration which are able to trigger neuronal action potentials within the cortex (Biagioni et al., 2018). Clinical effects of NIBS are attributed to complex phenomena including modulation of cortical excitability and neuronal connectivity. Long-term potentiation- and long-term depression-like phenomena are considered to be involved in the neuroplastic effects (Blandini et al., 2019). NIBS are promising techniques that show potential for maintaining physical

and/or cognitive function in PD patients. However, the currently available evidence supporting the beneficial neuromodulation effects of rTMS and tDCS is limited to small studies and heterogeneous experimental methodologies. The knowledge concerning the exact neurochemical mechanisms underlying those neuroplastic effects is still very limited (Biagioni et al., 2018). Further limitations exist for specificity due to low special resolution, inability to reach deep brain tissue and problems with pathological brains in which the neuronal conductivity can be profoundly altered. Also, the patients' pharmacological profile can affect the brains activation state and connectivity and critically influence the neurophysiologic effects of rTMS und tDCS (Hallett, 2000; Minjoli et al., 2017; Spagnolo et al., 2019).

Transcranial pulse stimulation (TPS) is a newly developed NIBS tool. It is based on single ultrashort ultrasound pulses that are delivered through the skull and mechanically stimulate nerve tissue (Beisteiner et al., 2019). The non-invasive application of ultrasound bears the potential for revolutionary therapy. Unlike electrophysiological brain stimulation techniques, TPS allows non-invasive deep brain stimulation and does not depend on intracerebral conductivities (Legon et al., 2018). First clinical data have shown that 2-4 weeks of TPS therapy improve functional networks and cognitive performance of Alzheimer's disease (AD) patients for up to 3 months (Beisteiner et al., 2019).

The underlying molecular mechanism remains elusive. Regarding how TPS may affect neurons and generate neuroplastic effects, current knowledge related to different ultrasound-based techniques indicates the following principles: Ultrasound directly activates neurons with evocation of action potentials (Weinreb & Moses, 2022). Ultrasound pulses also affect mechanosensitive ion channels in the neuronal membranes and thereby modify the cells' gating kinetics (d'Agostino et al., 2015). Furthermore, the ultrasound wave changes the membrane permeability by inducing membrane pore formation. The mechanical ultrasound stimulus is converted into biochemical responses by mechanotransduction leading to a change in humoral factor and neurotransmitter concentrations (Ingber, 2006; Zhang et al., 2017). Pulsed ultrasound therapy is shown to stimulate the vascular endothelial growth factor (VEGF) (Yahata et al., 2016) and the brain-derived neurotrophic factor (BDNF)

expression (Wang et al., 2017). VEGF stimulate neural cells to induce neuroprotective effects and thereby suppress nerve cell damage and death. In the treatment of PD VEGF is shown to have a potential neuroprotective effect (Yasuhara et al., 2004). BDNF plays an important role in central motor structures including the motor cortex and the BG. It has been shown to provide neurotrophic support for dopaminergic SNpc neurons and exerts direct electrophysiological effects (Howells et al., 2000). TPS may also increase nitric oxide production in cells which leads to increased metabolic activity and angiogenesis (Mariotto et al., 2005).

TPS has the potential to change neuronal activity and modulate pathological brain networks and may therefore be a promising technique for broad neuroscientific applications (Beisteiner & Lozano, 2020). To date, only a few research studies using transcranial ultrasound for neuromodulation have been performed on human subjects (Beisteiner et al., 2019; Jeong et al., 2022; T. Kim et al., 2021; Lee et al., 2021; Monti et al., 2016; Stern et al., 2021). Concerning PD, no study has been conducted in humans. TPS is a substantially safe and non-invasive add-on therapy which makes this new technique of appealing interest for the study and treatment of various neurological disorders (Beisteiner et al., 2019), including PD.

The overall aim of the present study was to investigate the effects of TPS on PD symptoms for the first time. Preliminary clinical safety and efficiency was examined by an uncontrolled pilot study in 29 and 17 patients, respectively. UPDRS motor scores and BDI-II scores were used to gauge the effects of pulsed ultrasound brain stimulation in patients with PD.

