Towards Increased Gait Flexibility in Parkinson's Disease

Clinical Implications & Neurophysiological Mechanisms



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"Kennis die je opdoet is sowieso een geweldig iets.
Het blijft altijd bij je en het houdt altijd zijn waarde in je leven."
– Coby Nijmeijer

"Knowledge you gain is a great thing anyway.
It always stays with you and it always holds its value in your life."
– Coby Nijmeijer

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List of Abbreviations

ANOVA	Analysis of variance
BA	Brodmann area
BBS	Berg balance scale
BF	Biceps femoris
BG	Basal ganglia
CBM	Cerebellum
CI	Confidence interval
CoM	Center of mass
CRUNCH	Compensation-related utilization of neural circuits
CV	Coefficient of variation
DBS	Deep brain stimulation
DT	Dual task
EEG	Electro-encephalography
EMG	Electromyography
ES	Effect size
ES (Chapter 5)	Erector spinae
FAB	Frontal assessment battery
FAB-S	Fullerton advanced balance scale
FES-I	Falls efficacy scale – International
fMRI	Functional magnetic resonance imaging
fNIRS	Functional near-infrared spectroscopy
FOG	Freezing of gait
GL	Gastrocnemius lateralis
GLM	General linear model
GLMM	Generalized linear mixed model
GP	Globus pallidus
GPi	Globus pallidus interna
GPe	Globus pallidus externa
HC	Healthy controls
HbO_2	Oxygenated hemoglobin
HHb	Deoxygenated hemoglobin
HOA	Healthy older adults
H&Y	Hoehn and Yahr
IMRAD	Introduction methods results and discussion
IQR	Inter quartile range
LEDD	Levodopa equivalent daily dosage
MCAR	Missing completely at random
MDS-UPDRS III	Movement Disorders Society – Unified Parkinson's disease rating scale part III
MiniBESTest	Mini balance evaluation systems test
MMSE	Mini-mental state examination
MoCA	Montreal cognitive assessment
MSN	Medium spiny neurons
NFOG-Q	New freezing of gait questionnaire
NNMF	Non-negative matrix factorization
PET	Positron emission tomography
PD	Parkinson's disease
PFC	Prefrontal cortex
PMC	Premotor cortex
PPC	Posterior parietal cortex
PwPD	People with Parkinson's disease
RCT	Randomized controlled trial
RF	Rectus femoris
RGPT	Repeated gait perturbation training
ROI	Region of interest
SB	Split-belt
SBT	Split-belt treadmill
SD	Standard deviation
SMA	Supplementary motor area
SMD	Standardized mean difference
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SPM	Statistical parametric mapping
ST	Single task
STN	Subthalamic nucleus
TA	Tibialis anterior
TB	Tied-belt
TBT	Tied-belt treadmill
TFL	Tensor fasciae latae
TMT	Trail making test
VAF	Variance accounted for
VL	Vastus lateralis

Glossary

Adaptive gait or Gait adaptability	A feature of walking capacity, which enables to make a quick initial response and appropriate adjustment of the gait pattern to withstand a sudden change in the environment, f. i. when needing to avoid an obstacle or perform a turn.
Gait adaptation	The concept of learning a new gait pattern during an adaptation paradigm, for instance during prolonged asymmetrical split-belt walking, where over time gait gradually adapts leading to a new symmetrical pattern.
Gait flexibility	The overarching concept of the ability to walk with sufficient flexibility to meet the requirements of the environmental demands in daily life, which results from several components including adaptive gait, gait adaptation, as well as other factors such as cognitive and visual skills, f.i. increasing speed to cross the street, avoiding to hit obstacles and changing direction to reach a goal.
The redundancy phenomenon	The phenomenon based on the notion that the central nervous system has an infinite number of ways to control bodily movements (degrees of freedom), and whereby it is assumed that there is a need to constrain these options to achieve motor efficiency while at the same time preserving motor flexibility.
Muscle synergy	The concept of muscle groups with a common distinct function and activation pattern during a task such as walking, in order to explain the redundancy phenomenon.
Neuromuscular control	The control of movement through coordinated muscle activity, governed by input from the nervous system.

