Treadmill walking in the gait rehabilitation in Parkinson's disease:

Neurophysiological mechanisms and their combination with the non-invasive brain stimulation

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Submitted:

Miguel Ángel Fernández del Olmo, PhD in Physical Education and Professor in the Department of Physical Education and Sport in the University of A Coruña, and Olalla Bello Rodriguez, PhD and Assistant Professor in the Faculty of Physical Therapy in the University of A Coruña.

DECLARE:

That the Bachelor in Physical Therapy Helena Fernández Lago, has developed under their supervision the work called "*Treadmill walking in the gait rehabilitation in Parkinson's disease: neurophysiological mechanisms and their combination with the non-invasive brain stimulation*". This work satisfies all the requirements for a dissertation to aim for the International PhD in the University of A Coruña.

Miguel A. Fernández del Olmo
PhD. Proffessor

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A mis padres y hermanos.

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“I have seen one, who was able to run, but not to walk.”

Gaubius, H. D.

SUMMARY

Parkinson's disease (PD) is a neurodegenerative disease characterized by a set of motor and non-motor symptoms. Gait disorders are among the most disabling motor impairments in PD patients, as they severely affect their quality of life.

In recent decades, the treadmill has been explored as a tool for improving gait in PD. Understanding the mechanisms underlying these improvements will ameliorate the efficacy and prescription of physical therapy in PD. However, in spite of the growing body of evidence that links gait to cognitive function, the role played by attention in the gait improvements associated to treadmill walking in PD is still unknown. In the same way, the possible associated neurophysiological mechanisms have never been explored.

However, transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation, has been explored recently in order to improve gait in PD, and offers a promising tool in increasing the efficacy of rehabilitative interventions. It could also enhance our understanding of PD-pathophysiology. Nevertheless, the combined use of tDCS and the treadmill in PD has not yet been investigated.

The work presented here consists of three studies. The first explores the attentional demands involved in gait improvements associated with the treadmill in PD. The second investigates the immediate kinematics and neurophysiological effects of a single treadmill walking session on PD. The third study investigates the kinematic and neurophysiological effects of the combined use of tDCS and treadmill walking in patients with PD.

The results of this thesis do not support attentional resources as a possible mechanism for treadmill-associated gait improvements in PD. Likewise, a specific therapeutic effect of a single treadmill walking session on gait in PD was observed, with no associated neurophysiological changes in the outcomes measured. However, the combination of tDCS and treadmill resulted in a specific spinal excitability modulation. Further studies would be recommended to explore the role of belt displacement and constant speed as

main underlying mechanisms. Additional studies are also needed to investigate the functional significance of the interaction of tDCS and treadmill walking in PD.

RESUMEN

La enfermedad de Parkinson (EP) es una enfermedad neurodegenerativa, caracterizada por un conjunto de síntomas motores y no motores. Los trastornos de la marcha se consideran entre los síntomas motores más incapacitantes que afectan severamente a la calidad de vida de los pacientes con EP.

En las últimas décadas, el tapiz rodante ha sido explorado como una herramienta que mejora la marcha de los pacientes con EP. Conocer los mecanismos subyacentes a estas mejoras, ayudará a aumentar la eficacia y la prescripción de la fisioterapia en la EP. Sin embargo, a pesar de la creciente evidencia que vincula la marcha a la función cognitiva, todavía no sabemos el papel de la atención como mecanismo subyacente a las mejoras de la marcha asociadas al tapiz rodante en la EP. De la misma manera, no se han explorado los posibles mecanismos neurofisiológicos asociados.

Por otro lado, se ha explorado recientemente la estimulación transcraneal de corriente continua directa (tDCS), una modalidad de estimulación cerebral no invasiva, para mejorar la marcha en la EP, ofreciendo una herramienta prometedora para potenciar la eficacia de las estrategias de rehabilitación, además de mejorar nuestra comprensión de la fisiopatología en la EP. Sin embargo, todavía no ha investigado la combinación de tDCS con el tapiz rodante en la EP.

El trabajo presentado aquí consta de tres estudios. El primero explora si las demandas atencionales están involucradas en las mejoras de la marcha asociadas con el tapiz rodante en la EP. El segundo investiga los efectos cinemáticos y neurofisiológicos inmediatos de una sesión de tapiz rodante en la EP. El tercer estudio explora los efectos cinemáticos y neurofisiológicos de la combinación de tDCS y tapiz rodante en la EP.

Los resultados de esta tesis indican que los recursos atencionales no explican las mejoras de la marcha asociadas sobre el tapiz rodante en la EP. Del mismo modo, se observó un efecto terapéutico específico de sola sesión de tapiz rodante sobre la marcha en la EP, sin cambios neurofisiológicos

asociados. Sin embargo, la combinación de tDCS y tapiz resultó en una modulación específica a nivel espinal. Recomendamos más estudios que exploren el papel del desplazamiento de la banda rodante y la velocidad constante como principales mecanismos subyacentes, así como, se necesitan más estudios para investigar la importancia funcional de la combinación de la tDCS y el tapiz rodante en la EP.

RESUMO

A enfermidade de Parkinson (EP) é unha enfermidade neurodexenerativa, caracterizada por un conxunto de síntomas motores e non motores. Os trastornos da marcha considéranse entre os síntomas motores máis incapacitantes que afectan severamente á calidade de vida dos pacientes con EP.

Nas últimas décadas, o tapiz rodante foi explorado como unha ferramenta que mellora a marcha na EP. Coñecer os mecanismos subxacentes a estas melloras, incrementará a eficacia e a prescrición da fisioterapia na EP. Con todo, a pesar do crecente corpo de evidencia que vincula a marcha á función cognitiva, aínda non se sabe se a atención xoga aquí un papel primordial. Da mesma maneira, os posibles mecanismos neurofisiolóxicos asociados nunca foron explorados.

Doutra banda, explorouse recentemente a estimulación cerebral transcraneal de corrente contínua directa (tDCS), unha modalidade de estimulación cerebral non invasiva, para mellorar a marcha na EP, ofrecendo unha ferramenta prometedora para potenciar a eficacia das intervencións de rehabilitación, ademais de mellorar a nosa comprensión da fisiopatoloxía na EP. Con todo, aínda non investigou a combinación de tDCS co tapiz rodante na EP.

O traballo presentado aquí consta de tres estudos. O primeiro explora se as demandas atencionais están involucradas nas melloras da marcha asociadas co tapiz rodante na EP. O segundo investiga os efectos cinemáticos e neurofisiolóxicos inmediatos dunha sesión de tapiz rodante na EP. O terceiro estudo explora os efectos cinemáticos e neurofisiolóxicos da combinación de tDCS e tapiz rodante na EP.

Os resultados desta tese indican que os recursos atencionais non explican as melloras da marcha asociadas sobre o tapiz rodante na EP. Do mesmo xeito, observouse un efecto terapéutico específico de soa sesión de tapiz rodante sobre a marcha na EP, sen cambios neurofisiolóxicos asociados. Con todo, a combinación de tDCS e tapiz rodante resultou nunha modulación

específica a nivel espinal. Recomendamos máis estudos que exploren o papel do desprazamento da banda rodante e a velocidade constante como principais mecanismos subxacentes, así como, necesítanse máis estudos para investigar a importancia funcional da combinación da tDCS e o tapiz rodante na EP.

PREFACE

The present work, the thesis titled *Treadmill walking in the gait rehabilitation in Parkinson's disease: neurophysiological mechanisms and their combination with the non invasive brain stimulation* contains experimental work performed between 2013 and 2017 at *Faculty of Sports Science and Physical Education of University of A Coruña, Department of Sports Science*. Also, some work was performed during an stance in the laboratoy at the *Department of Nutrition, Exercise and Sport Sciences at University of Copenhagen* under the supervision of Dr. Jens Bo Nielsen from September to December 2014.

Three original experimental studies are included. The first and third studies have already been published in the international peer review journal American Journal of Physical and Rehablitation Medicine. The second study is under review in the peer review journal Human Movement Science.

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INTRODUCTION

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ABBREVIATIONS

ANOVA	Repeated-measures analysis of variance
BDNF	Brain-derived neurotrophic factor
BG	Basal ganglia
COMT	Catechol o methyl transferase
CPG	Central Pattern Generator
CN	Cuneiform nucleus
CV	Coefficient of variation
DA	Dopamine
DBS	Deep brain stimulation
DLPC	Dorsolateral prefrontal cortex
DT	Dual task
EMG	Electromyography
FOG	Freezing of gait
GABA	Gamma aminobutyric acid
GDNF	Glial cell-derived neurotrophic factor
GPe	External pallidal segment
GPi	Internal pallidal segment
H&Y	Hoehn and Yahr scale
H-reflex	Hoffmann reflex
Hmax	Maximum H-reflex response
ICF	Intracortical facilitation
MAO-B	Monoamine oxidase B
MEP	Motor evoked potentials
MLR	Mesencephalic locomotor region
Mmax	Maximum M amplitude
MMSE	Mini Mental State Examination
MSNs	Medium spiny GABAergic neurons
MPTP	1-methyl-4-phenyl-1, 2, 3, 6,-tetrahydropyridine
MRgFU	Magnetic resonance–guided focused ultrasound
M1	Primary motor cortex
NIBS	Non-invasive brain stimulation
PD	Parkinson's disease

PPN	Pedunculopontine nucleus
QoL	Quality of life
REM sleep	Rapid eye movement sleep
RMT	resting motor threshold
rTMS	Repetitive transcranial magnetic stimulation
SEM	standard error of the mean
SICI	Short intracortical inhibition
SMA	Supplementary motor area
SNc	Substantianigrapars compacta
SNr	Substantianigraparsreticulata
SOL	Soleus
SPECT	Single photon emission computed tomography
ST	Single task
STN	Subthalamic nucleus
TA	Tibialis anterior
tDCS	Transcranial direct current stimulation
TES	Transcranial electrical stimulation
TMS	Transcranial magnetic stimulation
UPDRS	Unified Parkinson´s Disease Rating Scale
UPDRS-III	Unified Parkinson´s Disease Rating Scale Part III

LIST OF PUBLICATIONS INCLUDED IN THIS THESIS

STUDY 1 Fernández-Lago H, Bello O, López-Alonso V, Sánchez JA, Morenilla L, Márquez G, Fernández-del-Olmo M (2015). Gait Pattern and Cognitive Performance During Treadmill Walking in Parkinson Disease. American Journal of Physical Medicine and Rehabilitation.

STUDY 2 Fernández-Lago H, Bello O, Vidal-Salgado A, Fernández-del-Olmo M. Acute kinematic and neurophysiological effects of treadmill and overground walking in Parkinson´s disease. Under review in Human Movement Science.

STUDY 3 Fernandez-Lago H, Bello O, Mora-Cerdá F, Montero-Cámara J, Fernández-del-Olmo M (2017). Treadmill walking combined with anodal tDCS in Parkinson's Disease: A Pilot Study of Kinematic and Neurophysiological Effects. American Journal of Physical Medicine and Rehabilitation.

OTHER SCIETIFIC CONTRIBUTIONS

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Lorentzen J, Kirk H, **Fernandez-Lago H**, Frisk R, Nielsen NS, Jorsal M, Nielsen JB (2015). Treadmill training with an incline reduces ankle joint stiffness and improves range of movement during gait in adults with cerebral palsy. Disability and Rehabilitation.

Lorentzen J, Kirk H, **Fernandez-Lago H**, Pingel J, Frisk R, Nielsen JB (2016). Evaluation of plantar-flexor function in relation to daily treadmill training in adults with cerebral palsy - an RCT- study. Gait and Posture.

The autor also contributed with communications/posters to the following international congresses:

Fernandez-Lago H, Bello O, Fernández-del-Olmo M. "Spinal excitability modulation induced by treadmill walking in Parkinson's disease: A preliminary study". XX World Congress on Parkinson's Disease and Related Disorders Geneva (Switzerland, December 2013).

HONORATIONS AND AWARDS

The scientific production of this thesis have received the following award:

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CHAPTER 1.

INTRODUCTION

1.1 GENERAL OVERVIEW OF PARKINSON´S DISEASE

Parkinson's disease (PD) is a common progressive neurodegenerative disorder described in 1817 by James Parkinson in the classic "Essay on the Shaking Palsy" (Parkinson, 2002). The cardinal signs of PD are related to motor dysfunction, including resting tremor, bradykinesia, rigidity, gait disturbances and postural instability (Jankovic, 2008; Berardelli et al., 2013). Nevertheless, PD is characterized clinically by a combination of motor and non-motor symptoms that severely threaten the quality of life (QoL) (Schrag et al., 2000; Barone et al., 2009; Soh et al., 2011) and poses a significant economic burden on patients and society (Noyes et al., 2006; Findley, 2007).

The main pathological finding associated with the motor deficits of PD is the degeneration of the dopaminergic neurons of the substantia nigra pars compacta (SNc) (Halliday et al., 2011). The motor symptoms appear when at least 60% of dopaminergic neurons are lost and 80–85% of dopamine content in the striatum is depleted (Jankovic, 2008). Catecholaminergic and serotoninergic brainstem neurons may also degenerate. Lewy bodies (eosinophilic inclusion bodies containing many different proteins) are present mainly in the surviving neurons (Braak et al., 2003). The pathogenesis mechanisms that cause dopaminergic cell death may include defective handling of proteins, mitochondrial dysfunction, oxidative stress, and inflammation (Schapira & Jenner, 2011).

1.1.1 Epidemiology

PD is considered to be the second most common neurodegenerative disorder after Alzheimer's disease, and affects 0.3% of the entire population in industrialized countries. Standardized incidence rates of reported PD are 10-20 per 100,000 people per year (Twelves et al., 2003). Incidence varies depending on age, gender and ethnicity (Van Den Eeden, 2003). Incidence and prevalence are consistently higher in men than in women, and the average age of onset in PD is established at about 60 years old, so it is principally considered to be a disease of the elderly (Wirdefeld et al., 2011).

The number of patients is expected to rise considerably in the coming decades, due to the ageing of the population.

1.1.2 Etiology

The purely sporadic etiologic basis in PD has changed now to a view where both environmental and genetic factors contribute to the onset of the illness (Schapira & Jenner, 2011).

In 1980s was discovered the 1-methyl-4-phenyl-1, 2, 3, 6,-tetrahydropyridine (MPTP), a substance structurally similar to the herbicide paraquat, that could destroy dopaminergic neurons causing chronic parkinsonism (Langston & Irwin, 1984). MPTP finding was stimulated studies focus on environmental factors, and most important, generated novel experimental models of PD.

Environmental factors on the occurrence of PD range from the general, in terms of a potential role for industrialization, rural environment, well water, plant-derived toxins, and bacterial and viral infection, to the specific, as occurs with exposure to organic solvents, carbon monoxide, and carbon disulfide (Schapira & Jenner, 2011). Despite of the wide literature, the degree of evidence of some environmental factors, for instance, pesticides, metals or magnetic fields are few (Richardson et al., 2009; Kieburtz & Wunderle, 2013). The evidence for cigarette smoking and caffeine intake as protective factors appears clear, but there is still uncertainty over the role of others, for example, exercise, anti-inflammatories, antihypertensive (most notably calcium antagonists), and antilipidaemics (Warner & Schapira, 2003; Chin-Chan et al., 2015; Kalinderi et al., 2016).

On the other hand, the last decade has been characterized by a remarkable acceleration in the identification of genes that appear to cause an illness very similar to PD or PD itself (Wirdefeldt et al., 2011). So far 18 mutations in genes / loci have been identified, that include autosomal dominant forms (PARK1-4, PARK5, and PARK8), recessive autosomal forms (PARK2, PARK6, PARK7, and PARK9) locus and mutations of genes associated with PARK10-16. Despite of these, only 10 to 15% of patients with the disease

show a familiar form, indicating that more PD genes and loci remain to be identified. The increasing knowledge about genetics of PD has provided clues about the molecular mechanisms involved in its pathogenesis. For instance, Park1 and Park4 are α-synuclein genes located on chromosome 1 and 4 respectively, that carries the genetic code for the production of α-synuclein protein present in Lewy bodies (Fujioka &Wszolek, 2014).

1.1.3 Basal ganglia circuitry in PD

The term Basal Ganglia (BG) refers to a group of nuclei in the forebrain and midbrain that are extensively connected to different parts of the cerebral cortex, thalamic nuclei and specific mesencephalic structures. These sets of nuclei include the striatum (caudate and putamen), the globus pallidus pars externa (GPe) and pars interna (GPi), the subthalamic nucleus (STN) and the substantia nigra pars compacta (SNc), and pars reticulata (SNr). The organization of the connectional relationships of the BG has had a great impact on the insight into the pathophysiology of PD and other movement disorders.

The main BG inputs come from the cerebral cortex and from the thalamus, while the projections of the BG have an ascending component and a descending component. The ascending component, the so-called basal ganglia-thalamo-cortical loops, projects to the cortical areas. The BG loops are functionally subdivided as motor, oculo-motor, associative, limbic, and orbitofrontal, according to the main cortical projection areas (DeLong, 1990). This arrangement explains the influence of BG on sensorimotor, cognitive/executive and emotional-motivational functions. The descending component consists of the BG projections directed to mesencephalic structures and, in turn, to motor output structures in the lower brainstem and spinal cord. These projections form the neuronal basis for the influence of the BG on posture and balance, as well as on muscle tone.

The classical model of BG organization was also developed in the late 1980s and was focused on motor control (Albin et al., 1989; DeLong, 1990). This model was based on the following findings:

1) Cortical motor areas and the primary somatosensory cortex project to the striatum in a somatotopical manner.

2) Striatal efferent neurons are GABAergic medium spiny neurons (MSNs) that project to BG output, i.e., GPi and SNr, through two different pathways: ‘‘direct’’ and ‘‘indirect’’. MSNs of the “direct” monosynaptic pathway contain dopamine (DA) D-1 receptors, co-express the peptides substance-P and dynorphin, and project directly from putamen to GPi/SNr. MSNs of the “indirect” polysynaptic pathway contain DA D-2 receptors and co-express enkephalin, and pass to the GPe and from there to both output nuclei (GPi and SNr), either directly or via the intercalated STN.

3) DA modulates glutamatergic effects of corticostriatal inputs by exerting a dual effect on striatal neurons, exciting D1 neurons in the direct pathway and inhibiting D2 neurons in the indirect circuit (Gerfen & Surmeier, 2011), which in turn exert a tonic inhibition of the thalamus.

4) Reduced BG output leads to movement facilitation and increased BG activity to movement inhibition.

According to this model, DA deficiency in the parkinsonian state causes a hyperactivity of the indirect pathway and hypoactivity of the direct pathway (DeLong, 2001), promoting increased activity in the STN and GPi/ SNr, which in turn causes increased thalamic inhibition and finally a decrease in the excitation of the cortical motor systems and the brainstem (Obeso et al., 2000). Thus, the balance of BG activity shifts toward the “indirect” circuit, where the GPe-STN-GPi microcircuit plays a paramount role (Figure 1).

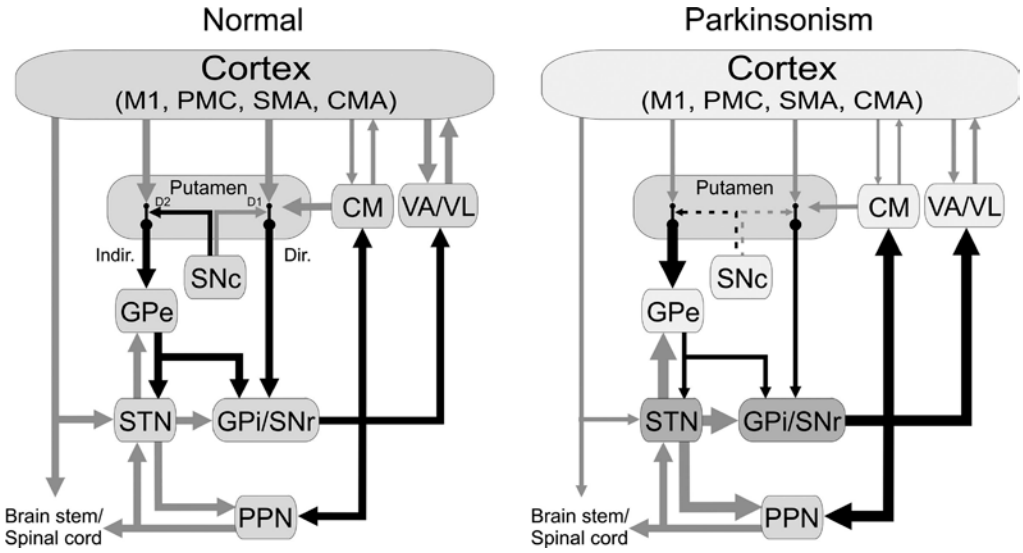


Figure 1 Parkinsonism-related changes in overall activity ("rate model") in the basal ganglia-thalamocortical motor circuit.

Black arrows indicate inhibitory connections; gray arrows indicate excitatory connections. Abbreviations: CM, centromedian nucleus of thalamus; CMA, cingulate motor area; Dir., direct pathway; D1, D2, dopamine receptor subtypes; Indir., indirect pathway; M1, primary motor cortex; Pf, parafascicular nucleus of the thalamus; PMC, premotor cortex; PPN, pedunculopontine nucleus; SMA, supplementary motor area; VA, ventral anterior nucleus of thalamus; VL, ventrolateral nucleus of thalamus (Wichmann, Delong, Guridi, & Obeso, 2011).

The classical model provides a reasonable explanation for the origin of akinetic features in PD, such as movement initiation, and the response to drugs and surgery. Other typical features in PD such as reduced blinking rate, a positive Meyerson's sign, and decreased arm swing are probably mediated by brainstem mechanisms, which are also functionally impaired by excessive basal ganglia inhibitory outputs.

However, despite the existence of more complex BG models, they do not provide a definitive explanation for the two other cardinal features of PD, that is, rigidity and tremor, as well as gait dysfunction, attention and learning deficits, emotional disturbances, and cognitive disorders, all of which have now been acknowledged to form part of the global clinical picture of PD (Marsden & Obeso, 1994; Obeso, Marin, et al., 2008; Obeso, Rodríguez-Oroz, et al., 2008).

1.1.4 Clinical manifestations

The clinical PD hallmarks include motor parkinsonism. Despite the diagnosis of PD being mainly dependent on motor symptoms, a period called the premotor phase has been described in which non-motor symptoms can precede PD motor impairments.

PD is diagnosed on clinical criteria and there is no definitive test for its diagnosis. However, there are some established criteria to make the diagnosis as objective as possible. According to the Parkinson's UK Brain Bank, the diagnosis of PD is based on the presence of at least two cardinal symptoms (bradykinesia combined with one of the three other cardinal signs, i.e., rigidity, resting tremor or postural instability) of progressive, unilateral onset and response to levodopa. In addition, none of a number of exclusion criteria must be present, and other typical PD signs might support the diagnosis.

1.1.4.1 ***Motor features***

Motor features comprise mainly the four cardinal signs of PD (resting tremor, bradykinesia, rigidity, and gait disturbances/postural instability), plus a widespread number of other motor abnormalities, called secondary motor symptoms, such as dysarthria, hypophonia, dysphagia and sialorrhoea, among others (Table 1). In addition, flexed posture, gait disorders and freezing (motor blocks) have been included among the classic features of PD (Shahed & Jankovic, 2007).

Table 1 PD motor symptoms.