2. Methods

2.1 Study design

This was an open label, uncontrolled, retrospective study to investigate the following questions: Is TPS safe and feasible in a broad range of PD patients? Are there indications for preliminary effects as examined by neuropsychological scores? The primary outcome measure was a change in the Unified Parkinson's Disease Rating Scale (UPDRS) part III after completion of TPS treatment compared with pre-treatment score. The secondary outcome measure evaluated the potential of TPS to improve depression as measured by the Beck Depression Inventory-II (BDI-II).

2.2 Participants

29 patients with idiopathic PD according to the British Parkinson's Disease Society (21 men, 8 women; mean age 56.5 ± 8.9 years; age range, 47-82 years; mean disease duration 43.2 ± 31.3 months; disease duration range, 4-120 months) were included. All patients requested TPS treatment as a therapeutic attempt and received 10 sessions of TPS intervention within two weeks at the TPS Therapy and Development Center – Prof. Beisteiner (Vienna) between February 2021 and June 2022. Written informed consent was obtained from each participant. All patients had optimized standard treatments before intervention and continued to take that medication throughout the study without dose adjustments. All study assessments took place in the "on" state. Common inclusion criteria were clinically stable patients and signed written informed consent. Common exclusion criteria were noncompliance with study protocol, relevant intracerebral pathology unrelated to PD (e.g. brain tumor), haemophilia or other blood clotting disorders, or corticosteroid treatment within the last six weeks prior to the first treatment.

2.3 TPS treatment and stimulation targets

Brain stimulation was performed using the NEUROLITH TPS system (Storz Medical AG, Tägerwilen, Switzerland). As previously described by Beisteiner et. al, the TPS system consists of a mobile single transducer generating the ultrasound pulses, and an infrared camera (Polaris Vicra System by Northern Digital Inc.) to track the position of

the handpiece and the patient's head via infrared markers and thereby enable MR based neuro-navigation (Beisteiner et al., 2019). Before TPS treatment each patient had a special MRI-scan for TPS navigation performed at the Radiology Center, Vienna. A neurologist (R.B.) defined individual regions of interest (ROIs) to target PD-relevant brain regions. Specifically, ROIs comprised bilateral motor area (MA) and the supplementary motor area (SMA). Patients received TPS always in the "on" state while seated in a chair. The TPS system enabled real-time tracking and documentation of the applied pulses. Thereby, the applied energy was evenly distributed within the ROIs. TPS intervention was performed with ultrashort (about 3µs) ultrasound pulses, 0.25 mJ/mm^2 energy flux density, pulse repetition frequency 4 Hz, pulse number per therapeutic session 4000 (thus a total of 40 000 pulses per subject in 10 sessions).

2.4. Safety

The study was carried out in a broad clinical setting for outpatients to assess safety and feasibility on a wide range of PD patients. Adverse events were monitored during the 2 weeks of TPS therapy. At each visit patients were interviewed for adverse events. At the end of each treatment session the patients evaluated their level of pressure and pain during the treatment using visual analogue scales (VAS; 0 = none, 10 = very strong pain/pressure).

2.5. Outcome measures

All neurologic and psychiatric scores were assessed in the medication "on" state by an independent investigator.

2.5.1. Primary outcome measure

UPDRS-III was used to assess a change in the motor status, as the primary outcome measure. UPDRS is a commonly used PD assessment to monitor the progression of PD symptoms. The PD rating scale is divided into 4 parts:

- I: Mentation, behavior, mood
- II: Activities of daily living
- III: Motor examination
- IV: Clinical fluctuations

All parts are scored on a 0-4 rating scale. Higher scores indicate more advanced symptoms. In studies especially the motor part (part III) is commonly used. It must be addressed that the UPDRS-assessment in the present study was performed by independent neurologists who used two different versions of the rating scale, namely UPDRS and the revised MDS-UPDRS (revision of the UPDRS by the Movement Disorder Society (MDS)). The newer MDS-UPDRS retained the original four-scale structure but added supplementary items (Goetz et al., 2008). To enable a consistent analysis of UPDRS-III the points of the supplementary items of the MDS-UPDRS part III were removed.

