São Paulo State University
"Júlio de Mesquita Filho"

Post Graduate Program in Human Movement Sciences – Interunit

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The effect of transcranial direct current stimulation and physical exercise on interlimb coordination during walking in people with Parkinson's disease

Thesis submitted to the post Graduate Program in Human Movement Sciences – Interunit, Bauru Campus, São Paulo State University "Júlio de Mesquita Filho," as part of the requirements for obtaining the Phd degree.

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE VINICIUS CAVASSANO ZAMPIER, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DO MOVIMENTO, DA FACULDADE DE CIÊNCIAS - CÂMPUS DE BAURU.

Aos 17 dias do mês de dezembro do ano de 2024, às 14h, por meio de Videoconferência, realizou-se a defesa de TESE DE DOUTORADO de VINICIUS CAVASSANO ZAMPIER, intitulada **The effect of transcranial direct current stimulation and physical exercise on interlimb coordination during walking in people with Parkinson's disease**. A Comissão Examinadora foi constituída pelos seguintes membros: Profa. Dra. PAULA FAVARO POLASTRI ZAGO (Participação Presencial) do(a) Departamento de Educação Física / UNESP - Faculdade de Ciências de Bauru - SP, Prof. Dr. LUIS MOCHIZUKI (Participação Virtual) do(a) Escola de Artes, Ciências e Humanidades / Universidade de São Paulo - SP, Prof. Dr. ALEXANDRE HIDEKI OKANO (Participação Virtual) do(a) Centro de Matemática, Computação e Cognição / Universidade Federal do ABC - Santo André/SP, Dr. DIEGO ORCIOLI DA SILVA (Participação Virtual) do(a) Educação física / Universidade Estadual Paulista Júlio de Mesquita Filho Instituto de Biociências de Rio Claro, Prof. Dr. PAULO ROBERTO PEREIRA SANTIAGO (Participação Virtual) do(a) Escola de Educação Física e Esporte de Ribeirão Preto (Eeferp) / USP. Após a exposição pelo doutorando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final:_A_P_R_O_VA_ D_O_. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Profa. Dra. PAULA FAVARO POLASTRI ZAGO

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Resumo

A estimulação transcraniana por corrente contínua (ETCC) e o exercício físico são terapias eficazes para melhorar a coordenação de movimentos e os parâmetros do andar em pessoas com doença de Parkinson (DP), geralmente acompanhadas por mudanças na atividade cortical. Contudo, a variabilidade nos protocolos de ETCC gera dúvidas sobre quais parâmetros são eficazes, e pouco se sabe sobre os efeitos da combinação de ETCC e exercício na locomoção e na atividade cortical em DP. Esta tese investigou essas lacunas em três estudos. O Estudo #1 sintetizou achados de estudos sobre protocolos de ETCC para melhorar a coordenação motora em parkinsonianos e idosos saudáveis por meio de uma revisão sistemática nas bases PubMed, Embase e Web of Science. O Estudo #2 investigou os efeitos agudos, e o Estudo #3 os efeitos crônicos, de um protocolo combinado de ETCC e exercício locomotor na coordenação intermembros, parâmetros do andar e atividade cortical de 22 indivíduos com DP. No protocolo de ETCC, o ânodo foi posicionado sobre o córtex motor contralateral ao lado mais afetado pela DP e o catodo na área supraorbital contralateral. A corrente elétrica de 2 mA foi aplicada por 20 minutos (ativo) ou 10 segundos (sham), concomitantemente ao exercício (20 minutos). No protocolo de exercício, os participantes percorreram um circuito de 18 m em duas condições: i) andar aumentando amplitude dos braços (10 min); ii) andar aumentando velocidade dos braços (10 min). No Estudo #2, os participantes realizaram ambas as condições de estimulação com sete dias de intervalo, sendo avaliados antes e após cada sessão. No Estudo #3, 22 participantes foram divididos em dois grupos (ativo, n = 12; sham, n = 10) e realizaram seis sessões em dias alternados. As avaliações ocorreram antes da primeira sessão, 48 horas após e 30 dias após a última sessão. O andar foi avaliado com o sistema GaitRite posicionado em uma passarela de 8 m. A coordenação intermembros foi analisada via fase relativa contínua entre sinais de acelerômetros nos punhos e tornozelos contralaterais. A atividade cortical foi analisada por EEG de 64 canais, considerando a densidade do poder espectral (alpha, beta e gamma) nos canais Fz, F3, F4, Cz, C3, C4, Pz, P3 e P4. O Estudo #1 mostrou que a ETCC melhora a coordenação motora, especialmente com protocolos de 20 min e 2 mA. O Estudo #2 revelou que uma sessão de ETCC combinada com exercício não potencializou os efeitos do exercício. O Estudo #3 mostrou que seis sessões potencializaram os efeitos do exercício, com menor variabilidade do ângulo de fase e redução do poder espectral na banda alfa do córtex pré-frontal no grupo ativo. Conclui-se que exercícios focados na amplitude e velocidade dos braços promovem melhorias na marcha e na atividade cortical em DP. Contudo, múltiplas sessões de ETCC são necessárias para potencializar esses efeitos, sugerindo que intervenções combinadas e repetidas são mais eficazes.

Palavras-Chave: Parkinson, Estimulação cortical, Locomoção, Eletroencefalograma.

Abstract

Transcranial direct current stimulation (tDCS) and physical exercise are effective therapies for improving motor coordination and gait parameters in people with Parkinson's disease (PD), often accompanied by changes in cortical activity. However, variability in tDCS protocols raises questions about which parameters are effective, and little is known about the effects of combining tDCS and exercise on locomotion and cortical activity in PD. This thesis investigated these gaps through three studies. Study #1 synthesized findings from studies on tDCS protocols aimed at improving motor coordination in individuals with parkinsonism and healthy older adults through a systematic review of PubMed, Embase, and Web of Science databases. Study #2 investigated the acute effects, and Study #3 the chronic effects, of a combined tDCS and locomotor exercise protocol on interlimb coordination, gait parameters, and cortical activity in 22 individuals with PD. In the tDCS protocol, the anode was positioned over the motor cortex contralateral to the most affected side and the cathode over the contralateral supraorbital area. A 2 mA current was applied for 20 minutes (active) or 10 seconds (sham) during 20 minutes of exercise. In the exercise protocol, participants walked an 18 m circuit under two conditions: i) walking with increased arm swing amplitude (10 min); ii) walking with increased arm swing speed (10 min). In Study #2, participants performed both stimulation conditions seven days apart, with assessments conducted before and after each session. In Study #3, 22 participants were divided into two groups (active, n = 12; sham, n = 10) and completed six sessions on alternate days. Assessments were performed before the first session, 48 hours after, and 30 days after the final session. Gait was assessed using the GaitRite system positioned on an 8 m walkway. Interlimb coordination was analyzed via continuous relative phase between signals from accelerometers placed on contralateral wrists and ankles. Cortical activity was analyzed using 64-channel EEG, considering spectral power density (alpha, beta, gamma) in channels Fz, F3, F4, Cz, C3, C4, Pz, P3, and P4. Study #1 showed that tDCS improves motor coordination, particularly with 20-minute, 2 mA protocols. Study #2 revealed that one tDCS session combined with exercise did not enhance exercise effects. Study #3 demonstrated that six sessions amplified exercise effects, with the active group showing lower phase angle variability and reduced alpha-band power in the prefrontal cortex. In conclusion, exercise protocols focusing on arm amplitude and speed improve gait and cortical activity in PD. However, multiple tDCS sessions are necessary to enhance these effects, suggesting that combined and repeated interventions are more effective.

Keywords: Parkinson, Cortical Stimulation, Locomotion, Electroencephalogram.

Summary

1. Chapter 1

General introduction

Locomotion is a type of motor behavior activated by signals sent from various cortical regions (Takakusaki, Tomita, & Yano, 2008). It requires visuomotor cognitive processing regulated by neural networks involving the cerebral cortex, basal ganglia, cerebellum, brainstem, and spinal cord (Takakusaki, 2017). The frontal areas of the cortex are responsible for visuospatial movement control, whereas motor areas, such as the supplementary motor area (SMA), premotor area, and primary motor cortex (M1), are involved in motor planning (Takakusaki, 2017). The cerebellum plays a role in executing cyclic movements following preestablished motor plans, controlled unconsciously (Linsberger & Thach, 2014). These regions are connected to basal ganglia structures such as the thalamus and substantia nigra pars reticulata (Takakusaki et al., 2004). The basal ganglia receive inputs from the cortex and send projections to brainstem structures, including the pedunculopontine nucleus, which regulates muscle tone, and the mesencephalic locomotor region, responsible for rhythmic movement control. Furthermore, they influence spinal cord structures such as the central pattern generator (CPG), which modulates rhythmicity by coordinating flexor and extensor muscle activity during gait (Pearson & Gordon, 2014; Takakusaki et al., 2003). When basal ganglia function is impaired, as in Parkinson's disease (PD), individuals exhibit movement execution impairments, including slowness (bradykinesia) and reduced amplitude (hypometria) (Takakusaki, 2017).

PD is characterized by progressive and asymmetric degeneration of dopaminergic neurons located in the substantia nigra pars compacta of the basal ganglia (Wichmann & DeLong, 2014). Dopamine is a critical neurotransmitter for regulating cortical activity. Its reduced production impairs inhibitory stimulus transmission from the substantia nigra pars compacta to the internal globus pallidus and substantia nigra pars reticulata, leading to increased inhibitory stimuli sent to the thalamus (Takakusaki, Tomita, & Yano, 2008). Consequently, there is greater inhibition of the thalamo-cortical pathway, reducing stimulus transmission to the cortex and decreasing basal ganglia activation (Blandini et al., 2000; Takakusaki, Tomita, & Yano, 2008). This dysfunction is associated with resting tremor, a hallmark symptom of PD. In gait-related circuitry deficits, inhibition of the

pedunculopontine nucleus and mesencephalic locomotor region is reduced. With diminished dopaminergic activity, these structures, which typically receive inhibitory projections from the basal ganglia, increase inhibitory signals sent to the reticular formation (Takakusaki et al., 2004). As a result, gait deficits arise, including reduced stride length and speed, increased double support time, decreased and asymmetric arm swing, and impaired interlimb coordination (Huang et al., 2012; Lewek et al., 2010). The dense connectivity between the cortex and basal ganglia explains why, in the presence of motor dysfunction, individuals with PD exhibit altered cortical activity (Karimi et al., 2022; Stuart et al., 2021). Compared to healthy older adults, individuals with PD show increased alpha-band (8–12 Hz) and beta-band (13–30 Hz) power in frontal, motor, and parietal cortical regions (Karimi et al., 2022; Stuart et al., 2021). This increased cortical activity is interpreted as a compensatory mechanism for the basal ganglia's deficient circuitry, aimed at maintaining performance during motor tasks such as gait (Stuart et al., 2018).

Several studies have focused on treating gait characteristics in individuals with PD (Li et al., 2020; Luna et al., 2020; Nonnekes et al., 2016). The most commonly used therapy for managing PD signs and symptoms involves medications that compensate for dopamine deficits (Tarazi et al., 2014). This pharmacological approach effectively alleviates symptoms such as resting tremor and gait deficits, resulting in increased stride length and speed in older adults with PD (Nonnekes et al., 2016). Regarding cortical activity, medication reduces alpha-band spectral power in the frontal cortex of individuals with PD, reflecting decreased executive control demand and restoration of automatic gait patterns (Orcioli-Silva et al., 2021). However, continuous use of these medications can lead to side effects such as hallucinations and dyskinesia, while failing to improve static and dynamic balance (Nonnekes et al., 2016; Smulders et al., 2016). Consequently, other therapies that complement medication are gaining prominence in treating PD symptoms. Studies have shown that various physical exercise protocols improve gait parameters in individuals with PD, including increased stride length and speed, as well as enhanced strength in the lower and upper limbs (Ni et al., 2018). However, many exercise protocols developed to date focus on lower limb movement, disregarding upper limb movement and the interaction between these two body segments.

The Arm movement plays a crucial role in controlling key aspects of locomotion, such as dynamic balance and energy efficiency during walking, counterbalancing leg

movement and reducing trunk rotation (Meyns et al., 2013; Pontzer et al., 2009). In neurodegenerative diseases such as PD, neural impairments affect arm movement, contributing to gait and movement coordination deficits (Espinosa et al., 2023). Ospinna et al. (2018) observed that individuals with PD exhibit reduced movement magnitude and speed and increased arm swing asymmetry compared to neurologically healthy older adults (Lewek et al., 2010). However, when individuals with PD are instructed to walk with increased arm movement amplitude and speed, improvements are observed in gait parameters such as stride length and speed (Zampier et al., 2018), interlimb coordination parameters such as decreased coordination pattern variability and increased arm-leg coupling (Zampier, 2019), accompanied by changes in alpha and beta bands in the supplementary motor area, indicating functional restoration of this region's activity (Wersink et al., 2020). Despite the relationship between upper and lower limb movements, the effects of exercise protocols targeting upper limb movement on gait parameters and interlimb coordination remain poorly understood.

In addition to physical exercise, other techniques have been explored to address gait deficits in older adults with PD, including invasive and non-invasive cortical stimulation techniques. Transcranial direct current stimulation (tDCS) (Beretta et al., 2020; Broeder et al., 2015; Lattari et al., 2017) is a non-invasive technique that applies low-intensity electrical currents (typically between 1–2 mA) through the scalp, altering neuronal membrane potentials and consequently increasing or decreasing excitability in specific cortical areas (Nitsche & Paulus, 2000). tDCS has gained popularity due to its ease of application, safety, and relatively low cost compared to other techniques, such as transcranial magnetic stimulation and deep brain stimulation (Broeder et al., 2015; Hanakawa, 2015). Studies suggest tDCS as a promising technique for improving movement coordination and gait in individuals with PD (Barreto et al., 2019; Pol et al., 2021). For instance, Leenus et al. (2014) reported that a 20-minute tDCS session over the prefrontal cortex reduced errors and increased accuracy in interlimb coordination tasks involving opposing arm and leg movements. Barreto et al. (2019) found that five consecutive sessions of cathodal tDCS over the motor cortex improved the coordination of rapid movements executed with a single limb. Additionally, Ferrucci et al. (2019) reported that a single tDCS session applied to the cerebellum improved coordination between movements of a single limb. In terms of gait, tDCS has been associated with increased step length and speed in older adults with PD (Pol et al., 2021). Although these

findings demonstrate tDCS's efficacy in improving coordination-related characteristics, varying results, procedures, and stimulated areas suggest a need for studies that consolidate this information to facilitate understanding and exploration of tDCS effects on coordination.

Given the positive effects of tDCS on motor aspects, studies have examined the effects of combining tDCS with physical exercise on locomotor characteristics and cortical activity in individuals with PD (Beretta et al., 2020). When combined with aerobic exercise, tDCS enhances prefrontal cortex activity in the stimulated region (Conceição et al., 2021). This cortical activity alteration is accompanied by reduced variability in step duration and reaction time (Conceição et al., 2021). Beyond gait and cortical activity changes, combining tDCS with exercise protocols also shows synergistic effects on upper limb function and postural control in older adults with PD (Beretta et al., 2020). However, the wide variety of tDCS protocols used makes findings on the combined effects of tDCS and physical exercise on locomotor aspects and cortical activity in individuals with PD inconsistent, highlighting the need for further research. Additionally, most studies combining tDCS with physical exercise have employed exercise protocols focusing on lower limb movement, such as cycling (Conceição et al., 2021), without considering upper limb movement. To date, no studies have reported on the combined effects of tDCS and arm-focused locomotor exercises on gait parameters, interlimb coordination, and cortical electrical activity in individuals with PD.

Considering these points, this doctoral thesis aims to address three primary questions regarding the type of tDCS protocol that should be employed to improve movement coordination in individuals with PD: (1) How does tDCS alone influence gait parameters and interlimb coordination in older adults with PD? (2) How do exercise protocols involving the arms affect interlimb coordination and cortical activity in individuals with PD? (3) How does a combined tDCS and arm-focused locomotor exercise protocol affect gait parameters, interlimb coordination, and cortical activity in older adults with PD?

- Study #1: Effectiveness of transcranial direct and alternate current stimulation on movement coordination in older people and individuals with Parkinsonism: A systematic review.

- Study #2: The acute effects of transcranial direct current stimulation combined with locomotor exercise on people with Parkinson's disease locomotion and cortical activity.

- Study #3: The chronic effects of transcranial direct current stimulation combined with locomotor exercise on people with Parkinson's disease locomotion and cortical activity.

2. Chapter 2

Study #1 - Effectiveness of transcranial direct and alternate current stimulation on movement coordination in older people and individuals with Parkinsonism: A systematic review.

Submitted in Human Movement Science Journal

Effectiveness of transcranial direct and alternate current stimulation on movement coordination in older people and individuals with Parkinsonism: A systematic review

Abstract

Movement coordination deficits such as the reduction on movement amplitude and speed are commonly observed in aging and neurological diseases such as parkinsonism. Although transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) emerges as promising therapies to improve movement coordination parameters evidence indicates mixed results and protocols' characteristics. To synthesize findings and guide healthcare professionals, we conducted a systematic review of the literature aiming to assess tDCS and tACS protocols focused on the improvement of movement coordination parameters and verify their effectiveness. We included seven English-controlled studies. These studies varied in sample sizes and stimulation parameters and they have at least one movement coordination variable such as movement amplitude and speed. The data bases considered were PubMed, Embase and Web of science. The risk of bias was evaluated through The Cochrane risk of bias assessment and the methodological quality through Physiotherapy Evidence Database. Most demonstrated promising outcomes in improving movement coordination, specifically in movement amplitude and speed. However, some showed no effects on the selected population's coordination. In summary, tDCS and tACS appear effective in enhancing movement coordination, but their efficacy in cyclical motor tasks like walking requires further investigation in clinical trials.

Keywords: Neurological disorders; Aging; Transcranial electrical stimulation; Movement coordination; Movement amplitude; Movement speed.

2. 1. Introduction

Movement coordination, defined as the phenomenon of bringing into proper relation multiple and different components of the body, is important for the success or failure of a motor task such as walking [1, 2]. Literature consistently notes age-related movement coordination deficiencies [2, 3] attributable to the gradual decline in sensory and motor systems during aging, affecting their integrative capacities [4]. Aging-induced poorer sensory integration (e.g., integration of proprioception and vision) is a possible explanation for deficits in movement coordination [5]. Hafer and Boyer [3] showed that older people present a decrease in movement coordination variability, which may indicate altered strategies or a reduced ability to control certain segments of motion during a motor task, such as walking. Movement coordination deficits, such as the reduction in movement amplitude and frequency are accentuated in neurodegenerative diseases such as parkinsonism [6, 7, 8, 9].

Parkinsonism is an overall term to describe a group of neurological diseases with signs and symptoms similar to Parkinson's disease such as rigidity, slowness, and tremor (https://jamanetwork.com/journals/jama/fullarticle/2760741). Less common parkinsonism includes neurological diseases such as cerebellar ataxia, progressive supranuclear palsy or multiple system atrophy [10]. Also, parkinsonism is a term to describe drug-induced parkinsonism and vascular parkinsonism. Due to the neural pathways affected in parkinsonism some brain regions responsible for coordinated movement control are affected, such as mesencephalic locomotor region and pontine peduncle nucleus [11]. The affected neural pathways also compromise the sensorial feedback related to continuous adjustments during cyclical tasks resulting in a poorer sensorial integration [5]. Poorer sensory integration is not improved by levodopa [12], as they are likely to depend on specific, time-dependent neural activity, which cannot be readily restored by increasing the gain of the corresponding neural pathway [5, 13]. Considering the lack of levodopa effectiveness on sensory integration, new therapy strategies have been emerging to improve movement coordination in older adults and people with Parkinsonism.