Tremor, bradykinesia, rigidity, postural instability
Hypomimia, dysarthria, dysphagia, sialorrhoea
Decreased arm swing, shuffling gait, festination difficulty arising from chair, turning in bed
Micrographia, cutting food, feeding, hygiene, slow activities of daily living
Glabellar reflex, blepharospasm, dystonia, striatal deformity, scoliosis, camptocormia

a) Bradykinesia

From a purist perspective, bradykinesia refers to slowness of movement, akinesia refers to poverty of spontaneous movement and the term hypokinesia means smaller amplitude of movement. However, in the literature the term bradykinesia tends to group these motor features within the same construct. Bradykinesia encompasses difficulties with planning, initiating and executing movement with performing sequential and simultaneous tasks (Berardelli et al., 2001). Bradykinesia is the motor sign that appears to correlate best with a degree of dopamine loss, due to the potentially correlations with disease severity and response to treatment (Rodríguez-Oroz et al., 2009).

b) Classic tremor

Classic tremor is the most common and easily recognized symptom of PD. It is characterized by a low frequency rhythmic oscillation within a bandwidth of 4 or 6 Hz that occurs at rest (Shahed & Jankovic, 2007). Hand-tremor is described as a supination-pronation tremor ("pill-rolling"), but rest tremor in PD can also involve the lips, chin, jaw and legs. This type of tremor is pathophysiologically separated from bradykinesia and rigidity, and the response of tremor to dopaminergic agents is less than bradykinesia (Jellinger, 2012). It is now realized that classical tremor in PD is mediated by an abnormal oscillatory activity in an extensive motor network that involves the BG, cerebellum, thalamus, and motor cortex (Hallett, 2014).

c) Rigidity

Rigidity is essentially an increase in resistance to passive movement. It has been thought for decades that rigidity should be related to an enhancement of stretch reflex excitability (Cantello et al., 1991; Delwaide et al., 1993; Moreau et al., 2002). It is known that rigid patients present an enhanced cortical excitability, coupled with DA depletion and increased BG output (Rodríguez-Oroz et al., 2009). However, it has not yet been clarified how BG changes associated with dopamine depletion modify the excitability of stretch reflex mechanisms.

d) Postural stability and gait disorders

Parkinson's patients have difficulties with balance, both sitting and standing, and also with walking. When PD patients are standing, they adopt a flexed posture, with the body inclined slightly forward, with flexed knees and arms in front of the body (Kim et al., 2013). The typical gait of these patients is slow with short, shuffling steps, swinging arms and a flexed posture. The pathophysiology of postural instability and gait problems is complex and is not caused by a single factor, but is the result of a combination that includes changes in postural reflexes (anticipatory postural reflexes and automatic postural reactions), rigidity and akinesia (Hanakawa et al., 1999b).

1.1.4.2 ***Non-motor features***

PD not only involves the degeneration of mesencephalic dopaminergic structures, but also of the peripheral autonomic nervous system, dorsal motor vagal nucleus, olfactory bulb, brainstem centers and the neocortex (Wolters et al., 2014).

Pending the exact localizations and severity of the pathological condition, autonomic nervous system dysfunctions, sleep-wake disorders, sensory disorders, and neuropsychiatric disorders are part of the non-motor symptomatology of PD (Table 2) (Chaudhuri et al., 2006).

Table 2 The non-motor symptom complex of PD.

Neuropsychiatric symptoms	▪ Depression, apathy, anxiety ▪ Anhedonia ▪ Cognitive impairment (executive dysfunction) ▪ Hallucinations, illusion, delusions ▪ Dementia ▪ Obsessional behaviour (usually drug induced), repetitive behaviour ▪ Confusion ▪ Delirium (could be drug induced) ▪ Panic attacks
Sleep disorders	▪ Restless legs and periodic limb movements ▪ Rapid eye movement (REM) sleep behaviour disorder and REM loss of atonia ▪ Non-REM-sleep related movement disorders ▪ Excessive daytime somnolence ▪ Vivid dreaming

	▪ Insomnia ▪ Sleep disordered breathing
Autonomic symptoms	▪ Bladder disturbances ▪ Urgency ▪ Nocturia ▪ Frequency ▪ Sweating ▪ Orthostatic hypotension ▪ Falls related to orthostatic hypotension ▪ Coat-hanger pain ▪ Sexual dysfunction ▪ Hypersexuality (likely to be drug induced) ▪ Erectile impotence ▪ Dry eyes (xerostomia)
Gastrointestinal symptoms (overlaps with autonomic symptoms)	▪ Dribbling of saliva ▪ Ageusia ▪ Dysphagia and choking ▪ Reflux, vomiting ▪ Nausea ▪ Constipation ▪ Unsatisfactory voiding of bowel ▪ Faecal incontinence
Sensory symptoms	▪ Pain ▪ Paraesthesia ▪ Olfactory disturbance
Other symptoms	▪ Fatigue ▪ Diplopia ▪ Blurred vision ▪ Seborrhoea ▪ Weight loss ▪ Weight gain (possibly drug induced)

Some non-motor symptoms, such as olfactory problems, constipation, depression, mid cognitive impairment (executive domain) and rapid eye movement disorder may precede the first motor manifestations (Marras & Chaudhuri, 2016). This primary clinical phase is called premotor stage (Table 3). For instance, cognitive dysfunction may be present from the early stages of PD and might involve a more extensive cognitive dysfunction in the future stages. Impairment may be mild or severe enough to justify the diagnosis of dementia (Tolosa & Pont-Sunyer, 2011). Mild cognitive impairment can include executive dysfunction and impairment of attention and working

memory, as well as deficits confined to language, memory, or visuospatial domains.

Table 3 Survey of primary (premotor) non-motor symptoms in PD.

Autonomic dysfunction	Parasympatheic cholinergic: Dry mouth, gastrioaresis, constipation, pollakisuria, incontinence, erectile dysfuncion, pupillomotor abnormalities
	Sympathetic cholinergic: thermoregulatory dysfunction, hypo/hyperhidrosos (drenching sweats)
	Sympathetic noradrenergic: cardiovascular dysfunction, baroreflex failure, orthostatic hypotension
Sleep-wake disorders	Insomnia and sleep fragmentation, fatigue, excessive daytime sleepiness and sleep attacks, REM sleep behavioral disporder
Sensory disorders	Pain, hyposmia, impaired color vision
Neuropsychiatric disorders	Apathy, anxiety and panic attacks, depression, mid cognitive impairment (executive domain) and psychosis

1.1.5 Treatment

Nowadays, treatment approaches in PD are focused on alleviating symptoms and maximizing functions. The mainstay of intervention for people with PD is medical management, including pharmacology and, to a lesser extent, surgical options (Kakkar & Dahiya, 2015). Nevertheless, patients require an holistic and multispecialty approach to maximize their QoL, and the QoL of their carers (Johnson, 2015).

In recent years, there have been remarkable advances in the knowledge of the etiology and pathophysiology of the disease, helping the development of new treatments. Although cellular and genetic research is advancing in the clinical domain, the current treatments for PD continue to show important limitations, such as medication-related complications and surgical risks. These facts highlight the need to improve the current treatments, and to investigate adjunctive therapies.

a) Pharmacological therapy

Levodopa is the precursor to DA, norepinephrine and epinephrine, and crosses the blood-brain barrier and is converted into DA in the nigrostriatal nerve terminals. Levodopa is usually administrated with carbidopa, a peripheral dopa decarboxylase inhibitor, which enhances the therapeutic benefits of levodopa. It remains the most potent drug for controlling symptoms in PD, principally bradykinesia.

Despite the advances in levodopa therapy, such as rapid-onset formulations and duodenal infusion therapy, the response to levodopa becomes less reliable and less predictable over time, and after 5 years of therapy, medication-related complications develop in the majority of patients (Jankovic, 2008). Motor complications, such as dyskinesia, wearing-off, and "on–off" fluctuations, are well-recognized (Nutt, 2001; Stacy, 2009).

DA agonists and non-dopaminergic therapy, such as catechol-o-methyl-transferase (COMT) inhibitors, Monoamine oxidase B (MAO-B) inhibitors, amantadine, etc. are other modalities in the pharmacological management of PD and may be used concomitantly or sequentially with levodopa. For instance, DA agonists activate DA receptors and only provide a modest improvement in parkinsonian symptoms, but this improvement may be sufficient in the early stages in order to delay the introduction of levodopa. COMT inhibitors prolong DA response, improving the percentage of levodopa that enters the brain. MAO-B inhibitors degrade dopamine and can be used to prolong the effect of dopaminergic agents or as a monotherapy (Katzenschlager et al., 2011).

Future pharmacological therapies involve the use of neurotrophic factors to enhance the survival of midbrain dopaminergic neurons in vitro and to save degenerating neurons in vivo (Zhang et al., 2012; De Munter et al., 2014).

b) Surgical treatment

Deep brain stimulation (DBS) is the gold standard for surgical treatment in PD, such as levodopa is for pharmacotherapy. DBS was introduced as a validated therapy in the 1990s. It uses a device called a neurostimulator to

deliver electrical signals to the different areas of the brain. The two main targets for DBS are STN and GPi. DBS of the STN leads to improvements in rigidity, tremor and bradykinesia, while dyskinesias are dramatically improved with GPi-DBS (Bronstein et al., 2011). A new target of DBS is the pedunculopontine nucleus (PPN), with promising results in gait function (Pierantozzi et al., 2008; Ferraye et al., 2010; Hariz et al., 2013). Nevertheless, DBS has some disadvantages, such as the need for careful patient selection to achieve favorable outcomes, and adverse effects, which include perioperative, hardware-associated complications and stimulation-induced complications (Huys et al., 2014).

Lesional procedures have been largely replaced by DBS, but under certain conditions, unilateral pallidotomy, unilateral thalamotomy, and even subthalamotomy remain useful alternatives. Other innovative surgical treatments include magnetic resonance–guided focused ultrasound (MRgFU), a new lesioning modality that may have some advantages over current lesioning procedures; and gene and cellular therapies, including stem cells, which remain avenues to be investigated (Fox et al., 2011).

Based on DBS studies, new treatment techniques for PD patients have been developed, such as repetitive transcranial magnetic stimulation (rTMS) (Benninger & Hallett, 2015) and transcranial direct current stimulation (tDCS) (Fregni et al., 2005a). The rationale behind these techniques, which are known as non-invasive brain stimulation (NIBS) techniques, is that they are based on attempting to reverse the clinical deficits of abnormal brain functioning and physiology. RTMS modulates cortical excitability. High-frequency (≥5 Hz) rTMS has proved to be facilitatory (Pascual- Leone et al., 1994), and low-frequency rTMS (≤1 Hz) has proved to be inhibitory (Chen et al., 1997). tDCS delivers a continuous current to the scalp that modulates membrane excitability and induces shifts in cortical excitability, with polarity defining the effects (Nitsche & Paulus, 2000; Lang et al., 2005; Nitsche et al., 2008). Recent studies have suggested that these techniques may have additional therapeutic potential that goes beyond that of conventional therapies (Fregni et al., 2005b; Elahi et al., 2009; Zhu et al., 2015). However, more studies are necessary in order to demonstrate substantial clinical effects.

c) Multispecialty care

Despite medical management, most patients with PD continue to experience a wide range of motor and non-motor symptoms. A multispecialty approach seems to be preferable over a single-clinician approach in order to improve QoL, motor functioning, day-to-day activities and the psychosocial burden involved. A wide range of disciplines might have potential value for PD care (Van der Marck & Bloem, 2014), including medical specialists, specialized nurses, and allied healthcare professionals (such as physical therapists, occupational therapists, speech–language therapists, dieticians, social workers, sexologists and neuropsychologists). In recent years, several allied health disciplines have become more evidence-based. The evidence grade is highest for physical therapy (Keus et al., 2009) and speech–language therapy (Ramig et al., 2001), followed by occupational therapy (Dixon et al., 2007).

1.2 GAIT DISORDERS OF PARKINSON´S DISEASE

1.2.1 Features of PD gait

Gait disturbances are one of the classic features of PD, which manifest themselves in almost all patients and often lead to a decline in their QoL (Ebersbach et al., 2013). Gait difficulties become progressively more levodopa-resistant, and thus are considered the hallmarks of the advanced stages of PD.

PD gait is mainly characterized by the inability to generate an appropriate stride length (Morris et al., 1996). Associated disturbances include a reduced gait speed, reduced arm swing (Wood et al., 2002), increased stride-to-stride variability, increased double limb support time, gait instability, stooping, difficulty turning, and forward-flexed posture (Nieuwboer et al., 2009; Huang et al., 2012; Ebersbach et al., 2013). In advanced stages, more complex gait disturbances may appear, including FOG, motor blocks, festination and disequilibrium. All these gait features cause significant disabilities resulting from falls, immobility, and loss of independence (Bloem et al., 2016). Falls are considered to be one of the most serious complications of motion in PD, with an incidence of 70% during a 1-year follow-up, when recurrent falls have been shown to occur in approximately 50% of cases (Bloem et al., 2004; Okuma, 2014).

a) Stride length, gait speed and cadence

PD patients typically show a reduced walking velocity, which is associated with reduced stride length and a compensatory increase in cadence. An abnormal slowness of gait speed has already been reported in early and middle stages of PD, compared with healthy subjects (Ebersbach et al., 2013). The reduced stride length of PD subjects is present even though the cadence (steps per minute) remains intact (Morris et al., 1996). Thus, the main problem of gait hypokinesia is PD subjects' particular difficulty in the internal regulation of stride length. PD subjects have higher cadence rate

than control subjects for any given velocity, but this is a compensation for reduced step size (Morris et al., 1994).

b) Gait variability

The ability to maintain a steady gait pattern is also impaired in PD subjects, which is thought to reflect reduced motor automaticity. Gait variability typically means walking fluctuations from one stride to the next. PD patients show a higher variability of stride duration, and even an increased variability of leg muscle activation during walking (Schaafsma, 2003; Baker et al., 2008; Lord et al., 2011). Increased gait variability is associated with an increased fall risk in older adults, as well as in patients with PD (Hausdorff, 2003).

c) Complex gait disturbances

As the disease progress, more complex gait disturbances can appear, such as FOG and festination. FOG is defined as a "brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk" (Bloem et al., 2004; Nutt et al., 2011). This definition includes episodes in which the patient cannot initiate gait ("start hesitation") and arrests forward progression during walking ("turn" and "destination" hesitation). Festination is the tendency to move the center of gravity forward, walking increasingly rapidly with smaller steps (Nutt et al., 2011). Festination is mostly shown in patients suffering from FOG; however, it may occur independently.

1.2.2 Associations between cognition and gait in PD

Gait impairments in PD include a reduced gait speed, increased stride-to-stride variability, and a specific difficulty in regulating stride length. In addition, people with PD suffer from a deficit in executive functions and attention abilities. A growing body of evidence links gait to cognitive function in healthy young subjects (Marquis et al., 2002; Verghese et al., 2002b; Hausdorff et al., 2005) and also in PD subjects (Amboni et al., 2010), suggesting that gait is not quite automatic, but consumes some attention resources (Camicioli et al., 1997; Brauer et al., 2002; Woollacott & Shumway-Cook, 2002). A 2-year follow-up study of 26 PD patients

established that “on” state FOG correlated with a faster progression of executive dysfunction. Thus, in early PD, better attention and executive function can compensate for a loss of gait automaticity, but this is no longer an efficient strategy when the disease progresses and both attention and executive function deteriorate too. These results emphasize the interrelation that exists between gait and cognitive symptoms in PD.

The dual-task (DT) paradigm has been used to study this dependence (Canning, 2005). Dual tasking is a component of the executive function (Della et al., 1995) and refers to the ability to divide attention between tasks that are performed at the same time (Yogev-Seligmann et al., 2008). Several theories have been proposed to explain DT interference, such as the “capacity sharing model” and the “bottleneck model”. All of them agree that when two tasks are performed concurrently, performance decrements in one or both tasks (Marois et al., 2005; Yogev-Seligmann et al., 2008). For instance, according to the capacity sharing model, when two attentionally demanding tasks are performed simultaneously, competition of attentional resources results in the deterioration of performance in one or both of the tasks (Tombu & Jolicœur, 2003).

It has been shown that gait impairments are exacerbated under DT conditions in PD. When PD patients perform an additional motor or cognitive task when walking, they manifest a slower gait speed, shorter strides, increased double support time, and increased stride-to-stride variability, exacerbating their risk of falling in DT situations (Morris et al., 1996; Bloem, 2000; Bond, 2000; O’Shea et al., 2002; Hausdorff, 2003; Galletly & Brauer, 2005; Yogev et al., 2005).

DT causes competition for attention, but also poses a challenge in prioritizing one of the two tasks. It has been shown that, while healthy controls give attentional priority to posture and gait, PD patients are at higher risk of falling because they use a “posture second” strategy (Bloem et al., 2006). Fallers had poorer scores on executive function tests than non-fallers, while the gait speed and coordination of fallers were worse than those of non-fallers, particularly under DT conditions (Plotnik et al., 2011).

When considering the effects of various interventions on PD gait, single-task walking in PD has been well described, but there is less research that examines the efficacy of different pharmacological, surgical, or rehabilitative therapies on DT walking in this population (Kelly et al., 2012). It is important to point out the functional significance of DT, since it is a common activity in daily life. Further studies are therefore required to evaluate the efficacy of the treatment on gait under DT conditions, and consequently, on the interaction of gait and executive functions.

1.2.3 The physiopathology of gait disorders in PD

Firstly, it is interesting to consider the pathogenesis of hypokinesia within the physiopathology of PD gait, since the reduced stride length is the main hallmark of PD gait disturbances (Morris et al., 1996). Studies on primates and in people with PD reported that the interaction between BG and the supplementary motor area (SMA) is disrupted during movement performance in hypokinesia. The reduced amplitude of a movement in hypokinesia could result from a disorder in a motor set-related activity within the BG, which is needed for the running of the entire sequences of a movement (Brotchie et al., 1991). The set-related activity in the BG contributes to set-related activity in the SMA, so as to maintain the entire sequence in preparedness for running (Cunnington et al., 1995; Iansek et al., 1995). Thus, an abnormal motor set-related activity from BG to the cortex, for a whole gait sequence, could affect the ability to elicit a normal step in PD subjects (Morris et al., 1994).

Several studies indicate that the cortico-frontal regions are likely to be involved in the physiopathology of gait disorders in PD patients. The hypoperfusion of the SMA and other parts of the frontal lobe were found to be associated with severe gait disturbances and bradykinesia in PD (Matsui et al., 2005; Mito et al., 2006; Nutt et al., 2011). SPECT has been used to investigate the mechanisms underlying the improvement of gait in PD patients when exposed to visual stimuli. In these conditions, it seems that PD patients can compensate for the impaired SMA function by the activation in the lateral premotor cortex (Hanakawa et al., 1999). Even, a dysfunctional striato-frontal (in the “executive-attention” networks) and downstream

cortico-pontine pathways may both play a critical role in the physiopathology of FOG (Herman et al., 2013).

Secondly, a subject's walking pattern is adjusted according to individual EMG bursts and relative timings. Orlovsky et al. (1999) stated (Orlovskiĭ et al., 1999).

"…the locomotor activity in humans has been much more thoroughly studied than its neural control and in this matter we have to rely mainly on extrapolations from simpler animal models".

The neural networks that generate locomotor bursts have been extensively investigated in animals. These networks are hierarchically structured, including: 1) Lower effector levels; 2) Center pattern generators (CPGs) for locomotion; 3) Several locomotor areas located in the brainstem, which controls CPGs; 4) Higher level control systems, such as basal ganglia and neocortex (Grabli et al., 2012). Thus, we should regard research in animals in order to understand the neural control of PD gait.

On the one hand, forward propulsion of the body is mainly powered by contraction of the calf muscles, with a contribution from the knee extensors (Neptune et al., 2001). Thus, it is important to highlight the reflex control of the ankle extensors on gait. The stretch reflex has been suggested as making a substantial contribution to motor output during animal and human walking (Andersen et al., 1994; Nielsen & Sinkjaer, 2002). The electrical stimulation of Ia fibers in peripheral nerves elicits the monosynaptic component of the stretch reflex, which can be recorded from the muscles as an action potential (H-reflex) (Capaday, 2002). For the interpretation of the H-reflex, it is important to note that its circuit is embedded in a complex neural system, and its behavior reflects the integral activity of the whole (Figure 2).

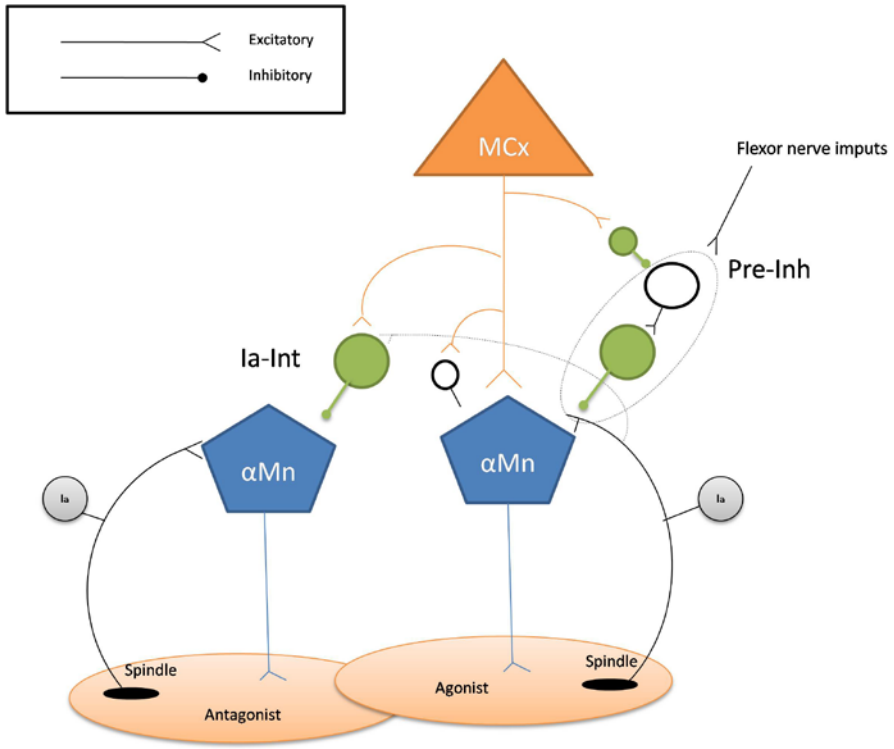


Figure 2 Basic spinal circutry and its corticospinal control.

At the peripheral end, the sensitivity of the receptor organ, the muscle spindle, to muscle stretch is controlled by the independent γ-motoneuron system (not shown). Centrally, collaterals of group Ia afferents from muscle spindles inhibit the antagonistic α-motoneuron pool, via Ia-inhibitory interneurons (Ia-Int.). These form part of the neural circuits that mediate reciprocal inhibition between antagonistic muscles. Additionally, a presynaptic inhibitory network (Pre-Inh.) controls the efficacy of synaptic transmission from the Ia-afferent terminals to α-motoneurons. The presynaptic inhibition circuit can be activated from the periphery by stimulation of muscle flexor nerves. The last order of interneurons inhibits Ia-neurons, and consequently reduce the reflex response. At the same time, stimulation of the motor cortex reduces presynaptic inhibition (i.e. inhibits presynaptic inhibition). Corticospinal neurons that project to a given motoneuron pool also inhibit the antagonistic motoneuron pool via Ia-inhibitory interneurons, adding an extra layer of complexity to the reciprocal inhibitory pathway. Several other circuits could be added to the figure, but what is important is that descending pathways act simultaneously on α-motoneurons and interneurons (Adapted from Capaday et al., 2002)

The amplitude of the H-reflex and its relationship with the background level of motor activity is strongly dependent on the motor task (Sinkjaer et al., 2000; Nielsen & Sinkjær, 2002). In human gait, neural mechanisms that modulate the SOL H-reflex during the normal step cycle include increased activity of α-motoneurons during the stance phase (Capaday & Stein, 1986),

increased postsynaptic inhibition of α-motoneurons during the swing phase (Lavoie et al., 2013), and a tonic increase in presynaptic inhibition of group Ia afferent terminals projecting to the α-motoneurons.