2.5.2. Secondary outcome measure

As depression is typical comorbidity of PD, the effect of TPS on depressive symptoms was monitored using the Beck Depression Inventory-II (BDI-II). BDI-II is a commonly used assessment tool for measuring the severity of depression. The self-report inventory exists of 21 questions relating to symptoms of depression. Each item is scored on a 0-3 rating scale with higher scores indicating more severe depression. To examine the effect of TPS on depressive symptoms BDI-II scores were assessed within four weeks before and within four weeks after TPS treatment.

2.6. Data analysis

Data was collected in Excel and statistical analyses were performed using IBM SPSS, version 28. Effects were considered statistically significant if a p-value < .05 was found. Primary and secondary outcome scores were tested for normality using the Kolmogorov-Smirnov test. Depending on the normal distribution of the variable considered, either a t-test for paired variables or a Wilcoxon test was performed. These statistical analyses were carried out on actual values of the scores. Correlation between pre/post TPS change in UPDRS-III and clinical-demographic parameters was calculated using the Pearson's test for parametric measures (age, disease duration, baseline UPDRS-III).

3. Results

3.1. Subject characteristics and TPS Safety

A total of 29 patients (21 men, 8 women; mean age 56.5 ± 8.9 years; age range, 47-82 years; mean disease duration 43.2 ± 31.3 months; disease duration range, 4-120 months) with state-of-the-art treatment were treated with TPS for 2 weeks. All patients completed the 10 sessions of TPS intervention and no serious adverse events occurred. 19 patients (65.5%) reported mild, self-limiting side effects within the 10 days of TPS treatment. 15 patients (51%) reported adverse effects on 1-3 days, 3 patients (10.3%) on 4-5 days and 1 patient reported side effects after all 10 sessions of TPS treatment. Fatigue, headache, and dizziness were the most common adverse events and reported by 12 (41.4%), 8 (27.6%) and 7 (24.1%) patients, respectively. Visual analogue scale evaluation (VAS 0-10) of within-treatment pain or pressure experience resulted in 91% VAS 0, 5% 1-3, 3% 4-6, and 1% 7-8 pressure (% off all TPS sessions). 1 patient reported pain during the treatment with a maximum of VAS 6.

3.2. Changes in primary outcome: UPDRS-III

Of the 29 patients who received TPS therapy, 17 took the UPDRS assessment within 4 weeks before (pre) and after (post) TPS treatment and were therefore included in further analyses. Demographic and clinical characteristics of those patients are shown in table 1. The change between pre and post UPDRS-III scores was normally distributed according to Kolmogorov-Smirnov test ($p=0.123$) and thus analysed using t-test for paired variables. The UPDRS-III score as the major outcome parameter for the patients' motor symptoms improved significantly after treatment (mean difference = -4.2 ± 2.8 points; 95% confidence interval [CI] -2.7 to -5.5, $p < 0.001$; figure 1). Analysis of the individual change in UPDRS-III total scores revealed that 16 patients exhibited a decrease in motor score (indicating an improvement in motor symptoms), whereas the UPDRS-III total score of one patient was similar pre and post brain stimulation (figure 2).

Table 1: **Demographic and clinical characteristics of patients**

Pt.no.	Gender	Age (years)	Disease duration (months)	UPDRS-III		BDI-II	
				pre	post	pre	post
P01	m	75	107	11	4	n.a.	n.a.
P02	m	64	52	38	33	n.a.	n.a.
P03	f	59	41	20	20	5	1
P04	m	69	17	9	6	n.a.	n.a.
P05	m	76	82	15	14	13	19
P06	m	72	34	36	30	11	5
P07	m	58	9	12	10	4	4
P08	m	62	47	11	8	27	14
P09	m	73	9	6	5	3	0
P10	m	69	120	11	4	2	1
P11	m	48	70	14	9	7	5
P12	f	73	36	17	8	12	9
P13	f	71	30	8	6	4	2
P14	m	72	45	23	14	n.a.	n.a.
P15	m	76	70	18	13	4	3
P16	m	76	45	13	10	0	2
P17	f	53	48	12	10	n.a.	n.a.
Mean ± SD	m:f=13:4	67.4 ± 8.6	50.7 ± 31	16.1 ± 9	12 ± 8.4	7.7 ± 7.3	5.4 ± 5.8

Abbreviations: BDI-II = Beck Depression Inventory-II; n.a. = not available; SD = Standard deviation; UPDRS-III = Unified Parkinson's Disease Rating Scale part III.