Recent studies have suggested non-invasive brain stimulation as a promising approach to improve movement coordination in neurologically healthy older adults and people with Parkinsonism. This technique induces cortical plasticity by stimulating

neurons from specific cortical regions, subsequently improving brain function [14]. Transcranial direct current stimulation (tDCS) and transcranial alternate current stimulation (tACS) have been shown promising results for treating parkinsonism symptoms [15], as such treatment reduces the time to perform upper limb movement sequences [16] and the movement amplitude variability during a finger tapping task [17]. Anodal tDCS increases neural excitability modulating the neuronal membrane potential towards its firing threshold while cathodal tDCS moves the neuronal membrane potential away from its firing threshold [18]. The tACS alternated current at certain frequencies affects ongoing rhythms in the cortex and improves motor learning [19]. In both techniques, electrodes are positioned over the scalp to emit a constant (tDCS) or an alternate (tACS) low-intensity electrical current (1 to 4 mA) [18]. Barreto et al., [20] demonstrated that five 20-minute tDCS sessions over the primary motor cortex improved gait parameters evaluated through the scale for Assessment and Rating of Ataxia (SARA) on patients with cerebellar ataxia [20]. In addition, a single tDCS session over the cerebellum improved movement coordination during the mechanical countertest in people with cerebellar ataxia [21]. Despite promising results, the vast number of available protocols makes it challenging for clinicians and healthcare professionals to define the most appropriate protocol to improve motor coordination in older adults and people with Parkinsonism. Defining when to use one or another tDCS and tACS protocol is important to ensure that these techniques are applied effectively and safely in accordance with the desired clinical objectives.

In light of the emerging use of transcranial electrical stimulation to enhance movement coordination in both healthy and clinical populations, a structured and comprehensive comparison among various stimulation protocols is lacking in previous studies. There exists variability in stimulation site (e.g., motor cortex, pre-frontal cortex), type (tDCS and tACS), intensity, and polarity of the stimulation current (anodal, cathodal, inhibitory). Consequently, our study aims to systematically review literature on brain electrical stimulation protocols involving tDCS and tACS in individuals with Parkinsonism and older adults. Through this review, we aim to propose recommendations concerning stimulated brain regions, session numbers, and durations to enhance movement coordination in these populations. The outcomes of our study could prove beneficial for therapists endeavoring to implement brain electrical stimulation to enhance their patients' movement quality and mobility. Additionally, these findings could serve as

guiding principles for future research endeavors seeking to advance knowledge on brain stimulation techniques.

2. 2. Methods

2.2.1. Protocol Registration and Search Strategy

We followed the Preferred reporting Items for systematic Reviews and Meta-Analyses (PRISMA) statements [22]. We conducted searches in May 2023 using three databases: Embase, PubMed and Web of Science. A protocol was prospectively registered with the International Prospective Register of systematic Reviews (PROSPERO: CRD42023396420) and approved for execution. The following Booleans terms composed the search strategy: ((((Parkin) OR Parkinson) OR Older) OR Elderly) AND (((((transcranial direct current stimulation) OR tDCS) OR transcranial electrical stimulation) OR transcranial alternating current stimulation) OR taCS) AND ((((((((((bimanual coordination) OR bilateral coordination) OR interlimb coordination) OR movement coordination) OR arm coordination) OR foot coordination) OR finger coordination) OR hand coordination) OR leg coordination) OR coupling) OR relative phase) OR coordination. We considered only papers in English. Table 1 shows the terms and synonyms used to search papers in the databases in the title, abstract or keywords from peer-reviewed studies. The search terms were verified at the Medical Subject Headings (MeSH).

2.2.2. Information Sources and Eligibility Criteria

One author VCZ created a search strategy that was revised and approved by the authors CKF and FAB to identify all potentially relevant studies. The manuscripts pointed out were downloaded through a reference manager software (Rayan). VCZ and CKF performed the initial screen by reviewing the titles and abstracts. When necessary FAB made the final decision. In cases where the study eligibility was not clear, a full-text analyses were performed. The included studies should i) investigate the effects of different transcranial electrical stimulation modalities (i.e. tDCS or tACS), combined or not with other intervention protocols on movement coordination variables; ii) be a peer-reviewed study written in English; iii) include at least one outcome measure related to the following movement coordination characteristics: movement amplitude and/or speed, continuous relative phase, continuous relative phase variability, movement coupling, and movement duration. Studies were excluded if they: i) were not in line with the objectives;

ii) were conducted in animals; (iii) were conducted in children/infants; iv) used invasive brain stimulation (e.g., deep brain stimulation); v) used transcranial magnetic stimulation as intervention; vi) were conducted in population not related to older people or parkinsonism.

Table 1. Search terms used in each database.

Population	**Intervention**	**Outcome**
T-A-Kw	**T-A-Kw**	**T-A-Kw**
Parkin	Transcranial direct current stimulation	Bimanual coordination
Parkinson	tDCS	Bilateral coordination
Older	Transcranial electrical stimulation	Interlimb coordination
Elderly	Transcranial alternating current stimulation	Movement coordination
	tACS	Arm coordination
		Foot coordination
		Finger coordination
		Hand coordination
		Leg coordination
		Coupling
		Relative phase
		Coordination

T: Title; A: Abstract; Kw: Key worlds.

2.2.3. Data extraction and synthesis

Data were extracted by two authors, VCZ and CKF, and synthesized in a table format. Data entry was confirmed by the author FAB. Data included in the table considered the following information: i) the First author and the year of publication; ii) the population characteristics (sample size, group characteristics and study design); iii) the study design; iv) the main objectives; v) the main outcomes (measurement characteristics); vi) the transcranial electrical stimulation characteristics (protocol information); vii) the comparison of interest (comparison between groups or periods); viii) conclusions (conclusions related to the effects of transcranial electrical stimulation on movement coordination variables).

2.2.4. Risk of bias across studies and methodological quality

VCZ and CKF assessed the methodological quality and the risk of bias of the selected studies using the Physiotherapy Evidence Database (PEDro) rating scale [23] and the Cochrane risk of bias assessment [24]. The author FAB solved inconsistencies.

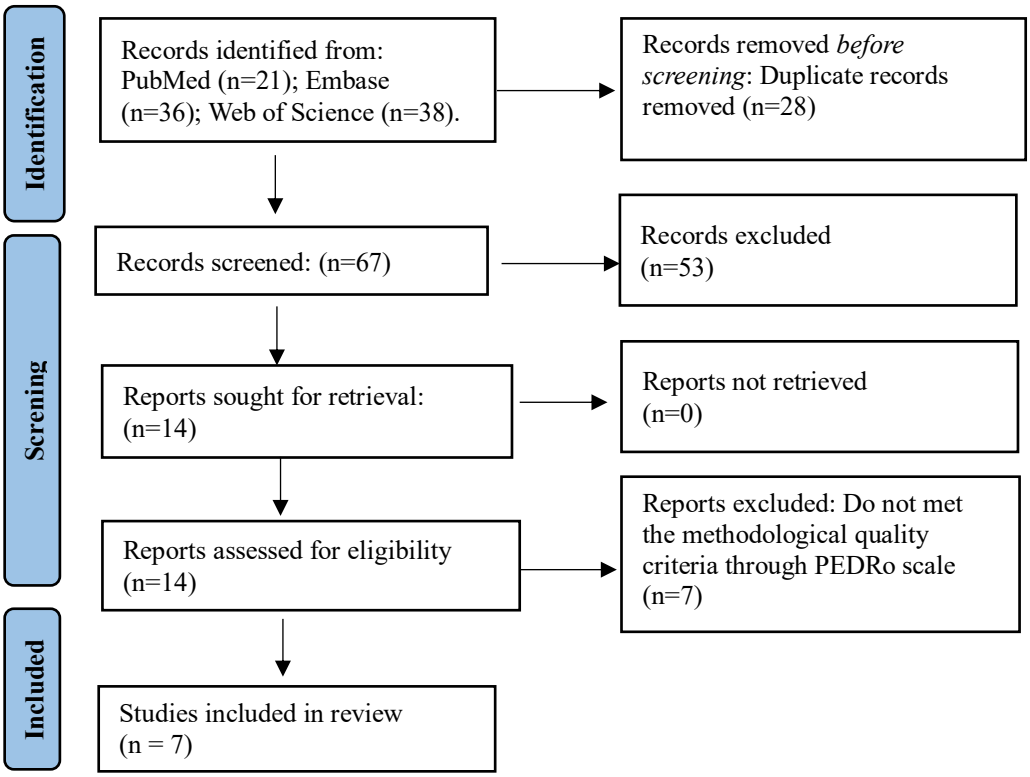
The studies' methodological quality were assessed through the 11 items of PEDro scale regarding group allocation, blinding, attrition, statistical analyses, and data variability [23]. Studies with scores of 9–10 were considered with excellent methodological quality, 6–8 good, 4–5 fair, and < 4 poor [23]. Only studies with scores ≥ 4 were considered in this study.

The risk of bias was assessed through The Cochrane risk of bias assessment which includes five domains to verify the selected studies' risk of bias [24]: i) bias arising from the randomization process; ii) bias due to deviations from intended interventions; iii) bias due to missing outcome data; iv) bias in the measurement of the outcomes; v) bias in selection of the reported result. Review Manager 5.4 software (The Cochrane Collaboration, 2020) was used to evaluate the risk of bias assessment which has three levels in each domain: low risk, high risk, and unclear. Only studies with low risk or unclear risk of bias were considered in this study.

2.3. Results

The flow chart containing the review searching process is shown in Figure 1, as suggested by the PRISMA guidelines. The search strategy found 95 entries from the different search databases, from which 28 duplicates were removed. After reviewing the titles and abstracts from the 67 remaining studies, 14 studies were included. After the full-text review of the 14 selected studies, seven met the methodological quality and the suggested risk of bias was assessed through the PEDro scale and the Cochrane risk of bias assessment and were included in further analyses.

Figure 1: Screening process



Flowchart with screening process following the PRISMA guidelines.

2.3.1. Included studies

Table 2 shows the extracted information regarding the population assessed, the design used and the objectives of the selected studies. The sample size varied from 7 to 19 participants (11.5 ± 5.8). Two studies investigated the effects of tACS in people with Parkinson's disease (PwPD) [17, 25]. One study investigated the effects of tACS in older people [26]. Four studies investigated the effects of tDCS in people with Parkinsonism [27, 20, 28, 29]. Specifically, they investigated the following diseases: Parkinson's disease [17, 25]; progressive supranuclear palsy [27] and cerebellar ataxia [20, 28, 29]. The participants' mean age varied from 29 to 67 years old.

2.3.2. Application of stimulation

Two studies verified the effects of tACS on finger-tapping task performance [17, 25]. One study verified the effects of tACS on Diadochokinesia task performance [17]. One study verified the effects of tDCS on the Symbol Coding-WAIS III performance task [27]. The Symbol Coding subtest is a component of the Wechsler Adult Intelligence Scale III. Participants task is to quickly write the corresponding numbers for each symbol within

a specified time limit. The Symbol Coding can be used to assesses visual-motor coordination. One study verified the effects of tACS on ankle and knee joint flexion and extension lag time [26]. Two studies verified the effects of tDCS on finger chase movement task performance [20, 28]. One study verified the effects of tDCS Nine-hole Peg Test performance [29]. On Nine-hole Peg Test the participant is required to pick up nine pegs, one at a time, from a container, and insert them into nine holes on a board as quickly as possible. After all pegs are inserted, the participant is then asked to remove them from the holes and place them back into the container. The test measures the time taken to complete these tasks. It is often used to evaluate manual dexterity and coordination.

2.3.3. Stimulation type and intensity

Table 3 shows the extracted information regarding the characteristics of the transcranial stimulation protocols, the comparison made, the main outcomes and the conclusions of the selected studies. Four studies adopted protocols based on tDCS [27, 20, 28, 29] and three studies adopted protocols based on tACS [17, 26, 25]. Three studies involving tDCS adopted an intensity of 2mA [27, 20, 28, 29] and one of them also adopted 1mA [28]. Regarding the tACS studies, two studies adopted an intensity of 1mA [17, 25] and one study adopted a 2mA current intensity [26].

2.3.4. Sessions number and duration

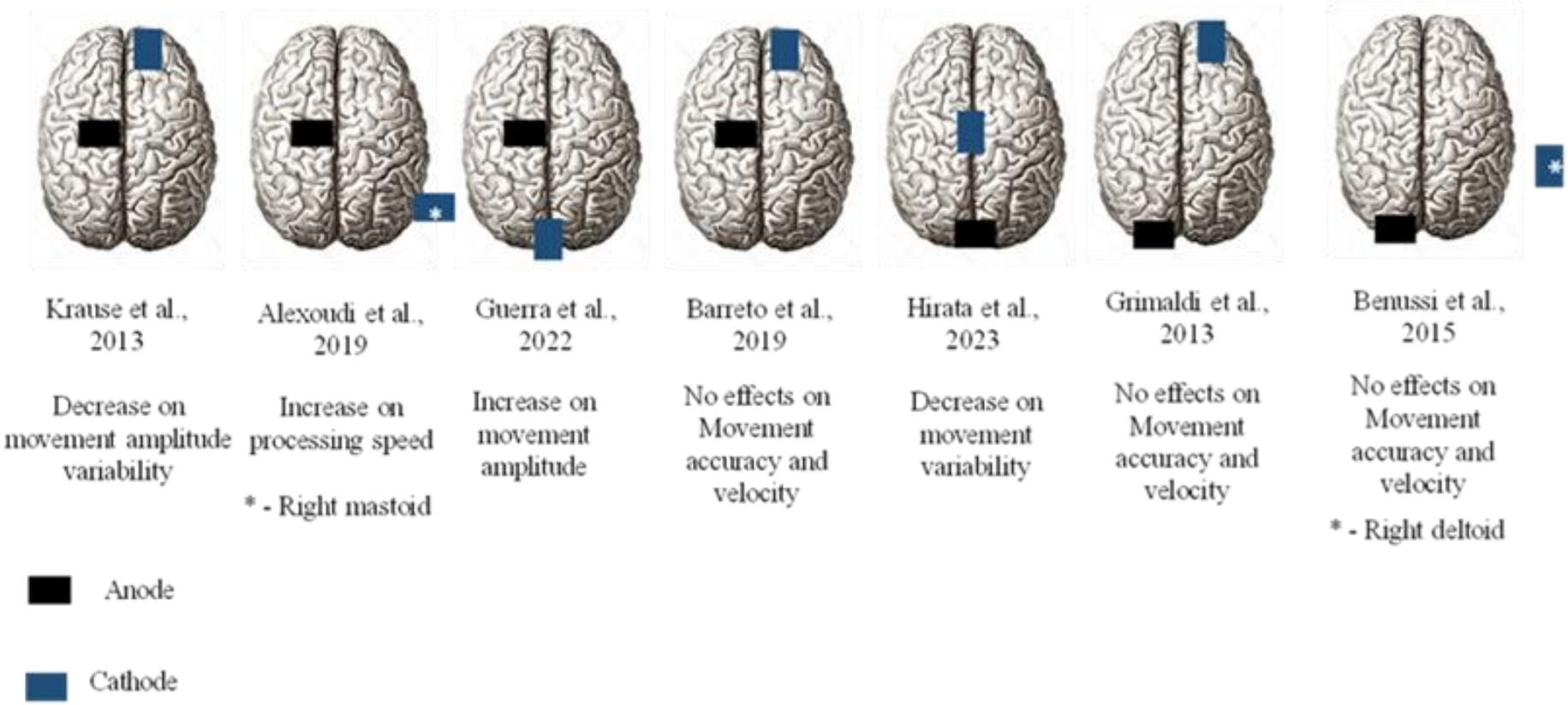
Two studies adopted one intervention day [17, 25], one study adopted two interventions days [29], one study adopted three-intervention days [26], one study adopted five-intervention days [20], one study adopted 6 intervention days [28] and one study adopted 10 intervention days [27]. One study adopted an intervention of 5min [26], one study adopted an intervention of 6min [25], one study adopted an intervention of 15min [17] three studies adopted an intervention of 20min [20, 28, 29] and one study adopted an intervention of 30min [27].

2.3.5. Cortical regions stimulated and electrode size

Figure 2 shows the stimulated areas adopted in each study. Four studies stimulated the motor cortex [17, 27, 25, 20] while the other three studies stimulated the cerebellum [26, 28, 29]. Krause et al., [17] positioned the anode at the motor cortex, with a 35cm^2

electrode size, and the cathode at the contralateral supraorbital area. Alexoudi et al., [27] positioned the anode at the motor cortex, with a 35cm^2 electrode size, and the cathode at the right mastoid. Guerra et al., [25] positioned the anode at the motor cortex, with a 25cm^2 electrode size, and the cathode at the occipital cortex. Barreto et al., [20] positioned the anode at the motor cortex, with a 35cm^2 electrode size, and the cathode at the contralateral supraorbital area. Hirata et al., [26] positioned the anode at the inion region, with a 25cm^2 electrode size, and the cathode at the vertex region. Grimaldi et al., [28] positioned the anode at the cerebellum, with a 20cm^2 electrode size, and the cathode at the contralateral supraorbital area. Benussi et al., [29] positioned the anode at the cerebellum, with a 35cm^2 electrode size, and the cathode at the right deltoid.

Figure 2. Transcranial stimulation positioning and effects



2.3.6. Main findings

Five studies observed improvements in movement coordination after transcranial electrical stimulation such as an increase in movement amplitude and velocity and a decrease in movement amplitude variability [17, 27, 26, 25, 29], two studies observed no effects of transcranial electrical stimulation on movement coordination [20, 28] (Table 3). Regarding tACS Krause et al., [17] observed that one tACS session over the motor cortex of 20Hz at 1mA for 15 minutes decreased the movement amplitude variation of finger taping task, and Guerra et al., [25] observed that one tACS session of 70Hz over the motor cortex at 1mA for 6 minutes increased movement amplitude of PwPD. Hirata et al., [26] observed that older people, after three tACS sessions of 5 minutes at 2mA over the cerebellum, decreased the temporal difference variability between ankle and knee joints during a flexion/extension movement. Regarding tDCS Alexoudi et al., [27] observed that ten tDCS sessions over the motor cortex for 30 minutes at 2mA increased visuo-motor coordination processing speed during the Symbol Coding-WAIS III test performance on people with PSP. Benussi et al., [29] observed that after two tDCS sessions of 20 minutes at 2mA over the cerebellum, people with PSP increased the arm and finger movement velocity during the nine-hole peg test. Although improvements were observed after tDCS or tACS application some of the selected studies did not observe improvements after transcranial electrical stimulation sessions. Barreto et al., [20] observed that five tDCS sessions of 20 minutes at 2mA over the motor cortex also did not improve the arm/finger movement velocity of people with Parkinsonism during a finger chase task. Grimald et al., [28] observed that six tDCS sessions over the cerebellum for 20 minutes at 2mA did not improve movement velocity during a finger click task of people with Parkinsonism.

2.3.7. Methodological quality assessment and risk of bias

The methodological quality assessment revealed that 37.5% of the reviewed studies were classified as excellent, 25% as good and 37.5% as fair according to the PEDro scale (Table 4). Regarding the risk of bias assessed through The Cochrane risk of bias assessment, using the RobVis tool, 2 studies were considered to have some concerns regarding the risk of bias and six studies were considered to have low risk of bias (Figure 3).

Table 2. Methodological characteristics and main outcomes of the reviewed studies.