In PD, only a few studies have evaluated some spinal reflex related to gait. Pierantozzi et al. (2008) showed that DBS of the PPN provides a distinctive influence on H-reflex, suggesting that it has a role in gait impairments in PD (Pierantozzi et al., 2008). Hiraoka et al. (2005) found a reduction of the SOL H-reflex during gait initiation in PD (Hiraoka et al., 2006) and, finally, Meunier and colleagues (2000) reported a correlation between the decreased reciprocal Ia-inhibition, at the onset of voluntary ankle dorsiflexion, with axial signs of PD patients (Meunier, et al., 2000a).

On the other hand, in non-mammalian vertebrates and quadrupedal mammals, such as rats and cats, the isolated spinal cord can generate spontaneous locomotor bursts in the complete absence of peripheral feedback. CPGs are the neural networks of the spinal cord that generate these bursts. At the same time, CPGs are regulated by descendent supraspinal signals and afferent input of the limbs (Golgi tendon organs, Ia-muscle spindles, mechanoreceptors in the foot), which regulate the stance phase and the swing phase of the step cycle (Crommertet al., 1998). In humans, there is indirect evidence of the existence of spinal CPGs, and if they exist, they are much less robust than in mammals.

Given the mechanical complexity of human bipedal locomotion, and that complete spinal cord lesion in humans leads to paralyses with no recovery of gait, it is often suggested that the corticospinal tract has a more predominant role in the control of walking in humans than in other animals. The involvement of cortical structures in the control of human gait can be assessed using several non-invasive methods, such as neuroimaging, transcranial magnetic and electrical stimulation (TMS and TES, respectively). As has been noted, neuroimaging studies showed the involvement of the SMA (among other regions) on hypokinetic PD gait. Moreover, recent TMS studies revealed abnormalities in intracortical facilitation (ICF) of the primary motor cortex that may be related to decreased stride length and slower gait speed in PD subjects (Vacherot et al., 2010a).

Finally, experiments in decerebrate cats demonstrated that electrical stimulation of an area of the brainstem region triggered walking and even galloping. This area is called the mesencephalic locomotor region (MLR), which is gaining importance in the physiology of gait. MLR is composed by the PPN and the cuneiform nucleus (CN). Both nuclei have reciprocal connections with BG (SNc, STN, pallidum) and have major outputs to the descending reticulo-spinal pathway and the ascending thalamo-cortical pathway. The neurons of the MLR are mainly cholinergic and GABAergic.

Recently the dysfunction of the MLR of the brainstem has been emphasized in the physiopathology of gait and balance disorders in PD (Pahapill & Lozano, 2000; Grabli et al., 2012). In normal monkeys, lesions in the PPN induce akinesia and gait and balance disorders. In people with PD, DBS of the PPT region can improve gait function (Alam, Schwabe, & Krauss, 2010). Furthermore, post-mortem studies have shown that the degree of cholinergic neuronal loss within the PPN in PD patients has a correlation with the level of dopamine cell loss (Zweig et al., 1989) and, importantly, with the occurrence of falls (Karachi et al., 2010).

In summary, the physiopathology of PD gait is certainly complex, and the neurophysiology of gait has not been extensively studied in people with PD, which hinders the targeting of successful treatment strategies. The inclusion of neurophysiological measures in studies related to PD gait can help to increase knowledge about it and, ultimately, to improve the effectiveness of medical and rehabilitation treatments.

1.2.4 Gait rehabilitation in PD

Although pharmacological therapies ameliorate many parkinsonian symptoms, especially in the early stages of the disease, as the disease progresses the effectiveness of pharmacological therapy is diminished, leading to the worsening of gait disorders and the appearance of more complex gait symptoms. Even certain temporal aspects of parkinsonian gait disorder remain therapeutically resistant, both in the short-and long- term (Pötter-Nerger & Volkmann, 2013) with other gait difficulties persisting or

worsening after surgical treatment, such as FOG and postural instability. Therefore, the treatment of gait disorders is crucial in PD patients' rehabilitation programs in order to maximize exercise tolerance, improve the gait pattern, maintain or increase independence regarding mobility, and reduce the risk of falls (Gage & Storey, 2004; Keus et al., 2009; Van der Eijk et al., 2011).

The most widely used form of non-pharmacological therapy for improved gait in PD is physical therapy. In 2000, Morris was the first to describe a theoretical framework supporting the use of physical therapy in PD (Morris, 2000). Morris described specific approaches to improve the performance of functional motor tasks, with emphasis on gait, postural instability and prevention of falls. These strategies incorporate the use of external cues (visual, auditory, or proprioceptive cues) and cognitive strategies, such as attentional strategies, in order to activate alternative pathways in the brain bypassing the defective BG circuitries of PD patients (Morris et al., 1996).

A Cochrane meta-analysis that included 33 randomized controlled trials with a total of 1,518 participants, showed that physical therapy provides short-term benefits in the treatment of PD gait (Tomlinson et al., 2012). The trials included were categorized as follows: general physical therapist, exercise, treadmill, cueing, dance, and martial arts. Significant benefits on gait with clinical relevance after physical therapy intervention compared with a placebo or no intervention were considered in this review for the two- or six-minute walk test, speed, Berg Balance Scale, and Unified Parkinson´s Disease Rating Scale (UPDRS) total. Improvements were also demonstrated for other walking outcomes, such as stride length (Fisher et al., 2008a; Sage & Almeida, 2009; Boehm et al., 2011; Almeida & Bhatt, 2012). Later, Gisbert et al. (2015) published a patient- intervention-comparison-outcome analysis, showing that the results of the Cochrane review could be applied to patients (Gisbert, 2015).

1.3 TREADMILL WALKING IN GAIT REHABILITATION IN PD

The treadmill is a device used for walking or running while staying in the same place. The motorized treadmill began to be employed in the medical field for ergometry studies in the middle of the 20th century (Yu et al., 1951). Nowadays, the treadmill is a familiar device employed in gyms, hospitals and research centers.

In the physiological field the treadmill has been used to explore the neurophysiology of locomotion in mammals. Pioneering studies in spinalized cats showed that these animals could step on the treadmill with their hind-limbs, if their body weight was externally supported (Edgerton et al., 1992). On the basis of these experiments, the same approach was applied to humans with spinal cord injury (Wernig & Müller, 1992). A harness that supported a part of the patient's body weight was used in order for them to maintain an erect posture when they walked on the treadmill. More recently, the use of the treadmill has been introduced for gait rehabilitation in other neurological pathologies such as PD.

1.3.1 Treadmill walking studies in gait rehabilitation in PD

Over the last few decades, the treadmill has become a therapeutic tool for gait rehabilitation in PD. Miyai et al. (2000) conducted the two initial studies to study treadmill training with supported body weight in people with PD, and found improvements in UPDRS, ambulation speed and number of steps (Miyai et al., 2000, 2002). Later, their findings were replicated and extended. Several weeks of treadmill training without body weight support led to improvements in some lower limb tasks, such as walking along a corridor (Kurtais et al., 2008), and also in specific gait parameters of PD patients, such as enlarged stride length, increased speed, decreased double support time, and reduced swing time variability. In 2010, Mehrholz et al. (2010) conducted a meta-analysis that included 8 trials with a total of 203 participants. The review provided evidence of the use of treadmill training in

patients with PD in order to improve gait parameters that included speed and stride length (Mehrholz et al., 2010).

One important feature of treadmill training programs is the reported long-lasting effects (Miyai et al., 2002; Toole et al., 2005; Herman et al., 2007; Bello, 2013; Nadeau, 2014). After 4 weeks of treadmill training, improvements in balance, gait, range of motion and motor UPDRS were maintained for one month (Toole et al., 2005). Recently, improvements in speed, cadence and stride length have been reported that persisted until 6 months after the treadmill training (Nadeau et al., 2014).

In addition to the long-term effects, the immediate effects of one treadmill session have also been investigated. Gait speed, stride length and double stance improved immediately after one session of treadmill walking (Miyai et al., 2000; Pohl et al., 2003). Improvements in gait speed were also found 10 minutes after a treadmill gait session, where advanced PD patients increased their gait speed as a result of an improvement in stride length (Bello et al., 2008).

In order to discover the effect of the treadmill in gait rehabilitation in PD patients, it is necessary to determine the differences between treadmill walking and overground walking. When walking on the treadmill, a group of advanced PD patients (H&Y 3) improved their step length while walking in comparison with overground walking (Bello et al., 2008). Moreover, PD patients increased their step length, but no other gait parameters, after treadmill training in comparison with overground training (Bello, 2013). The immediate effects of one treadmill session in comparison with one session of overground walking have not yet been investigated. This is of importance in order to determine whether the treadmill has a specific therapeutic effect in PD.

1.3.2 Mechanisms implicated in treadmill gait benefits in PD

An understanding of the mechanisms underlying the gait improvements associated with the treadmill will improve the prescription and efficacy of physical therapy in PD. Several mechanisms have been proposed (Bello & Fernández-Del-Olmo, 2012). Some of them are related to the different contexts in which the walking took place, such as the external cues provided by the treadmill and the attentional strategies used by PD patients; and others are related to the features of the treadmill itself, such as constant speed or the use of handrails (see Table 4).

Table 4 Summary of the main mechanisms implicated in treadmill gait benefits in PD (Adapted from Bello et al., 2012).

Mechanism	Arguments to Support this Theory	Arguments to Question this Theory
Central Pattern Generator	Treadmill training could provide adequate sensory inputs, which may stimulate the spinal locomotor circuitry	It is undetermined whether CPG is relevant in the gait improvement of PD patients
External sensory cues	Treadmill walking provides propioceptive signals that may be used by PD patients, triggering intact circuits and by- passing the defective pallidocortical circuit, in order to control movement	Changes in regional flow evaluated with SPECT during treadmill walking, showed absence of the lateral premotor cortex-parietal overactivation that seems to accompany externally triggered movements in PD patients, indicating that treadmill-walking has an internally driven mechanism
Visual feedback	Modifications in the optical flow lead to modification in the gait pattern. Absence of visual flow may force the subject to look for another source of visual information, as an anchor for maintaining a stable position	Only the absence of visual flow do not increase the step length in PD patients

Attentional strategies	PD patients can use attentional strategies and compensate for the damaged automaticity	
Imposed and constant gait speed	The constant speed of the treadmill could reduce the de- grees of freedom and help to minimize stride-to-stride variations in gait timing	
Hand support	Walking aids that have hand support, can improve balance and mobility in older adults and people with other clinical conditions	PD patients reduce their speed and stride length when they walk with a wheeled walker in comparison with normal gait.
Motor learning	Improvement in gait is sustained several months after the treadmill training is completed.	

The visual cues and the handrail support are two of the possible explanations that have already been rejected. A recent study that used a treadmill simulator built by extracting the belt showed that visual feedback was not the main mechanism involved in the step length increase in PD patients during treadmill walking (Bello et al., 2010). Moreover, the fact that the patients held their arms on the handrail of the treadmill could improve their balance and may account for the adaptation of the step length observed over the treadmill (Bateni & Maki, 2005). However, two studies have shown that PD patients reduce their speed and stride length when they walk with a wheeled walker in comparison to normal gait (Frenkel-Toledo et al., 2005; Bello et al., 2010).

One of the most plausible explanations so far is related to belt movement and proprioceptive signals. It is well known that PD patients can generate a normal gait pattern in the presence of adequate regulatory sensory stimulation. Lines placed on the floor at the desired step length, or rhythmic auditory cues, can assist the initiation and execution of gait in PD. These cues

would bypass the defective internal pallidocortical projections in PD, activating compensatory cortical pathways, possibly via the lateral premotor cortex, which controls externally guided movements (Hanakawa et al., 1999a; Fukuyama et al., 2004). Frenkel-Toledo et al. (2005) suggested that the treadmill provides external cues to reduce gait variability (Frenkel-Toledo et al., 2005). Moreover, a recent study confirmed that improvements in stride length in PD are due to the belt movement itself, probably due to proprioceptive afferents generated by belt displacement (Bello et al., 2010). Thus, treadmill walking can provide proprioceptive signals that could bypass the defective pallidocortical circuit in PD.

In this way, the proprioceptive afferents generated by the belt movement could provide suitable sensory inputs for the stimulation of the CPGs, i.e., the spinal locomotor circuitry (see 1.2.3) (Protas et al., 2005; Herman et al., 2007; Fisher, 2008).The positive effect of the treadmill in humans with a spinal lesion has been attributed to the activation of the CPGs (Dietz, 2003; Shepherd, 1999). Thus, treadmill walking would stimulate the spinal locomotor circuitry in PD. Another explanation for the treadmill improvements is related to the assumption that treadmill gait can induce motor learning in PD (Protas et al., 2005; Herman et al., 2007; Fisher, 2008). This could explain why the improvement in gait is sustained several months after the treadmill training is completed (Herman et al., 2007). The motor learning induced by treadmill training can also be mediated by CPGs, since learning occurs at the spinal level in PD. Another possibility is that the neural changes may occur at cortical level. A recent study reported normalization of corticomotor excitability after treadmill walking exercise at high-intensity, as well gait improvements in early PD (Fisher et al., 2008).

Besides belt movement and proprioceptive signals, attentional resources have also been proposed as a mechanism involved in the gait improvements associated with treadmill use in PD (Bello & Fernández-Del-Olmo, 2012). Although regulation of gait pattern is an automatic process that does not require attention in healthy adults, people with PD are able to improve their gait when they direct their attention to their walking movement, suggesting an impairment in the automatic control of their gait (Morris et al., 1996). Thus, it is plausible that the stable environment and absence of distracters

associated with walking over the treadmill could allow PD patients to allocate attention to their gait in comparison with an overground walking condition, and consequentially, bypass the defective pallidocortical circuit in PD.

Having reported the several hypotheses underlying the improvements associated with the use of a treadmill in PD, it must be emphasized that there are no studies that have explored the role of attention as an underlying mechanism, notwithstanding the current association between attention and gait in PD. In the same way, there are no studies that investigate the possible neural mechanisms involved in treadmill gait benefits in PD.

1.4 TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) IN GAIT REHABILITATION IN PD

1.4.1 Basis of application of tDCS in PD

TDCS is a model of NIBS that could enhance or reduce cerebral excitability by the use of low direct current delivered via two surface electrodes on the head, anode and cathode. Although the scalp possesses high impedance, sufficient intracranial current flows to produce changes in membrane resting thresholds within the cortex beneath each electrode. This results in an increase in activity under the anode and a decrease in activity beneath the cathode (Nitsche et al., 2005).

TDCS has the capability to promote motor learning and consolidation, and may enhance long-term retention in healthy subjects (Nitsche et al., 2003). This provides the rationale for combining tDCS with a rehabilitative intervention, and has been shown to promote motor recovery in chronic stroke (Hummel et al., 2005). In recent years, there has been increased interest in tDCS as an intervention in PD.

In PD, cortical excitability is increased during rest and decreased during voluntary activity which corresponds to reduced facilitation (Cantello et al., 1991; Valls-Solé et al., 1994; Cantello et al., 2002). Impaired facilitation likely results from a deficient thalamo-cortical drive, while the increased activity during rest may be compensatory (Berardelli et al., 1996). The first placebo-controlled study that investigated the effects of a single tDCS session in PD reported that anodal tDCS of the primary motor cortex (M1) results in a motor function enhancement, along with a restoration of the reduced activity in motor and prefrontal cortices (Fregni et al., 2006).

The physiological effects of anodal tDCS in PD have not been extensively investigated. RTMS release dopamine in the caudate and putamen corresponding to their cortico-striatal projections and could contribute to the acute effects of transcranial stimulation. Anodal tDCS causes widespread activation (Lang et al., 2005) that may trigger similar effects.

In view of the above, the use of tDCS in PD offers a promising rationale for improving the efficiency of rehabilitative interventions, as well as possibly enhancing our understanding of PD pathophysiology.

1.4.2 tDCS studies in gait function in PD

he rationale behind the application of tDCS on gait in PD comes from the DBS of the PPN (Plaha & Gill, 2005; Stefani et al., 2007), which has been reported to improve gait disturbances refractory to conventional therapy. In addition, tDCS has the capability to modulate spinal reflexes such as reciprocal Ia-inhibition in healthy people, demonstrating that the effects of the low direct current at the cortex reach spinal levels.

Several studies have evaluated the effect of anodal tDCS on gait function in PD. TDCS has been suggested by some to improve gait, while others have found no benefit in PD. Several sessions of anodal tDCS delivered over the M1 and the premotor area improved upper-extremity bradykinesia and, to a lesser extent, walking time (Benninger et al., 2010). In a cross-over RCT, 5 sessions of anodal tDCS of M1 in 10 patients had a beneficial effect on gait, FOG and motor performance, and these effects lasted throughout the observation period of 1 month (Valentino et al., 2014). However, a single session of anodal tDCS over M1 did not induce improvements on gait function measured with the 10-m walking test (Verheyden et al., 2013).

It has also recently been shown that anodal tDCS, combined with physical training, increased gait velocity and improved balance in PD more than isolated physical training or tDCS alone, which did not produce motor improvements (Kaski, et al., 2014).

Another possibility that has not been studied is the combination of treadmill walking and tDCS. This combined therapy could increase the effects of treadmill walking or tDCS alone on gait function in PD.

CHAPTER 2.

QUESTIONS OF RELEVANCE

After a brief review of the current data related to the topic of this thesis, some questions of interest remain unresolved:

1) Are attentional demands a reliable underlying mechanism related to the gait improvements associated to treadmill walking in PD?

2) What is the cognitive cost of treadmill walking and overground walking under dual-task conditions in PD?

3) Could one session of treadmill walking lead to gait improvements in PD patients in comparison with one session of overground walking?

4) What are the neural mechanisms that underlie gait improvements related to treadmill walking in individuals with PD?

5) What are the effects of combining tDCS and treadmill walking in gait function in PD subjects?

6) What are the effects of combining tDCS and treadmill walking in the neurophysiological function of PD subjects?

This piece of research is an attempt to address these questions. Questions 1 and 2 will be addressed in the first study. Question 3 and 4 will be addressed in the second study. Questions 5 and 6 will be addressed in the third study.

CHAPTER 3.

HYPOTHESIS AND MAIN AIMS OF THE STUDIES

3.1 Study I: Gait Pattern and Cognitive Performance During Treadmill Walking in Parkinson's Disease

3.1.1 Hypothesis

Performance of a cognitive task during treadmill walking will lead to an impairment in one or both of the tasks in PD subjects.

3.1.2 Aims

- To explore whether attentional demands are involved in gait improvements in PD patients when they walk on a treadmill.
- To investigate the gait pattern in PD subjects and controls when walking over the treadmill and when walking overground during the performance of a concurrent cognitive task.
- To explore the cognitive performance in PD subjects and controls when walking over the treadmill and when walking overground during the performance of a concurrent cognitive task.

3.2 Study II: Acute kinematic and neurophysiological effects of treadmill and overground walking in Parkinson´s disease

3.2.1 Hypothesis

A single treadmill walking session, unlike a single overground walking session, would lead to gait improvements in PD, accompanied by walking-related neurophysiological modulations.

3.2.2 Aims

- To explore the short-term effects of a single session of either treadmill or overground walking on kinematics in PD patients.
- To address the immediate effects of a single session of either treadmill or overground walking on the excitatory and inhibitory cortical networks in PD patients.
- To address the immediate effects of a single session of either treadmill or overground walking on spinal excitability in PD patients.

3.3 Study III: Treadmill walking combined with anodal tDCS in Parkinson's Disease: kinematic and neurophysiological effects

3.3.1 Hypothesis

The combination of treadmill walking with tDCS enhances the gait improvements associated with treadmill walking in PD subjects.

3.3.2 Aims

- To explore the acute effect of treadmill walking combined with anodal tDCS on gait function both on overground and treadmill walking in individuals with PD.
- To investigate the possible spinal and cortical neural mechanisms involved in the effects of this combined therapy in PD individuals.

CHAPTER 4.

STUDIES

4.1 STUDY I: Gait Pattern and Cognitive Performance During Treadmill Walking in Parkinson's Disease

4.1.1 Abstract

The aim of this study was to explore whether attentional demands are involved in gait improvements in PD patients when they walk on a treadmill. Nineteen individuals with idiopathic PD and 19 age-matched healthy controls participated in this study. Participants walked on a treadmill and on overground under single task (walk only) and DT (walk performing a simultaneous cognitive task) conditions. The DT paradigm was used to reveal the attention allocation behaviour. Gait pattern and cognitive performance was measured. The PD group showed reduced gait variability when walking on a treadmill in comparison with overground. However, this reduction did not deteriorate during the DT. Moreover, there were no differences in the cognitive performance between treadmill and overground walking. This study does not support the proposition attentional resource allocation as a possible mechanism for the treadmill-associated gait improvements observed in PD.

4.1.2 Introduction

Gait disorders are among the most significant impairments in PD that severely affect the individual's QoL (Keus et al., 2009). PD gait is characterized by a reduced gait speed, increased stride-to-stride variability and a specific difficulty to regulate stride length (Keus et al., 2009). In addition, people with PD suffer a deficit in executive functions and attention abilities, (see Dirbenger and Jahanshahi (2013), for an extensive review of executive deficits in PD; and Yogev-Seligmann et al. (2008), for a review of the role of executive function and attention in gait) (Yogev-Seligmann et al., 2008; Dirnberger & Jahanshahi, 2013). These cognitive dysfunctions could explain the exacerbated gait impairments in PD subjects (i.e. reduced walking speed and stride length, and increased stride-to-stride variability) when they perform a concurrent task (O'Shea et al., 2002; Rochester et al., 2004; Yogev et al., 2005). Previous studies have examined the relationship between cognition and gait performance in people with PD using a secondary task during walking (DT paradigm) (Yogev et al., 2005; Yogev-Seligmann et al., 2012; Kelly et al., 2012; Brauer & Morris, 2010; Chawla et al., 2015). DT is a component of executive function, which refers to the ability to allocate attention to tasks that are performed at the same time (Ivanoff, 2005). Although, several theories have been proposed to explain DT interference, all of them agree that when two tasks are performed concurrently, performance decrements are observed in one or both tasks (Marois et al., 2005; Kelly et al., 2012). For instance, and according to the capacity sharing model (O'Shea et al., 2002; Herman et al., 2007), when two attentionally demanding tasks are performed simultaneously, competition of attentional resources results in the deterioration of performance in one or both of the tasks. Therefore, it is important to explore the role of attention associated with therapeutic strategies to improve the gait in subjects with PD.

In the last decade, several studies have shown the therapeutic use of treadmill training for gait rehabilitation in PD (Mehrholz et al., 2010). Several weeks of treadmill training lead to an improvements in several gait parameters such as enlarged stride length, increased speed, decreased double support, reduced swing time variability and reduced stride length

variability between others (Herman et al., 2007; Bello, 2013; Nadeau et al. 2014; Tseng et al. 2015). Notably, subjects with PD increased their stride length after 5 weeks of treadmill training but not when the training consisted of walking overground (Bello, 2013). A recent study showed improvements in walking speed and walking endurance even 6 month after the treadmill training (Nadeau et al., 2014). In addition to these long term effects of treadmill training, immediate improvements on overground gait, such as faster speed and longer step length, have been reported after a single treadmill session in PD (Bello et al., 2008).