Chapter 1

General Introduction

Preamble

Being able to adjust gait flexibly to sudden changes in our environment is essential during daily life ambulation and is something most of us can do automatically in most situations. For people with Parkinson's disease (PD), however, promptly adjusting gait to an unexpected situation is not so evident, as it exacerbates their existing gait deficits. Therefore, this thesis set out to investigate the potential of split-belt (SB) treadmill training as a new rehabilitative tool to improve gait adaptability in people with PD (PwPD), and to unravel its underlying working mechanisms. This thesis consists of two parts: Part I focusses on the current literature about adaptive gait training and describes the results of a randomized controlled clinical trial on SB-training in PD, while Part II encompasses two more fundamentally-oriented studies investigating the working mechanisms of SB-walking.

The first section of the general introduction will describe the epidemiology, clinical presentation, pathology of PD and the current treatment options. Next, the adaptive gait deficits of PwPD and their repercussions for daily life mobility will be discussed in more detail, as well as, the lack of sufficient rehabilitation interventions aimed at improving adaptive gait. In the second section of the introduction, literature regarding the underlying neural and neuromuscular mechanisms of adaptive gait in PD will be discussed. In the last section, the outline of the different chapters of this doctoral thesis together with its main objectives and hypotheses will be introduced.

Parkinson's disease

Epidemiology

Parkinson's disease (PD) is the fastest growing neurodegenerative disorder in the world and one of the principle causes of disability worldwide [1]. The number of people living with PD has doubled between 1990 and 2015 [1], and is expected to double again to more than 12 million people by 2040 [2]. The incidence of PD increases substantially after the age of 60 [3], which is concerning given the global ageing population [4]. As PD is a progressive disease for which there currently is no cure, its burden on patients, their caregivers and society as a whole increases over time [5,6]. This emphasizes the need for the development of rehabilitation interventions aimed at relieving symptoms and improving daily functioning.

Clinical presentation

James Parkinson first described PD in detail in 1817 in his work the 'Shaking Palsy'. The cardinal motor symptoms of PD include resting tremor ('*shaking*'), bradykinesia (*'palsy'*), as well as muscle rigidity [7]. Bradykinesia refers to slowness of movement [8], and is the hallmark symptom of PD. Bradykinesia, together with one or more of the other cardinal motor symptoms allows for the clinical diagnosis of PD [9,10]. Besides the cardinal motor symptoms, PwPD experience a multitude of gait and balance problems that increase the risk of falls. Indeed, approximately 60% of PwPD report at least one fall per year [11] and 39% even report repeated falls [12]. The characteristic Parkinsonian gait is slow with small, narrow-based and sometimes shuffling steps [13] or even outright freezing of gait episodes, where people try to move but have the feeling that their feet are glued to the floor [14]. Their posture is typically stooped with flexion at the hip and neck and reduced arm and trunk mobility [15,16]. Next to the more well-known motor symptoms, PwPD can also experience difficulty with writing (micrographia), other fine motor skills (e.g. buttoning a shirt), speech (monotonous, soft and unarticulated) and swallowing [17–19].

PD has a very heterogeneous clinical presentation. Indeed, besides motor symptoms, there are also several non-motor symptoms which are often already present in the prodromal phase up to 20 years before the PD diagnosis (**Figure 1**) [20]. Non-motor symptoms are prevalent and can include: poor sense of smell, constipation, sleep disorders, autonomic dysfunction, pain, fatigue, cognitive impairment and psychiatric symptoms comprising of anxiety, apathy, depression and hallucinations [20]. Although these non-motor symptoms often get overshadowed by the more visible motor symptoms, they have a large impact on people's wellbeing [21,22]. Regarding the symptoms discussed above, it is important to note that not everyone will experience all or the same symptoms [23,24]. Often a distinction is made based on the predominance of tremor [20,23]. People with a tremor dominant subtype generally

present with a milder and slower disease progression [20,25]. The non-tremor dominant subtype is often divided into two groups based on the presence of: (1) Rigidity and lack of spontaneous movement or (2) gait problems with postural instability [20].

In the early disease stage (0-5 years post clinical diagnosis), people generally experience mild non-motor and motor symptoms and dopamine replacement therapy relieves symptoms considerably [20]. With disease progression (5-10 years), however, motor symptoms worsen, more non-motor symptoms appear and medication efficacy and reliability decline with increased complications such as motor fluctuations and troublesome dyskinesia [20]. Unfortunately, symptom progression will continue inevitably and eventually leads to considerable loss of independence. In the more advanced stage (usually >15 years), the combination of immobility, non-motor issues and severe complications of chronic medication use makes clinical management often only possible in residential care facilities or professional in-home care [20].