Study	Population	Design	Main objective	Main Outcomes
First author (year)	**- Population (older people; PD; Parkinsonism)** **- Groups (male or female; mean ± standard deviation (age)**	**- Study design (e.g., Parallel arm, cross-over, randomized, double-blind, sham-controlled)**	**- General objectives** **- Specific objectives**	**-Outcome domain (bimanual; bilateral; between arms; between legs; between arm and leg; between fingers; between feet)** **-Measurement (movement duration; movement frequency/ amplitude; continuous relative phase; muscle activity; force application)** **-Task (walking; finger taping; Diadochokinesia; test name)**
Krause (2013)	-PD -PD group (n=10, 49.4 ± 3.1, 5M/5F); CG group (n=10, 47.8 ± 4.3, 5M/5F)	- Within–subject, double blind, randomized, sham-controlled.	- Investigate the effects of tACS on cortical muscular coupling, on fast finger taping and on diadochokinesia. - Investigate whether stimulation effects may vary depending on the exact stimulation frequency.	- Hand and finger coordination - Movement amplitude and frequency - Finger taping and Diadochokinesia
Alexoudi (2019)	- Parkinsonism -PSP group (n=8, 67.4 ± 7.4, 4M/4F)	-Parallel arm	- Investigate the effects of tDCS on patients with PSP motor condition. - Investigate the effects of tDCS on motor outcome as it is reflected on a disease-specific rating scale.	-Visuomotor coordination -Information processing speed -Symbol Coding-WAIS III
Hirata (2023)	-Older people -Healthy young adults group (n=12, 23.8 ± 4.1, 3M/9F); Healthy older adults group	- Parallel arm	- Investigate the effects of cerebellar tACS on the coordination of multi-joint movements of neurologically healthy older people. - To test the effects of synchronized and unsynchronized cerebellar activity,	-Leg coordination -Difference of mean lag time from the final to the first trial; Variation of the lag time from the final to the first trial - Ankle and knee joint flex/extension

	(n=17, 72.8 ± 5.5, 6M/9F)		through tACS, on point tracking, finger taping and eye-tracking tasks.	
Guerra (2022)	-PD -Health age matched Elderly (n=16, 67.4 ± 9.8, 12M/4F); PD group (n=18, 67.4 ± 9.8, 14M/4F)	- clinical trial, double-blind, sham-controlled	- Investigate the role of altered oscillatory activity within the basal ganglia and primary motor cortex on the PD sign and symptom of bradykinesia. - The study assessed whether driving beta and gamma cortical rhythms through non-invasive brain stimulation using tACS modulates movement amplitude in PwPD.	-Hand coordination -Difference in movement amplitude and frequency between stimulation condition -Finger tapping
Barreto (2019)	-Parkinsonism - Young adult with cerebellar ataxia (n=7, 36.57 ± 21,5, 4F/3M)	- Cross-over, double-blinded, auto-matched, pilot study	- To investigate if tDCS could be used as a rehabilitation tool for patients with cerebellar ataxia. - To measure the effects of tDCS on gait, posture, and coordination of movements in subjects with cerebellar ataxia.	-Hand coordination, intersegmental coordination -Difference in movement velocity -Finger movement (Finger chase, Nose finger Test, Fast alternating hand movements – SARA scale), Standing up a chair (maximum movement velocity – CvMob)
Grimaldi (2013)	-Parkinsonism - Young adult with cerebellar ataxia (n=9, 51.33 ± 14.04, 7M/2F)	- Double-Blind, sham-controlled	- To investigate the effects of anodal tDCS over the cerebellum in a group of cerebellar ataxia patients. - To investigate the effects of cerebellar tDCS on short and long-latency stretch responses, quantitative coordination task in upper limbs and posture of people with cerebellar ataxia.	-Upper limb coordination -Difference in movement velocity - Finger movement velocity (number of clicks during the MTC test)
Benussi (2015)	-Parkinsonism	- Cross-over, double-blind,	- To assess the effects of a single session of anodal cerebellar tDCS on clinical performance in patients with ataxia.	-Upper limb coordination -Difference in movement velocity

	- Young adult with cerebellar ataxia (n=19, nr, nr)	sham-controlled		-Arm and finger movement velocity (time to complete the Nine-hole Peg Test)

PD: Parkinson's disease; CG: Control group; PSP: Progressive supranuclear palsy; MTC: Mechanical counter test; SARA: Scale for the Assessment and Rating of Ataxia; tDCS: transcranial direct current stimulation; tACS: Transcranial alternated current stimulation.

Table 3. Methodological characteristics and conclusions of the reviewed studies.

Study	**Transcranial stimulations characteristics**	**Comparison**	**Conclusions**
First author (year)	**-Stimulation type (tDCS; tACS)** **-Polarity of stimulation current (anodal, cathodal; inhibitory, excitatory)** **- target area stimulated** **- Reference electrode** **- Electrode/coil size** **- Duration** **- Intensity** **- Number of sessions** **- Additional intervention (yes or no)**	**-active vs sham or experimental vs control group or pre vs post** **- Assessment period (pre; post and follow–up)**	**- The conclusions suggested by the authors regarding the effects of transcranial stimulation on movement coordination**
Krause (2013)	-tACS -Excitatory -M1 (most affected) -Contralateral supraorbital area -35cm2 -15min -1mA -1 -No	-PD 10Hz vs PD 20Hz; PD 10Hz vs PD sham; PD 20Hz vs PD sham; PD 20Hz vs CG 20HZ; -Pre and post	- tACS at 20Hz decreased the movement amplitude variation during fast finger taping in people with PD

Alexoudi (2019)	-tDCS -Anodal -Motor and premotor cortex -Right mastoid -35cm2 -30min -2mA -10 -No	- Baseline vs post1 (1 day after the end of tDCS sessions) vs follow up1 (30 days after the end of tDCS sessions) vs follow up 2 (90 days after the end of tDCS sessions) -Baseline (day1), post day11), follow up1 (day 30) and follow up2 (day90)	-Individuals' processing speed increased only at post moment by the increase in the number of items done at Symbol Coding-WAIS III test.
Hirata (2023)	-tACS -Excitatory -Inion (cerebellum) -Vertex -25cm2 -5min -2mA -3 -No	-Young vs older adults in synchronized tACS with the target condition vs Young vs adults in unsynchronized tACS with the target condition vs Young vs adults in sham tACS condition -pre-test with 3 conditions	- Neurological health older adults showed a reduction in 32ynchronization variation in synchronized tACS condition.
Guerra (2022)	-tACS -Excitatory -M1 -Occipital cortex -25cm2 -6min -1mA -1 -No	-Health subjects vs PD group (On X OFF medication) in 20Hz stimulation condition vs 70Hz stimulation condition vs Sham stimulation condition -pre-test with three conditions	- 20Hz-tACS reduced movement velocity and 70Hz-tACS increased movement amplitude in patients, while the sequence effect did not change during b- and c-tACS.
Barreto (2019)	-tDCS -Anodal -M1	-tDCS active vs tDCS sham	-Patients did not show a significant difference on CvMob analyses and on Finger

	-Contralateral supraorbital area -35cm2 -20min -5 -2mA -No		Chase, Nose finger Test and Fast alternating hand movements on the SARA scale, but they improved their gait parameters.
Grimaldi (2013)	-tDCS -Anodal -Cerebellum -Contralateral supraorbital area -20cm2 -20min -6 -1mA, 2mA -No	-Baseline vs tDCS active vs tDCS sham	-Anodal tDCS of the cerebellum does not improve a functional coordination task in the upper limbs (MTC test).
Benussi (2015)	-tDCS -Anodal -Cerebellum -Right deltoid -35cm^2 -20min -2 -2mA -No	-Baseline vs tDCS sham -Baseline vs tDCS active	-tDCS anodal improved the SARA, ICARS scores, 9HPT, and 8MT in the whole cohort of patients, demonstrating significant improvement in posture and gait and limb coordination subscore

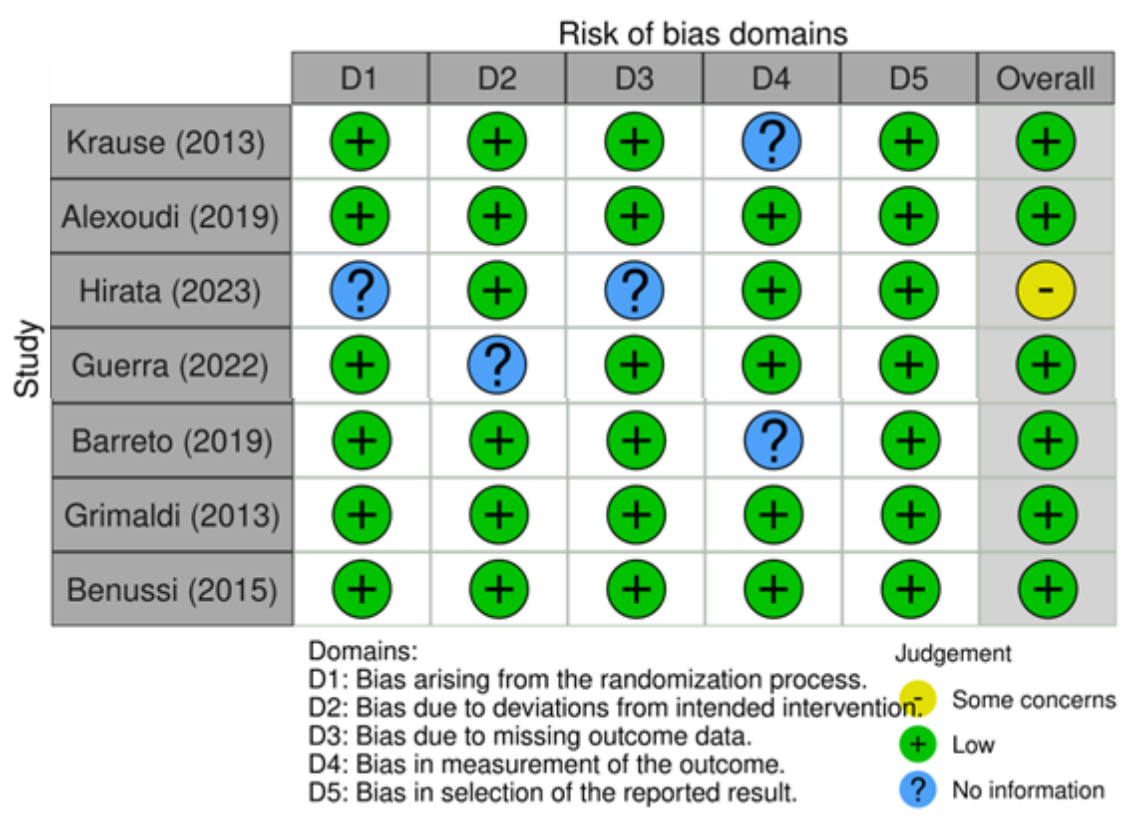
PD: Parkinson's disease; CG: Control group; MTC: Mechanical counter test; SARA: Scale for the Assessment and Rating of Ataxia; tDCS: transcranial direct current stimulation; tACS: Transcranial alternated current stimulation.

Table 4. Methodological quality assessment.

PEDro score – items	Studies						
	Krause (2013)	**Alexoudi (2019)**	**Hirata (2023)**	**Guerra (2022)**	**Barreto (2019)**	**Grimaldi (2013)**	**Benussi (2015)**
1	1	1	1	1	1	1	1
2	1	0	1	1	0	0	1
3	1	0	1	1	0	0	1
4	1	1	0	1	0	1	1
5	1	0	1	1	1	1	1
6	0	0	0	0	0	0	1
7	1	0	0	1	1	0	1
8	1	1	1	1	1	0	1
9	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1
total	9	5	6	9	6	5	10

PEDro: Physiotherapy Evidence Database rating scale; 1: Eligibility criteria were specified? 2: Subjects were randomly allocated to groups? 3: allocation was concealed? 4: The groups were similar at baseline regarding the most important prognostic indicators. 5: There was blinding of all subjects? 6: There was blinding of all therapists who administered the therapy. 7: There was blinding of all assessors who measured at least one key outcome? 8: Measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups. 9: All subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analyzed by "intention to treat"? 10: The results of between-group statistical comparisons are reported for at least one key outcome? 11: The study provides both point measures and measures of variability for at least one key outcome**.**

Figure 3. Risk of bias assessment



2.4. Discussion

The present study aimed to propose recommendations regarding brain regions to stimulate, as well as the number of sessions and their durations to improve movement coordination in people with Parkinsonism and older adults. We assessed various stimulation protocols, encompassing the current type (tDCS or tACS), number of sessions, stimulation target, current intensity, and session duration. From the studies reviewed, positive effects of tACS or tDCS on movement coordination emerged in five studies, as evidenced by increased movement amplitude [25], decreased movement amplitude variability [17, 26], and enhanced movement speed [27, 29]. However, two studies reported no significant effects on movement coordination [20, 28], although one of these reported improvements in specific gait parameters like increased gait speed [20].

2.4.1. tDCS and tACS positive effects

The aging process and the changes caused by Parkinsonism lead to a decrease in movement speed and amplitude [30, 31]. The reduction in movement amplitude and speed during locomotor tasks is related to impairments in brain functional connectivity in people with parkinsonism and neurologically healthy older people [32], and to abnormal neural oscillations in people with parkinsonism [33]. tDCS can modulate cortical excitability [18] and improve brain functional connectivity [34, 35]. Those positive effects caused by tDCS on brain function might be related to the increase in movement processing speed observed by Alexoudi et al., [27] and to the improvement of limb coordination sub score on the SARA scale observed by Benussi et al., [29]. tACS alternated current can modulate and improve neural oscillations at different frequencies [36, 33]. These positive effects of tACS on neural oscillation might be related to the decrease in movement amplitude variability observed by Krause et al., [17], the improvement in joint synchronization observed by Hirata et al., [26] and the increase in movement amplitude observed by Guerra et al., [25].

2.4.2. Electrical current intensity

Two studies adopted a current intensity of 1mA and five studies adopted a 2mA current intensity. Positive results were observed in both current intensities. The literature suggests that the cortical excitability might be related to the current intensity, which means that the neurons of the stimulated cortical region became more excitable at greater current intensity [18, 36]. This fact explains why there were a greater number of studies that adopted a 2mA current intensity. However, there might be some current intensity-associated disadvantages, such as problems with blinding the participants since that at greater current intensities tDCS or tACS became more perceptible and as problems related to pain and skin irritability [36].

2.4.3. Protocols duration

Three studies adopted protocols of 20 minutes [20, 28, 29], one study adopted a 15-minute protocol [17], one study adopted a 30 minutes protocol [27], one study adopted a 5 minutes protocol [26] and one study adopted a 6 minutes protocol [25]. The duration of transcranial electrical stimulation protocol directly influences the intensity and retention of stimulation effects [37, 38]. In stimulation protocols up to 10 minutes were

observed less pronounced effects on cortical plasticity when compared to protocols up to 20 minutes [37]. However, is noteworthy that the effects observed in protocols with 30 minutes were less pronounced than the effects of 20 minutes protocols [37, 38]. These effects are observed due to impairments in neural adaptation caused by long periods of transcranial electrical stimulations [18]. Those findings suggest that does not mean that the longer the protocol lasts, the more pronounced its effects will be.

2.4.4. Target areas

The choice of stimulation targets is pivotal, and understanding this selection along with the observed outcomes is essential. Four studies focused on stimulating the motor cortex [17, 27, 20, 25] and three studies targeted the cerebellum [26, 28, 29]. The rationale for these choices is rooted in the specific neurological conditions under investigation and their associated mechanisms. Parkinson's disease affects two distinct cortical pathways: one involving the basal ganglia and brainstem responsible for resting tremors (the "direct pathway") and another involving the basal ganglia and motor cortex contributing to issues in walking and coordination (the "indirect pathway") [39, 40]. Notably, two motor cortex stimulation studies included individuals with Parkinson's disease [17, 25], one involved progressive supranuclear palsy [27], and another featured cerebellar ataxia [20]. These studies observed significant improvements in motor symptoms, such as bradykinesia, likely linked to modulating abnormal basal ganglia activity through motor cortex stimulation. However, the study involving cerebellar ataxia [20] did not demonstrate improvements in movement coordination-related characteristics. Cerebellar ataxia is characterized by deficits in movement coordination and walking, with various affected structures, including the cerebellar vermis, cerebellar hemispheres, and deep cerebellar nuclei [41]. Benussi et al. [29] and Hirata et al. [26] applied cerebellar stimulation in patients with cerebellar ataxia and neurologically healthy older people and reported positive outcomes, including improvements in signs and symptoms associated with cerebellar ataxia and aging, such as impairments in movement velocity and amplitude. These effects can be attributed to the cerebellum's role in fine-tuning motor control. However, Grimaldi et al. [28] did not observed improvements in movement coordination-related characteristics. In summary, the stimulation targets was guided by the specific characteristics of the neurological conditions under study and the underlying physiological mechanisms. Studies stimulating the motor cortex yielded positive effects in improving motor symptoms in Parkinson's disease patients, potentially through the

modulation of basal ganglia activity. Conversely, studies targeting the cerebellum reported positive outcomes related to motor coordination, suggesting a role in compensating for cerebellar deficits. These findings underscore the importance of tailoring stimulation targets based on the specific condition being addressed.

2.4.5. Positive stimulation effects on movement coordination

Overall, the selected studies showed positive effects of transcranial electrical stimulation (e.g., tDCS or tACS) increasing movement amplitude and speed. The relation between movement amplitude and speed significantly influences the coordination pattern in motor tasks [20]. The increase of arm swing amplitude and frequency during gait generates positive effects on walking parameters, including increased stride length and velocity [42], on interlimb coordination, such as a decrease in the coordination pattern variability [43], and an increase on brain functional connectivity [32]. It is noteworthy that the selected studies observed the effects of tDCS or tACS on movements of discreet skills such as finger taping [17], leg flexion/ extension [26], and single limb movement such as nose finger test [29]. However, the positive effects of tDCS and tACS on movement amplitude and speed of discreet skills lead us to speculate that transcranial electrical stimulation might be a good strategy to increase movement amplitude and frequency during a complex skill such as walking.

2.4.6. Limitations of the current study

The limited number of eligible studies hindered our ability to discuss the optimal stimulation sections required for enhancing movement coordination in PwPD and neurologically healthy older individuals. Additionally, a dearth of research investigating transcranial electrical stimulation effects during complex tasks like walking in these populations was noted. Consequently, further studies should explore varied stimulation protocols' effects on movement coordination during complex tasks. Among the selected studies, limitations were highlighted. Alexoudi et al. [27] and Benussi et al. [29] reported small sample sizes, impacting result generalizability. Barreto et al. [20] utilized a two-phase design, complicating the isolation of tDCS effects. Grimald et al. [28] included a sample size that might not fully capture tDCS effects in cerebellar ataxia. Guerra et al. [25] focused solely on Parkinson's disease, limiting findings' applicability. Hirata et al. [26] mentioned limitations related to age representation, potentially limiting

understanding of cerebellar rhythm across lifespans. Krause et al. [17] faced limitations due to a narrow condition focus, necessitating further research for confirmation.

2.5. Conclusions

tDCS and tACS can improve movement coordination in neurologically healthy older people and individuals with parkinsonism. Following our systematic review, we conclude that an effective stimulation target to improve movement coordination in neurologically healthy older people and individuals with Parkinsonism should i) consider the participants' status, ii) have a current intensity of 2mA, and iii) last at least 20 minutes.

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3. Chapter 3

Study #2 - The acute effects of transcranial direct current stimulation combined with locomotor exercise on people with Parkinson's disease locomotion and cortical activity

The acute effects of transcranial direct current stimulation combined with locomotor exercise on people with Parkinson's disease locomotion and cortical activity

Abstract

Transcranial direct current stimulation (tDCS) and locomotor exercise are promising tools for addressing locomotor deficits and cortical dysfunction in people with Parkinson's disease (PwPD). Combining tDCS with locomotor exercise may have synergistic effects on gait and cortical activity. However, most protocols focus primarily on lower-limb functions, neglecting the contribution of arm movements to PwPD gait. This study investigated the acute effects of a combined tDCS and locomotor exercise protocol, emphasizing increased arm swing amplitude and speed, on cortical activity, interlimb coordination, and gait parameters in PwPD. Twenty-two participants were tested in a sham-controlled, randomized, crossover design. Disease severity and cognitive status were assessed using the MDS-UPDRS and Mini Mental State Examination (MMSE). Participants attended two sessions, spaced one week apart, performing active or sham tDCS protocols combined with locomotor exercises on each day. tDCS was applied at 2mA for 20 minutes (active condition) or 10 seconds (sham condition) during the locomotor exercise, with the anode placed over the motor cortex contralateral to the most affected side and the cathode over the contralateral supraorbital area. Locomotor exercises involved walking along an 18-meter circuit under two conditions: increased arm swing amplitude (10 minutes) and speed (10 minutes). Gait was assessed using the GaitRite system, interlimb coordination via wrist and ankle accelerometers with continuous relative phase analysis, and cortical activity using a 64-channel EEG with power spectral density (PSD) measured in alpha, beta, and gamma bands at pre-frontal, motor and occipital cortex. A two-way ANOVA (group: active/sham tDCS; moment: pre/post) showed improvements in stride length ($p<0.001$), velocity ($p<0.001$), and reduced double support time ($p<0.001$) in both groups. These changes were accompanied by decreased gamma PSD in the motor cortex ($p=0.010$) and reduced alpha ($p=0.008$), beta ($p=0.001$), and gamma PSD in the occipital cortex. These findings suggest that a single session of locomotor exercise targeting arm swing can improve gait and cortical activity in PwPD. However, multiple tDCS sessions may be necessary to achieve synergistic effects on locomotor behavior and cortical function on PwPD.