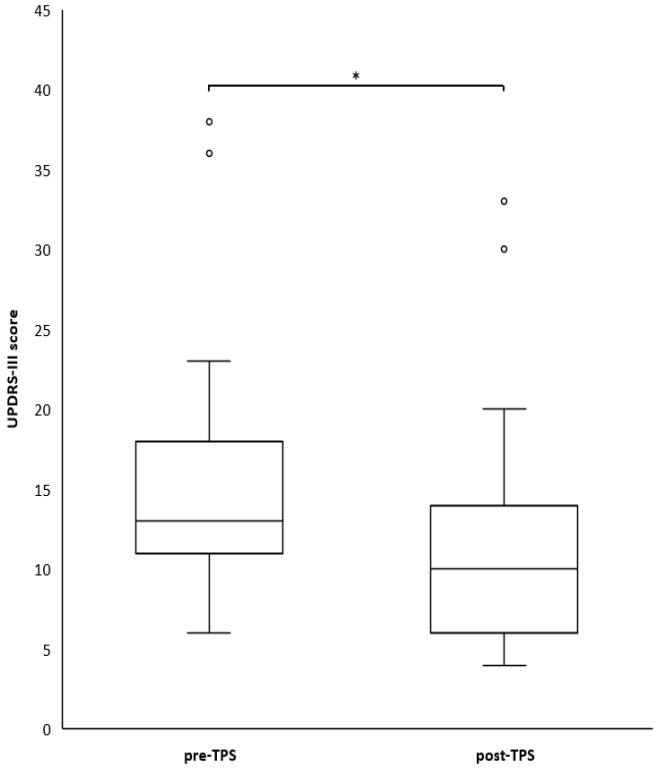

Figure 1: **Comparison of Unified Parkinson's Disease Rating Scale part III (UPDRS-III) total scores pre and post transcranial pulse stimulation (TPS).** Boxplots represent the medians, and the 25th and 75th percentiles, whereas error marks demonstrate the minimum and maximum values. Motor symptoms improved significantly after TPS (*p< .05; paired t-test).

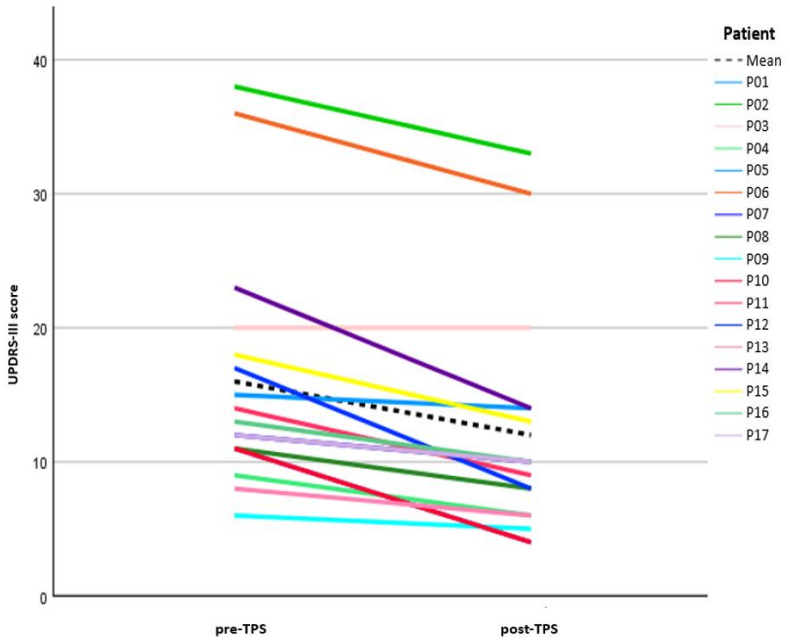

Figure 2: **Individual change of Unified Parkinson's Disease Rating Scale part III (UPDRS-III) total score after transcranial pulse stimulation (TPS).** Each patient is indicated by a different color; the mean value is marked as dashed line. The UPDRS-III total score decreased in 17 out auf 18 patients after TPS.