The therapeutic effect of treadmill could be explained as a specific gait modulation in PD subjects when walking over this device. PD subjects walk on a treadmill with lower stride-to-stride variability (Frenkel-Toledo et al., 2005) and higher length step in comparison with walking overground (Bello et al., 2008). However, the mechanisms underlying these positive treadmill effects in PD remain unknown, even though several theories have been postulated (Bello et al., 2010; Bello & Fernández-Del-Olmo, 2012). One of these theories refers to attentional resources. Although, regulation of gait pattern is an automatic process that does not require attention in healthy adults (Yogev et al., 2005), people with PD are able to improve their gait when they direct their attention to their walking movement (Morris et al., 1996; Canning, 2005) suggesting an impairment in the automatic control of the gait. Thus, it is plausible that the stable environment and absence of distracters associated with walking over the treadmill allow PD patients to allocate attention to their gait in comparison with an overground walking condition. However, to the best of our knowledge there are no studies that have explored the role of attention during treadmill walking in subjects with PD.

Therefore, the main goal of this study was to investigate whether attention could be an underlying mechanism for the gait improvements observed during treadmill walking in PD. To this end, and using a DT paradigm, the present work compared the gait pattern in PD and controls, when walking over the treadmill and when walking overground during the performance of a concurrent cognitive task. The proposed hypothesis was that if the gait improvements observed while walking over a treadmill depend on attentional

resources, performance of a cognitive task will lead to impairment in one or both of the tasks.

4.1.3 Material and methods

Subjects

Nineteen individuals diagnosed with idiopathic PD by a neurologist, according to the United Kingdom Bank Criteria (Hughes et al., 1992) (11 males and 8 females, mean age = 59.79 ± 12.63), and nineteen age-matched healthy controls (11 males and 8 females, mean age = 59.53 ± 12.1) were recruited for the study from a local community. Inclusion criteria for participants with PD was: diagnosis of idiopathic PD, the ability to walk for 10 min without stopping or walking assistance, absence of neurologic disorders other than PD and absence of orthopedic, cardiovascular or visual disturbances that could affect gait. Healthy controls were included if they did not have history of neurological pathology or other disease that could affect the ability to walk. All participants did not use a treadmill for at least 12 months prior to the experiment. No participant showed dementia as assessed by a mini-mental state examination (MMSE). The level of severity of motor signs associated with PD was measured in ON state (45 minutes – 1.5 hours after medication intake) using the Unified Parkinson´s Disease Rating Scale Part-III (Fahn et al.*; 1987*) (UPDRS-III) and Hoehn and Yahr scale (Hoehn MM, 1967) (H&Y). A neurologist confirmed the ON state. All participants gave their written informed consent according to the Declaration of Helsinki (1964), before entering the study. The experimental procedures were approved by the local ethics committee.

Apparatus

A treadmill with handrails (SportsArt 6300, Sports Arts Fitness) was used. Gait performance overground and on the treadmill was recorded using an optical detection system (Optogait, Microgait, USA). This optical and modular system included transmitting and receiving bars of infrared LEDs. The apparatus detected the interruptions of the communication between the bars

during walking. Wireless headphones (Philips Hi-Fi, SHD8600UG) were used for the cognitive task.

Testing procedure

All participants were tested in four conditions: overground single task (ST: walk only), overground dual task (DT: walk and perform a cognitive task), treadmill ST and treadmill DT. Single and dual task conditions were arranged in a random order. However, overground and treadmill conditions were not counterbalanced since it has been reported that treadmill walking has significant and long lasting effects on the speed and frequency of consequent overground walking(Bello et al., 2008). The experimental sessions were carried out while patients were ON medication, confirmed by a neurologist evaluating the motor items of the UPRDS scale (Figure 3).

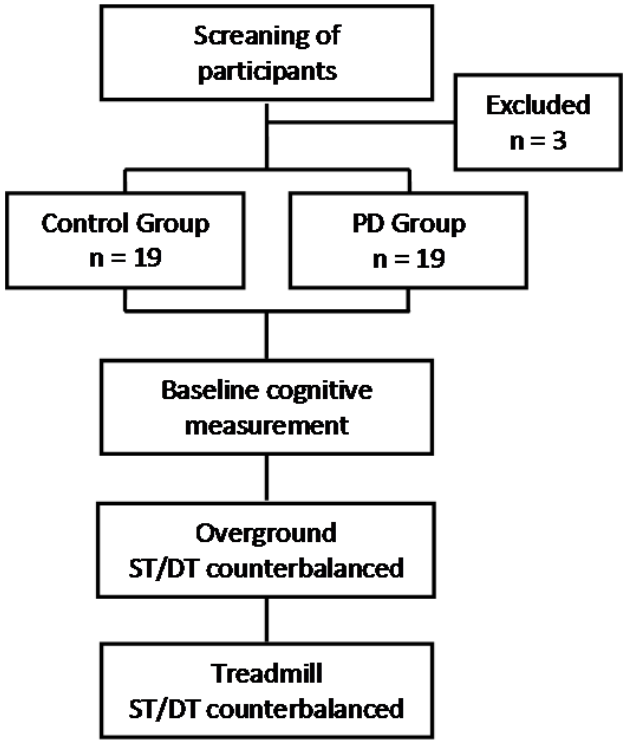


Figure 3 Flow chart of Study I.

The cognitive task consisted of a phoneme monitoring paradigm (Connine, 1996). The phoneme monitoring task has been previously used in several studies of PD and gait (Springer et al., 2006; Bello, 2013; Wild et al., 2013). Participants were asked to listen to a 1-minute-long narration through wireless headphones and they had to count the number of times that two pre-specified words were repeated in the narration. At the end of the task, participants reported the number of times the words were repeated in the text. They were not allowed to use their voice or fingers to help in the count. The subjects were not given any instructions or information regarding prioritization of the tasks. Baseline measurement of the cognitive task (without walking) was performed in a sitting position at the beginning of the session. A total number of fourteen texts were used, that were arranged in a randomized order for the different cognitive tests (baseline measurement, overground DT and treadmill DT). The two pre-specified words differed for each text. For the DT condition, the subjects were not given any instructions or information regarding prioritization of the tasks.

The overground walking tests started with a practical trial of 3 minutes to familiarize the subjects with the walkway and with them self-select comfortable speed. Participants were tested at their self-selected comfortable speed, up and down an 8 meters walkway, for a total time of 1 minute. The gait parameters were recorded during the straight walk but not during the turns. Thus, each overground condition (ST or DT) was repeated twice to obtain the same number of steps to that in the treadmill conditions. The only instruction that participants received was to walk at their preferred speed.

The treadmill walking tests started with a period of familiarization of 3 minutes to reach, on the treadmill, the gait speed previously established during the overground condition (ST). During the familiarization period, all participants were instructed to keep their steps close to the front of the treadmill to keep their body erected. The treadmill tests consisted of two new blocks of 3-minutes for ST and DT conditions. During the first minute, belt speed was increased to reach the overground speed. The measurements were taken in the second minute of each block (ST and DT). All participants walked on the treadmill holding the handrails (Bello, 2013; Bello et al., 2010) since some subjects, specifically individuals with PD, did not feel safe to walk

without the handrail support. Participants walked on the treadmill under the close supervision of a physical therapist. To minimize fatigue effects, rest periods of 3 minutes between treadmill blocks and over-ground measures were included.

Data analysis

The following variables of gait were evaluated: overground speed (m/s), stride length (m), stride frequency (Hz), coefficient of variation (CV) of stride length (%) and CV of stride frequency (%). Coefficients of variation were calculated using the following formulas: (standard deviation/ stride length) x100; (standard deviation/stride frequency) x100. The outcomes of gait measurements were recorded during the tests with Software OptoGait v.1.9.9.0, (Microgait, USA) and were exported to excel format for offline analysis.

The variable of the cognitive task was called “cognitive performance”. The cognitive performance was evaluated by calculating the percentage of mistakes made when counting the two pre-specified words.

Statistical analysis

To determine group and gait differences, a *t-test* was performed using the demographic and anthropometric measures. A repeated-measures analysis of variance (ANOVA) was carried out to compare the changes in gait in different conditions. Two-way ANOVA was used for gait speed (analysed only overground), with “task” as the within subject factor and “group” as the between subject factor. For the remaining variables of gait, three-way ANOVA was used with “surface” (overground and treadmill) and “task” (single task and dual task) as within-subject´s factors, and “group” (PD and control) as between subject´s factor. Post Hoc t-tests were computed. Wilcoxon rank test was applied for each group to compare cognitive performance between baseline, over-ground and treadmill conditions. Mann-Whitney test was used to compare cognitive performance between PD patients and controls for baseline, overground and treadmill conditions. All statistical analysis was performed using PASW Statistics 18. With the exception of percentage of

mistakes, none of the data violated the normality assumption according to Shapiro-Wilk test. A *P* value ≤ 0, 05 was considered statistically significant.

4.1.4 Results

T-tests showed no significant differences for age, leg length, weight and height between PD and control groups (Table 5).

Table 5. Characteristics of PD and control groups.

	PD	CONTROL	Group Differences
Age (years)	59.68 ± 12.81	59.53 ± 12.10	NS
Weight (Kg)	79.91 ± 14.66	73.63 ± 8.6	NS
Leg length[a] (m)	87.37 ± 7.22	85.64 ± 6.97	NS
Height (m)	1.65 ± 0.072	1.65 ± 0.08	NS
MMSE	29.38 ± 1.02	29.52 ± 0.89	NS
Disease duration (years)	5.08 ± 3.83	__	__
UPDRS-III	21.06 ± 10.69	__	__
H&Y stage (subjects in each stage)	1 (n= 4); 1.5 (n=3); 2 (n=6); 2.5 (n= 6)	__	__

Values are mean ± SD. *t -test* analysis for group differences. [a]Distance from the great trocanter to the floor. PD, Parkinson´s Disease; MMSE, Mini-Mental State Examination; H&Y, Hoen and Yahr; UPDRS-III, Unified Parkinson´s Disease Rating Scale motor section; NS, Not Significant; n; sample.

Two-way ANOVA for gait speed showed a significant main effect for group and task factors (Table 6B), with higher overground speed in control participants compared with PD patients and also during ST compared with DT. No significant task*group interaction was found.

Three-way ANOVA for stride frequency showed a significant main effect for group factor (Table 6B). The control group walked with a higher stride frequency than the PD group.

Three-way ANOVA for stride length revealed a significant surface*task interaction (Table 6B). The Post Hoc analysis showed that for the ST condition, there were no significant differences in stride length between surfaces. However, the stride length was shorter for DT than for ST, while walking overground ($F = 44.30$; $P < 0.001$), but there were no differences between DT and ST conditions during treadmill walking. On the treadmill, stride length for the DT condition was larger than for overground walking ($F = 24.26$; $P < 0.001$).

Table 6 Means, standard deviations and ANOVA results of gait variables.

A. Gait variables in PD and control groups

	PD				CONTROL			
	Overground		Treadmill		Overground		Treadmill	
	ST	DT	ST	DT	ST	DT	ST	DT
Gait speed (m/s)	1.07 ± 0.23	1.01 ± 0.24	1.07 ± 0.23	1.07 ± 0.23	1.33 ± 0.20	1.25 ± 0.22	1.33 ± 0.20	1.33 ± 0.20
Stride length (m)	1.18 ± 0.20	1.13 ± 0.20	1.20 ± 0.23	1.19 ± 0.24	1.35 ± 0.17	1.31 ± 0.17	1.34 ± 0.18	1.36 ± 0.16
Stride frequency (Hz)	0.90 ± 0.07	0.93 ± 0.15	0.90 ± 0.07	0.93 ± 0.09	0.98 ± 0.07	0.95 ± 0.09	1.00 ± 0.09	0.98 ± 0.08
CV of stride length (%)	4.27 ± 2.52	4.63 ± 2.44	1.73 ± 0.93	1.54 ± 0.76	2.65 ± 1.00	3.35 ± 1.41	1.56 ± 0.84	1.56 ± 0.83
CV of stride frequency (%)	2.60 ± 0.80	2.96 ± 0.94	1.90 ± 1.12	1.88 ± 1.07	1.84 ± 0.53	2.37 ± 0.91	2.91 ± 1.97	2.80 ± 2.01

Values are mean ± SD. PD, Parkinson´s disease; ST, single task; DT, dual task; CV, coefficient of variation.

B. ANOVA results of gait variables

	Surface	Task	Group	Surface× Task	Surface× Group	Task× Group	Surface× Task× Group
Gait speed (m/s)	––	F=46.76 P<0.001	F=12.03 P=0.001	––	––	NS	NS
Stride length (m)	F=8.00 P=0.008	F=21.32 P<0.001	F=6.73 P=0.01	F= 29.77 P<0.001	NS	NS	NS
Stride frequency (Hz)	NS	NS	F=7.37 P=0.01	NS	NS	NS	NS
CV of stride length (%)	F=73.35 P<0.001	NS	F=4.11 P=0.05	F=5.72 P=0.02	F=7.67 P=0.009	NS	NS
CV of stride frequency (%)	NS	NS	NS	F=4.65 P=0.04	F=11.71 P=0.002	NS	NS

CV, coefficient of variation; NS, Not significant.

The analysis of the CV of stride frequency showed significant surface*group and surface*task interactions (Table 6B) (Figure 4). Therefore, there were no specific group effects of the task in this parameter. The Post Hoc analysis of the surface*group interaction showed that overground PD patients walked with a higher CV of stride frequency than control subjects ($F = 8.67$; $P = 0.006$). However, PD patients showed a lower CV than control subjects while walking on the treadmill ($F = 4.10$; $P = 0.050$) due to a decrease of the CV values in the PD group ($F = 6.93$; $P = 0.01$) and an increase of CV in control subjects when walking on the treadmill in comparison with walking overground ($F = 4.87$; $P = 0.03$). The Post-Hoc analysis of the surface*task interaction indicated that the CV of stride frequency was higher during DT than during ST in the overground condition ($F = 11.39$; $P = 0.002$) but not in the treadmill condition.

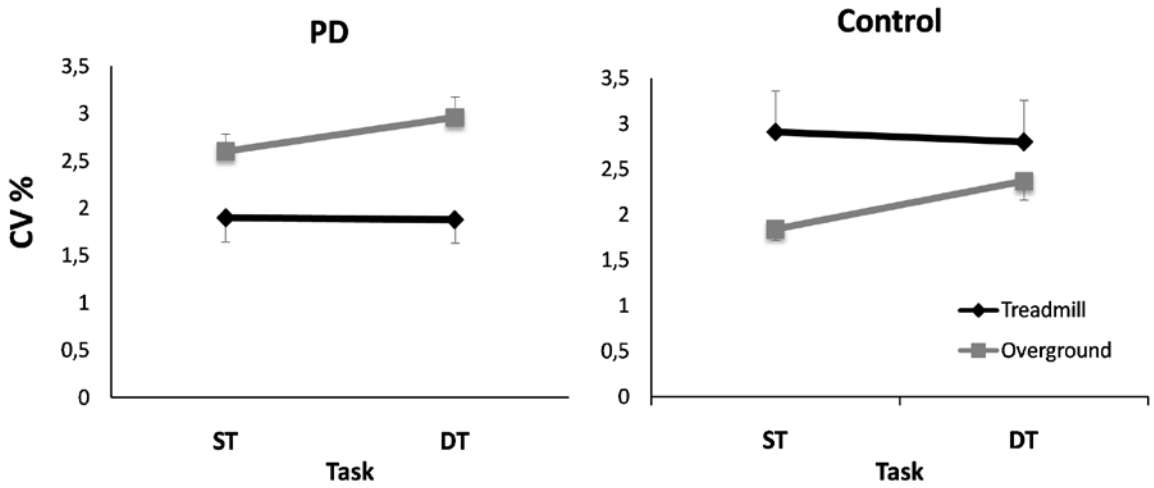


Figure 4 Comparison of the coefficient of variation of stride frequency between single and dual task, in PD and control groups.

PD indicates Parkinson disease; CV, coefficient of variation; ST, single task; DT, dual task.

ANOVA showed significant surface*group and surface*task interactions (Table 6B) for CV of stride length (Figure 5). These results indicated there were no specific group effects of the task on the CV of stride length. The Post Hoc analysis of the surface*group interaction showed that in overground condition PD subjects walked with a higher CV of stride length compared with

control participants (F = 6.21; P = 0.017). However, these differences were not significant on the treadmill. This was due to a significant reduction of the CV, in both PD and control subjects, for treadmill versus overground walking (F = 64.23; P < 0.001 and F = 16.79; P < 0.001). The Post Hoc analysis of the surface*task interaction showed that the CV of stride length was higher for DT compared with ST while walking overground (F = 4.39; P = 0.04), but there were no significant differences between the tasks on the treadmill. For treadmill walking the CV was significantly lower than for overground walking for both the ST and DT condition (F = 48.20; P < 0.001 and F = 66.45; P < 0.001, respectively).

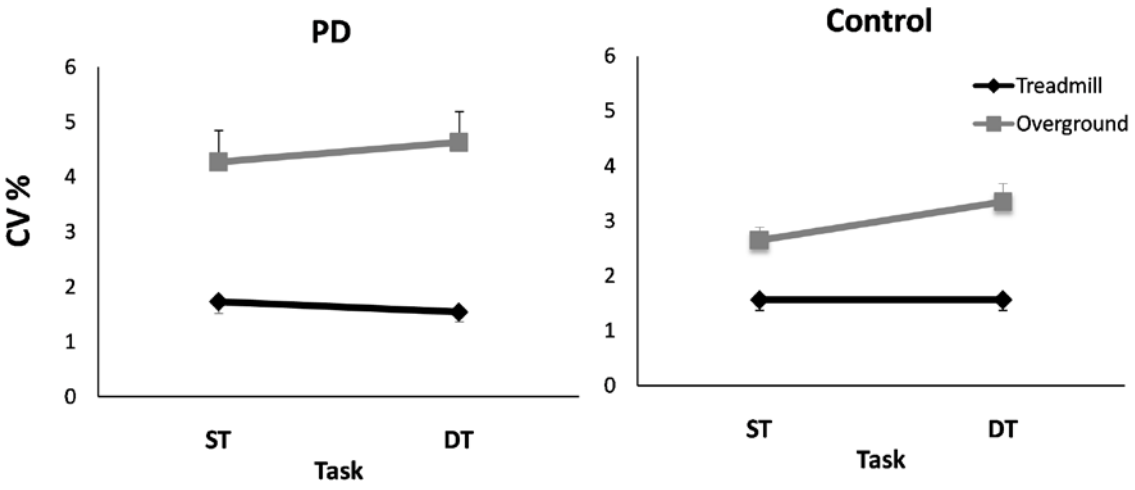


Figure 5 Comparison of the coefficient of variation of stride length between single and dual task, in PD and control groups.

PD indicates Parkinson disease; CV, coefficient of variation; ST, single task; DT, dual task.

The Wilcoxon test in the control group did not show differences on cognitive performance between baseline, overground and treadmill conditions. However, the PD group showed significant differences between baseline and treadmill (Z = 2.550; P = 0.011). PD patients had a greater percentage of mistakes during treadmill walking compared with baseline. No differences were found between baseline and overground (Z = 1.221; P = 0.222) or between overground and treadmill walking (Z = 0.101; P = 0.92) in the PD group. Mann-Whitney tests did not show cognitive differences between

groups in the three task conditions. However, there was a tendency for a higher percentage of mistakes in PD participants in comparison with controls during overground (Z = 1.929; P=0.054) and treadmill (Z =1.875; P = 0.061) walking (Figure 6).

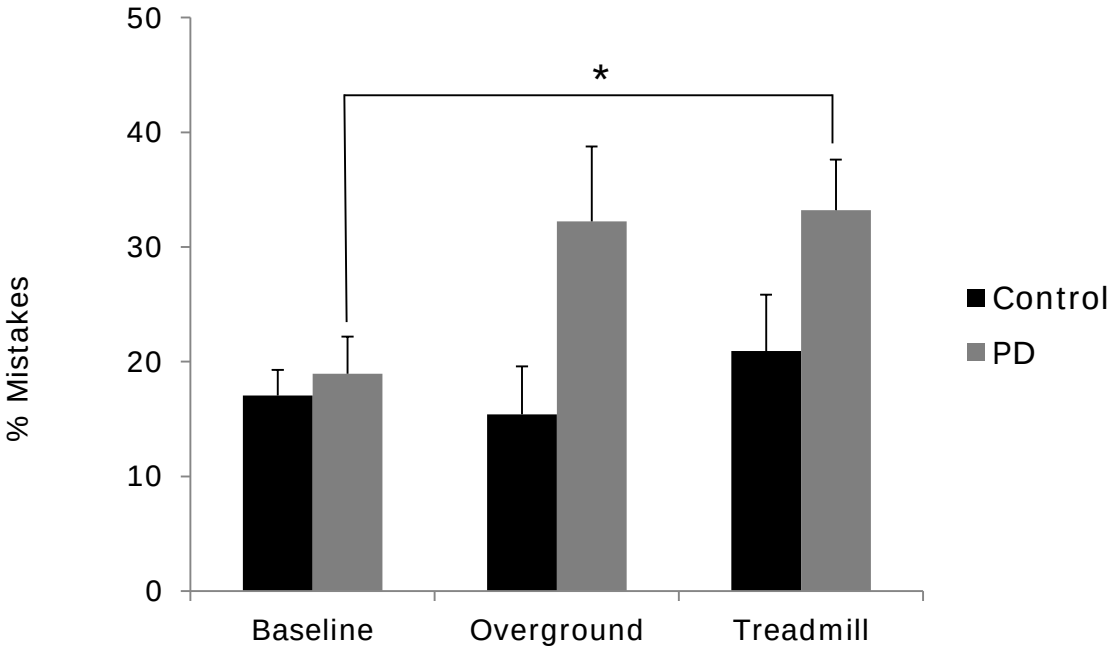


Figure 6 Comparison of cognitive performance between control and PD subjects for baseline, overground, and treadmill conditions.

4.1.5 Discussion

The main goal of this study was to explore whether attentional resources may explain the gait improvements observed during treadmill walking in PD. The results show that during treadmill walking with and without the cognitive tasks, individuals with PD reduced their gait variability. These gait improvements did not deteriorate during the performance of a concurrent cognitive task. Moreover, cognitive performance did not show statistical differences between treadmill and overground walking. Therefore, these findings suggest that improvements of gait in PD patients during treadmill walking are independent of attentional demands to the task of walking.

Differences in gait parameters between surfaces

The results showed that the PD group demonstrated a shorter stride length, lower velocity and higher gait variability compared with the control group while walking overground. However, the PD group reduced the CV of stride frequency and the CV of stride length when walking on the treadmill. The reduction of the stride frequency variability during treadmill walking has been previously reported in PD (Frenkel-Toledo et al., 2005). However, to our knowledge, the current study is the first to report a reduction in the variability of stride length. This is of relevance since a high stride to stride variability was associated with an increased risk of falls in PD (Schaafsma, 2003; Hausdorff et al., 2007). Therefore, the present investigation extends the benefits of this device, suggesting that walking on the treadmill could normalize the temporal variability as well as spatial gait variability in PD.