Keywords: Neuro-degenerative diseases; Brain Stimulation; Cortical activity; Walking

3.1. Introduction

Parkinson's disease (PD) is a neurodegenerative condition characterized by the degeneration of dopaminergic neurons in the substantia nigra within the basal ganglia (Wichmann & DeLong, 2014). This dopamine loss leads to an increased basal ganglia inhibition of brain areas involved in cyclic movements and muscle tone regulation, such as the mesencephalic locomotor region and the pedunculopontine nucleus (Blandini et al., 2000; Takakusaki, 2017). Consequently, people with PD (PwPD) exhibit locomotor impairments, including reduced stride length and speed, increased arm swing asymmetry, and impaired interlimb coordination (Takakusaki et al., 2008; Ospina et al., 2018; Winogrodzka et al., 2005). Due to the basal ganglia-cortex connectivity, PwPD presents higher cortical activity in areas like the prefrontal cortex (PFC) and motor cortex (M1) as a compensatory response to the dopaminergic losses (Stuart et al., 2018; Takakusaki, 2017). Standard PD treatment is based on medications that improve gait and restore cortical activity (Orcioli-Silva et al., 2021) but, over time, can cause side effects such as dyskinesia, raising the need for complementary therapies (Jankovic, 2000; Voon et al., 2009).

Physical exercise offers benefits for various functional aspects in PwPD and older adults (Ni et al., 2018; Casas-Herrero et al., 2022). Studies show that five weeks of walking training can enhance PwPD stride length and velocity (Bello et al., 2013), while six weeks of neuromuscular training improves coordination stability in young adults (Sheikhi et al., 2021). Yet, most PD exercise studies emphasize the effects on lower-limb movements, often overlooking upper-limb contributions to gait. Arm movement deficits, including reduced and asymmetrical swing (Ospina et al., 2018), affect locomotion and interlimb coordination in PwPD (Zampier et al., 2018; Winogrodzka et al., 2005) and are linked to M1 power frequency changes (Borhanazad et al., 2024). Instructions to increase arm swing amplitude and frequency (Zampier et al., 2018), potentially act as motor memory facilitators, and could enhance motor skill consolidation as seen in similar studies (Taubert et al., 2015; Statton et al., 2015). These findings suggest that arm swing characteristics, such as amplitude and speed, positive influence PwPD locomotion and may impact motor and cortical parameters when increased (Wersink et al., 2020; Zampier et al., 2018). However, there is no stablished knowledge about the effects of locomotor exercises, focused on the increase of arm swing amplitude and frequency, on PwPD gait, coordination parameters and cortical activity.

Transcranial direct current stimulation (tDCS) is another promising PD therapy (Broeder et al., 2015). This non-invasive brain stimulation technique uses scalp-applied currents to modulate neuron membrane potentials, facilitating (anodal tDCS – atDCS) or inhibiting (cathodal tDCS – ctDCS) the neurons fiering threshold in targeted brain regions (Nitsche & Paulus, 2000). A single tDCS session over the supplementary motor area (SMA) improves young adults' movement coordination (Carter et al., 2017), and tDCS applied on PFC enhances interlimb accuracy (Leenus et al., 2014). Studies suggest that combining tDCS and exercise has synergistic effects on cognition, mobility, and gait in PwPD (Beretta et al., 2020) and causes an increase in PFC activity (Conceição et al., 2021).

In terms of cortical activity, both tDCS and exercise have positive effects by increasing the connectivity between brain areas (Baxter et al., 2017; Calabro et al., 2019). Arm swing instructions also appear to generate positive effects on cortical activity by increasing event related desynchronization of alpha band on M1 (Wersink et al., 2020). Yet, the effect of combining tDCS with an exercise protocol focused on arm swing amplitude and frequency on PwPD cortical activity is still unknown. Understanding these effects could reveal significant insights into motor-related electroencephalography (EEG) activity that are related to walking regulation.

Facing those gaps, this study aimed to assess the acute effects of a combined tDCS and locomotor exercise protocol focused on arm swing amplitude and frequency on PwPD walking parameters, interlimb coordination, and cortical activity. Given prior findings, our hypothesis is that this protocol will improve gait and interlimb coordination through changes in cortical activity in PwPD.

3.2. Methods

3.2.1. Study design and participants

This randomized, double blind, cross-over and sham-controlled study was retrospectively registered in the Brazilian Registry of Clinical Trials (ReBEC - RBR-10v4dxgm). The study was carried out at Posture and Gait Laboratory Study and in Human Movement Research Laboratory, São Paulo State University (Unesp). PwPD residents at the local community that participates in the physical activity programs at the same Labs were recruited to participate in this study. Sample size determination was based on a priori analysis using the G*Power software. The analysis indicated that 18

participants would be needed to detect the difference between factors using a two-way ANOVA (α<0,05) and a (1-β) of 0.80. The sample size analysis was preformed based on the stride length variable ($F_{2,68} = 25.503$; $p < 0.001$; $\eta p^2 = 0.429$) from our previous study (Zampier et al., 2018). Thus 22 PwPD were recruited to participate in this study. The randomization was performed by a member of the Posture and gait laboratory study research group that was not involved in the data collection.

As the inclusion criteria, PwPD should have the disease diagnosed according to the UK Brain Bank criteria, age ≥ 60 years, to continuously use the disease specific medication, score between 1 and 3 in the Hoehn & Yahr scale (H&Y) (Schenkman et al., 2001) and had independent locomotion. The following exclusion criteria were adopted i) severe cognitive decline (score < 20 in Mini-Mental State Examination – MMSE) (Brucki et al., 2003); ii) Changes in PD medication during the study period; iii) musculoskeletal and not corrected visual impairments that could the experimental protocol performance; iv) presence of any uncontrolled disease that could affect peripheral sensory function (e.g., diabetes); and v) risk of receiving tDCS (neural implants, pacemaker, history of seizures and epilepsy). Participants were also excluded if they did not complete the experimental protocol (tDCS sessions and assessments). The study aims and experimental protocol were described to all participants during the recruitment. This study was approved by the São Paulo State University ethics committee (CAAE: 58135122.2.0000.5465). All PwPD who participated in this study signed the consent statement before the experiments began.

3.2.2. Study Protocol

The experimental protocol was conducted in 2 days with one washout week between them. On the first day, before the beginning of the experimental procedures, clinical and cognitive assessments for the sample characterization were performed. On each day participants performed the walking, interlimb coordination and cortical activity assessments immediately before (pre) and after (post) the combined tDCS and locomotor exercise protocol. At least two team members that were blinded to the group allocation conducted the walking, interlimb coordination and cortical activity assessments.

3.2.3. Clinical, cognitive and walking assessments

An experienced evaluator applied the Movement Disorders Society – Unified Parkinson's Disease Rating Scale – motor section (MDS-UPDRS III) to assess the disease

motor severity (Goetz et al., 2008) and the MMSE to assess the participants cognitive status (Brucki et al., 2003). For the walking assessment participants walked over an 8m strait pathway after an auditory cue "*Ready Go*" under their preferred speed for five trials. Before each trial participants were instructed to stand as quiet as possible for 30 seconds in order to normalize their cortical activity. After that participants were asked to start the walking over a 8m pathway. To assess the walking variables such as stride length, velocity and width and double support time a 5,74m pressure sensor carpet (GAITRite®, CIR Systems Inc.) with a sample frequency set at 200Hz was placed in the middle of the pathway in order to avoid the acceleration and de-acceleration periods (Zampier et al., 2018).

3.2.4. Interlimb coordination assessment

To evaluate interlimb coordination among participants, accelerometers (Trigno™ wireless system, Delsys Inc., Natick, USA) operating at a 148.148 Hz sampling frequency were placed on each participant's wrists and ankles (Figure 1a). Acceleration signals were recorded using EMG acquisition software (Delsys Inc., Natick, USA) and processed with MATLAB scripts (2022 version, MathWorks, MA, USA). Continuous relative phase (CRP) analysis was conducted to assess phase angles and variability (V-CRP) between the vertical acceleration signals of contralateral wrists and ankles (Wagenaar & Emmerik, 2000; Winogrodzka et al., 2005). Initially, signals were low-pass filtered using a fourth-order Butterworth filter with a 5 Hz cut-off frequency. Gait events—heel strike (HS) and toe-off (TO)—were identified from ankle acceleration signals for each leg, defining each stride cycle for the CRP analysis (Figure 1b). HS and TO events in the ankle acceleration signal were determined through a parallel experiment, where the ankle acceleration signals of 17 healthy young adults were cross-referenced with a force plate signal (AccuGait, Advanced Mechanical Technologies, 200 Hz) during walking (Figure 2a). This procedure enabled us to define a consistent cyclical event in the ankle acceleration signal that temporally corresponded to HS and TO events. The same cyclical events were observed in the PwPD participants in this study (Figure 2b). After defining stride cycles for each leg, the signals were time-normalized to represent a gait cycle percentage (0–100%). The ankle and corresponding wrist acceleration signals for each cycle were then transformed using the Hilbert transformation (Lamb & Stöckl, 2014) (Figure 1c). Following this, CRP was calculated between the normalized signal pairs (right wrist with left ankle, and left wrist with right ankle) (Winogrodzka et al., 2005) (Figure 1d). CRP

variability (V-CRP) was calculated as the standard deviation of the mean CRP values for each stride (Wagenaar et al., 2000). An average CRP and V-CRP value was obtained for each participant, in each pair, and in each moment. The mean CRP and V-CRP values for each pair was used in the statistical analysis. Finally, the mean CRP and average V-CRP for each participant were compared across moments and groups.

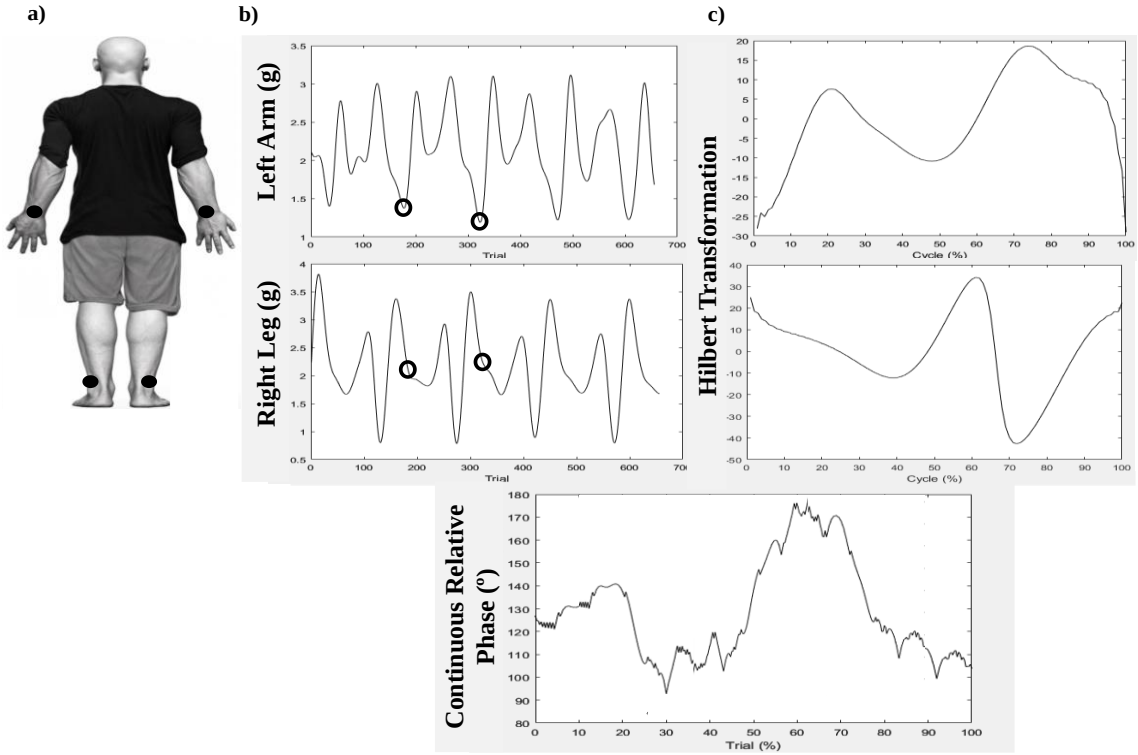

Figure 1. a: Accelerometer Placement over the wrists and ankles; **b:** Filtered acceleration data; **c:** Normalized cycle after the Hilbert Transformation; **d:** Continuous Relative Phase data during the trial.

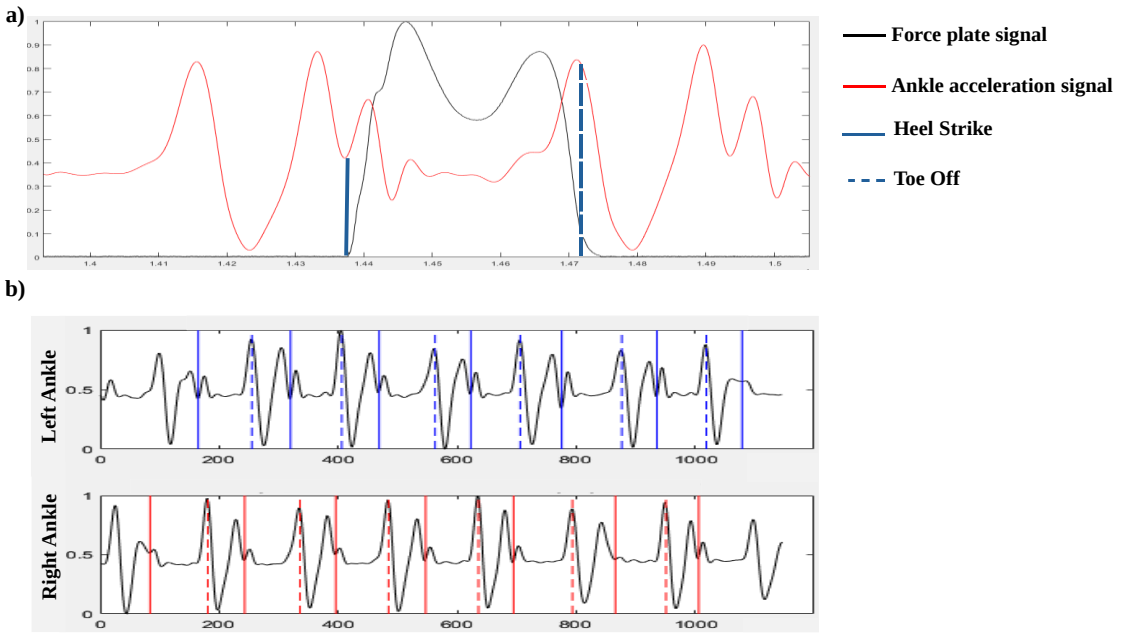

Figure 2. a: Ankle acceleration signal crossed with force plate signal of a healthy young adult with the heel strike and toe-off events; **b:** Ankle acceleration signal with the heel strike and toe-off events of one of this study participants.

3.2.5. Cortical Activity Assessment

Cortical activity was recorded using electroencephalogram (EEG) equipment (eego™sports, ANT Neuro, Enschede, Netherlands, 1024 Hz), following procedures recommended by the EEG manufacturer. The EEG system included a cap with 64 passive electrodes placed on the participants' scalps according to the international 10–20 electrode placement system, with electrode impedance kept below 10 KΩ. To ensure accurate temporal alignment between EEG and movement coordination data, the EEG and accelerometer signals were synchronized via a common event marker. EEG analysis (Figure 3) was conducted using the open-source software EEGLAB (Delorme et al., 2011). The EEG signal was band-pass filtered (4–50 Hz) and pre-processed with the PREP pipeline (Bigdely-Shamlo et al., 2015) to remove line noise (60 Hz), re-reference and interpolate channels. Artifact Subspace Reconstruction (ASR) (Jacobsen et al., 2021) was used to remove flat-line segments and low-correlation channels. After pre-processing, the interested channels were selected included three for the prefrontal cortex (PFC) area (Fz, F3, F4), three for the motor cortex (M1) (Cz, C3, C4), and three for the occipital cortex (POz, PO3, PO4). Independent Component Analysis (ICA) was then performed to identify data-relevant components. The components were labeled using the ICLabel toolbox (Pion-Tonachini et al., 2019), and those with a probability ≥70% of being

related to muscle, eye, heart, or line noise were removed (Guo et al., 2023). Following component removal, Power Spectral Density (PSD) analysis was conducted for each electrode, targeting the α (8–12 Hz), β (13–30 Hz), and γ (31–50 Hz) frequency bands (Faria et al., 2023). The PSD data from each electrode were averaged across prefrontal, motor, and occipital regions for analysis (Tropini et al., 2011). If any selected channels were excluded during pre-processing, participant's data was not included in the analysis. All EEG data analysis was conducted by an experienced researcher.

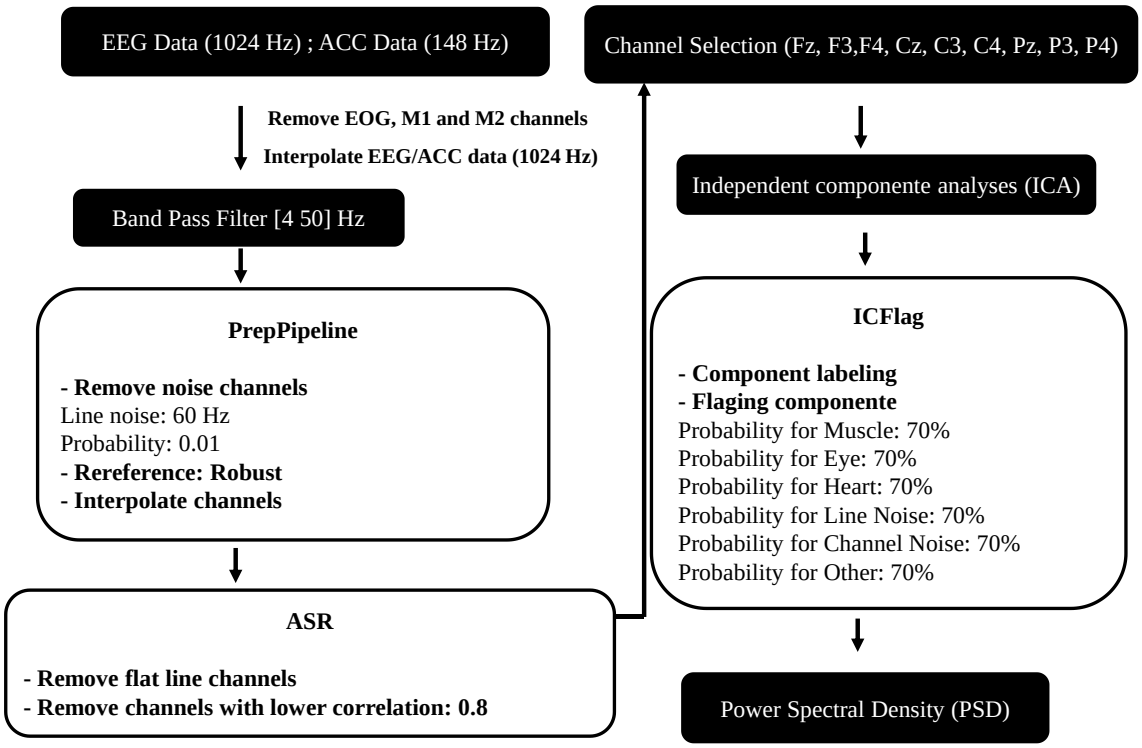

Figure 3. EEG analysis pipeline.