3.3 Changes in secondary outcome: BDI-II

12 patients were able to complete the BDI-II questionnaires within 4 weeks pre and post TPS treatment. On average, BDI-II score was 7.67 (standard deviation [SD]=7.34, n=12) at baseline and 5.42 (SD=5.82, n=12) after TPS intervention. The change of BDI-II scores was not normally distributed, according to Kolmogorov-Smirnov test (*p*=0.48). Comparison between pre and post BDI-II total scores using the non-parametric Wilcoxon-test for two paired variables showed a tendency of decline in BDI-II scores (i.e. improvement of depressive symptoms), but below significance (*p*=0.092, two-tailed; figure 3). Analysis of individual BDI-II scores pre and post stimulation (figure 4) showed improvement of depressive symptoms in 9 patients. 1 patient exhibited a similar BDI-II score pre and post treatment. 2 participants showed a higher BDI-II total score after brain simulation compared to baseline, indicating worsening of depressive symptoms.

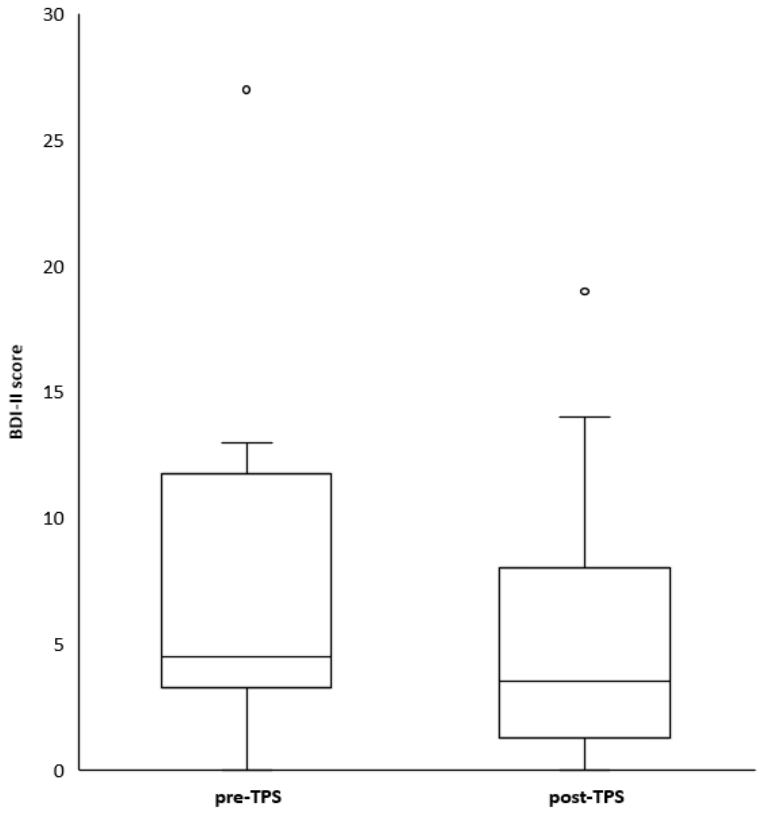

Figure 3: **Comparison of Beck Depression Inventory-II (BDI-II) total scores pre and post transcranial pulse stimulation (TPS).** There was no significant improvement in BDI-II total scores after TPS (*p*=0.092, Wilcoxon-test). Depressive symptoms showed a tendency to decline. Boxplots represent the medians, and the 25th and 75th percentiles, whereas error marks demonstrate the minimum and maximum values.