Differences in gait between tasks

The present data clearly demonstrate that during the performance of a secondary cognitive task all the participants displayed a decrease in the length of the step and speed together with an increase in the spatial and temporal variability of overground gait. This is in line with previous studies that showed a deterioration of gait disturbances in people with PD when they walk and perform a secondary task at the same time (Canning, 2005; Springer et al., 2006; Yogev-Seligmann et al., 2008; Bello et al., 2010). Other studies have also shown alterations of the gait pattern in healthy older adults while simultaneously performing a cognitive task, suggesting an age-related reduction in dual task capacity (Morris et al., 1996; Rochester et al., 2014). Moreover, there was a non-significant tendency, in PD participants, to perform the cognitive task during walking worse than control subjects. This tendency is in agreement with previous studies showing that PD individuals require more attention to regulate the gait pattern in order to compensate their damaged automaticity (Morris et al., 1996). Therefore, this report reinforces the hypothesis that walking during the performance of a cognitive task requires greater levels of cognitive function in PD patients (Plotniket al., 2011).

The cognitive task did not affect any gait parameters during treadmill walking, in PD and control subjects. Overall, the gait variability reduction in PD participants, as a result of walking on the treadmill, did not deteriorate during the performance of the cognitive task. Therefore, the present study suggests that the adaptive changes of the gait pattern while walking on a treadmill are not related to increased attentional demands in comparison with walking overground.

In the present work PD patients showed deterioration in their cognitive performance during treadmill walking in comparison with performing a cognitive task in a static position (baseline cognitive measurement). Previous studies showed that when the environment becomes more demanding, focusing on the cognitive task becomes too risky and the attention is allocated toward postural stability in order to reduce the likelihood of falling and secure safety (Yogev-Seligmann et al., 2012). Although, the handrail support during treadmill walking could improve the postural stability, it is likely that this effect was counteracted by the balance instability induced by the belt movement. This study suggests that, despite the handrail support, DT on a treadmill could be more demanding for PD participants compared with performing a cognitive task in a rest position without a simultaneous motor task.

Mechanisms of treadmill walking in PD

This article provides additional data in order to elucidate the role of attentional resource allocation in the treadmill associated gait improvements observed in patients with PD.

One of the hypotheses to explain the gait improvements in PD subjects is that they are able to allocate more attention to walking on a treadmill than walking overground (Bello & Fernández-Del-Olmo, 2012). Previous studies showed that people with PD can normalize gait pattern when focusing attention on gait (Morris et al., 1996). However, during the performance of daily activities, distracters impede attention allocation with the consequent gait deterioration (Rochester et al., 2004). During treadmill walking, the environment is more stable and the absence of distracters could help PD

subjects to maintain their focus on the gait. The present study does not support this hypothesis. The results reported that CV of stride frequency was reduced during treadmill walking in both ST and DT conditions (Fig. 4). Moreover, an absence of differences in cognitive performance between treadmill and overground walking in PD was observed. Therefore, these findings suggest that attentional demands do not contribute to the gait improvements in PD.

Alternative explanations for the gait improvements are the belt displacement and the constant speed provided by the treadmill (Frenkel-Toledo et al., 2005; Bello et al., 2010; Bello & Fernández-Del-Olmo, 2012). The belt displacement enhances the hip extension movements during treadmill walking. These proprioceptive inputs (repeated contraction and relaxation of muscles groups) may act as an external rhythmical cue bypassing the defective pallidocortical projections, resulting in a normalized gait pattern (Bello et al., 2010). In addition, the propioceptive inputs during treadmill walking could stimulate the CPG. CPG refers to neural spinal circuits that can produce rhythmic movements. However, the role of CPG in human control remains unknown (Dietz, 2003). The constant speed provided by the treadmill could also contribute to minimizing the stride-to-stride variation in gait timing (Frenkel-Toledo et al., 2005), in contrast to overground walking where there are ongoing fluctuations in gait speed. The study results showed a reduction in both stride length and frequency variability on the treadmill in PD patients, in comparison with overground. Thus, PD patients could benefit from a constant speed, which improves gait timing. A recent study has suggested that the improvement in the step length may be due to the belt movement but that changes in variability may be influenced further by the imposed treadmill speed (Bello et al., 2010).

Although, all subjects walked on the treadmill with the handrail support, it is unlikely that this could account for the gait improvement in subjects with PD. A previous study from our group clearly showed that, when PD subjects walked with a treadmill simulator without the belt but with hand support, stable environment and imposed speed, their step length did not improve in comparison with normal overground walking (Bello et al., 2010). Thus, hand

support may not be responsible for the gait improvements during treadmill walking that were observed in the present study.

In summary, this is the first study that explores the role of attention in the gait improvements observed in PD subjects during treadmill walking. The present results show that PD subjects walking on a treadmill, while simultaneously performing a cognitive task, are able to maintain the reduction of their gait variability. Moreover, their cognitive execution was similar during treadmill and overground walking. These findings suggest that attentional resources are not involved in the gait treadmill improvements in PD subjects and point to belt displacement and constant speed as alternative explanations.

Clinical implications

The present study provides additional information that may be useful when considering the treadmill as a therapeutic tool to improve the gait in subjects with PD. The reduction of gait variability walking on a treadmill did not deteriorate during the performance of a concurrent cognitive task. Therefore, therapists may include performance of cognitive tasks during the treadmill walking in order to simulate real life conditions (i.e. walking while keeping a conversation). In addition, we recommend further studies that investigate the role of belt displacement and constant speed in gait improvements using treadmill walking in PD and to determine how these improvements translate to real life conditions.

4.2 STUDY II: Acute kinematic and neurophysiological effects of treadmill and overground walking in Parkinson's disease

4.2.1 Abstract

The use of the treadmill as a gait rehabilitation tool has provided novel options for treatment of gait impairments in PD. However, the neural mechanisms underlying these therapeutic effects in PD remain unknown. Our goal was to examine the immediate short-term effects of a single session of treadmill and overground walking on gait, spinal and corticospinal parameters in PD. Fifteen PD participants were evaluated in two separate sessions and walking conditions: walking over a treadmill and walking overground. The following measurements were evaluated before and after each condition: overground walking performance, the Soleus (SOL) H-reflex, Reciprocal Ia-Inhibition from the TA to the SOL muscle, ICF and Short Intracortical Inhibition (SICI) of the TA muscle. We found that treadmill walking, but not overground walking, lead to an improvement in the stride length and gait speed in the PD patients. Both walking conditions modulated spinal and corticospinal parameters in a similar way. This study provides evidence of a specific therapeutic effect of a single session of treadmill walking on gait in PD. Further studies are needed to explore other possible neural mechanisms.

4.2.2 Introduction

Gait disturbances are one of the principal and most incapacitating symptoms of PD (Keus et al., 2009). PD gait is characterized by the inability to regulate an appropriate stride length, a reduced gait speed and an increased stride-to-stride variability (Ebersbach et al., 2013). Gait disorders in PD may also include festination, start hesitation during gait initiation, freezing of gait and falls (Ebersbach et al., 2013). The physiopathology of these gait disorders has not been studied extensively and only a few studies have investigated the neurophysiological aspects of parkinsonian gait. A recent study showed a reduction of the SOL H-reflex during gait initiation in PD (Hiraoka et al., 2006). Others have reported a correlation between the decreased reciprocal Ia-inhibition at the onset of a voluntary ankle dorsiflexion during axial swings in PD patients (Meunier et al., 2000a). In addition, TMS studies revealed abnormalities in ICF, that may be related with the decreased stride length and the slower gait speed in PD (Vacherot et al., 2010a).

In recent years, there has been an increased interest in the treadmill as a potential gait rehabilitation tool in PD. Several studies have shown that treadmill training enlarge stride length, increase gait speed, decrease double support and reduce gait in PD subjects (Miyai et al., 2000, 2002; Bello et al., 2010; Bello, 2013). Gait benefits have been observed immediately after a single session of treadmill walking. For example, after walking on a treadmill for 20 minutes, PD patients were able to walk overground with lower stride-to-stride variability (Frenkel-Toledo et al., 2005) and higher stride length (Fernández-Lago et al., 2015). Although, these short-term gait improvements were attributed to the treadmill walking, the studies did not examine whether these effects could also be achieved by a single session of overground walking. This is of relevance in order to determine whether the treadmill has a specific therapeutic effect in PD.

In addition, although several hypotheses have been suggested in order to explain the beneficial effects associated with the use of a treadmill in PD (see review of Bello et al. 2012) (Bello & Fernández-Del-Olmo, 2012), the

neural mechanisms underlying these improvements still remain largely unknown.

The aim of the current study was to explore the immediate effects of a single session of either treadmill or overground walking on gait, spinal and corticospinal measurements in PD. We hypothesized that only the treadmill walking session would lead to an improvement in the gait kinematics in PD subjects, as well as walking-related neurophysiological modulations.

4.2.3 Material and methods

Participants

Fifteen individuals, diagnosed with idiopathic PD by a neurologist according to the United Kingdom Bank Criteria (Hugheset al., 1992), were recruited for the study from a local community. Inclusion criteria for participants was diagnosis of idiopathic PD, the ability to walk for 10 minutes without stopping or walking assistance, absence of neurologic disorders other than PD, not being treated with deep brain stimulation, and absence of orthopaedic, cardiovascular or visual disturbances that could affect gait. The participants did not use a treadmill for at least 12 months before the experiment. No participant showed dementia as assessed by the MMSE. The level of severity of the motor signs associated with PD was measured using the UPDRS-III (Fahn et al.,1987) and H&Y scale (Hoehn, 1967). Tests were conducted with the patients in the “ON” state (45 minutes – 1.5 hours after medication intake) when they were moving freely and easily without dystonia, excessive rigidity or tremor. All participants gave their informed consent according to the Declaration of Helsinki (1964), before entering the study. The experimental procedures were approved by the local ethics committee. Details of participants are shown in Table 7.

Table 7 Details of PD participants.

	Sex	Age (Yr)	Weight (Kg)	Height (cm)	Leg length (cm)	Disease Duration (yr)	H&Y	UPDRS III	MMSE	More symptomatic or onset side	Medication
1	M	45	84	170	87.5	7	2	30	30	L	Levodopa/Carbidopa 750/187.5, Entacapone 1000, Pramipexole 2.1, Rasagiline 1
2	M	85	69	180	90.1	16	3	58	27	R	Levodopa/Carbidopa 600/150, Entacapone 800
3	M	42	87	175	92	2	1.5	18	28	L	Levodopa/Carbidopa 200/50, Entacapone 200
4	F	67	51	151	80.5	4	1.5	21	29	L	Levodopa/Benserazide 250/50, Rasagiline 1
5	F	51	62	160	88	10	2	21	29	I	Levodopa/Carbidopa 800/400, Pramipexole 3.15, Amantadine 300
6	M	67	81	174	91	8	1.5	9	30	L	Levodopa/Carbidopa 450/112.5, Entacapone 600, Pramipexole 3.6
7	F	45	64	171	93	7	1.5	21	30	L	Levodopa/Carbidopa 150/25, Trihexyphenidyl 2
8	M	60	85	165	82	5	1.5	23	30	R	Levodopa/Benserazide 175/43.75, Pramipexole 2.1
9	M	36	86	168	90	4	1	9	29	R	Levodopa/Carbidopa 150/37.5 Entacapone 600, Rotigotine 4, Rasagiline 1
10	M	59	75	170	83.5	2	1.5	32	30	R	Rasagiline 1, Pramipexole 1
11	M	56	75	173	90	3	2.5	26	30	L	Levodopa/Carbidopa 350/75 Rasagiline 1, Rotigotine 8
12	F	67	67	147	74	3	1.5	18	29	R	Rasagiline 1
13	F	49	73	150	77.5	8	1.5	12	26	R	Levodopa/Carbidopa 500/125 Pramipexole 1
14	F	62	76	160	85	10	1.5	12	30	L	Levodopa/Benserazide 900/225, Ropinirole 16, Rasigiline 1
15	M	59	75	165	91.1	9	2	16	27	R	Levodopa/Carbidopa 300/75, Rasagiline 1, Ropinirole 6
Mean		56.67	74.00	154.18	86.35	6.53	1.68	21.23	28.93		
DS		12.48	10.13	43.32	5.73	3.86	0.46	12.20	1.33		

Yr, years; M, male; F, female; R, right; L, left.

Study protocol

All participants performed three sessions, one familiarization session with the treadmill and two experimental sessions corresponding to the two walking conditions: overground walking and treadmill walking. The experimental sessions were arranged in random order and separated by a period of one week. A summary of the protocol is shown in Figure 7.

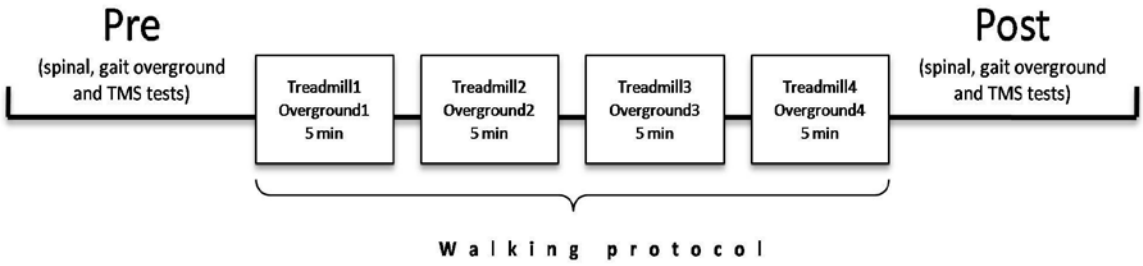


Figure 7 Scheme of an experimental session of Study II.

Walking protocol consisted of 4 blocks (5 minutes per block) of either treadmill or overground walking, with 3 minutes of rest between blocks.

Spinal measurements, gait overground test performance and corticospinal measurements were recorded, in that order, before (pre) and after (post) each walking condition. Spinal measurements were tested first, to exclude the possibility that the post-measurements of the gait overground test may mask possible spinal modulations. Corticospinal parameters were measured at the end, to ensure that the post-measurements of the gait overground test took place at least 10 minutes after each of the walking conditions (Bello et al., 2008). The self-selected gait speed obtained during the first gait overground test, was used for the subsequent walking conditions.

The treadmill walking condition consisted of four 5-minutes blocks of treadmill walking with a 3-minutes rest period between blocks. During the first minute of each block, the belt speed was increased to the overground

self-selected, preferred speed. All the participants walked on the treadmill holding the handrails since some participants did not feel safe to walk without the handrail support. The subjects walked on a motorized treadmill (SporsArt 6300, Sports Arts Fitness) under the close supervision of a physical therapist.

The overground condition consisted of four 5-minutes blocks of overground walking with a 3-minutes rest period between blocks. The overground walking session was conducted in an indoor facility. PD participants had to walk forming a square marked with cones (20x20m). The walking direction was alternated in each block, either clockwise or counter-clockwise. The walking speed was monitored during the session, in order to confirm that each patient maintained the overground walking speed obtained at the beginning of the experimental session.

Outcome measures

The gait overground test

Gait performance was recorded overground using an optical detection system (Optogait, Microgait, USA), after a familiarization trial. Participants were recorded walking up and down an 8 meters walkway at their self-selected comfortable speed, for a total time of 2 minutes. The gait parameters were recorded during the straight walking portion, but not during the turns.

The following gait variables were evaluated: speed (m/s), stride length (m), stride frequency (Hz), CV of the stride length (%) and of the stride frequency (%). CV is an indicator of variability, where CV = (standard deviation/ mean) x100.

Neurophysiologic measurements

Spinal and corticospinal recordings were performed at rest before and after each walking condition. Subjects were seated comfortably in a reclining armchair; with the feet resting on a foot support so that the hips were flexed at a 120 degrees, the knees semi-flexed at 160 degrees, and the ankles were positioned at 110 degrees of a plantar flexion.

Electromyographic (EMG) was recorded by a pair of adhesive surface electrodes 2-cm apart (bipolar), placed over the SOL and Tibial Anterior muscles bellies, according to SENIAM recommendations (Hermens et al., 2000). Muscles from the more symptomatic side of the PD patients were recorded. The reference electrode was placed over the medial malleolus bone surface. The recording sites were shaved, abraded and cleaned with isopropyl alcohol to obtain low impedance (Z, 5kΩ). The EMG signals were simultaneously digitized using an acquisition car at a sampling rate of 5kHz per channel (Digitimer D360, Welwyn Garden City, UK), and then recorded using a Signal script software (Cambridge Electronic Devices, Cambridge, UK).

Hmax/Mmax ratio

Transcutaneous electrical stimulation of the posterior tibial nerve was used to elicit the H-reflex in the SOL muscle using a Digitimer stimulator (model DS7, Welwyn Garden City, UK). The optimum site of nerve stimulation was first located using a hand-held electrode. The cathode (2 cm diameter brass hemisphere) was placed on the popliteal fossa and the anode (5 cm^2) above the patella. The adhesive electrodes were fixed with an elastic strap. The stimulus that was used was a rectangular pulse with a duration of 1 ms. The maximum H-reflex response (Hmax) and the maximum M amplitude (Mmax) were recorded.

Reciprocal Ia-inhibition

To evaluate the reciprocal Ia-inhibition from the TA to the SOL muscles, the size of the SOL control H-reflex was adjusted to Hmax/2 (Crone, 1990) and to 20- 25% of the Mmax, and kept constant throughout the experiment. The conditioning stimulus was applied to the common peroneal nerve through bipolar electrodes placed at the neck of the fibula. Rectangular pulses of 1 ms duration were used. The conditioning stimulus was adjusted to the Tibialis Anterior (TA) motor threshold intensity. Special care was taken to ensure a pure TA contraction (Meunier et al., 2000b). The conditioning–test interstimulus interval was determined using 0.5 ms steps until the maximum reciprocal Ia-inhibition of SOL H-reflex response was reached, and this value was then kept constant throughout the experiment. Ten unconditioned and

ten conditioned reflexes were recorded. Mean as well as standard error of the mean (SEM) values are reported. The amount of inhibition was defined as: [(mean control H value – mean conditioned H value)/mean control H value)] ×100.

Corticospinal measurements

The following measures, elicited by TMS, were recorded: the motor evoked potential (MEP), SICI and ICF of the TA muscle. TMS was delivered using a double cone coil connected to a Magstim 200 magnetic stimulator (Magstim, Dyfed, United Kingdom). The optimal scalp location was determined by placing the coil over the inter hemispheric scissura and by moving it around until the hotspot that was contralateral to the most affected side, was located. We determined the resting motor threshold (RMT) in accordance with the International Guidelines (Rossini et al., 1994): the nearest 1% of the maximum stimulator output was defined as the minimum stimulus intensity required to produce MEPs of >50 μV in at least 5 of 10 consecutive trials. In the paired-pulse TMS recordings, a subthreshold conditioning stimulus was delivered at 80% of the RMT, following a suprathreshold test stimulus intensity set at 130% of the RMT. Based on results observed by Vacherot et al. (2010), SICI was elicited at an interstimulus interval of 3 ms, whereas ICF was elicited at an interstimulus interval of 15 ms (Vacherot et al., 2010a). A total of 10 tests, 10 SICIs and 10 ICFs stimuli were randomly delivered and recorded in a single block (Kujirai et al., 1993). SICI and ICF amplitudes were expressed as the percentage of the mean amplitude of the unconditioned MEP.

Statistical analysis

To explore the changes in walking performance and of the neurophysiological parameters before and after each walking condition, a two-way ANOVA, with “condition” (treadmill and overground) and “time” (pre and post) as the main factors, was performed for each of the following variables: gait speed, stride length, stride frequency, CV of stride length and CV of stride frequency, Hmax/Mmax ratio, Reciprocal Ia-inhibition, MEP, ICF and SICI.

Post Hoc t-tests were computed. Variables did not violate assumption of normality, except for the SICI and ICF variables. The analyses of these variables were conducted using logarithmic transformation values. All statistical analyses were performed using PASW Statistics 18. A *P* value ≤ 0, 05 was considered statistically significant.

4.2.4 Results

The gait overground test

The results of the gait parameters are shown in Table 8.

Table 8 Mean and standard deviations of gait variables before, and after a single session intervention.

	TREADMILL		OVERGROUND	
	PRE	POST	PRE	POST
Gait speed (m/s)	1.17±0.26	1.25±0.24	1.21±0.19	1.20±0.22
Stride length (m)	1.23±0.19	1.29±0.18	1.26±0.17	1.23±0.16
Stride frequency (Hz)	0.94±0.09	0.95±0.09	0.95±0.07	0.97±0.09
CV of stride length (%)	4.52±2.01	3.94±1.78	4.30±1.78	4.22±2.29
CV of stride frequency (%)	3.08±1.41	2.84±1.06	2.90±1.32	2.85±1.47

PRE, pretest; POST, postest; CV, coefficient of variation.

The analysis of the overground gait speed showed a significant condition×time interaction (F=6.981, p=0.019) without significant main effects for condition and time. Post hoc analysis showed that gait speed was faster for post-treadmill compared with pre-treadmill (p=0.001). No significant gait speed changes were found for the pre vs. post-overground condition (Figure 8).

The analysis of the stride length also showed a significant condition×time interaction ($F=4.94$, $p=0.043$) without significant main effects for condition and time. Post hoc analysis showed that the stride length was larger post-treadmill compared with pre-treadmill ($p=0.007$). No significant changes were found for the overground condition (Figure 8). The analysis for the remaining gait parameters did not show significant main effects or interactions (Figure 8).

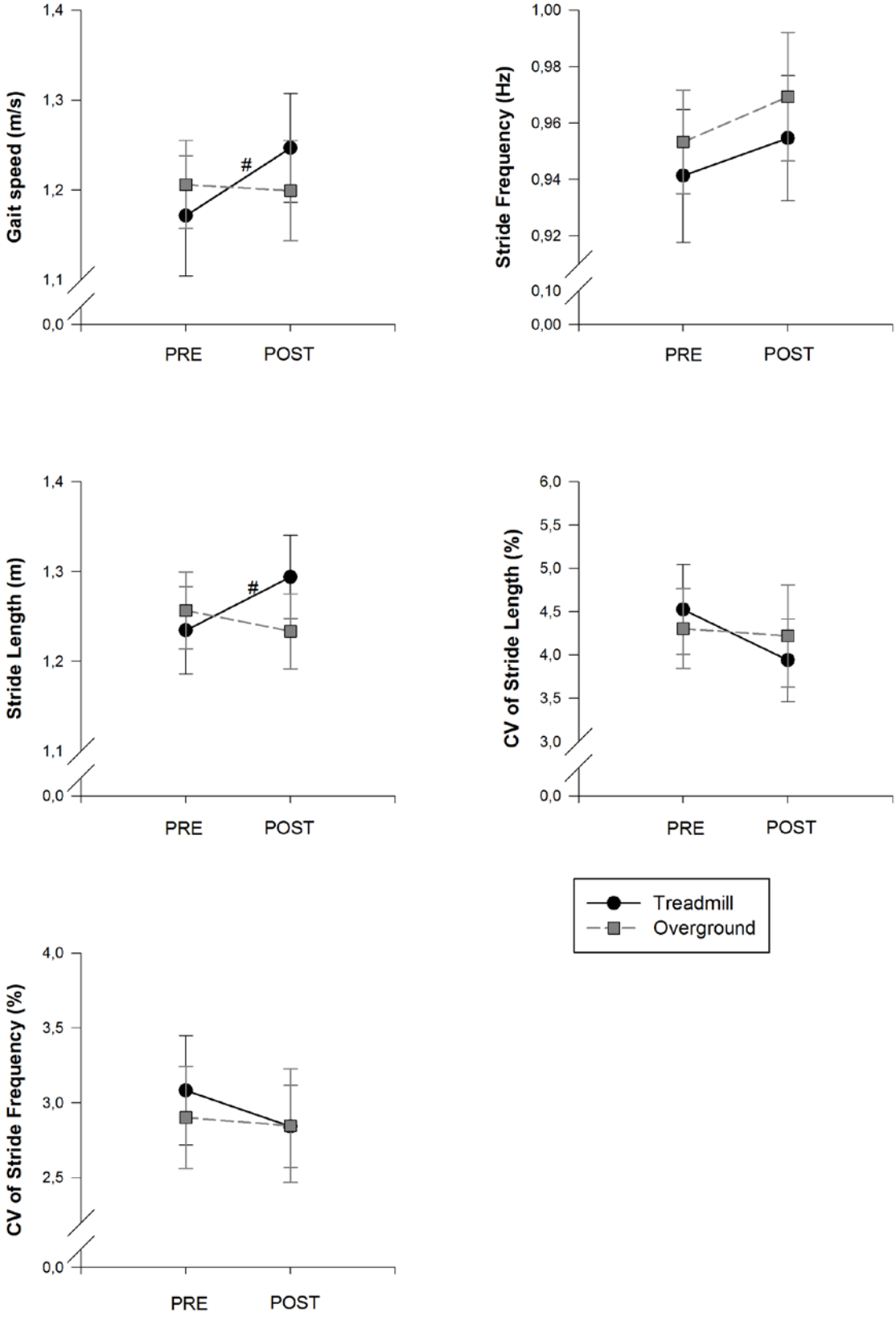


Figure 8 Results of walking performance.