3.2.6. Combined tDCS and Locomotor Exercise Protocol

For the tDCS protocol on the first day participants were randomly assigned in two groups, the active tDCS group (a-tDCS) and the control (sham) tDCS group (s-tDCS). After one wash out week participants groups were inverted. The tDCS protocol was the same in all sessions for all participants. tDCS was applied using a Microestim GENIUS (NKL Electronic Products) via conductive-rubber electrodes, placed in two saline-soaked sponges (35 cm2) while participants performed the locomotor exercise. After the tDCS device was prepared it was placed in a backpack that did not limit the participants' movements. Anodal electrode was placed over the M1 area (C3/C4 – EEG positioning system), and the reference electrode (cathode) was placed over the contralateral

supraorbital region (Nonnekes et al., 2014; Vaseghi et al., 2015). Anodal stimulation was placed in the most affected cerebral hemisphere of PwPD determined by the MDS-UPDRS items (Beretta et al., 2020; Cosentino et al., 2017; Beretta et al., 2015). The same tDCS protocol montage was performed in both active (a-tDCS) and control (s-tDCS) groups. However, for the active tDCS a continuous electrical current with 2mA was applied for 20 minutes with a 30-second ramp-up at the beginning and a 30-second ramp-down at the end of the stimulation period. For the s-tDCS group the same 30 seconds of ramp up/ramp down were performed, but the stimulation remained at 2 mA between these periods for 10 seconds (Beretta et al., 2020). The locomotor exercise protocol focused on the increase of participants arm swing amplitude and speed during walking (Zampier et al., 2018; 2019). After the tDCS placement, participants were instructed to walk over a 18m rectangular circuit with 2 parallel lines of 8m for 20 minutes (online with tDCS protocol) in two conditions, each lasting 10 minutes. In one condition participants were instructed to walk at preferred speed increasing their arm swing amplitude, on the other condition participants were instructed to walk at preferred speed increasing their arm swing speed. Both a-tDCS and s-tDCS groups performed the same locomotor exercise protocol. The order in which the participants began the protocol was randomized. After each tDCS session a structures questionnaire was applied to identify tDCS side effects in both groups (Beretta et al., 2020).

3.2.7. Statistical analysis

Statistical analyses were performed using IBM® SPSS® software (Amos™; Version 22), with a significance level maintained at 0.05. Normality, sphericity and homogeneity were verified by Shapiro-Wilk, Mauchley's and Levene test, respectively. The side effect of tDCS was analyzed by the Mann-Whitney U test. Descriptive statistics were computed using the mean and standard deviation values of each variable considered in this study. The comparisons were performed using two-way ANOVA procedures, with factors for group (a-tDCS and s-tDCS) and moment (pre and post intervention) with repeated measures for the last factor. The Greenhouse-Geisser correction was adopted for the degrees of freedom. Bonferroni post hoc test was applied when interaction between factors were pointed out. Eta-square (η^2) were determined for all main effects, which was categorized as follows: large (> 0.14), medium (0.13 – 0.06), and small (0.05 – 0.01); negligible (< 0.01) (Fritz et al., 2012).

3.3. Results

Table 1 shows the mean and standard deviation for the clinical and cognitive and demographic assessments.

Table 1. Means and standard deviation for PwPD clinical, cognitive and demographic characteristics.

	PwPD (22)
Gender	F(5); M(17)
Age (years)	69.53 ± 3.62
Height (cm)	168.65 ± 18.76
Body mass (Kg)	71.81 ± 12.82
MMEE (pts)	26.61 ± 1.18
MDS-UPDRS III (pts)	29.93 ± 7.99
MDS-UPDRS total (pts)	45.33 ± 14.55
Hoen Yahr	2(21); 2.5(1)
Most affected side	18(Left); 4(Right)

PwPD: People with Parkinson's disease; MMEE: Mini Mental Estate Examination; MDS-UPDRS: Unified Parkinson's disease rating scale adopted by Movement Disorders Society.

3.3.1. tDCS side effects

No difference between groups regarding tDCS side effects was evidenced (Table 2). The main reported sensation was tingling (86% for both groups), 45% of a-tDCS and 41% of s-tDCS group reported itching, 13% of a-tDCS and 9% of s-tDCS group reported burning sensation, 4% of s-tDCS group reported trouble to concentrating and was observed skin redness in 13% of a-tDCS and 9% of s-tDCS group (Table 2). The scores of each stimulation condition for each sensation were mild from the adapted questionnaire. No accidents such as falls occurred during the experimental protocol.

Table 2. Median and quartiles of tDCS side effects score.

	a-tDCS (n= 22)	s-tDCS (n= 22)	p value
Participant's report	**Median (quartiles) percentage**	**Median (quartiles) percentage**	
Headache	0 (0-0) 0%	0 (0-0) 0%	1
Neck pain	0 (0-0) 0%	0 (0-0) 0%	1
Scalp pain	0 (0-0) 0%	0 (0-0) 0%	1
Tingling	1 (0.75-1.25) 86%	1 (0.75-1.25) 86%	0.897
Itching	0 (0-1) 45%	0 (0-1) 41%	0.443
Burning sensation	0 (0-1) 13%	0 (0-1) 9%	0.641
Sleepiness	0 (0-0) 0%	0 (0-0) 0%	1
Metallic/iron taste	0 (0-0) 0%	0 (0-0) 0%	1
Fatigue	0 (0-0) 0%	0 (0-0) 0%	1
Trouble concentrating	0 (0-0) 0%	0 (0-1) 4%	0.935
Acute mood change	0 (0-0) 0%	0 (0-0) 0%	1
Investigator's reports			
Skin redness	0 (0-0) 13%	0 (0-0) 9%	0.841
Skin irritation	0 (0-0) 0%	0 (0-0) 0%	1

Note: Median and quartiles values were calculated from the scores of each participant in each session. 0 = none (I did not feel the sensation addressed); 1 = mild (I mildly felt the sensation addressed); 2 = moderate (I felt the sensation addressed); 3 = strong (I felt the sensation addressed to a considerable degree).

3.3.2. Gait performance

Gait parameters are presented in Figure 4. Participants from both groups presented an increase in stride length ($F_{1.42}$= 22.702; p<0.001; ηp^2= 0.351) and velocity ($F_{1.42}$= 48.145; p< 0.001; ηp^2 = 0.534) and a decrease in double support time ($F_{1.42}$= 17.793; p<0.001; ηp^2 = 0.299) in the post moment. However no moment main effect was observed for stride width ($F_{1.42}$= 3.652; p= 0.099; ηp^2= 0.063). Statistical analysis did not pointed group main effect for stride length ($F_{1.42}$= 2.025; p= 0.788; ηp^2= 0.065), velocity ($F_{1.42}$= 1.956; p= 0.795; ηp^2 = 0.055), width ($F_{1.42}$= 1.652; p= 0.859; ηp^2= 0.043) and double support time ($F_{1.42}$= 1.938; p= 0.841; ηp^2 = 0.039).

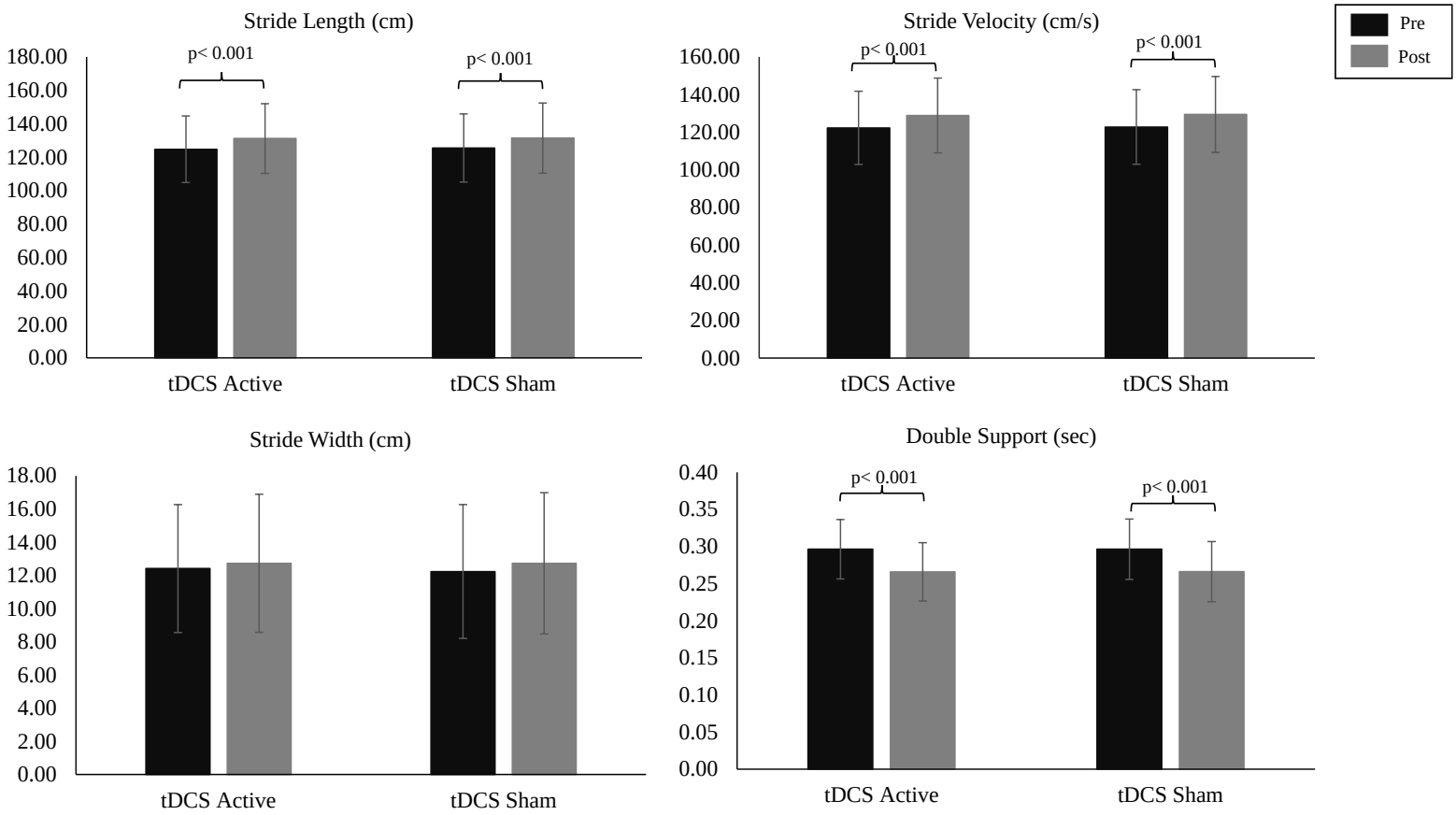

Figure 4. Mean and standard deviation of gait parameters for a-tDCS and s-tDCS groups in pre and post moments.

3.3.3. Interlimb coordination during walking

CRP and V-CRP values are presented in Figure 5. Statistical analysis did not pointed moment main effect for CRP ($F_{1.42}$= 3.652; p= 0.099; ηp^2= 0.063) and V-CRP

($F_{1.42}$= 1.181; p= 0.673; ηp^2 = 0.014), also did not pointed group main effect for CRP ($F_{1.42}$= 1.652; p= 0.859; ηp^2= 0.043) and V-CRP ($F_{1.42}$= 1.498; p= 0.489; ηp^2 = 0.012).

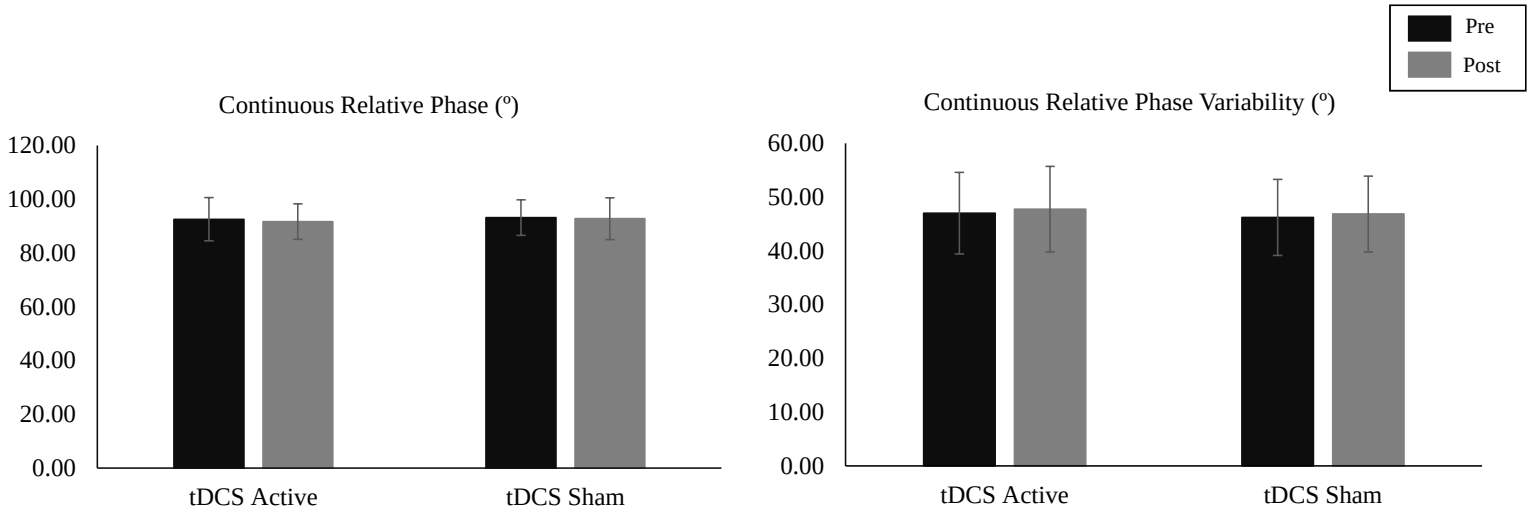

Figure 5. Mean and standard deviation of CRP and V-CRP values for a-tDCS and s-tDCS groups in pre and post moments.

3.3.4. EEG PSD during walking

Regarding PFC PSD (Figure 6a), statistical analysis did not indicatemoment main effect in alpha band ($F_{1.42}$= 3.060; p= 0.087; ηp^2= 0.068), in beta band ($F_{1.42}$= 2.168; p= 0.145; ηp^2=0.046) and in gamma band ($F_{1.42}$= 3.332; p= 0.078; ηp^2= 0.076). Also, ANOVA did not pinted group main effect in alpha band ($F_{1.42}$= 1.652; p= 0.859; ηp^2= 0.043), in beta band ($F_{1.42}$= 1.068; p= 0.800; ηp^2= 0.013) and in gamma band ($F_{1.42}$= 1.907; p= 0.170; ηp^2= 0.044).

Regarding M1 PSD (Figure 6b) statistical analysis did not pointed moment main effect in alpha band ($F_{1.42}$= 2.197; p= 0.147; ηp^2= 0.058) and in beta band ($F_{1.42}$= 2.133; p= 0.155; ηp^2=0.048) but poited a decrase in both groups gamma band PSD in the moment post ($F_{1.42}$= 5.933; p= 0.010; ηp^2= 0.124). Also, ANOVA did not pinted grouo main effect in alpha band ($F_{1.42}$= 1.017; p= 0.929; ηp^2= 0.023), in beta band ($F_{1.42}$= 1.196; p= 0.280; ηp^2= 0.043) and in gamma band ($F_{1.42}$= 1.147; p= 0.590; ηp^2= 0.054).

Regarding occipital cortex PSD (Figure 6c) participants from both groups presented a decrease in alpha band PSD ($F_{1.42}$= 7.666; p= 0.008; ηp^2= 0.124), in beta band PSD ($F_{1.42}$= 11.687; p= 0.001; ηp^2= 0.218) and in gamma band PSD ($F_{1.42}$= 8.967; p= 0.001; ηp^2= 0.086) in the moment post. However, ANOVA did not pointed group main

effect for alpha band PSD ($F_{1.42}$= 1.136; p= 0.914; ηp^2= 0.019), beta band PSD ($F_{1.42}$= 1.148; p= 0.640; ηp^2= 0.011) and gamma band PSD ($F_{1.42}$= 2.447; p= 0.140; ηp^2= 0.080).

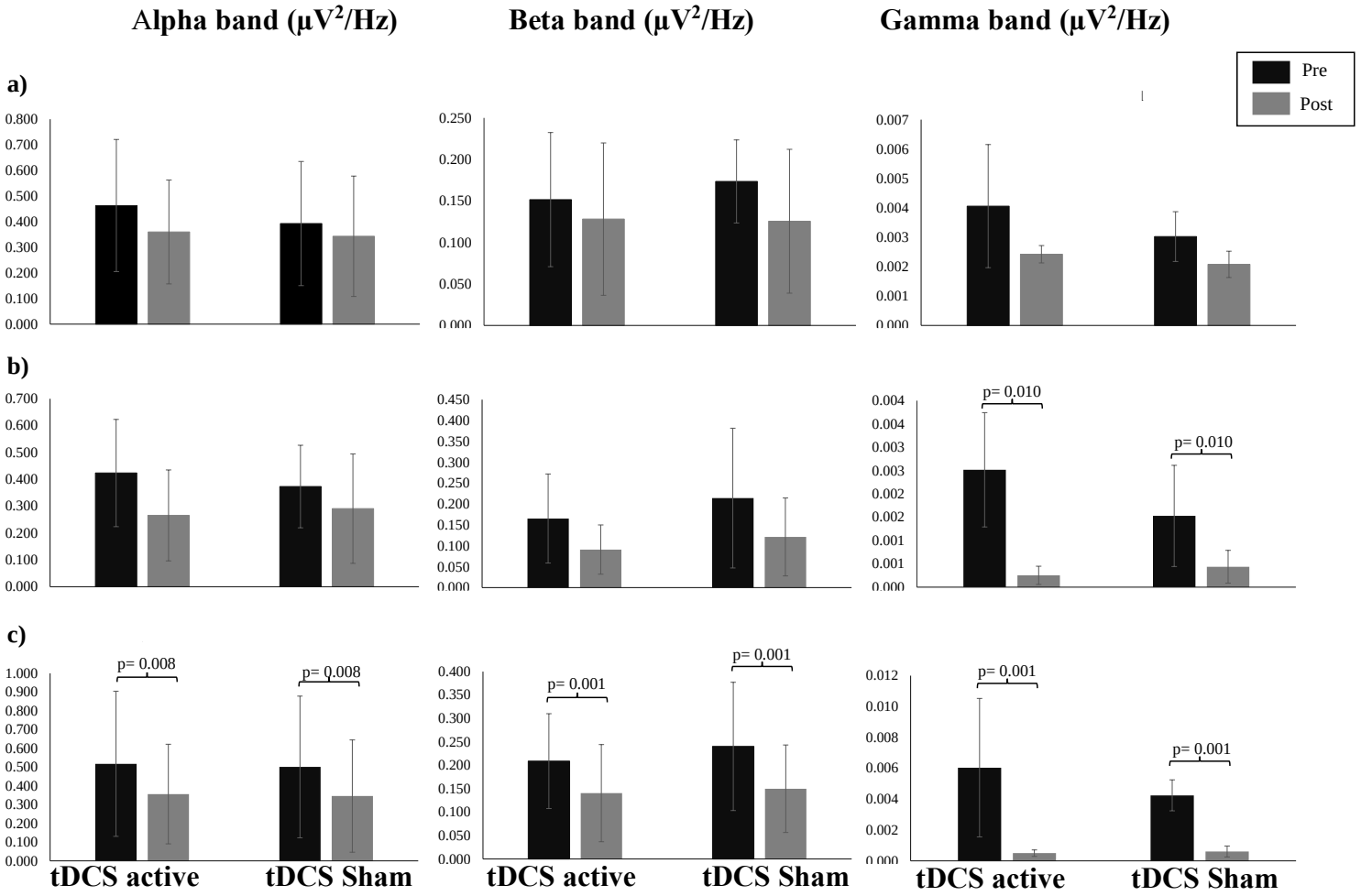

Figure 6. Means and standard deviation of EEG PSD of **a)** PFC, **b)** M1 and **c)** Occipital cortex at the moments pre and post test for a-tDCS and s-tDCS.

3.4. Discussion

This study investigated the acute effects of a combined tDCS and locomotor exercise protocol on PwPD gait parameters, interlimb coordination and cortical activity. Contrary to our hypothesis, the findings did not support the hypothesis that a combined tDCS and locomotor exercise protocol—targeting increased arm swing amplitude and speed—would exert synergistic effects on gait and interlimb coordination parameters or modify cortical activity. Our results suggest that the locomotor exercise protocol focused on enhancing arm swing amplitude and speed had beneficial effects on PwPD gait parameters and cortical activity, evidenced by increases in stride length and velocity, reductions in double support time, and decreases in the PSD of the gamma band in M1

and in the alpha, beta, and gamma bands in the occipital cortex. However, there were no main group effects observed across any of the analyzed variables, and no significant group or time main effects were found in CRP and V-CRP measurements.