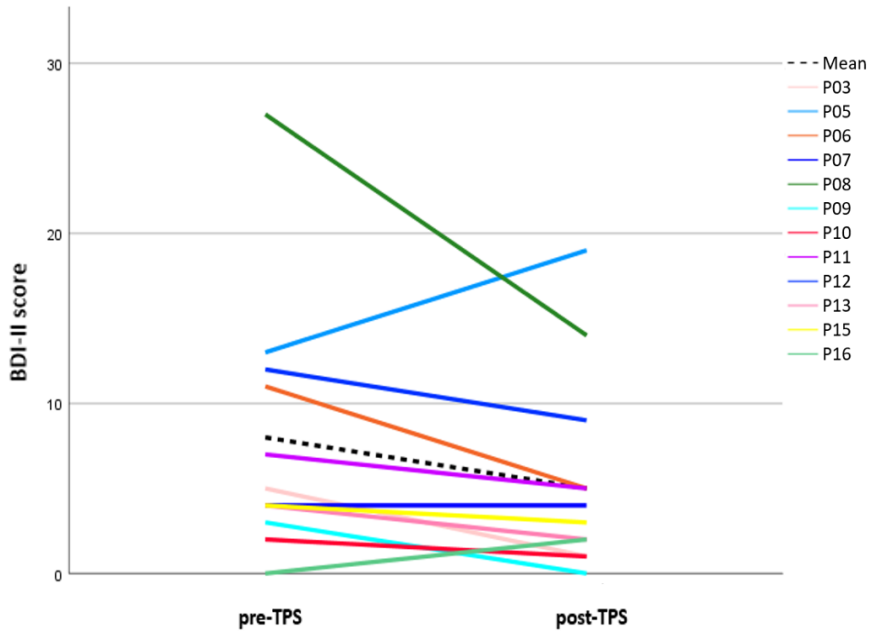

Figure 4: **Individual change of Beck Depression Inventory-II (BDI-II) total scores pre and post transcranial pulse stimulation (TPS).** Each patient is indicated by a different color; the mean value is marked as dashed line. Depressive symptoms decreased in 9 out of 12 patients after TPS.

3.4. Subgroup analysis

Given the above finding of improved motor function, additional analyses were performed to better understand this result. No significant correlation emerged between changes of BDI-II scores and TPS motor effect, indicating that motor improvement was not driven by change of depressive symptoms and vice versa. Exploratory post hoc subgroup analysis of mild PD (baseline UPDRS-III ≤ 13, medial score for the study population) versus more advanced PD (baseline UPDRS-III >13) showed no difference in the delta of UPDRS-III total scores. Also, there was no difference in change of UPDRS-III in younger patients (age ≤ 71 years, medial score for the study population) compared to older patients (> 71 years). Likewise, the duration of the disease (≤ 4 years compared to > 4 years; medial score for the study population) made no difference regarding the UPDRS-III change.

4. Discussion

The present study indicates that 10 sessions of TPS within 2 weeks are likely to improve motor symptoms in patients with PD. Further, the presented results suggest that ultrasound brain stimulation has the potential to alleviate PD depression. The effects were achieved in PD patients already receiving optimized standard treatment. Therefore, this study provides evidence that non-invasive ultrasound pulse stimulation is a good candidate as an independent add-on therapy for motor symptoms in PD.

4.1. High treatment tolerability

TPS applied over the cortical MA and SMA was well tolerated by the participants. This finding is consistent with prior data from preclinical experiments and first clinical application of TPS in patients with Alzheimer's disease (Beisteiner et al., 2019). No serious or severe adverse events were reported and the intensity of pressure and pain during the intervention was rated low. In total 19 patients reported at least one mild, self-limiting side effect, most dominant fatigue, and headache. Although the majority of the patients reported at least one adverse effect within the intervention period, only 20% of the patients reported side effects on more than 3 days. Also worth mentioning is placebo-controlled studies of comparable NIBS show that the proportion of subjects experiencing adverse events do not significantly differ between verum and sham group (Brys et al., 2016; Padala et al., 2020). Overall, TPS applied over MA and SMA appears to be safe with few, generally mild adverse effects.