PRE, pretest; POST, postest; #, ($p<0.005$).

Neurophysiological measurements

The analysis of the Hmax/Mmax ratio revealed a significant main effect for time ($F=23.308$, $p<0.001$), without a significant main effect for condition nor a significant condition×time interaction. The Hmax/Mmax ratio was lower after than before the two walking conditions (Figure 9). No significant main effects or interactions were found for the reciprocal Ia-inhibition.

The analysis of the absolute MEP amplitudes for single TMS pulses did not show significant main effects nor a significant interaction. The analysis of ICF values showed a significant main effect for time ($F=7.053$, $p=0.019$), without a significant effect of condition or a condition×time interaction. ICF values decreased after, compared with before, the two walking conditions (Figure 9). No significant main effects or interactions were found for measurements of SICI (Figure 9).

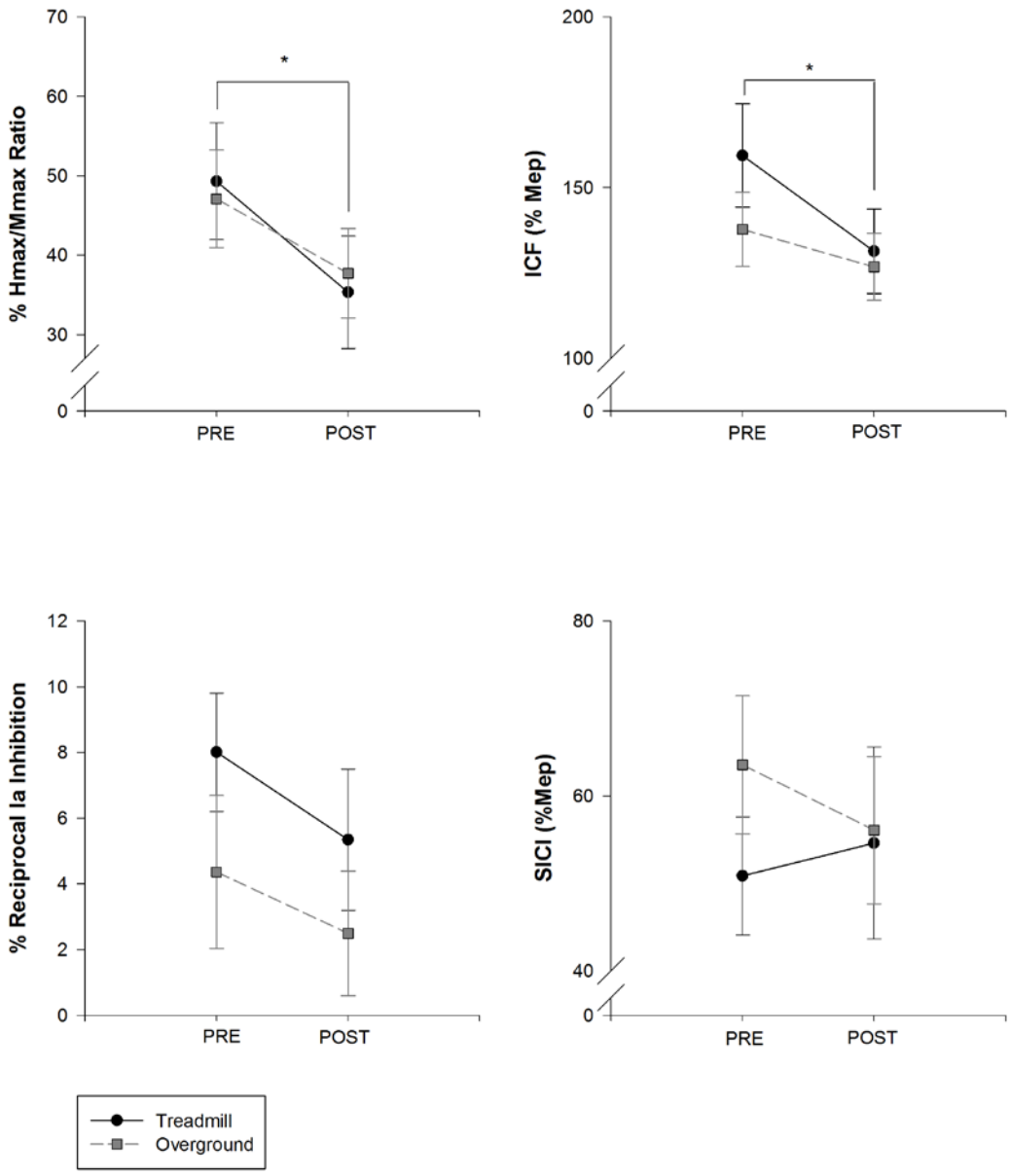


Figure 9 Results of spinal and cortical excitability.

PRE, pretest; POST, postest; ICF, intracortical facilitation; *, significant main effect ($p<0.005$).

4.2.5 Discussion

The current study demonstrated that a single session of treadmill walking, but not of overground walking, lead to immediate gait improvements in PD patients. However, the spinal and corticospinal modulations that were observed in our study were not specific to the therapeutic effect of treadmill walking.

Our study showed that PD participants increased their overground gaits peed and stride length after, compared to before, a 20 minutes session of treadmill walking. Previous findings have reported similar improvements in PD gait after a single session of treadmill walking (Miyai et al., 2000; Bello et al., 2008; Kurtais et al., 2008). For instance, overground gait speed, stride length and double stance duration have been shown to improve after a single session of treadmill walking with weight support. However, none of these studies have compared a single session of treadmill walking with a single session of overground walking. To our knowledge the current study is the first to show that 20 minutes of overground walking does not improve gait in PD. Therefore, our results suggest a specific therapeutic effect of treadmill walking in PD subjects.

The current investigation showed that the changes in the neurophysiological parameters were comparable across the two walking conditions, i.e. treadmill and overground walking. We found that ICF values decreased after both walking interventions in PD patients. Thus, the observed ICF reduction was not specific to the treadmill, but instead as a result of the walking movement itself. The ICF of the lower limb areas has not been investigated extensively in PD, and to date only one study has reported ICF impairments in the TA muscle (Vacherot et al., 2010). In this study PD patients manifested an abnormal reduced ICF that correlated with the observed shortened stride length and reduced gait velocity. However, it is difficult to compare the results of this study with our current findings since, unlike our study, ICF values were measured before any walking activity was performed and half of the patients manifested freezing of gait (Vacherot et

al., 2010b). Our findings suggest that ICF is not a mechanism underlying the gait improvement associated with treadmill walking in PD.

The current study showed that the SICI values did not change after a single session of treadmill and overground walking. SICI has been shown to be mediated by cortical $GABA_a$ activity (Werhahn et al., 1999; Ziemann et al., 1996) and can be modulated by a short period of motor skill training involving ankle muscles (Perez et al., 2004). Several studies have suggested that gait improvements associated with treadmill walking may be related to motor learning mechanisms, since the improvements are sustained for several months after the treadmill training has been completed (Protas et al., 2005; Herman et al., 2007; Fisher et al., 2008b). However, our SICI findings suggest that it is unlikely that the gait improvements, observed after a single session of treadmill walking, are related to the acquisition of a motor skill.

In addition, we observed spinal modulations after both the treadmill and walking sessions as indicated by a reduction of the Hmax/Mmax ratio values. However, we did not observe any significant changes in the reciprocal Ia-inhibition. A previous study showed that the SOL Hmax/Mmax ratio and the recruitment curve of H-reflex at rest are similar in PD and control subjects (Dietrichson, 1971). However, several other studies have suggested that this reflex has a role in the gait impairments that are observed in PD (Hiraoka et al., 2005; Hiraoka et al., 2006; Pierantozzi et al., 2008). There is some evidence to suggest that the depression of the SOL H-reflexes can occur after different training tasks such as a single training session of cycling (Meunier et al., 2007), balance (Freyler et al., 2014), and co-contraction (Perez et al., 2007) in healthy adults. Thus, it is likely that the depression of the Hmax/Mmax ratio observed in the present study may be due to the actual walking activity. Our findings suggest that both the H-reflex and the reciprocal Ia-inhibition are not involved in the gait improvements associated with a single session of treadmill walking in PD. Further studies are needed to elucidate the neural mechanisms involved in treadmill walking improvements in PD, perhaps using other neurophysiologic parameters.

4.3 STUDY III: Treadmill walking combined with anodal tDCS in Parkinson's Disease: kinematic and neurophysiological effects.

4.3.1 Abstract

The use of treadmill as a gait rehabilitation tool and advances in NIBS has provided novel and low-risk options for treatment of gait impairments in PD. We tested the hypothesis that combining treadmill walking with tDCS enhances the gait improvements associated with treadmill walking in PD subjects. We explored the effects of these combined methodologies on spinal and corticospinal parameters. Eighteen PD participants were evaluated in separate sessions under three treadmill walking conditions: treadmill walking alone (treadmill), treadmill walking combined with anodal tDCS (AtDCS+treadmill), and treadmill walking combined with sham tDCS (StDCS+treadmill). Overground walking performance, the SOL H-reflex, Reciprocal Ia-Inhibition from the TA to the SOL muscle, ICF and SICI of the TA muscle, were measured before and after each treadmill condition. The SOL H-reflex and walking performance on the treadmill were also evaluated. All treadmill conditions improved walking performance and modulated spinal and corticospinal parameters in a similar way. However, AtDCS+treadmill lead to a different modulation of Reciprocal Ia-Inhibition from the TA muscle to the SOL in comparison with the other treadmill conditions. Although, a single session combining treadmill walking and anodal tDCS did not enhance the improvements of gait parameters associated with treadmill walking in PD, the specific modulation of the Reciprocal Ia-Inhibition point out to an interaction of the effects of these tools. Further studies are needed to explore the functional significance of this interaction.

4.3.2 Introduction

Gait disturbances are among the most significant impairments in PD that severely affect the individual's QoL (Keus et al., 2009). PD gait is characterized by a particular difficulty with the internal regulation of stride length. This is often accompanied by a reduced gait speed, and an increase of the double support phase and stride variability (Ebersbach et al., 2013).

Gait disturbances in PD may be linked with the degeneration of the PPN of the MLR (Grabli et al., 2013), coupled with bidirectional disruptions of signals to the BG (Mena-Segovia et al., 2004). However, neurophysiological features associated with parkinsonian gait have been not been investigated extensively. Findings suggest that PD subjects show an inhibited SOL H-reflex during gait initiation (Hiraoka et al., 2006). This H-reflex inhibition is removed after deep brain stimulation of the PPN (Pierantozzi et al., 2008), suggesting a role of this reflex in the gait impairments in PD. In addition, abnormalities in ICF detected by TMS may also be involved in the shortened stride length and reduced gait speed that are observed in PD patients (Vacherot et al., 2010a).

Although, dopaminergic therapies ameliorate many of the parkinsonian symptoms especially in the early stages of the disease, as the disease progresses, effectivity of pharmacological therapy is diminished (Katzenschlager & Lees, 2002). Therefore, treatment of gait symptoms continues to be a challenge. As a result, a significant number of rehabilitation strategies have been explored in order to improve the gait in this population (Tomlinson, 2012). The use of treadmill as a gait rehabilitation tool and advances in NIBS has provided novel and low-risk options for treatment of gait impairments in PD.

Several studies have shown that treadmill training leads to improvements of gait parameters in people with PD, such as enlarged stride length, increased gait speed, decreased double support and reduced gait variability (Miyai et al., 2000; Kurtais et al., 2008; Fisher, 2008; Bello, 2013). These improvements could even occur after one single treadmill walking session in PD (Bello et al., 2008) and may be related with the gait modulation that takes

place when the patients walk on the treadmill. Treadmill walking has been shown to be associated with longer stride length and a more stable gait pattern in PD patients, compared with overground walking at an identical speed (Frenkel-Toledo et al., 2005; Fernández-Lago et al., 2015).

TDCS is a NIBS technique that consists of delivering weak currents through a pair of electrodes placed on the scalp, with the capability to modulate corticomotor excitability (Nitsche et al., 2008) and spinal reflexes such as reciprocal Ia-inhibition in healthy people (Roche et al., 2012). In PD, tDCS application over motor cortex produced a polarity-dependent effect on corticospinal motor excitability that seemed to correlate with motor function enhancements (Fregni et al., 2006). Anodal tDCS over motor cortex resulted in modest improvements of gait function in PD subjects (Benninger et al., 2010). In addition, the combination of anodal tDCS and physical training seems to be more efficient than the application of tDCS alone (Kaski et al., 2014). Therefore, combining treadmill walking with tDCS may be an effective strategy for enhancing the therapeutic effects of treadmill walking in PD patients.

The aim of the current study was to explore the effects of a single session of anodal tDCS during treadmill walking on gait parameters in PD. In addition, we recorded spinal and corticospinalparameters in order to investigate the possible neural mechanisms involved in the effects of the combined therapy. Our hypothesis was that anodal tDCS will enhance the effect of treadmill walking on gait parameters in PD.

4.3.3 Material and methods

Participants

Eighteen individuals diagnosed with idiopathic PD by a neurologist, according to the United Kingdom Bank Criteria (Hughes et al., 1992) were recruited for the study from a local community. Inclusion criteria for participants were: diagnosis of idiopathic PD, the ability to walk for 10 minutes without stopping or walking assistance, absence of neurologic disorders other than PD, not being treated with deep brain stimulation, and

absence of orthopaedic, cardiovascular or visual disturbances that could affect the gait. No participant showed dementia as assessed by the MMSE. The severity of the motor signs associated with PD was measured using the UPDRS-III (Fahn et al., 1987) and H&Y scale (Hoehn, 1967). Tests were conducted with the patients in the “ON” state (45 minutes – 1.5 hours after medication intake) when they were moving freely and easily without dystonia, excessive rigidity or tremor. All participants gave their informed consent according to the Declaration of Helsinki (1964) before entering the study and the protocol were approved by the local ethics committee. Details of participants are shown on Table 9.

Table 9 Details of participants.

	Sex	Age (Yr)	Weight (Kg)	Height (cm)	Leg length (cm)	Disease Duration (yr)	H&Y	UPDRS III	MMSE	Body side more affected	Medication
1	M	59	74	165	83.5	5	1.5	24	29	R	Levodopa/Carbidopa 600/150, Rasagiline 1, Rotigotine 14
2	M	63	79	166	82	6	1.5	13	29	R	Pramipexole 3.15, Levodopa/Carbidopa 100/25, Rasagiline 1
3	M	45	84	170	87.5	7	2	30	30	L	Levodopa/Carbidopa 750/187.5, Entacapone 1000, Pramipexole 2.1, Rasagiline 1
4	M	85	69	180	90.1	16	3	58	27	R	Levodopa/Carbidopa 600/150, Entacapone 800
5	M	42	87	175	92	2	1.5	18	28	L	Levodopa/Carbidopa 200/50, Entacapone 200
6	F	67	51	151	80.5	4	1.5	21	29	L	Levodopa/Benserazide 250/50, Rasagiline 1
7	F	51	62	160	88	10	2	21	29	I	Levodopa/Carbidopa 800/400, Pramipexole 3.15, Amantadine 300
8	M	67	81	174	91	8	1.5	9	30	L	Levodopa/Carbidopa 450/112.5, Entacapone 600, Pramipexole 3.6
9	F	45	64	171	93	7	1.5	21	30	L	Levodopa/Carbidopa 150/25, Trihexyphenidyl 2
10	M	60	85	165	82	5	1.5	23	30	R	Levodopa/Benserazide 175/43.75, Pramipexole 2.1
11	M	36	86	1.68	90	4	1	9	29	R	Levodopa/Carbidopa 150/37.5 Entacapone 600, Rotigotine 4, Rasagiline 1
12	M	59	75	170	83.5	2	1.5	32	30	R	Rasagiline 1, Pramipexole 1
13	M	56	75	173	90	3	2.5	26	30	L	Levodopa/Carbidopa 350/75 Rasagiline 1, Rotigotine 8
14	F	67	67	147	74	3	1.5	18	29	R	Rasagiline 1
15	F	49	73	150	77.5	8	1.5	12	26	R	Levodopa/Carbidopa 500/125 Pramipexole 1
16	F	62	76	160	85	10	1.5	12	30	L	Levodopa/Benserazide 900/225, Ropinirole 16, Rasigiline 1
17	M	59	75	165	91.1	9	2	16	27	R	Levodopa/Carbidopa 300/75, Rasagiline 1, Ropinirole 6
18	F	48	56	1.68	91.5	2	1.5	18	27	L	Levodopa/Benserazide 100/25, Ropirinole 16
Mean		56.67	73.28	146.96	86.23	6.17	1.65	21.17	28.83		
DS		11.63	10.23	53.60	5.49	3.65	0.42	11.31	1.29		

Yr, years; M, male; F, female; R, right; L, left.

Study protocol

All participants performed four sessions, one familiarization session with the treadmill and tDCS, and three experimental sessions corresponding to three treadmill walking conditions: treadmill walking alone (treadmill), treadmill walking combined with anodal transcranial direct current stimulation (AtDCS+treadmill), and treadmill walking combined with sham stimulation (StDCS+treadmill). The experimental sessions were arranged in random order and separated by a period of one week.

A description of the experimental procedure is summarized in Figure 10. Spinal, walking overground performance and corticospinalmeasurements were recorded, in that order, before (pre) and after (post) the treadmill walking conditions. Cortical measurements were tested at the end, since it has been shown that the maximum increase in cortical excitability occurs 15 minutes post-AtDCS stimulation (López-Alonso et al., 2015). The individual velocity obtained during overground walking at the beginning of each experimental session was used for the subsequent treadmill walking conditions. Subjects walked on a motorized treadmill (SporsArt 6300, Sports Arts Fitness) under the close supervision of a physical therapist.

The treadmill conditions consisted on four 5 minutes blocks (T1–T4) of treadmill walking holding the handrails with a rest period between blocks of 3 minutes. In all treadmill blocks, during the first minute, the belt speed was progressively increased to the overground speed.

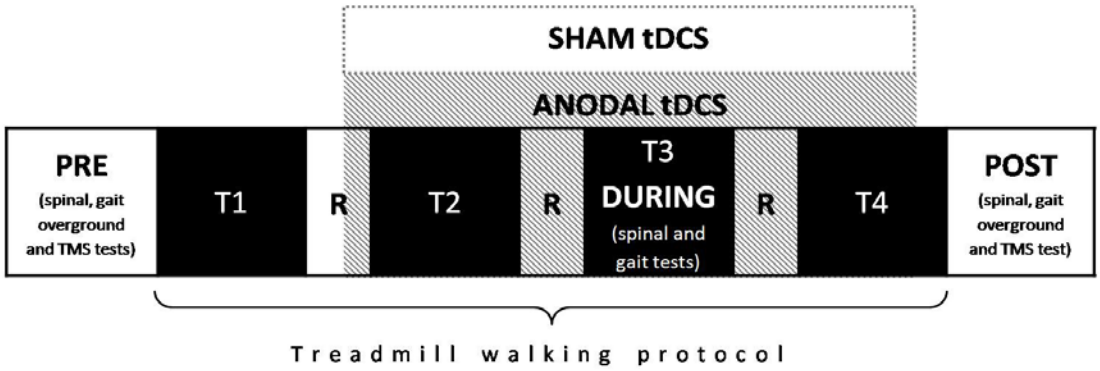
The AtDCS+treadmill condition was the same as the treadmill condition but was combined with a 20 minutes session of anodal tDCS stimulation. Anodal tDCS was delivered at 2 mA through a pair of saline-soaked sponge surface electrodes (3.5 cm^2) connected to a DC stimulator (neuroConn, Germany). The active electrode (anode) was placed over the motor cortex and positioned in the hotspot of the TA muscle contralateral to the most affected body side. This area was localized using a TMS procedure. The cathode was placed contralateral to the anode, over the supraorbital region. The stimulator was turned on at the first rest period. Subjects received a continuous 20 minutes anodal tDCS stimulation session (during the

corresponding blocks and rest periods). The current was faded in and faded out for 8 seconds.

The StDCS+treadmill condition was identical to the AtDCS+treadmill condition except that the current of the anodal tDCS was switched off after 8 seconds of stimulation.

Walking performance and H-reflex amplitudes were evaluated during each treadmill walking condition (during block T3 at 3 and 4 minutes for walking performance and H-reflex, respectively).

Figure 10 Scheme of one experimental session od Study III.



PRE, overground pre-treadmill; DURING, tests during treadmill walking; POST, overground post-treadmill. Treadmill walking protocol consists of 4 blocks (5 minutes per block) of treadmill walking (T1, T2, T3, T4) and 3 periods of 3 minutes of rest between blocks (R). Hatched part of the scheme represents 20 minutes of brain stimulation (ANODAL tDCS).

Outcome measurements

Walking performance

Gait performance was recorded overground and on the treadmill using an optical detection system (Optogait, Microgait, USA). Overground gait was evaluated after a familiarization trial. Participants walked up and down an 8 meters walkway, for a total time of 2 minutes, at a self-selected comfortable speed. The gait parameters were recorded during the straight walking portion and not when the subjects were turning.

The following variables of gait were evaluated: speed (m/s), stride length (m), stride frequency (Hz), CV of the stride length (%) and of the stride frequency (%). CV is an indicator of variability, where CV = (standard deviation/ mean) x100.

Neurophysiologic measurements

EMG: A pair of adhesive surface electrodes 2-cm apart (bipolar) were placed over the muscle bellies of the SOL and TA muscles (on the symptomatic side). The reference electrode was placed over the medial malleolus bone surface. EMG signals were amplified and filtered with a bandwidth frequency ranging from 10 Hz to 1 kHz. The EMG signals were recorded at a sampling rate of 5 kHz per channel (Digitimer D360, Welwyn Garden City, UK).

Spinal and corticospinalmeasurements were obtained at rest position before and after each treadmill walking condition. Subjects were comfortably seated in a reclining armchair; with the hips and the knees semi-flexed at 120 degrees and 160 degrees, respectively, and the ankles at 110 degrees plantar flexion with the feet resting on a foot support. Subjects remained in this position during the recordings, ensuring that there was no voluntary activity or excessive tremor.

Hmax/Mmax ratio: Transcutaneous electrical stimulation of the posterior tibial nerve was used to elicit the SOL H-reflex using a Digitimer stimulator (model DS7, Welwyn Garden City, UK). The optimum site of nerve stimulation was located using a hand-held electrode. The cathode (2 cm diameter brass

hemisphere) was placed in the popliteal fossa and the anode (5 cm2) above the patella. These adhesive electrodes were fixed with an elastic strap. The stimulus used was a rectangular pulse of 1 ms duration. The Hmax and the Mmax were recorded.