Numerous studies have established the positive impact of locomotor exercises on PwPD gait parameters, highlighting improvements such as increased stride length and velocity, enhanced lower limb strength, and better dynamic balance and movement coordination (Ni et al., 2018; Casas-Herrero et al., 2022; Bello et al., 2013). Although these benefits are well-documented, most of these protocols have focused on lower limb movements, often employing visual or auditory cues (Ni et al., 2018), which limits understanding of how upper limb-focused locomotor exercises may influence gait in PwPD. Previous studies have reported a strong association between arm swing characteristics (e.g., amplitude and frequency) and gait parameters in PwPD (Zampier et al., 2018; Ospina et al., 2018), indicating that increased arm swing amplitude and speed correlate with improved stride length and velocity. These findings suggest that locomotor exercise protocols should consider emphasizing these arm swing characteristics (Zampier et al., 2018). Our results showing increased stride length and velocity and reduced double support time in both a-tDCS and s-tDCS groups, corroborate with previous literature on the benefits of locomotor exercises for PwPD gait parameters and further suggest that a protocol focused on enhancing arm swing amplitude and speed may be a promising approach for improving gait in PwPD as suggested by Zampier et al., 2018.

Compared to healthy older adults, PwPD exhibit increased cortical activity as a compensatory mechanism for dopaminergic depletion and reduced movement automaticity (Takakusaki et al., 2008). This heightened cortical activity is also observed during adaptive walking. For instance, PwPD shows elevated gamma band activity in motor and occipital brain regions during obstacle avoidance (Orcioli-Silva et al., 2021), and increased alpha and beta band activity in the occipital cortex during visually cued walking (Stuart et al., 2018). These findings support the notion of reduced automaticity in PwPD, highlighting an increased reliance on cognitive resources to perform locomotor tasks (Takakusaki et al., 2008). In our results, both groups demonstrated a post-intervention decrease in gamma band activity in M1, as well as reductions in alpha, beta, and gamma band activity in the occipital cortex, which were accompanied by improvements in gait parameters. These findings suggest that the locomotor exercise

protocol focused on increasing arm swing amplitude and speed may promote healthier gait parameters in PwPD by restoring cortical activity in to normal patterns.

Transcranial direct current stimulation (tDCS) has been proposed as a promising therapy to enhance cortical activity and improve locomotion in individuals with Parkinson's disease (PwPD) (Boonstra et al., 2016; Beretta et al., 2018). Anodal tDCS, in particular, can increase the excitability of neurons in the targeted region by bringing their membrane potentials closer to their firing threshold (Nitsche & Paulus, 2000). In PwPD, this form of stimulation has also been shown to elevate extracellular dopamine levels in the striatum, potentially offsetting the heightened GABAergic activity characteristic of the disease (Stagg et al., 2009; Filmer et al., 2014). Notably, evidence suggests that multiple tDCS sessions may produce more robust and sustained effects than a single session, likely due to the greater neuroplastic changes facilitated through repeated stimulation (Pol et al., 2021; Beretta et al., 2024). In contrast, a single session may not provide sufficient stimulus for the brain to consolidate these tDCS-induced changes effectively. The absence of significant group effects in our results aligns with these findings, suggesting that a single tDCS session might not be enough to induce measurable changes in cortical activity or locomotion-related parameters in PwPD. This observation leads us to hypothesize that more than one combined tDCS and locomotor exercise session may be necessary to achieve meaningful improvements in locomotion and cortical activity for PwPD.

A key strength of this study is the simultaneous assessment of EEG activity across different brain areas and locomotor parameters in individuals with Parkinson's disease (PwPD) following a combined tDCS and locomotor exercise protocol, providing precise temporal alignment between the two measurements. Despite the valuable insights, our study has some limitations. The controlled laboratory setting limited participants to an 8-meter walking distance, restricting our ability to generalize findings to longer walking distances for PwPD. Additionally, the time required to prepare participants (e.g., setting up EEG, adjusting electrode impedance, positioning accelerometers) and conduct experimental procedures constrained the number of trials we could safely perform within the PD ON-medication stage.

In conclusion, this study demonstrates that a locomotor exercise protocol aimed to increase arm swing amplitude and speed can elicit improvements in gait parameters

and beneficial changes in cortical activity of PwPD. However, it appears that more than one tDCS session may be needed to achieve significant, synergistic effects on both locomotion and cortical activity in PwPD.

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4. Chapter 4

Study #3 - The chronic effects of transcranial direct current stimulation combined with locomotor exercise on people with Parkinson's disease locomotion and cortical activity

The chronic effects of transcranial direct current stimulation combined with locomotor exercise on people with Parkinson's disease locomotion and cortical activity

Abstract

Transcranial direct current stimulation (tDCS) and locomotor exercise are promising tools for addressing locomotor deficits and cortical dysfunction in people with Parkinson's disease (PwPD). Combining tDCS with locomotor exercise may have synergistic effects on gait and cortical activity. However, most protocols focus primarily on lower-limb functions, often neglecting the role of arm movements in PwPD gait. This study aimed to investigate the chronic effects of a combined tDCS and locomotor exercise protocol, emphasizing increased arm swing amplitude and speed, on cortical activity, interlimb coordination, and gait parameters in PwPD. Twenty-two participants were enrolled in a sham-controlled, randomized, parallel-arm design and allocated into two groups: active tDCS (n = 12) and sham tDCS (n = 10). Disease severity and cognitive status were assessed using the MDS-UPDRS and Mini-Mental State Examination (MMSE), respectively. Participants completed six tDCS sessions, spaced 48 hours apart, performing either active or sham tDCS protocols combined with locomotor exercises in each session. Active tDCS was applied at 2 mA for 20 minutes, while sham tDCS was applied for 10 seconds. The anode was positioned over the motor cortex contralateral to the most affected side, and the cathode over the contralateral supraorbital area. Locomotor exercises involved walking along an 18-meter circuit under two conditions: increased arm swing amplitude (10 minutes) and increased speed (10 minutes). Gait parameters were assessed using the GaitRite system, interlimb coordination through wrist and ankle accelerometers with continuous relative phase (CRP) analysis, and cortical activity using 64-channel EEG. Power spectral density (PSD) in alpha, beta, and gamma bands was analyzed in prefrontal, motor, and occipital regions.A two-way ANOVA (group: active/sham tDCS; moment: pre/post/follow-up) revealed that participants in the active tDCS group showed a significant reduction in prefrontal cortex alpha-band PSD ($p = 0.004$) and a decrease in CRP variability ($p < 0.001$) post-intervention. Additionally, participants in both groups demonstrated reductions in prefrontal cortex beta-band PSD ($p = 0.002$) and motor cortex alpha-band PSD ($p = 0.024$), increases in CRP values ($p < 0.001$), decreases in CRP variability ($p < 0.001$), as well as improvements in stride length ($p < 0.001$), walking velocity ($p < 0.001$), and decreases in double support time ($p < 0.001$).These findings suggest that multiple sessions of tDCS combined with locomotor exercises targeting arm swing may have synergistic effects, enhancing locomotion and cortical activity in PwPD.

Keywords: Neuro-degenerative diseases; Brain Stimulation; Cortical activity; Walking

4.1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder marked by the progressive loss of dopaminergic neurons in the substantia nigra, a crucial structure within the basal ganglia (Wichmann & DeLong, 2014). This dopamine depletion disrupts the balance of neural activity, increasing basal ganglia inhibition of motor regions such as the mesencephalic locomotor area and pedunculopontine nucleus, which regulate cyclic movements and muscle tone (Blandini et al., 2000; Takakusaki, 2017). These changes manifest as motor impairments in individuals with PD (PwPD), including shorter stride lengths, reduced walking speed, asymmetrical arm swing, and impaired interlimb coordination (Takakusaki et al., 2008; Ospina et al., 2018; Winogrodzka et al., 2005). Compensatory mechanisms further result in heightened activity in cortical regions like the prefrontal cortex (PFC) and motor cortex (M1) due to impaired basal ganglia function (Stuart et al., 2018; Takakusaki, 2017).

Although medications are a primary treatment for PD and can restore gait and cortical activity (Orcioli-Silva et al., 2021), long-term use often leads to side effects like dyskinesia, necessitating complementary approaches (Jankovic, 2000; Voon et al., 2009). Exercise emerges as a promising non-pharmacological intervention, offering significant functional improvements in PwPD and older adults (Ni et al., 2018; Casas-Herrero et al., 2022). Research shows that walking programs enhance stride length and velocity in PwPD (Bello et al., 2013), while neuromuscular training improves coordination in younger populations (Sheikhi et al., 2021). However, most studies focus predominantly on lower-limb movements, overlooking the critical role of upper limbs in locomotion. PwPD often experience deficits in arm swing, such as reduced amplitude and asymmetry (Ospina et al., 2018), which impair gait and interlimb coordination (Zampier et al., 2018; Winogrodzka et al., 2005) and are associated with altered cortical activity in M1 (Borhanazad et al., 2024). Increasing arm swing amplitude and frequency has been shown to positively influence motor memory and facilitate skill consolidation (Taubert et al., 2015; Statton et al., 2015), potentially enhancing gait and cortical function in PwPD (Wersink et al., 2020; Zampier et al., 2018). Despite these findings, the impact of exercise protocols targeting arm swing on gait, coordination, and brain activity in PwPD remains underexplored.

Transcranial direct current stimulation (tDCS) also holds potential as a complementary therapy for PD. This non-invasive brain stimulation technique applies weak electrical currents to the scalp to modulate neuronal activity, enhancing or suppressing excitability depending on electrode placement (Nitsche & Paulus, 2000). Evidence suggests that tDCS improves coordination and accuracy in motor tasks, with applications over regions like the supplementary motor area (SMA) and PFC yielding positive results in movement and interlimb coordination (Carter et al., 2015; Leenus et al., 2014). Combining tDCS with exercise has shown synergistic effects, improving mobility and cortical connectivity in PwPD (Beretta et al., 2020; Conceição et al., 2021). Furthermore, arm swing exercises themselves may enhance cortical activity, as evidenced by increased alpha-band desynchronization in M1 (Wersink et al., 2020).

However, little is known about the effects of combining tDCS with an exercise protocol specifically focused on arm swing amplitude and frequency in PwPD. Investigating these effects could provide valuable insights into the motor and cortical mechanisms underpinning gait regulation. Thus, this study aimed to evaluate the chronic effects of a combined tDCS and arm-focused locomotor exercise protocol on gait, interlimb coordination, and cortical activity in PwPD. Based on previous research, we hypothesized that this intervention would improve locomotion and coordination of PwPD by modulating cortical activity.

4.2. Methods

4.2.1. Study design and participants

This randomized, double blind, parallel arm and sham-controlled study was retrospectively registered in the Brazilian Registry of Clinical Trials (ReBEC - RBR-10v4dxgm). The study was carried out at Posture and Gait Laboratory Study and in Human Movement Research Laboratory, São Paulo State University (Unesp). PwPD residents at the local community that participates in the physical activity programs at the same Labs were recruited to participate in this study. Sample size determination was based on a priori analysis using the G*Power software. The analysis indicated that 18 participants would be needed to detect the difference between factors using a two-way ANOVA ($\alpha<0,05$) and a ($1-\beta$) of 0.80. The sample size analysis was preformed based on the stride length variable ($F_{2,68} = 25.503$; $p < 0.001$; $\eta p^2 = 0.429$) from our previous study (Zampier et al., 2018). Thus 22 PwPD were recruited to participate in this study. The

randomization was performed by a member of the Posture and gait laboratory study research group that was not involved in the data collection.

As the inclusion criteria, PwPD should have the disease diagnosed according to the UK Brain Bank criteria, age ≥ 60 years, to continuously use the disease specific medication, score between 1 and 3 in the Hoehn & Yahr scale (H&Y) (Schenkman et al., 2001) and had independent locomotion. The following exclusion criteria were adopted i) severe cognitive decline (score < 20 in Mini-Mental State Examination – MMSE) (Brucki et al., 2003); ii) Changes in PD medication during the study period; iii) musculoskeletal and not corrected visual impairments that could the experimental protocol performance; iv) presence of any uncontrolled disease that could affect peripheral sensory function (e.g., diabetes); and v) risk of receiving tDCS (neural implants, pacemaker, history of seizures and epilepsy). Participants were also excluded if they did not complete the experimental protocol (tDCS sessions and assessments). The study aims and experimental protocol were described to all participants during the recruitment. This study was approved by the São Paulo State University ethics committee (CAAE: 58135122.2.0000.5465). All PwPD who participated in this study signed the consent statement before the experiments began.

4.2.2. Study Protocol

The experimental protocol was conducted in eight days and included three assessments of gait, interlimb coordination, and cortical activity (pre, post, and follow-up). On the first day, clinical assessments were conducted before the pre-assessment, followed by the first combined tDCS and locomotor exercise session. Subsequently, participants returned to the laboratory for five additional sessions, with a 48-hour interval between each. The post-assessment took place 48 hours after the final tDCS session, while the follow-up assessment occurred approximately one month (30–31 days) after the last session. All assessments were conducted by at least two team members blinded to group allocation.

4.2.3. Clinical, cognitive and walking assessments

An experienced evaluator applied the Movement Disorders Society – Unified Parkinson's Disease Rating Scale – motor section (MDS-UPDRS III) to assess the disease motor severity (Goetz et al., 2008) and the MMSE to assess the participants cognitive status (Brucki et al., 2003). For the walking assessment participants walked over an 8m

strait pathway after an auditory cue "*Ready Go*" under their preferred speed for five trials. Before each trial participants were instructed to stand as quiet as possible for 30 seconds in order to normalize their cortical activity. After that participants were asked to start the walking over a 8m pathway. To assess the walking variables such as stride length, velocity and width and double support time a 5,74m pressure sensor carpet (GAITRite®, CIR Systems Inc.) with a sample frequency set at 200Hz was placed in the middle of the pathway in order to avoid the acceleration and de-acceleration periods (Zampier et al., 2018).

4.2.4. Interlimb coordination assessment

To evaluate interlimb coordination among participants, accelerometers (Trigno™ wireless system, Delsys Inc., Natick, USA) operating at a 148.148 Hz sampling frequency were placed on each participant's wrists and ankles (Figure 1a). Acceleration signals were recorded using EMG acquisition software (Delsys Inc., Natick, USA) and processed with MATLAB scripts (2022 version, MathWorks, MA, USA). Continuous relative phase (CRP) analysis was conducted to assess phase angles and variability (V-CRP) between the vertical acceleration signals of contralateral wrists and ankles (Wagenaar & Emmerik, 2000; Winogrodzka et al., 2005). Initially, signals were low-pass filtered using a fourth-order Butterworth filter with a 5 Hz cut-off frequency. Gait events—heel strike (HS) and toe-off (TO)—were identified from ankle acceleration signals for each leg, defining each stride cycle for the CRP analysis (Figure 1b). HS and TO events in the ankle acceleration signal were determined through a parallel experiment, where the ankle acceleration signals of 17 healthy young adults were cross-referenced with a force plate signal (AccuGait, Advanced Mechanical Technologies, 200 Hz) during walking (Figure 2a). This procedure enabled us to define a consistent cyclical event in the ankle acceleration signal that temporally corresponded to HS and TO events. The same cyclical events were observed in the PwPD participants in this study (Figure 2b). After defining stride cycles for each leg, the signals were time-normalized to represent a gait cycle percentage (0–100%). The ankle and corresponding wrist acceleration signals for each cycle were then transformed using the Hilbert transformation (Lamb & Stöckl, 2014) (Figure 1c). Following this, CRP was calculated between the normalized signal pairs (right wrist with left ankle, and left wrist with right ankle) (Winogrodzka et al., 2005) (Figure 1d). CRP variability (V-CRP) was calculated as the standard deviation of the mean CRP values for each stride (Wagenaar et al., 2000). An average CRP and V-CRP value was obtained for

each participant, in each pair, and in each moment. The mean CRP and V-CRP values for each pair was used in the statistical analysis. Finally, the mean CRP and average V-CRP for each participant were compared across moments and groups.

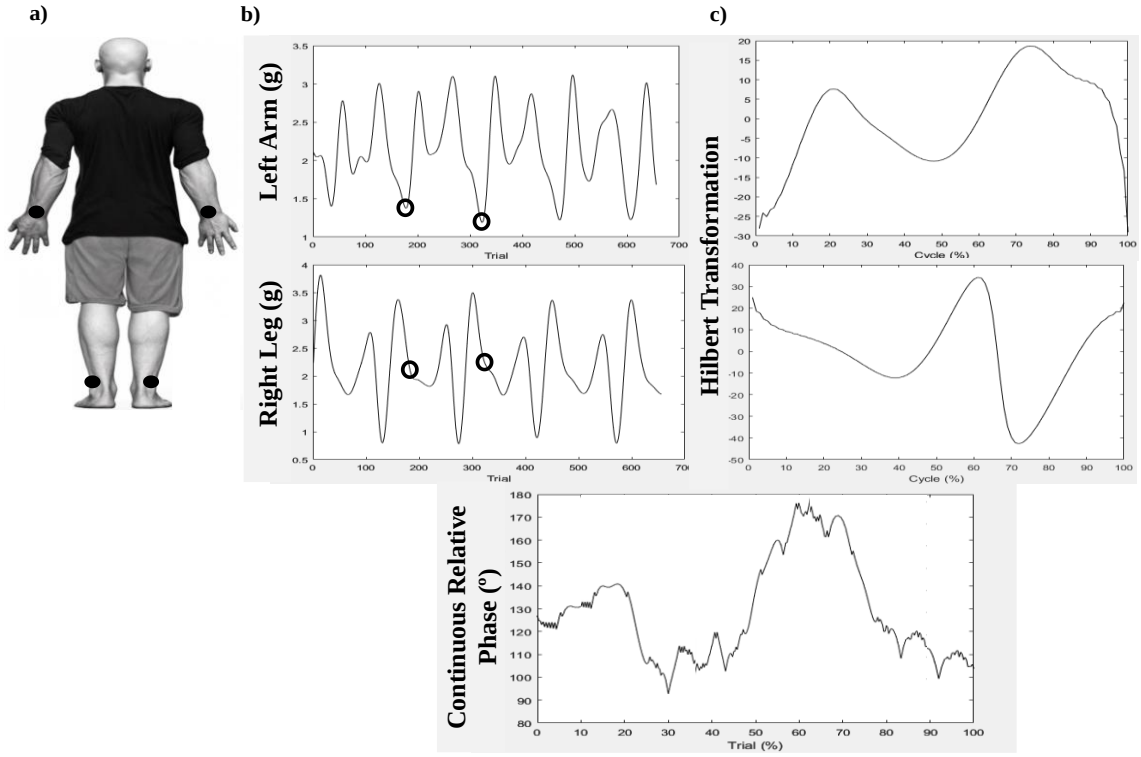

Figure 1. a: Accelerometer Placement over the wrists and ankles; **b:** Filtered acceleration data; **c:** Normalized cycle after the Hilbert Transformation; **d:** Continuous Relative Phase data during the trial

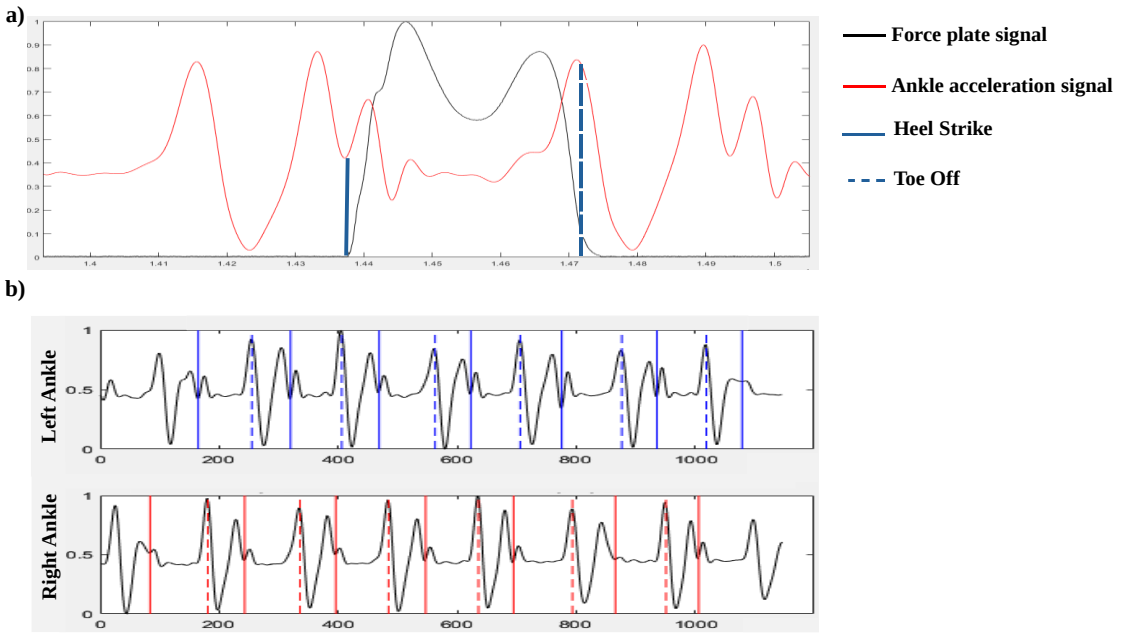

Figure 2. a: Ankle acceleration signal crossed with force plate signal of a healthy young adult with the heel strike and toe-off events; **b:** Ankle acceleration signal with the heel strike and toe-off events of one of this study participants.