4.2. Evidence for motor effects of TPS

The results of recent studies show preliminary efficacy of TPS for neuromodulation and sham-controlled evidence of long-term effects (Beisteiner et al., 2019; Matt, Kaindl, et al., 2022). This is the first demonstration of using TPS in PD patients. The choice to target MA and SMA as stimulation sites was based on previous research: The cortical MA and SMA are crucial components of the impaired BG-thalamo-cortical-circuit of PD. MA and SMA excitability is altered by the dopaminergic impairment within the BG of PD patients (Casarotto et al., 2019; Swanson et al., 2021). The MA is

the predominant source input to the pyramidal tract and associated with the planning and execution of movement. Changes in the electrophysical behaviour of MA-neurons result in the impaired voluntary movements in PD patients (Underwood & Parr-Brownlie, 2021). The SMA plays an important role in linking cognition to action and generally shows a decreased activity in PD patients (Haslinger et al., 2001; Playford et al., 1992). Dysfunction of the SMA is associated with impairments in motor sequencing, gait, and temporal processing (Rahimpour et al., 2022). NIBS studies over the MA and the SMA have been shown to improve motor symptoms in patients with PD (Brys et al., 2016; Hamada et al., 2008; Rahimpour et al., 2022; Yokoe et al., 2018).

This is the first demonstration of ameliorating PD symptoms using non-invasive ultrasound stimulation. The UPDRS-III data observed in this study demonstrated a clear pattern of motor improvement after TPS intervention. In total 16 patients achieved an improvement in the motor score of the UPDRS after TPS therapy. Only one patient showed the same UPDRS-III score pre and post brain stimulation and therefore did not verifiably benefit from the intervention. The mean improvement of -4.12 points in the motor part of the UPDRS is slightly below the minimal clinically important difference (MCID), which is set at -4.83 points, according to Schrag et al. (Schrag et al., 2006). Even though the mean improvement is slightly below the MCID a total of 8 patients exhibited a clinically important improvement of motor signs (delta UPDRS-III > 4 points) whereas no patient experienced worsening of motor symptoms.

4.3. Tendency of PD depression improvement after TPS

The primary goal in the treatment of PD is to improve the patient's overall quality of life. According to Aarsland et al., neuropsychiatric disturbances are often experienced as more distressing and problematic than the motor features of PD (Aarsland et al., 2009). Not only the improvement of motor signs but also the management of non-motor features of the disorder is of major clinical importance. A particular focus must be placed on alleviating depressive symptoms, since depressive disturbances are the most frequently reported neuropsychic comorbidity in PD and occur in 40-50% of patients with PD (Reijnders et al., 2008). Further, depressive disturbances negatively influence other clinical aspects such as motor and cognitive deficits and functional disability. Additionally, improved depression is associated with reduced physical

disability and improved quality of life in PD patients (Ravina et al., 2007). Therefore, the aim of the secondary measurement was to evaluate the effect of TPS on mood and depressive symptoms in patients with PD.

The pathophysiology of PD-depression is still poorly understood (Aarsland et al., 2009). There are two different models describing the etiology of depression (D'Ostilio & Garraux, 2016). (1) The biochemical model claims that depression is secondary to the neurodegeneration and the consequent decrease in monoamine levels (Chan-Palay & Asan, 1989; Mayeux et al., 1984; Remy et al., 2005). (2) An alternative hypothesis is the network model of depression in PD: According to this model, disturbances in neuronal plasticity within mood relevant neuronal networks – namely the corticolimbic circuits - might underly PD depression (Castrén, 2005; Castreń, 2013). Based on the network model of depression, NIBS-driven network reorganization could be an effective approach in treating depression (D'Ostilio & Garraux, 2016). In a recent study, evidence for TPS induced connectivity-change and its correspondence to improvement of AD depression was presented (Matt, Dörl, et al., 2022). Also, in PD comparable NIBS-techniques show potential for treating depression (Xie et al., 2015).