Reciprocal Ia-inhibition: To record reciprocal Ia-inhibition from the TA to the SOL muscle, the size of the SOL control H-reflexes was adjusted to Hmax/2 and to the 20-25% of Mmax, and kept constant throughout the experiment (Crone et al., 1990). The conditioning stimulus of the common peroneal nerve was elicited through bipolar electrodes at the neck of the fibulae. Rectangular pulses of 1 ms at TA motor threshold intensity were used. Special care was taken to ensure a pure TA muscle contraction without any peroneal muscle contraction (Meunier et al., 1993). The conditioning–test interstimulus interval was determined using 0.5 ms steps until the maximum reflex response was reached and this value was then kept constant throughout the experiment. Ten unconditioned and 10 conditioned reflexes were recorded in randomized order. The amount of inhibition was defined as: [(mean control H amplitude – mean conditioned H amplitude)/mean control H amplitude)] ×100.

H-reflex amplitude during walking: The SOL H-reflex was evoked by stimulation of the posterior tibial nerve. Test stimuli were 1 ms rectangular pulses delivered every 5 s. The H-reflexes during walking were elicited in 2 cycle phases: in the middle of the stance phase and in the middle of the swing phase. To determine the electric stimulus in the stance and swing phases, the latency between the stride marker and the stimulus was adjusted to each stride cycle. The beginning of a stride cycle was defined as the time when a stride marker, placed on the heel of the shoe, makes contact with the ground. To acquire H-reflexes evoked by the same stimulus intensity at each phase of the step cycle, the corresponding M-waves were used as indicators of the effective stimulus strength. The peak-to-peak amplitude of M-wave between 25% of Mmax was used to check the stability of stimulation conditions within the given phase of step cycle (Capaday & Stein, 1986). Mmax amplitude was measured during rest with the patients in a standing position. H-reflexes were elicited after 2 minutes of treadmill walking (T3). The amplitude of 10-H reflex was averaged in each step phase.

Corticospinal measurements: MEP, SICI and ICF of the TA muscle elicited by TMS were recorded. A double cone coil connected to a Magstim 200 magnetic stimulator (Magstim, Dyfed, United Kingdom) was used. The coil was placed over inter hemispheric scissura and moved around until the hotspot of the TA muscle, contralateral to the affected side, was localized. RMT was determined to the nearest 1% of the maximum stimulator output and defined as the minimum stimulus intensity required to produce MEPs of >50 μV in at least 5 of 10 consecutive trials (Rossini et al., 1994). In the paired-pulse TMS recordings, a subthreshold conditioning stimulus was delivered at 80% of the RMT, following a suprathreshold test stimulus intensity set at 130% of the RMT. SICI and ICF were elicited at an interstimulus intervals of 3 ms and 15 ms, respectively (Vacherot et al., 2010a). A total of 10 tests, 10 SICIs and 10 ICFs stimuli were randomly delivered and recorded in one single block (Kujirai et al., 1993). SICI and ICF amplitudes were expressed as a percentage of the mean amplitude of the unconditioned MEP.

Statistical analysis

To compare the gait pattern between overground pre-treadmill walking and the treadmill walking conditions, three-way ANOVAs with “treadmill condition” (AtDCS+treadmill, StDCS+treadmill and treadmill) and “time” (pre and during) as main factors were performed for the following variables: stride length, stride frequency, CV of stride length and CV of stride frequency.

To explore the changes in overground gait and of the neurophysiological parameters before and after each treadmill walking condition, three-way ANOVAs, with “treadmill condition” (AtDCS+treadmill, StDCS+treadmill and treadmill) and "time" (pre and post) as main factors, were used for the following variables: speed, stride length, stride frequency, CV of stride length and CV of stride frequency, Hmax/Mmax ratio, reciprocal Ia-inhibition, MEP, ICF and SICI.

To analyse the modulation of the H-reflex amplitudes of the gait phases during the treadmill walking conditions, a three-way ANOVA, with “treadmill

condition" (AtDCS+treadmill, StDCS+treadmill and treadmill) and "phase" (stance and swing) as main factors was conducted.

Post Hoc t-tests were computed when required. Variables did not violate assumption of normality, except for SICI and ICF. Therefore, analyses of these variables were conducted on logarithmic transformation values. All statistical analyses were performed using PASW Statistics 18. A P value ≤ 0, 05 was considered statistically significant.

4.3.4 Results

Pre-treadmill overground walking vs. Treadmill walking

Results of gait parameters are shown in Table 10.

Table 10 Results of gait parameters.

	AtDCS+Treadmill			StDCS+treadmill			Treadmill		
	PRE	DU	POST	PRE	DU	POST	PRE	DU	POST
Gait speed (m/s)	1.18± 0.21		1.24± 0.21	1.17± 0.25		1.21± 0.26	1.16± 0.25		1.23± 0.23
Stride length (m)	1.24± 0.16	1.28± 0.18	1.29± 0.16	1.24± 0.19	1.27± 0.21	1.27± 0.19	1.23± 0.18	1.29± 0.21	1.29± 0.17
Stride frequency (Hz)	0.95± 0.08	0.90± 0.18	0.96± 0.09	0.94± 0.1	0.90± 0.09	0.94± 0.1	0.94± 0.09	0.88± 0.12	0.94± 0.08
CV of stride length (%)	4.35± 2.21	2.16± 1.41	4.17± 2.33	4.1±1. 76	2.51± 1.42	4.60± 2.93	4.37± 1.91	3.69± 5.95	3.90± 1.69
CV of stride frequency (%)	2.78± 1.19	1.96± 2.13	2.65± 1.09	2.71± 0.78	1.74± 1.27	3.07± 1.42	2.99± 1.31	2.85± 5.66	2.83± 0.97

PRE, pretests; DU, tests during treadmill walking; POST, postests; CV, coeffiecient of variation.

The analysis of the stride length showed a significant main effect for time ($F=16.211$, $p=0.001$), without a significant treadmill condition effect or a condition×time interaction. The stride length was longer during the treadmill conditions compared with pre-treadmill overground walking (Figure 11). For the stride frequency, the analysis showed a significant main effect for time ($F=9.538$, $p=0.007$). However, there was no significant effect for treadmill condition nor any significant interactions. Stride frequency decreased during all treadmill walking conditions compared with pre-treadmill overground walking (Figure 11). The ANOVA for CV of stride length showed a significant main effect for time ($F=5.693$, $p=0.034$), without a significant effect for treadmill condition, nor any significant interactions. The CV of stride length was smaller during all treadmill walking conditions in comparison with pre-treadmill walking (Figure 11). No significant main effects or interactions were found for CV of stride frequency.

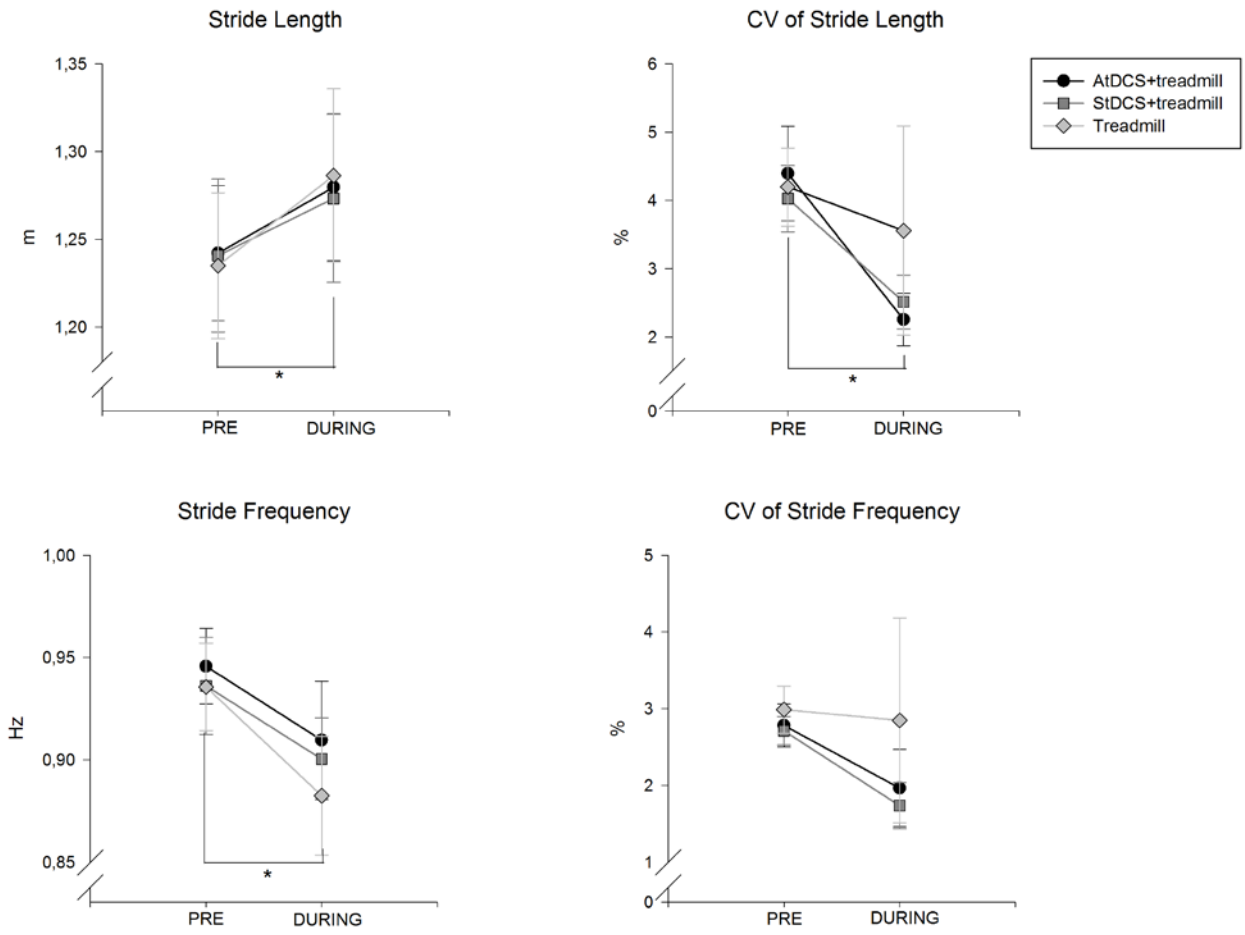


Figure 11 Results of walking performance before and during treadmill walking conditions.

PRE, overground pre-treadmill; DURING, during treadmill walking; *, significant main effect ($p<0.005$).

Overground gait pre-treadmill vs. post-treadmill

The analysis of the gait speed showed a significant main effect for time ($F=11.524$, $p=0.003$), without significant effect for treadmill condition or a significant condition×time interaction. Overground gait speed was greater post-treadmill compared with pre-treadmill walking (Figure 12). For the stride length, the analysis showed a significant main effect for time ($F=16.211$, $p=0.001$), without a significant treadmill condition effect or a condition×time interaction. PD participants walked with a longer stride length post-treadmill compared with pre-treadmill walking (Figure 12). The analysis for the remaining gait parameters did not show significant main effects or interactions (Figure 12).

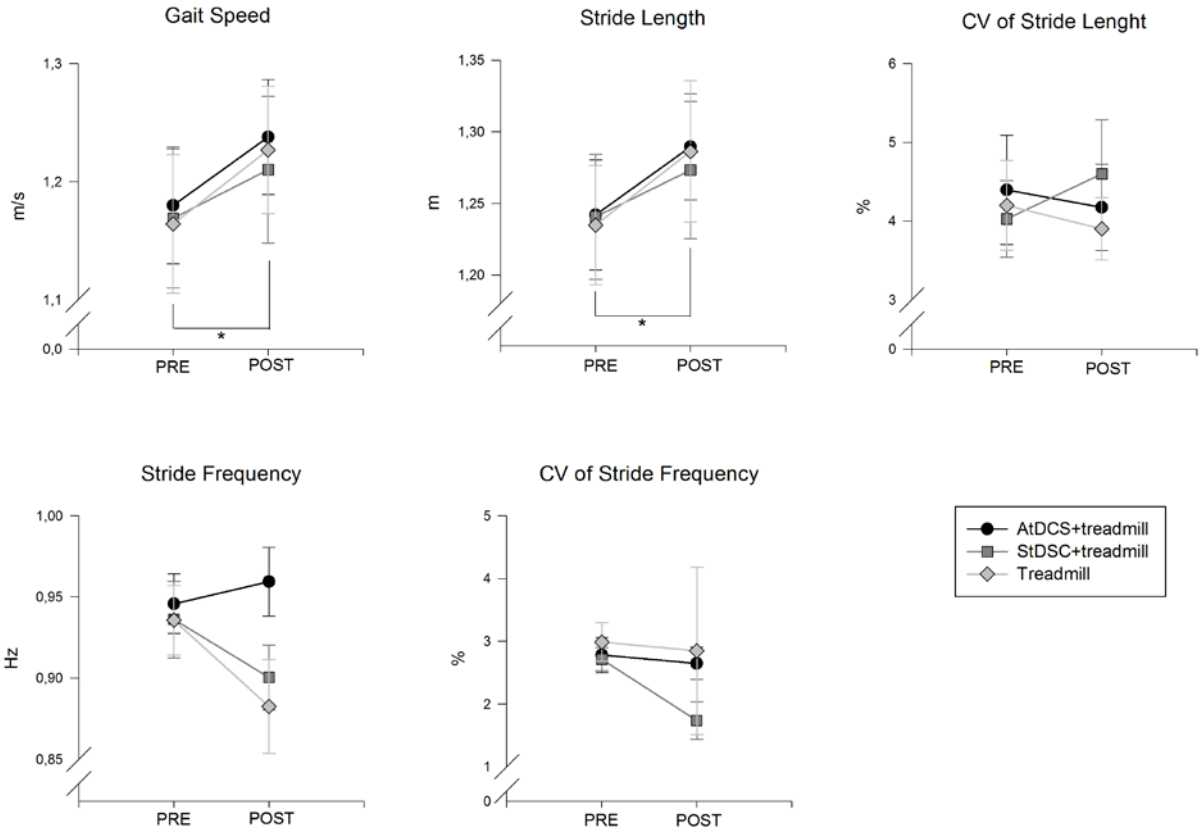


Figure 12 Results of walking performance before and after treadmill walking conditions.

PRE, overground pre-treadmill; POST, overground post-treadmill; *, significant main effect ($p<0.005$).

Neurophysiological measurements

The analysis of the Hmax/Mmax ratio revealed a significant main effect for time ($F=25.451$, $p<0.001$), without a significant effect of treadmill condition nor a significant interaction. The Hmax/Mmax ratio was lower post-treadmill compared with pre-treadmill walking (Figure 13A).

For the reciprocal Ia-inhibition, the ANOVA showed a significant condition×time interaction ($F=4.429$, $p=0.02$). However, there were no significant main effects for treadmill condition and time. Post hoc analysis showed that the reciprocal Ia-inhibition significantly decreased after the StDCS+treadmill ($p=0.019$) and treadmill ($p=0.048$) conditions compared with pre-treadmill walking. No significant reciprocal Ia-inhibition changes were found pre vs post the AtDCS+treadmill condition (Figure 13B).

The analysis of the absolute MEP amplitudes for single TMS pulses did not show significant main effects nor a significant interaction. The analysis of the ICF showed a significant main effect for time ($F=7.506$, $p=0.016$), without a significant effect for the treadmill condition or a condition×time interaction. ICF values decreased post-treadmill compared with pre-treadmill walking (Figure 13C). The analysis of the SICI revealed a significant main effect for time ($F=6.446$, $p=0.024$), without a significant treadmill condition effect nor any significant interactions. The SICI increased post-treadmill compared with pre-treadmill walking (Figure 13D).

The analysis of the H-reflex amplitude during treadmill walking showed a significant main effect for phase ($F=83.824$; $p<0.001$), without a significant effect for treadmill condition or a significant interaction. Greater H-reflex amplitudes were observed during the stance phase compared with the swing phase across treadmill walking conditions (Figure 13E).

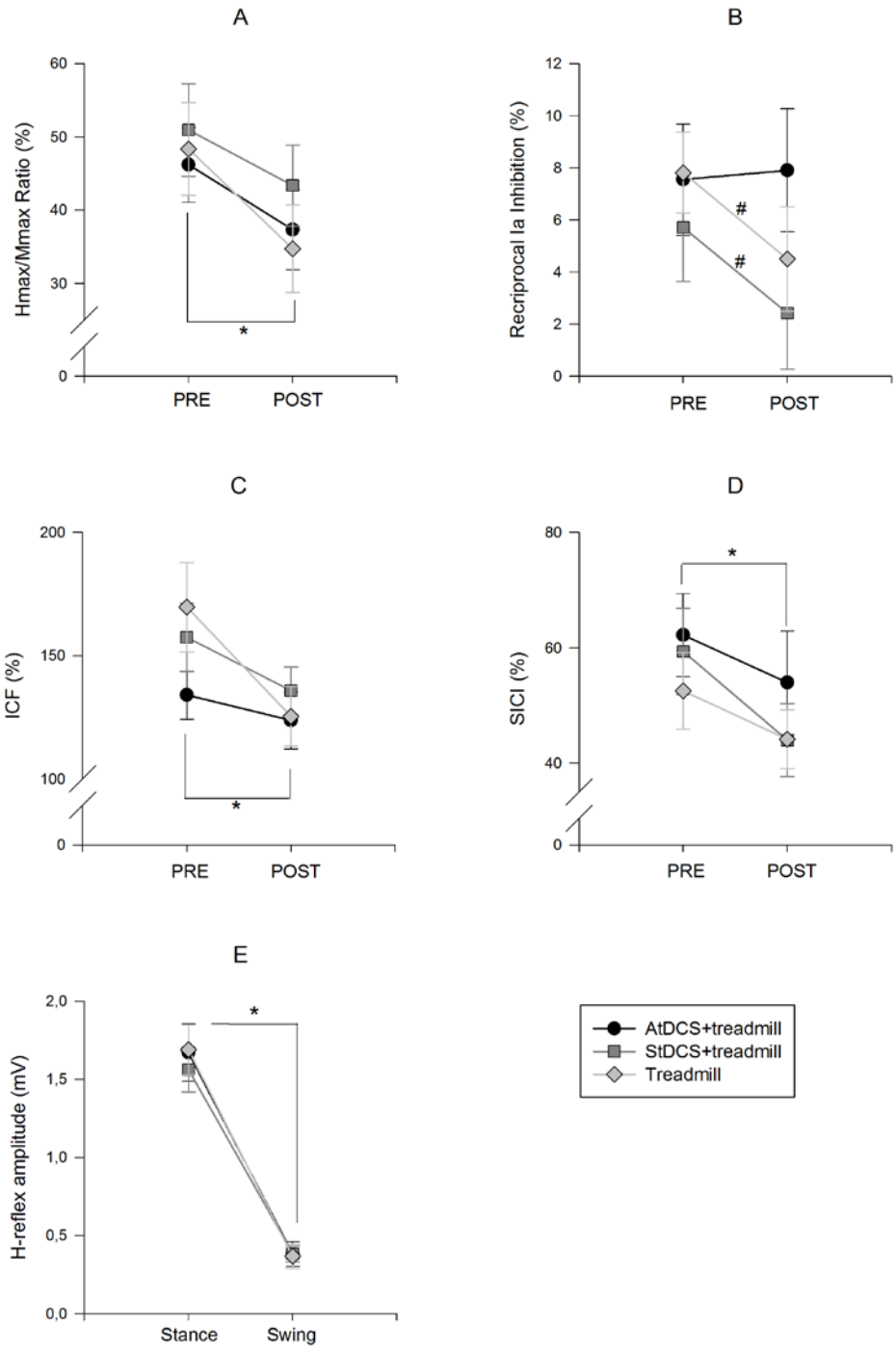


Figure 13 Results of neurophysiological measurements.

A-D: Spinal and cortical measurements at rest. E: H-reflex during treadmill walking. PRE, overground pre-treadmill; POST, overground post-treadmill; SICI, intracorticalinhibition; ICF, intracortical facilitation; Stance, stance phase; Swing, swing phase; *, significant main effect ($p<0.005$); #, significanton post hoc test ($p<0.005$).

4.3.5 Discussion

The current study demonstrated that a single session of treadmill walking combined with anodal tDCS did not enhance the effects of treadmill walking on gait parameters in PD. Concurrently, the application of anodal tDCS to the leg motor cortex during treadmill walking modulated the reciprocal Ia-inhibition reflex in the patients. As far as we are aware, this is the first randomised, sham controlled study that evaluated gait and neurophysiologic effects of treadmill walking combined with anodal tDCS in PD.

The current investigation showed that PD participants increased their overground gait speed and stride length after 20 minutes of three different treadmill walking conditions: treadmill, AtDCS+treadmill and StDCS+treadmill. These results reinforce previous findings showing immediate improvements in gait speed, stride length and double stance support time during overground walking, following a single session of treadmill walking, in PD patients (Miyai et al., 2000; Bello et al., 2008; Kurtais et al., 2008). Moreover, the PD participants in our study walked with longer stride length, reduced stride frequency and reduced gait variability (CV of stride length) during the three treadmill walking conditions, compared with overground walking at the same speed. Only two studies have investigated differences between treadmill and overground walking, showing that people with PD walked with a more stable pattern over the treadmill (Frenkel-Toledo et al., 2005; Bello et al., 2008). Therefore, our data support the evidence that during and after treadmill walking, people with PD improve their gait performance.

Our results showed that the combination of treadmill walking with anodal tDCS stimulation did not lead to enhanced improvements in PD gait (compared with those observed with treadmill walking alone or with sham tDCS stimulation). This finding does not support our initial hypothesis that the combination of both techniques could be a more efficient strategy compared with the single use of a treadmill device. Given the novelty of this approach and thus, the lack of similar studies, we can only speculate as to the explanation for this finding. Several studies have reported that motor training programs with anodal tDCS lead to significantly greater

improvements in motor function compared with motor training alone, in healthy, stroke and PD subjects (Filmer et al., 2014; Kaski et al., 2014; Márquez et al., 2015). Therefore, it is possible that more sessions would be needed in order to obtain an additive effect of the tDCS stimulation on treadmill walking. In addition, several factors related with the tDCS technique could have influenced the current results, such as the electrode configuration/location and the inter-individual variability demonstrated in response to tDCS (Van Asseldonk, 2015).

In addition to the improvements in gait parameters, changes in neurophysiological parameters were, with the exception of the reciprocal Ia-inhibition, comparable across treadmill conditions.

All treadmill conditions lead to SICI increases and ICF decreases in the TA muscle in comparison with values recorded before the treadmill interventions. The increase in SICI suggests that the mechanisms involved in intracortical inhibition are also involved in the gait improvements associated with treadmill walking. Improvements in PD gait together with an increase in intracortical inhibition of the first digital interosseous muscle have been reported following a high intensity treadmill training program (Fisher, 2008). The authors interpreted these changes as a normalization of cortical excitability since SICI is abnormally reduced in PD (Valls-Solé & Valldeoriola, 2002). Although, they related this increment to the intensity of the exercise, it may also have been due to the treadmill walking itself. The decrease in ICF after treadmill walking that we observed is difficult to interpret since a previous study found that ICF reductions in the TA muscle correlated with stride length and velocity impairments in PD (Vacherot et al., 2010a). However, half of the PD subjects included in that study manifested freezing of gait episodes, which reflect a more severe gait impairment than that observed in the participants of the current study. It is possible that the severity of the disease may affect intracortical facilitation mechanisms and could thus account for the contrasting findings. Further studies are needed to explore the relationship between ICF and gait parameters in PD.