4.2.5. Cortical Activity Assessment

Cortical activity was recorded using electroencephalogram (EEG) equipment (eego™sports, ANT Neuro, Enschede, Netherlands, 1024 Hz), following procedures recommended by the EEG manufacturer. The EEG system included a cap with 64 passive electrodes placed on the participants' scalps according to the international 10–20 electrode placement system, with electrode impedance kept below 10 KΩ. To ensure accurate temporal alignment between EEG and movement coordination data, the EEG and accelerometer signals were synchronized via a common event marker. EEG analysis (Figure 3) was conducted using the open-source software EEGLAB (Delorme et al., 2011). The EEG signal was band-pass filtered (4–50 Hz) and pre-processed with the PREP pipeline (Bigdely-Shamlo et al., 2015) to remove line noise (60 Hz), re-reference and interpolate channels. Artifact Subspace Reconstruction (ASR) (Jacobsen et al., 2021) was used to remove flat-line segments and low-correlation channels. After pre-processing, the interested channels were selected included three for the prefrontal cortex (PFC) area (Fz, F3, F4), three for the motor cortex (M1) (Cz, C3, C4), and three for the occipital cortex (POz, PO3, PO4). Independent Component Analysis (ICA) was then performed to identify data-relevant components. The components were labeled using the ICLabel toolbox (Pion-Tonachini et al., 2019), and those with a probability ≥70% of being

related to muscle, eye, heart, or line noise were removed (Guo et al., 2023). Following component removal, Power Spectral Density (PSD) analysis was conducted for each electrode, targeting the α (8–12 Hz), β (13–30 Hz), and γ (31–50 Hz) frequency bands (Faria et al., 2023). The PSD data from each electrode were averaged across prefrontal, motor, and occipital regions for analysis (Tropini et al., 2011). If any selected channels were excluded during pre-processing, participant's data was not included in the analysis. All EEG data analysis was conducted by an experienced researcher.

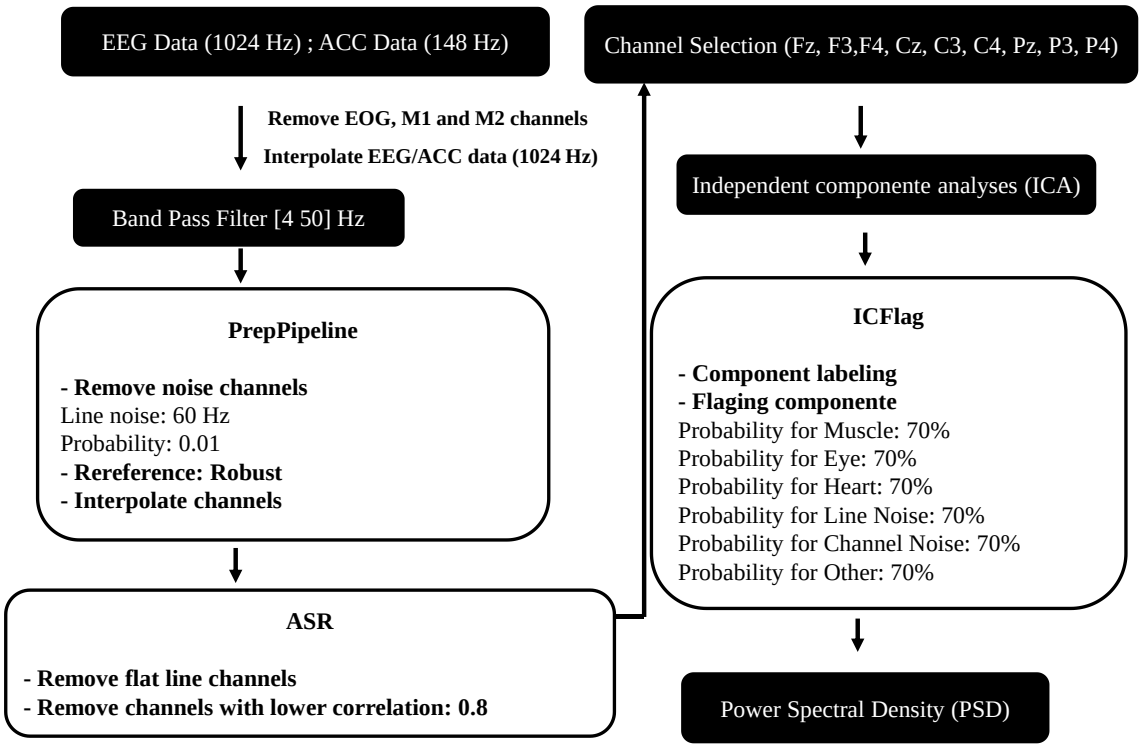

Figure 3. EEG analysis pipeline.

4.2.6. Combined tDCS and Locomotor Exercise Protocol

For the tDCS protocol on the first day participants were randomly assigned in two groups, the active tDCS group (a-tDCS - 12) and the control (sham) tDCS group (s-tDCS - 10). The tDCS protocol was the same in all sessions for all participants. tDCS was applied using a Microestim GENIUS (NKL Electronic Products) via conductive-rubber electrodes, placed in two saline-soaked sponges (35 cm2) while participants performed the locomotor exercise. After the tDCS device was prepared it was placed in a backpack that did not limit the participants' movements. Anodal electrode was placed over the M1 area (C3/C4 – EEG positioning system), and the reference electrode (cathode) was placed over the contralateral supraorbital region (Nonnekes et al., 2014; Vaseghi et al., 2015).

Anodal stimulation was placed in the most affected cerebral hemisphere of PwPD determined by the MDS-UPDRS items (Beretta et al., 2020; Cosentino et al., 2017; Beretta et al., 2015). The same tDCS protocol montage was performed in both active (a-tDCS) and control (s-tDCS) groups. However, for the active tDCS a continuous electrical current with 2mA was applied for 20 minutes with a 30-second ramp-up at the beginning and a 30-second ramp-down at the end of the stimulation period. For the s-tDCS group the same 30 seconds of ramp up/ramp down were performed, but the stimulation remained at 2 mA between these periods for 10 seconds (Beretta et al., 2020). The locomotor exercise protocol focused on the increase of participants arm swing amplitude and speed during walking (Zampier et al., 2018; 2019). After the tDCS placement, participants were instructed to walk over a 18m rectangular circuit with 2 parallel lines of 8m for 20 minutes (online with tDCS protocol) in two conditions, each lasting 10 minutes. In one condition participants were instructed to walk at preferred speed increasing their arm swing amplitude, on the other condition participants were instructed to walk at preferred speed increasing their arm swing speed. Both a-tDCS and s-tDCS groups performed the same locomotor exercise protocol. The order in which the participants began the protocol was randomized. After each tDCS session a structures questionnaire was applied to identify tDCS side effects in both groups (Beretta et al., 2020).

4.2.7. Statistical analysis

Statistical analyses were performed using IBM® SPSS® software (Amos™; Version 22), with a significance level maintained at 0.05. Normality, sphericity and homogeneity were verified by Shapiro-Wilk, Mauchley's and Levene test, respectively. Student t-test for independent samples, Mann-Whitney U and Chi-Square tests were conducted to analyze the clinical and cognitive sample characteristics. The side effect of tDCS was analyzed by the Mann-Whitney U test. Descriptive statistics were computed using the mean and standard deviation values of each variable considered in this study. The comparisons were performed using two-way ANOVA procedures, with factors for group (a-tDCS and s-tDCS) and moment (pre, post and follow-up intervention) with repeated measures for the last factor. The Greenhouse-Geisser correction was adopted for the degrees of freedom. Bonferroni post hoc test was applied when interaction between factors were pointed out. Eta-square (η^2) were determined for all main effects, which was categorized as follows: large (> 0.14), medium (0.13 – 0.06), and small (0.05 – 0.01); negligible (< 0.01) (Fritz et al., 2012).

4.3. Results

Table 1 shows the mean and standard deviation for the clinical and cognitive and demographic assessments.

Table 1. Means and standard deviation for PwPD clinical, cognitive and demographic characteristics.

	a-tDCS (n= 12)	s-tDCS (n= 10)	p value
Gender	F (3); M (9)	F (2); M (8)	0.645
Age (years)	68.25 ± 4.75	69.75 ± 2.40	0.886
Height (cm)	163.25 ± 13.60	166.15 ± 11.81	0.732
Body mass (Kg)	69.44 ± 10.32	73.33 ± 12.44	0.789
MMEE (pts)	26.12 ± 1.15	26.88 ± 1.45	0.943
MDS-UPDRS III (pts)	28.95 ± 4.79	30.93 ± 9.12	0.713
MDS-UPDRS total (pts)	42.61 ± 11.66	48.23 ± 16.22	0.659
Hoen Yahr	2(11); 2.5 (1)	2(10)	0.433
Most affected side	10(Left); 2(Right)	8(Left); 2(Right)	0.523

PwPD: People with Parkinson's disease; MMEE: Mini Mental Estate Examination; MDS-UPDRS: Unified Parkinson's disease rating scale adopted by Movement Disorders Society.

4.3.1. tDCS side effects

No difference between groups regarding tDCS side effects was evidenced (Table 2). The main reported sensation was tingling (86% for a-tDCS and 80% for s-tDCS), 45% of a-tDCS and 41% of s-tDCS group reported itching, 13% of a-tDCS and 9% of s-tDCS group reported burning sensation, 4% of s-tDCS group reported trouble to concentrating and was observed skin redness in 13% of a-tDCS and 9% of s-tDCS group (Table 2). The scores of each stimulation condition for each sensation were mild from the adapted questionnaire. No accidents such as falls occurred during the experimental protocol.

Table 2. Median and quartiles of tDCS side effects score.

	a-tDCS (n= 12)	s-tDCS (n= 10)	p value
Participant's report	**Median (quartiles) percentage**	**Median (quartiles) percentage**	
Headache	0 (0-0) 0%	0 (0-0) 0%	1
Neck pain	0 (0-0) 0%	0 (0-0) 0%	1
Scalp pain	0 (0-0) 0%	0 (0-0) 0%	1
Tingling	1 (0.75-1.25) 86%	1 (0.75-1) 80%	0.441
Itching	0 (0-1) 45%	0 (0-1) 40%	0.843
Burning sensation	0 (0-1) 16%	0 (0-1) 10%	0.341
Sleepiness	0 (0-0) 0%	0 (0-0) 0%	1
Metallic/iron taste	0 (0-0) 0%	0 (0-0) 0%	1
Fatigue	0 (0-0) 0%	0 (0-0) 0%	1
Trouble concentrating	0 (0-0) 0%	0 (0-1) 10%	0.225
Acute mood change	0 (0-0) 0%	0 (0-0) 0%	1
Investigator's reports			
Skin redness	0 (0-1) 13%	0 (0-1) 20%	0.622
Skin irritation	0 (0-0) 0%	0 (0-0) 0%	1

Note: Median and quartiles values were calculated from the scores of each participant in each session. 0 = none (I did not feel the sensation addressed); 1 = mild (I mildly felt the sensation addressed); 2 = moderate (I felt the sensation addressed); 3 = strong (I felt the sensation addressed to a considerable degree).

4.3.2. Gait performance

Gait parameters are presented in Figure 4. Participants from both groups exhibited an increase in stride length ($F_{2,40} = 10.039$; $p = 0.001$; $\eta p^2 = 0.344$) and velocity ($F_{2,40} = 18.179$; $p < 0.001$; $\eta p^2 = 0.475$) during the post moment. Furthermore, stride velocity was significantly higher ($p < 0.001$) and double support time was shorter ($F_{2,40} = 10.039$; $p = 0.001$; $\eta p^2 = 0.344$) at the follow-up moment compared to the pre moment. However, no main effect of moment was observed for stride width ($F_{2,40} = 3.045$; $p = 0.167$; $\eta p^2 = 0.090$). Similarly, statistical analyses revealed no significant main effect of group on stride length ($F_{1,20} = 1.876$; $p = 0.168$; $\eta p^2 = 0.086$), velocity ($F_{1,42} = 1.916$; $p = 0.359$; $\eta p^2 = 0.086$), width ($F_{1,42} = 1.083$; $p = 0.773$; $\eta p^2 = 0.045$), or double support time ($F_{1,42} = 1.776$; $p = 0.846$; $\eta p^2 = 0.022$).

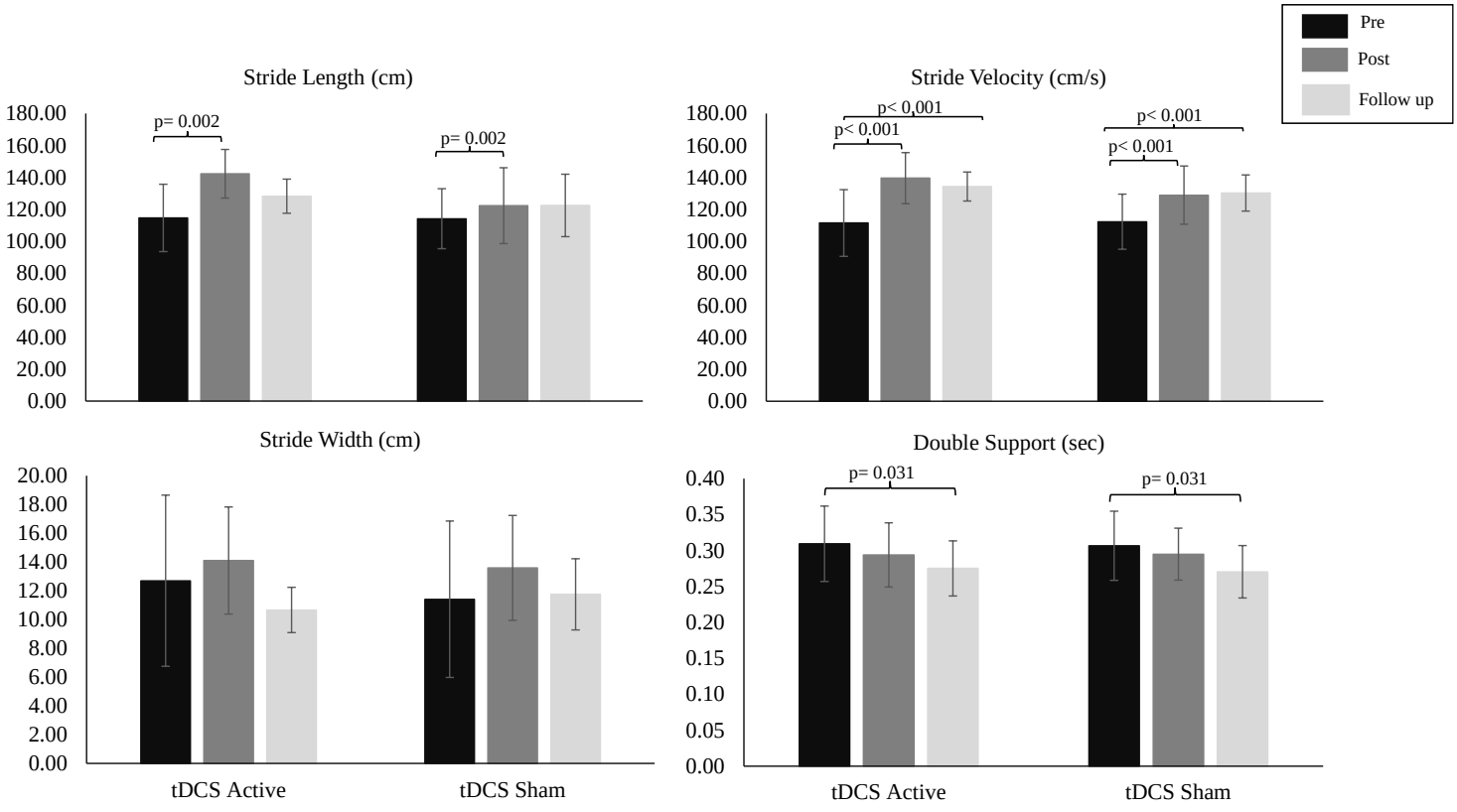

Figure 4. Mean and standard deviation of gait parameters for a-tDCS and s-tDCS groups in pre, post and follow-up moments.

4.3.3. Interlimb coordination during walking

CRP and V-CRP values are presented in Figure 5. Statistical analysis identified a significant interaction between group and moment for V-CRP ($F_{1,42} = 6.047$; $p = 0.005$; $\eta p^2 = 0.232$). Bonferroni post-hoc tests revealed that only the a-tDCS group showed a

significant reduction in V-CRP during the post moment compared to the pre moment ($p < 0.001$). Additionally, ANOVA indicated that participants from both groups demonstrated an increase in CRP and a decrease in V-CRP at the follow-up compared to the pre moment. However, no significant main effect of group was observed for CRP ($F_{1,42} = 3.655$; $p = 0.070$; $\eta p^2 = 0.115$).

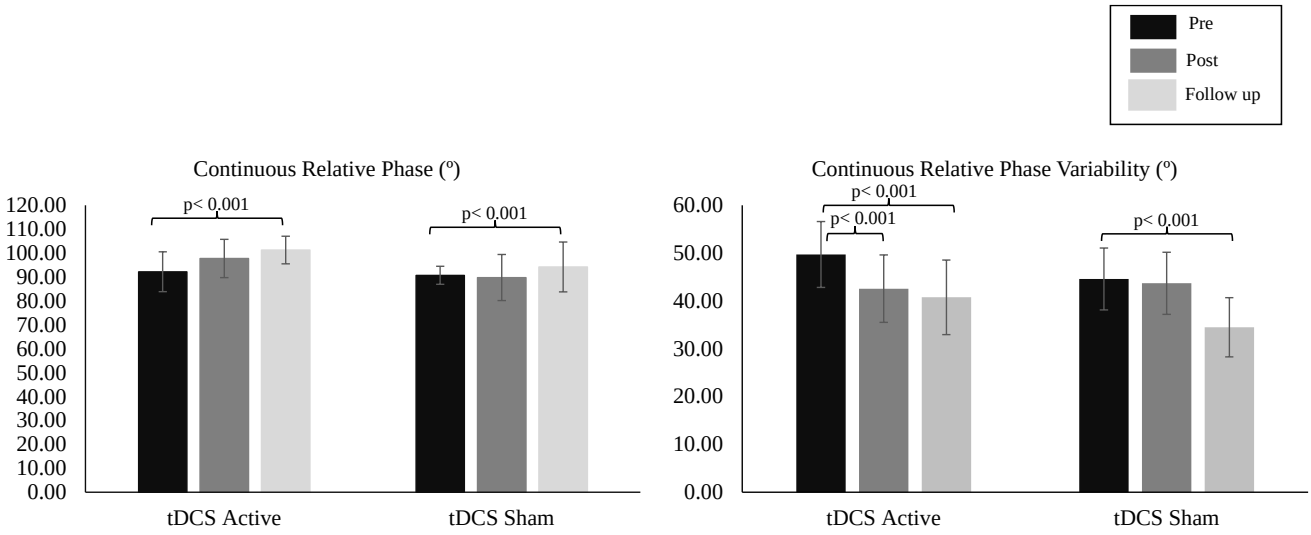

Figure 5. Mean and standard deviation of CRP and V-CRP values for a-tDCS and s-tDCS groups in pre, post and follow-up moments

4.3.4. EEG PSD during walking

Results for prefrontal cortex PSD are shown in Figure 6a. Statistical analysis revealed a significant interaction between group and moment ($F_{2,40} = 6.868$; $p = 0.004$; $\eta p^2 = 0.389$). Bonferroni post-hoc tests indicated that only participants in the a-tDCS group showed a decrease in alpha-band PSD during the post ($p = 0.004$) and follow-up moments ($p = 0.009$) compared to the pre moment. Additionally, a significant main effect of moment was observed ($F_{2,40} = 9.801$; $p = 0.002$; $\eta p^2 = 0.329$), with participants from both groups showing reduced beta-band PSD during the post ($p = 0.024$) and follow-up moments ($p = 0.002$) compared to the pre moment. No significant main effect of moment was observed for gamma-band PSD ($F_{2,40} = 2.011$; $p = 0.373$; $\eta p^2 = 0.329$). Furthermore, ANOVA showed no significant group main effects for beta-band PSD ($F_{2,40} = 1.504$; $p = 0.457$; $\eta p^2 = 0.109$) or gamma-band PSD ($F_{2,40} = 1.239$; $p = 0.673$; $\eta p^2 = 0.308$).