In the present study the brain stimulation led to an improvement in BDI-II evaluations. However, no significant effect could be demonstrated, and the size of mean improvement of 2 points was slightly below the level of MICD, which is most likely between 3 and 6 points, according to Hengartner & Plöderl (Hengartner et al., 2022). It must be emphasized that the generalizability of the findings is limited by (1) the small sample size, (2) the fact that this was a PD patient sample with mild depressive symptoms, and (3) the possibility of biases due to the non-controlled and nonblinded design. Still, in total 9 out of 12 patients exhibited an improvement in BDI-II scores after TPS, 5 of which even scored a clinically important improvement (delta BDI-II > 3 points). Considering mood improvement in patients with AD (Matt, Dörl, et al., 2022), depressed students (Reznik et al., 2020), and healthy subjects (Konishi et al., 2020) there are now numerous lines of evidence suggesting that precisely 3D-naviated ultrasound brain stimulation may be effective as an add-on therapy for depression.

While promising, further research is required to gain a better understanding and identify optimal ROIs for treating motor and non-motor features in PD patients.

4.4 Limitations

There are some limitations that need to be addressed. First, this PD patient pilot study was performed with an uncontrolled design. Therefore, a possible placebo effect must be taken into consideration and the results must be seen as preliminary. Placebo effect may be triggered by the expectation of therapeutic benefit from brain stimulation which can lead to the release of striatal dopamine as demonstrated by neuroimaging techniques (Ji et al., 2008; Strafella et al., 2006). Further randomized, placebo-controlled studies are required to confirm the stimulation effects. Also, any premature conclusions on the generalizability of the findings are limited by the small sample size (albeit comparable to other research in this field (M. S. Kim et al., 2015; Matt, Dörl, et al., 2022; Yokoe et al., 2018)). The lack of proper follow-up and thus, precluding the possibility to investigate the duration of clinical efficacy on PD motor and mood signs is a further limitation of this study. Evidence for persisting neuroplastic changes up to one week was provided in a sham-controlled study with healthy subjects (Matt, Kaindl, et al., 2022). In addition, a first uncontrolled study provides clinical data that indicate TPS induced cognitive improvements in AD patients up to three months and therefore argues for long-term effects of TPS (Beisteiner et al., 2019). However, regarding PD patients not much is known about TPS long-term efficacy. Since this was the first time that the effect of TPS on PD symptoms was assessed, only existing NIBS studies like rTMS and tDCS could be used to act as a comparison for the results. However, the mechanism of TPS, namely brain stimulation by ultrashort ultrasound waves, differs from existing NIBS technologies, which use electrical stimulation for neuromodulation. A clear understanding of the cellular mechanisms underlying the neuroplastic effects of TPS stimulation is lacking and further investigations are needed to expand the knowledge. Our experimental design is only the first step in determining the optimal TPS application mode for PD. In this study the focus was on TPS as an add-on therapy rather than as a medication replacement. Therefore, all measurements and treatments were performed in the “on” state in PD. Further investigations are required to supplement these limitations

in the future. Follow up studies should compare patient subgroups regarding disease stage, comorbidities, and extent of antiparkinsonian therapy for a more detailed assessment of clinical efficacy.

4.5. Conclusion

TPS is a promising novel brain stimulation technique. The presented results support and extend the understanding of the safety and efficacy profile of TPS in the treatment of neurodegenerative diseases. Sham-controlled studies with larger sample size are needed to further expand the knowledge on this approach, including long-term effects. However, the findings of this pilot study represent a strong argument to further investigate the value of TPS as a novel add-on therapy for PD.

5. References

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6. List of abbreviations

AD	Alzheimer's disease
BDI-II	Beck Depression Inventory-II
BDNF	Brain-derived neurotrophic factor
BG	Basal ganglia
MA	Motor area
MCID	Minimal clinical important difference
MDS	Movement Disorder Society
NIBS	Non-invasive brain stimulation
PD	Parkinson's disease
ROI	Region of interest
rTMS	Repetitive transcranial magnetic stimulation
SMA	Supplementary motor area
SD	Standard deviation
SNpc	Substantia nigra pars compacta
tDCS	Transcranial direct current stimulation
TPS	Transcranial pulse stimulation
US	Ultrasound
UPDRS	Unified Parkinson's Disease Rating Scale
VAS	Visual analogue scale
VEGF	Vascular endothelial growth factor

7. List of Figures and Tables