We found that the H-reflex modulations were similar across the three treadmill conditions. During all treadmill walking conditions H-reflex

amplitudes were larger in the stance phase compared to the swing phase. In addition, values of the SOL muscle Hmax/Mmax ratio at rest were lower post-treadmill compared with pre-treadmill walking. The modulation that we observed in PD patients during walking is similar to that reported in healthy subjects (Capaday & Stein, 1986). This is in line with previous findings showing that the recruitment curve and the Hmax/Mmax ratio at rest in PD patients are not significantly different from normal subjects (Dietrichson, 1971; Krassoievitch and Tissot, 1971). However, our study is the first study that has recorded the H-reflex during treadmill walking. Our findings suggest that the contribution of this reflex pathway to treadmill walking is not impaired in PD.

The reciprocal Ia-inhibition from the TA muscle to the SOL muscle decreased after treadmill walking alone and after treadmill walking combined with sham tDCS, compared with pre-treadmill walking. This reduction may indicate abnormalities of the spinal interneuron activity in PD that are related to a dysfunction in the descending reticulospinal tract, directly influencing Ia and Ib spinal interneurons (Delwaide et al., 2000). For instance, Meunier and colleagues (2000) found a reduction of the reciprocal Ia-inhibition from the TA to the SOL muscle at the onset of a voluntary ankle dorsiflexion, which correlated with axial signs in PD patients (Meunier et al., 2000a). Interestingly, in our study treadmill walking in combination with anodal tDCS did not induce a reduction in this reflex. This specific effect may result from an interaction between anodal tDCS and treadmill walking since anodal tDCS by itself, applied to the leg motor cortex, decreases reciprocal Ia-inhibition from the TA to the SOL muscle (Roche et al., 2011). However, since the combination of treadmill and anodal tDCS did not lead to enhanced improvements in walking compared with the other conditions, the functional meaning of this reflex modulation remains unknown. Rather than being controversial, we view such puzzling finding as a motivation to keep exploring the combination of treadmill and anodal tDCS in futures studies.

CHAPTER 5.

LIMITATIONS

There are several limitations in this thesis that must be addressed.

- The severity of PD symptoms in participants was moderate. Gait improvements as a result of treadmill training have been shown to be more marked in the advanced stages when compared with subjects with moderate PD (Bello et al., 2008). Moreover, it has been reported that anodal tDCS combined with physical training gives enhanced gait improvements in advanced PD patients (Kaski et al., 2014). There may be a ceiling effect of gait improvements and of neural effects in moderate PD patients. Thus, it would be important to replicate the studies included in this thesis in more advanced PD patients.

- PD patients were tested only in the “on” state. “On” medication may have impacted cognitive and gait performance, since it is known that dual-task walking deficits are improved by anti-Parkinson medications (Lord et al., 2011). In addition, the effects of combined AtDCS stimulation and treadmill walking may not be applicable to patients in the “off” state. However, from a rehabilitation point of view, it is more informative to explore this in the “on” state, rather than in the “off” state.

- In studies 2 and 3 control subjects were not included, since one of the main goals of this study was to compare neurophysiological parameters in PD patients across different walking conditions, rather than with healthy subjects. However, future studies including control subjects are warranted in order to determine whether the spinal and cortical modulations that are associated with treadmill, overground walking and treadmill combined with tDCS, are specific to PD.

- Several neurophysiological parameters (i.e. SICI and ICF) were obtained at rest rather than during walking. However, it was thought that these parameters would be functionally more informative if recorded during movement rather than at rest, as it would have been a methodological challenge to do so while the patients were walking on the treadmill.

CHAPTER 6.

CONCLUSIONS

- Attentional resources do not influence the gait improvements observed in individuals with PD during treadmill walking.

- The improvements of gait in PD patients during treadmill walking are independent of attentional demands to the task of walking.

- The belt displacement and constant speed are the main underlying mechanisms of treadmill walking improvements in PD subjects.

- A single session of treadmill walking, but not of overground walking, led to gait improvements in PD patients, suggesting a specific therapeutic effect of treadmill walking in PD subjects.

- The measurements of spinal and cortical modulations included in this thesis, such as monosynaptic stretch reflex and reciprocal inhibition reflex, as well as SICI and ICF, are not associated with the specific therapeutic effect of treadmill walking in PD patients.

- The modulation of the H-reflex in PD patients during treadmill walking is similar to that reported in healthy subjects, thus the contribution of this reflex pathway to treadmill walking is not impaired in PD.

- A single session of treadmill walking combined with anodal tDCS stimulation of the primary motor cortex does not enhance the improvements of gait parameters associated with treadmill walking in PD subjects.

- The combination of both techniques resulted in a specific modulation of the reciprocal Ia-inhibition from the TA to the SOL muscle. Further studies are needed to explore the functional significance of this interaction.

CHAPTER 7.

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CHAPTER 8.

APPENDIX

8.1 Resumen

La enfermedad de Parkinson (EP) es una enfermedad neurodegenerativa, caracterizada por un conjunto de síntomas motores y no motores. Los trastornos de la marcha se consideran entre los síntomas motores más incapacitantes que afectan severamente a la calidad de vida de los pacientes con EP.

En las últimas décadas, el tapiz rodante ha sido explorado como una herramienta que mejora la marcha de los pacientes con EP. Varias semanas de entrenamiento en tapiz rodante condujeron a mejoras en varios parámetros cinemáticos, tales como un aumento de la longitud de zancada y la velocidad de la marcha, como también una menor variabilidad temporal de la marcha (Bello, 2013, Nadeau Et al., 2014, Tseng et al., 2015). Un estudio reciente mostró que las mejoras de la marcha perduraron incluso 6 meses después del entrenamiento en tapiz rodante (Nadeau et al., 2014). Además de estos efectos a largo plazo, también se han reportado mejoras inmediatas después de una sola sesión de tapiz rodante en la EP (Bello et al., 2008). Conocer los mecanismos subyacentes a estas mejoras, ayudará a aumentar la eficacia y la prescripción de la fisioterapia en la EP. Sin embargo, a pesar de la creciente evidencia que vincula la marcha a la función cognitiva, todavía no sabemos el papel de la atención como mecanismo subyacente a las mejoras de la marcha asociadas al tapiz rodante en la EP. De la misma manera, no se han explorado los posibles mecanismos neurofisiológicos asociados.

Por otro lado, se ha explorado recientemente la estimulación transcraneal de corriente continua directa (tDCS), una modalidad de estimulación cerebral no invasiva, para mejorar la marcha en la EP, ofreciendo una herramienta prometedora para potenciar la eficacia de las estrategias de rehabilitación, además de mejorar nuestra comprensión de la fisiopatología en la EP. Sin embargo, todavía no ha investigado la combinación de tDCS con el tapiz rodante en la EP.

Por lo tanto, pese a la literatura existente en torno al uso del tapiz rodante en la rehabilitación de la marcha en la EP, algunas cuestiones de interés siguen sin resolverse:

1) ¿Las demandas atencionales son un mecanismo subyacente a las mejoras de la marcha asociadas al uso del tapiz rondante en la EP?

2) ¿Cuál es el coste cognitivo de la marcha en el tapiz rodante y en el suelo bajo la condición de doble tarea en la EP?

3) ¿Podría una sesión de tapiz rodante conducir a mejoras en la marcha en pacientes con EP en comparación con una sesión de marcha sobre el suelo?

4) ¿Cuáles son los mecanismos neurales que subyacen a las mejoras de la marcha relacionadas con el tapiz rondate en individuos con EP?

5) ¿Cuáles son los efectos de la combinación de la tDCS y el tapiz rodante sobre la marcha de personas con EP?

6) ¿Cuáles son los efectos neurofisiológicos de la combinación de la tDCS y el tapiz rodante en personas con de EP?

Para responder a estas preguntas se han llevado a cabo tres estudios. Las preguntas 1 y 2 se abordarán en el primer estudio. Las preguntas 3 y 4 se abordarán en el segundo estudio. Las preguntas 5 y 6 se abordarán en el tercer estudio.

8.2 Estudio 1: Patrón de la marcha y rendimiento cognitivo durante la marcha en tapiz rodante en la EP

El objetivo del primer estudio fue explorar si las demandas atencionales están involucradas en las mejoras de la marcha en pacientes con EP cuando caminan en un tapiz rodante.

Para ello se contó con una muestra de 19 individuos con EP idiopático y 19 controles sanos. Los participantes caminaron sobre un tapiz rodante y sobre el suelo bajo las condiciones de tarea simple (caminar solamente) y doble tarea (DT) (caminar realizando una tarea cognitiva simultánea). El paradigma de la DT se utilizó para evaluar a la influencia de la atención sobre la marcha. La tarea cognitiva utilizada fue el "paradigma del monitoreo de fonemas". Éste consistió en contar las veces que se repetían dos palabras, especificadas previamente, en un texto que se escuchaba a través de unos auriculares.

Se midieron la velocidad, la longitud de zancada, la frecuencia de zancada, el CV de la longitud de zancada y el CV de la frecuencia de zancada. También se evaluó el rendimiento cognitivo, medido en función del porcentaje de errores cometidos en la tarea cognitiva. Nuestra hipótesis inicial fue que el desempeño de una tarea cognitiva durante la marcha en tapiz rodante conduciría a un deterioro en una o ambas de las tareas en los individuos con EP. En cuanto al análisis estadístico se utilizó una ANOVA de medidas repetidas de tres factores y comparaciones post hoc, para las variables de la marcha. Se utilizó la prueba de rango de Wilcoxon y la prueba de Mann-Whitney para la variable de no paramétrica del rendimiento cognitivo.

Los resultados revelaron que el grupo con EP caminó con una menor variabilidad temporal y espacial de la marcha sobre el tapiz rodante en comparación con el suelo. Sin embargo, esta reducción de la variabilidad no se deterioró durante la DT. Por otra parte, no hubo diferencias en el rendimiento cognitivo entre el tapiz y el suelo (TablaA1).

Tabla A1. Media, desviación típica y resultados de la ANOVA de las variables de la marcha.

A. Variables de la marcha en los grupos EP y control.

	EP				CONTROL			
	Suelo		Tapizl		Suelo		Tapiz	
	ST	DT	ST	DT	ST	DT	ST	DT
Velocidad (m/s)	1.07 ± 0.23	1.01 ± 0.24	1.07 ± 0.23	1.07 ± 0.23	1.33 ± 0.20	1.25 ± 0.22	1.33 ± 0.20	1.33 ± 0.20
Longitud de zancada (m)	1.18 ± 0.20	1.13 ± 0.20	1.20 ± 0.23	1.19 ± 0.24	1.35 ± 0.17	1.31 ± 0.17	1.34 ± 0.18	1.36 ± 0.16
Frecuencia de zancada (Hz)	0.90 ± 0.07	0.93 ± 0.15	0.90 ± 0.07	0.93 ± 0.09	0.98 ± 0.07	0.95 ± 0.09	1.00 ± 0.09	0.98 ± 0.08
CV de la longitud de zancada (%)	4.27 ± 2.52	4.63 ± 2.44	1.73 ± 0.93	1.54 ± 0.76	2.65 ± 1.00	3.35 ± 1.41	1.56 ± 0.84	1.56 ± 0.83
CV de la frecuencia de zancada (%)	2.60 ± 0.80	2.96 ± 0.94	1.90 ± 1.12	1.88 ± 1.07	1.84 ± 0.53	2.37 ± 0.91	2.91 ± 1.97	2.80 ± 2.01

Los valores son media ± SD. EP, Enfermedad de Parkinson; ST, Tarea simple; DT, doble tarea; CV, Coeficiente de Variación.

B. Resultados de la ANOVA de las variables de la marcha.

	Superficie	Tarea	Grupo	Superficie × Tarea	Surperficie× Grupo	Tarea× Grupo	Superficie × Tarea× Grupo
Velocidad (m/s)	––	F=46.76 P<0.001	F=12.03 P=0.001	––	––	NS	NS
Logitud de zancada (m)	F=8.00 P=0.008	F=21.32 P<0.001	F=6.73 P=0.01	F= 29.77 P<0.001	NS	NS	NS
Frecuencia de zancada (Hz)	NS	NS	F=7.37 P=0.01	NS	NS	NS	NS
CV de la longitud de zancada (%)	F=73.35 P<0.001	NS	F=4.11 P=0.05	F=5.72 P=0.02	F=7.67 P=0.009	NS	NS
CV de la frecuencia de zancada (%)	NS	NS	NS	F=4.65 P=0.04	F=11.71 P=0.002	NS	NS

CV, Coeficiente de Variación; NS, No significativo.

Por lo tanto, los resultados de este estudio mostraron que durante la marcha en el tapiz rodante, los individuos con EP redujeron su variabilidad de la marcha. Redujeron tanto la variabilidad temporal, de acuerdo con estudios previos, como espacial, siendo este el primer estudio que lo indica. Por lo tanto, el presente trabajo extiende los beneficios del tapiz rodante en la EP.

Además, las mejoras de la marcha observadas no se deterioraron con el desempeño de una tarea cognitiva concurrente. Y, por otra parte, el rendimiento cognitivo no mostró diferencias estadísticas entre la el tapiz y suelo. Lo que sugiere que las mejoras la marcha observadas en este estudio en personas con EP no se deben únicamente a los mecanismos atencionales.

8.3 Estudio 2: Efectos cinemáticos y neurofisiológicos inmediatos de la marcha sobre el tapiz rodante y en suelo en la EP

El uso del tapiz rodante como una herramienta de rehabilitación de la marcha ha proporcionado nuevas opciones para el tratamiento de los desórdenes de la marcha en la EP. Sin embargo, los mecanismos neuronales subyacentes a estos efectos terapéuticos en la EP siguen siendo desconocidos.

El objetivo del segundo estudio fue examinar los efectos inmediatos a corto plazo de una sola sesión de marcha en tapiz y de una sesión de marcha en suelo sobre los parámetros espinales y corticales en la EP.

Quince participantes con EP idiopático se evaluaron bajo estas dos condiciones de marcha, en sesiones separadas: marcha en tapiz rodante y marcha en suelo. Se realizaron las siguientes mediciones antes y después de cada condición: se evaluó la cinemática de la marcha en el suelo, el reflejo H de músculo sóleo, el reflejo de inhibición recíproca-Ia del músculo tibial al músculo sóleo, la facilitación intracortical (ICF) y la inhibición intracortical (SICI) del músculo tibial (Figura A1). En cuanto al Análisis estadístico se utilizó una ANOVA de medidas repetidas y comparaciones post hoc.

Figura 1A. Esquema de una sesión experimental del estudio II..

El protocolo de la marcha consistió en 4 bloques (5 minutos por bloque) de narcha en tapiz y marcha en suelo, con tres minutos de descanso entre bloques.

Se observó que la velocidad de la marcha aumentó significativamente después de la sesión tapiz y no después de la sesión suelo. Lo mismo ocurrió con la longitud de zancada, que fue significativamente mayor después de que los participantes caminaran en el tapiz, pero no después de que caminasen en suelo. El resto de las variables de la marcha no mostraron diferencias significativas. Por otra parte, en cuanto a las variables neurofisiológicas, el reflejo H del sóleo, expresado como el ratio entre la onda H máxima de la respuesta motora refleja y la máxima onda M, de la respuesta de las motoneuronas alfa estimuladas directamente, disminuyó significativamente después de ambas sesiones. Lo mismo ha ocurrido con la ICF, cuyos valores fueron menores después de la sesión tapiz y de la sesión suelo. Mientras que la SICI y el reflejo de inhibición recíproca-Ia, no mostraron diferencias significativas.

Según nuestro conocimiento, el estudio actual es el primero en demostrar que 20 minutos de marcha en suelo no produce mejoras en la marcha en los sujetos con EP, sugiriendo un efecto terapéutico inmediato específico del tapiz rodante en la EP. Sin embargo, ambas condiciones de marcha modulaban los parámetros espinales y corticales de una manera similar. Al no haber diferencias entre las superficies, estos hallazgos sugieren que los cambios observados tanto el reflejo H como la facilitación intracortical, se deben al simple hecho de caminar y no están relacionados con las mejoras de la marcha asociadas a una sesión de tapiz rodante en la EP.

Por lo tanto, este estudio proporciona evidencia de un efecto terapéutico específico de una sola sesión de marcha en tapiz rodante en la EP, siendo necesarios más estudios para explorar otros posibles mecanismos neuronales.

8.4 Estudio 3: La marcha en tapiz rodante en combinación con la tDCS en la EP: Un estudio piloto de los efectos cinemáticos y neurofisiológicos

El uso del tapiz rodante como una herramienta de rehabilitación de la marcha y los avances en la estimulación cerebral no invasiva han proporcionado opciones nuevas y de bajo riesgo para el tratamiento de los desórdenes de la marcha en la EP.

En el presente estudio se ha investigado si la tDCS en combinación con el tapiz rodante potencia las mejoras en la marcha asociadas a caminar sobre este dispositivo en la EP. Se exploraron los efectos de la terapia combinada en los parámetros cinemáticos de la marcha y en los neurofisiológicos, tanto espinales como corticales.

Se evaluaron 18 participantes con EP idiopático en sesiones separadas, bajo tres condiciones de marcha en tapiz: marcha en tapiz rodante solamente (tapiz), marcha en tapiz rodante combinada con tDCS anódica (AtDCS + tapiz) y marcha en tapiz rodante con tDCS simulada (stDCS + tapiz rodante). Se midió la cinemática de la marcha en suelo, el reflejo H del SOL, el reflejo de inhibición recíproca-Ia del músculo TA al SOL, la ICF y SICI del músculo TA. También se evaluó el reflejo H del SOL y la cinemática de la marcha sobre el tapiz rodante. En cuanto al análisis estadístico se utilizó una ANOVA de medidas repetidas y comparaciones post hoc.

En cuanto a los resultados de la cinemática de la marcha, se observó que la longitud de zancada aumentó significativamente durante la marcha en tapiz, en comparación con la marcha previa en suelo, sin distinción entre las sesiones. Mientras que el CV de la longitud de zancada disminuyó durante la marcha en tapiz en comparación con la marcha en suelo medida previamente, para todas las sesiones.

Por otra parte, se observó que la longitud de zancada y la velocidad de la marcha aumentaron significativamente después de las sesiones para todas las condiciones.

Por lo tanto, estos datos apoyan la evidencia de que durante y después de una sesión sobre el tapiz rodante, las personas con EP mejoran el rendimiento de su marcha. Sin embargo, la combinación de marcha en tapiz rodante y tDCS anodal no condujo a unas mejoras mayores.

En cuanto a los parámetros neurofisiológicos, observamos que la ICF disminuyó significativamente después de todas las sesiones en comparación con antes, mientras que el SICI aumentó significativamente después de todas las sesiones en comparación con antes. El reflejo H, expresado como el ratio entre la onda H máxima y la onda M máxima, disminuyó significativamente después en comparación con antes, de nuevo sin distinción entre las sesiones. De nuevo, la excitabilidad del reflejo H durante la marcha en tapiz mostró un comportamiento similar entre las tres sesiones. Por lo tanto, todas las condiciones de marcha en tapiz mejoraron la cinemática de la marcha y modularon los parámetros espinales y corticales de manera similar

Sin embrago, la inhibición recíproca del músculo TA al SOL disminuyó significativamente después de la sesión tDCS simulada y de la sesión tapiz solamente. Pero, curiosamente, la sesión combinada con tDCS anodal, no indujo una reducción en este reflejo. Este efecto específico puede ser el resultado de una interacción entre el tDCS anodal y la marcha en tapiz rodante.

Por lo tanto, a pesar de que una sola sesión combinada de marcha en tapiz y tDCS anodal no potenció las mejoras de los parámetros de la marcha asociados con la marcha en tapiz en la EP, la modulación específica del refejo de Inhibición Recíproc-Ia apunta a una interacción de los efectos de estas herramientas. Se necesitan más estudios para explorar el significado funcional de esta interacción.

8.5 Conclusiones

- Los recursos atencionales no influyen en las mejoras de la marcha observadas en los individuos con EP cuando caminan sobre un tapiz rodante.
- Las mejoras de la marcha en pacientes con EP durante la caminata en tapiz rodante son independientes de la focalización de la atención sobre la tarea de caminar.
- El desplazamiento de la banda rodante y la velocidad constante son los principales mecanismos subyacentes a las mejoras de la marcha asociadas al tapiz rodante en personas con EP.
- Una sola sesión de marcha en tapiz rodante, pero no de marcha sobre el suelo, condujo a unas mejoras de la marcha en sujetos con EP, lo que sugiere un efecto terapéutico específico del tapiz en la EP.
- Las medidas de las modulaciones espinales y corticales incluidas en esta tesis, como el reflejo de estiramiento monosináptico y reflejo de inhibición recíproca, así como SICI e ICF, no están asociadas con el efecto terapéutico específico de la marcha en tapiz rodante en pacientes con EP.
- La modulación del reflejo H en los pacientes con EP durante la marcha en tapiz rodante es similar a la reportada en sujetos sanos, por lo que la contribución de esta vía refleja durante la marcha en el tapiz rodante no se ve afectada en la EP.
- Una sola sesión de marcha en tapiz rodante combinada con la tDCS anodal aplicada sobre la corteza motora primaria no potencia las mejoras de los parámetros de la marcha asociados con la marcha en tapiz rodante en sujetos con EP.
- La combinación de ambas técnicas resultó en una modulación específica del reflejo de inhibición recíproca Ia del músculo TA al músculo SOL. Se necesitan más estudios para explorar el significado funcional de esta interacción.

8.6 Limitaciones

Hay varias limitaciones en esta tesis que deben ser abordadas.

- La gravedad de los síntomas de la EP en los participantes fue moderada. Se ha demostrado que las mejoras en la marcha como resultado del entrenamiento en tapiz rodante son más marcadas en las etapas avanzadas en comparación con sujetos con EP moderada (Bello et al., 2008). Además, se ha informado de que la tDCS anodal combinado con el entrenamiento físico proporciona mejoras en la marcha en pacientes con EP avanzado (Kaski et al., 2014). Puede haber un efecto techo sobre las mejoras en la marcha y de los efectos neurales en pacientes con EP moderado. Por lo tanto, sería importante replicar los estudios incluidos en esta tesis en pacientes con EP avanzado.
- Los pacientes de EP se sometieron a las pruebas sólo en estado "ON". La medicación puede haber influido en el rendimiento cognitivo y de la marcha, ya que se sabe que los desórdenes de la marcha durante la doble tarea se mejoran con los medicamentos antiparkinsonianos (Lord et al., 2011). Además, los efectos de la marcha en tapiz combinada con la tDCS de tipo anodal no pueden ser aplicables a pacientes en estado "OFF". Sin embargo, desde el punto de vista de la rehabilitación, es más informativo explorar esto en el estado "ON".
- En los estudios 2 y 3 no se incluyeron los sujetos control, ya que uno de los principales objetivos fue comparar parámetros neurofisiológicos en pacientes con EP en diferentes condiciones de la marcha, en lugar de en sujetos sanos. Sin embargo, estudios futuros incluyendo sujetos controles sanos, están justificados para determinar si las modulaciones espinales y corticales son específicas para la EP.
- Varios de los parámetros neurofisiológicos medidos (concretamente, SICI e ICF) se obtuvieron en reposo. A pesar de que se contempló que estos parámetros serían funcionalmente más informativos si se registraran durante el movimiento, supuso un

reto metodológico hacerlo mientras los pacientes estuvieran caminando sobre el tapiz rodante.