Findings for motor cortex PSD are shown in Figure 6b. Participants from both groups exhibited a decrease in alpha-band PSD ($F_{2,40} = 5.693$; $p = 0.026$; $\eta p^2 = 0.190$). However, no significant main effect of moment was found for beta-band PSD ($F_{2,40} =$

4.051; p = 0.052; ηp^2 = 0.081) or gamma-band PSD ($F_{2,40}$ = 1.239; p = 0.673; ηp^2 = 0.308). Similarly, no significant group main effects were observed for alpha-band PSD ($F_{2,40}$ = 1.788; p = 0.338; ηp^2 = 0.097), beta-band PSD ($F_{2,40}$ = 1.094; p = 0.646; ηp^2 = 0.082), or gamma-band PSD ($F_{2,40}$ = 1.947; p = 0.273; ηp^2 = 0.108).

Results for occipital cortex PSD are displayed in Figure 6c. No significant main effect of moment was observed for alpha-band PSD ($F_{2,40}$ = 1.962; p = 0.392; ηp^2 = 0.057), beta-band PSD ($F_{2,40}$ = 1.713; p = 0.496; ηp^2 = 0.050), or gamma-band PSD ($F_{1,42}$ = 1.594; p = 0.470; ηp^2 = 0.049). Similarly, no significant group main effects were identified for alpha-band PSD ($F_{1,42}$ = 1.650; p = 0.471; ηp^2 = 0.094), beta-band PSD ($F_{1,42}$ = 2.094; p = 0.623; ηp^2 = 0.140), or gamma-band PSD ($F_{1,42}$ = 1.025; p = 0.753; ηp^2 = 0.054).

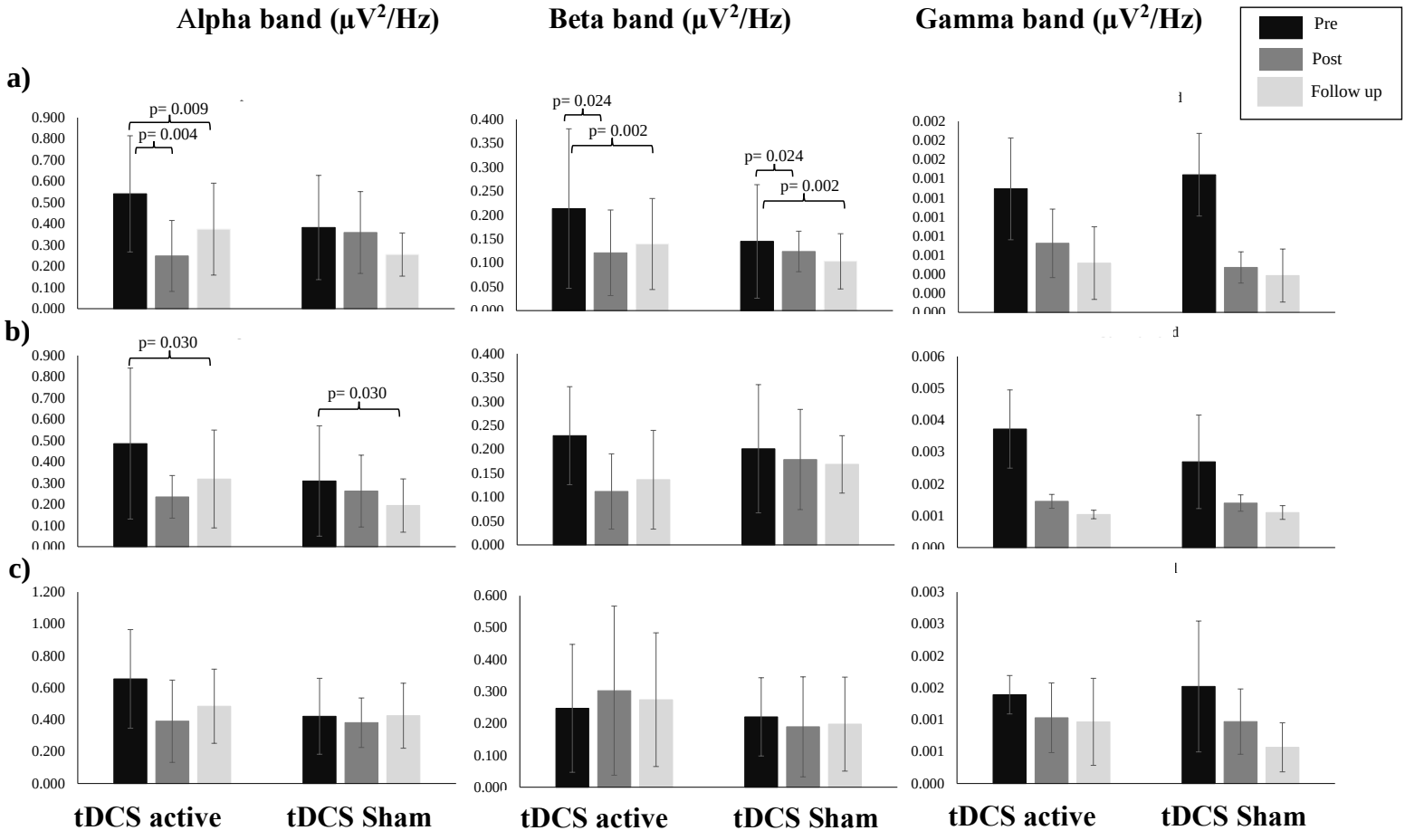

Figure 6. Means and standard deviation of EEG PSD of **a)** PFC, **b)** M1 and **c)** Occipital cortex at the moments pre, post and follow-up tests for a-tDCS and s-tDCS.

4.4. **Discussion**

This study investigated the chronic effects of six non-consecutive sessions of a combined tDCS and locomotor exercise protocol on gait parameters, interlimb coordination, and cortical activity in PwPD. Our findings support the hypothesis that combining tDCS with locomotor exercise aimed at increasing arm swing amplitude and speed produces synergistic effects, leading to improvements in locomotion and cortical activity in PwPD. Specifically, our results show that only the a-tDCS group exhibited a decrease in V-CRP and a reduction in PFC alpha band PSD after the intervention. Additionally, the data suggests that six sessions of the locomotor exercise protocol had beneficial effects on gait parameters, interlimb coordination, and cortical activity. These effects were evidenced by increased stride length and velocity, reduced double support time, increased CRP, decreased V-CRP, and reductions in the PSD of alpha and beta bands in the PFC, as well as the alpha band in M1, in both groups.

The reduction in dopamine levels in the basal ganglia caused by Parkinson's disease (PD) leads to increased inhibitory activity in structures such as the thalamus. This compromises pathways like the thalamus-motor cortex connection, as well as the pedunculopontine nucleus and the mesencephalic locomotor region, which are responsible for inhibitory muscle tone control and rhythmic movement regulation, respectively (Takakusaki et al., 2008; 2017). As a result of these changes in subcortical structures, PwPD exhibit heightened cortical activity, particularly in frontal areas, as a compensatory mechanism for the loss of movement automaticity (Stuart et al., 2018). This compensatory activity is associated with hypokinesia, increased movement variability, and impaired interlimb coordination during locomotor activities (Hoang et al., 2022; Mirelman et al., 2019; van Emmerik & Wagenaar, 1996; Yogev et al., 2007; Plotnik et al., 2007). In our study, following six sessions of a combined active tDCS and locomotor exercise protocol aimed at increasing arm swing amplitude and speed, PwPD demonstrated a decrease in prefrontal cortex (PFC) alpha-band PSD and reduced variability in contralateral arm and leg movement coordination 48 hours post-intervention while the s-tDCS group presented a decrease in PFC alpha-band PSD only 30 days post-intervention. These findings suggest that multiple active tDCS sessions produced a synergistic effect with the locomotor exercise protocol (Hoang et al., 2022), potentially restoring cortical activity and enhancing locomotor function in PwPD faster than the group who were submitted only to the exercise protocol. This was evidenced by the

adoption of a safer arm- leg coordination pattern with less variability (van Emmerik & Wagenaar, 1996; Yogev et al., 2007).

The sign and symptom of hypokinesia in PwPD can be observed as a reduction in movement amplitude and speed (Hoang et al., 2022). Specifically, decreased arm swing amplitude and speed (Ospina et al., 2018) are associated with walking deficits, such as reduced stride length and velocity (Lewek et al., 2010), and impaired coordination, evidenced by diminished segmental coupling and increased variability in movement patterns (van Emmerik & Wagenaar, 1992; 1996). These deficits are linked to a higher risk of falls in this population (Ford et al., 2007; Huang et al., 2012). Previous studies have demonstrated that voluntarily increasing arm swing amplitude and speed leads to improved locomotor behavior in PwPD, including enhanced stride length and velocity (Zampier et al., 2018), reduced variability in arm-leg coordination, and greater arm-leg coupling, as reflected by higher CRP values (Zampier, 2019). These findings suggest that interventions targeting arm movements during walking, particularly addressing impairments like reduced amplitude and frequency, could offer a safe, practical strategy to enhance PwPD functional capacity. The findings from this study further support this notion, showing that participants in both groups (a-tDCS and s-tDCS) exhibited lasting improvements in locomotor behavior, such as increased stride velocity, decreased V-CRP, and increased CRP, 30 days after completing the locomotor exercise protocol.

The automaticity loss in PwPD has been linked to increased brain activity, particularly in frontal areas, as a compensatory mechanism for deficits in subcortical regions caused by PD (Takakusaki et al., 2017; Stuart et al., 2018). Conventional treatments for PD symptoms typically involve medication, which alleviates motor deficits and restores brain activity in PwPD by reducing PFC alpha-band absolute power (Orcioli-Silva et al., 2021). In our study, after six non-consecutive sessions of a combined tDCS and locomotor exercise protocol focused on increasing arm swing amplitude and speed, participants in the a-tDCS group exhibited a decrease in PFC alpha-band PSD. Additionally, participants from both groups demonstrated decreases in beta-band PSD in the PFC and alpha-band PSD in M1. These brain activity changes suggest that the locomotor improvements observed in PwPD during this study may be related to a partial restoration of the automatic locomotion control system, utilizing pathways not compromised by PD.

A key strength of this study is the simultaneous assessment of EEG activity across different brain areas and locomotor parameters in individuals with Parkinson's disease (PwPD) following a combined tDCS and locomotor exercise protocol, providing precise temporal alignment between the two measurements. Despite the valuable insights, our study has some limitations. The controlled laboratory setting limited participants to an 8-meter walking distance, restricting our ability to generalize findings to longer walking distances for PwPD. Additionally, the time required to prepare participants (e.g., setting up EEG, adjusting electrode impedance, positioning accelerometers) and conduct experimental procedures constrained the number of trials we could safely perform within the PD ON-medication stage.

In conclusion, this study demonstrates that multiple sessions of a combined active tDCS and locomotor exercise protocol focused on the increase of arm swing amplitude and speed is promising tool that can be used as a coadjutant treatment to alleviate PD signs and symptoms. Also, this study can be used as a guidance for health care professionals by showing synergetic effects between tDCS and locomotor exercise on PwPD locomotion performance.

4.5. Acknowledgements

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5. Chapter 5

5.1. Final Comentes

Table 1 summarizes the main findings e perspectives for further studies. The present thesis investigated the effects of transcranial direct current stimulation (tDCS) on gait parameters, interlimb coordination, and cortical activity in older adults with Parkinson's disease (PD). The study was guided by three key questions regarding the methods and effects of tDCS on locomotion and cortical activity in older adults with PD: i) What are the characteristics of tDCS protocols (number and duration of sessions, type of stimulation, and current intensity) that should be applied to improve movement coordination in individuals with and without neurodegenerative diseases? ii) What are the effects of a single session of tDCS combined with a locomotor exercise protocol focused on arm movements on gait parameters, interlimb coordination, and cortical activity in individuals with PD? iii) What are the effects of multiple sessions of tDCS combined with a locomotor exercise protocol focused on arm movements on gait parameters, interlimb coordination, and cortical activity in individuals with PD? Based on the findings of this thesis, it can be concluded that:

- The appropriate placement of tDCS electrodes should consider the specific brain regions affected by each pathology.

- A current intensity of 2mA is more effective than higher or lower intensities.

- Sessions lasting 20 minutes are more effective than shorter sessions.

- Multiple sessions of tDCS combined with locomotor exercises demonstrate greater efficacy in promoting synergistic and long-lasting effects on locomotion and cortical activity in individuals with PD compared to single sessions. These benefits include a reduction in alpha spectral power density in the prefrontal cortex and decreased variability in the coordination pattern between arms and legs, observed exclusively in the group that received active tDCS, 48 hours and 30 days after the last session.

- Locomotor exercises focused on increasing the amplitude and speed of arm movements are effective in promoting both acute and chronic improvements in gait parameters, interlimb coordination, and cortical activity in individuals with PD. Improvements in gait were evidenced by increased stride length and speed,

as well as reduced double support phase, observed in both groups (active tDCS and sham tDCS) after a single session and after six sessions of arm-focused locomotor exercises. Improvements in coordination were demonstrated by increased phase angle and reduced variability in the coordination pattern between arm and contralateral leg movements after six non-consecutive sessions. Cortical activity changes included a reduction in gamma spectral power density in the motor cortex, as well as reductions in alpha, beta, and gamma spectral power densities in the occipital cortex, after a single session. After six sessions, reductions were also observed in beta spectral power density in the prefrontal cortex and alpha spectral power density in the motor cortex.

Studies have shown that both tDCS and locomotor exercise are promising, safe, and cost-effective tools for treating the signs and symptoms of PD (Ni et al., 2018; Broeder et al., 2015). Moreover, the combination of tDCS and physical exercise has been investigated, with evidence suggesting that tDCS can, in some cases, enhance the effects of exercise (Bereta et al., 2020; Conceição et al., 2021). However, findings on the effects of this combination remain inconsistent, raising questions about whether a single session of tDCS combined with exercise is sufficient to produce synergistic effects. Although there is a strong relationship between arm movements and gait parameters in individuals with PD (Zampieri et al., 2018; Ospina et al., 2018; Lewek et al., 2010; Ford et al., 2007), many studies investigating the effects of various exercise protocols on locomotor parameters in older adults with PD focus exclusively on modulating the lower limbs. Examples include interventions such as stationary cycling (Conceição et al., 2021) and visually guided walking (Ni et al., 2018), often neglecting the potential benefits of locomotor interventions focused on arm movements.

The results of this thesis support the literature highlighting the benefits of locomotor exercise on gait and motor coordination in older adults with PD. The study demonstrates that after engaging in a protocol of exercises aimed at increasing the amplitude and speed of arm movements, individuals with PD showed improvements in gait parameters and interlimb coordination. Furthermore, this work advances understanding of the neural mechanisms involved in gait control in individuals with PD by showing that these locomotor improvements are accompanied by reduced activity in motor and sensory cortical areas. Finally, the findings suggest that multiple sessions of tDCS are necessary to produce synergistic effects beyond those promoted by exercise

alone on locomotion and cortical activity in individuals with PD. Thus, it can be concluded that locomotor exercises focused on increasing the amplitude and speed of arm movements are effective for improving locomotion in individuals with PD, and that combining this protocol with multiple sessions of tDCS amplifies these effects.

5.1.1. Practical Applications

The findings of this thesis are highly relevant for healthcare professionals, providing important insights into the application and effects of tDCS on locomotor characteristics and cortical activity in individuals with PD. This thesis demonstrates that tDCS protocols with a duration of 20 minutes and a current intensity of 2mA yield positive and consistent results in the literature. Moreover, more than one session of tDCS combined with locomotor exercise is required to produce synergistic effects on locomotor parameters and cortical activity in individuals with PD. Finally, this thesis shows that locomotor exercises focusing attention on increasing the amplitude and speed of arm movements can be considered an effective strategy for treating locomotor deficits caused by PD.

5.1.2. Limitations and Future Studies

Despite the significant findings, this thesis has some limitations. The facility where data were collected only allowed participants to walk a distance of 8 meters without making turns, limiting the assessment of gait parameters, interlimb coordination, and cortical activity. Future studies should consider using longer distances to evaluate changes in locomotor parameters and cortical activity in PD individuals during longer tasks. Additionally, the time required for patient preparation (e.g., EEG and accelerometer placement and removal) and the execution of the combined tDCS and locomotor exercise protocol limited the number of trials and the overall protocol length, as there was a risk of participants transitioning out of the ON-medication state. Future studies should consider using a tDCS system integrated with an EEG system, allowing the entire experimental protocol to be conducted without removing the EEG equipment during the procedure.

Table1. Summary of main findings and suggestions for future studies

Current Literature	Main Findings	Future Studies
Study 1		
tDCS has been suggested as a promising therapy to improve movement coordination (Alexoudi et al., 2019).	- The electrode placement should consider the participant individual status; - The current intensity at 2mA seems to be more effective than lower or higher currents intensities; - Protocols lasting 20 minutes appear to be more effective than shorter protocols.	- Future studies should consider: - investigating the effects of transcranial electrical stimulation on locomotor parameters (e.g. movement coordination and gait parameters) during complex tasks such as walking.
The vast number of available protocols makes it difficult to define the most appropriate protocol to improve motor coordination in older adults and people with Parkinsonism.		
Study 2		
Locomotor exercise and tDCS have been show as effective alternative therapies to improve locomotion and to generate changes on cortical activity of PwPD (Broeder et al., 2015; Ni et al., 2018)	- Only one session of combined tDCS and locomotor exercise had no synergetic effect on PwPD walking, interlimb coordination and cortical activity parameters; - One session of locomotor exercise protocol focused on the increase of arm swing amplitude and speed generated improvements on PwPD gait parameters, such as the increase in stride length and velocity and decrease double support time and a decrease on their occipital cortex activity.	Future studies should consider: - investigate the effects of multiple sessions of a combined tDCS and locomotor exercise focused on arm swing amplitude and speed protocols on PwPD locomotor behavior - Adopt longer walking distances to acquire data from PwPD locomotion and cortical activity.
Despite the strong relation between arm movement and walking performance, no study yet investigate the effects of locomotor exercise focused on arm swing amplitude and speed on PwPD locomotor behavior. (Zampier et al., 2018)		
It has been suggested that tDCS generates synergetic effects on PwPD locomotor behavior when combined with physical exercise. However, no study yet investigated the acute effects of a combined tDCS and over ground locomotor exercise on PwPD interlimb coordination and cortical activity during walking. (Beretta et al., 2020; Conceição et al., 2021).		

Study 3		
The literature suggest that when combined with other therapies tDCS generates synergetic effects on PwPD motor parameters (Beretta et al., 2020; Conceição et al., 2021). However the literature lacks the information regarding the effects of multiple sessions of combined tDCS with over ground locomotor exercise on PwPD locomotor behavior and cortical activity.	The combination between tDCS and locomotor exercise focused on the increase of arm swing amplitude and speed had synergetic effects on PwPD locomotor behavior and cortical activity. The a-tDCS group presented a decrease on arm-leg movement coordination followed by a decrease in PFC alpha band PSD.	Future studies should consider: - Adopt longer walking distances to acquire data from PwPD locomotion and cortical activity. - Perform gait event related EEG analysis to verify the brain activity changes in different moments during the walking task

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