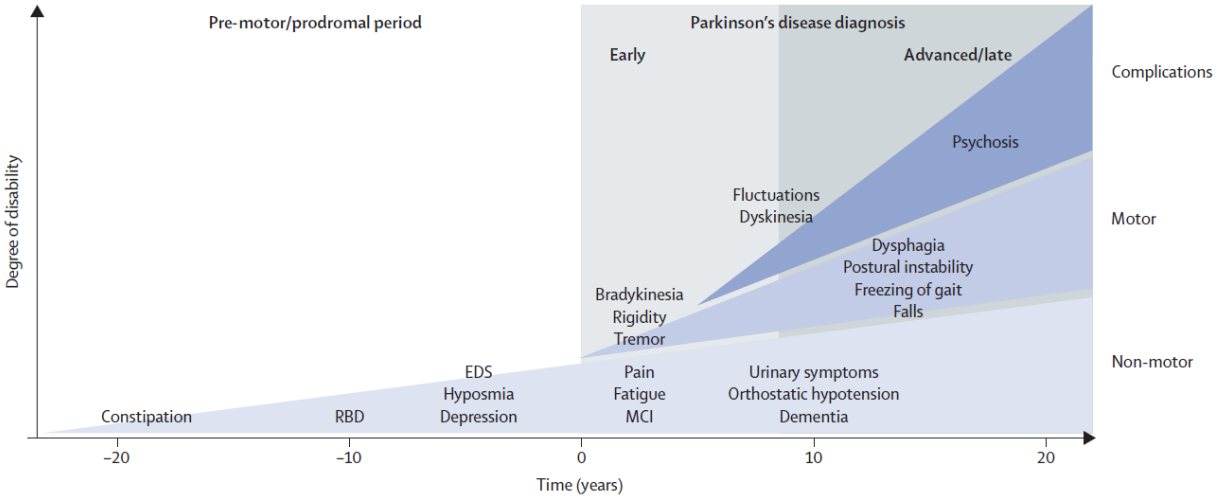


Figure 1. Clinical symptoms and time course of Parkinson's disease progression.
Before the clinical diagnosis is made, people with PD often experience several pre-motor symptoms which can already present up to 20 years before the onset of cardinal motor symptoms. Over the disease course, both motor and non-motor symptoms further develop. In the advanced stages complications due to medication are frequently encountered.
RBD, rapid eye movement (REM) sleep behavior disorder; EDS, excessive daytime sleepiness; MCI, mild cognitive impairment. Source: Kalia and Lang, The Lancet 2015 [20].

Pathology

The most distinct pathological feature of PD is the progressive loss of neurons in the substantia nigra pars compacta that produce the neurotransmitter dopamine. The neuronal loss is associated to the aggregation of misfolded α-synuclein protein, the deposits of which are called Lewy bodies [26]. There are likely multiple factors contributing to this cell death including other misfolded proteins (e.g. Tau, β-amyloid), mitochondrial dysfunction, neuroinflammation and impaired cellular transport processes [20]. The progression of PD pathology throughout the

brain has been described based on the location of these misfolded proteins by Braak and colleagues (2003) [26]. It is thought that the initial aggregations (Braak stage 1) occur in the brain stem and the olfactory nucleus [26], explaining why people can experience constipation, RBD, depression and hyposmia years before the diagnosis. The pathology progresses upwards via the pons, affecting the midbrain and SNr, and eventually almost all brain regions, including the neocortex [26]. Motor symptoms start to occur in stage 3, when the Lewy bodies progressed to the mid-brain, including the Subtantia Nigra pars compacta (SNc), affecting dopamine production [26]. However, the brain is incredibly resilient as symptoms only start to emerge after 50-80% of the dopaminergic neurons are lost [27,28]. It is important to note that several other neurotransmitter systems get affected by PD, including glutamate, norepinephrine, GABA, serotonin and acetylcholine [20,29] and contribute to the disturbed signal processing and divers symptoms described earlier. The Braak hypothesis assumes a 'brain-first' propagation of pathology. More recently, another hypothesis on the spread of pathology has emerged, the so called 'body-first' propagation model [30]. This model poses that pathology ascends from the gut via the vagus nerve to the central nervous system. Intensive study is ongoing to verify whether these different pathological routes underlie different subtypes of the disease [30].

Disturbed basal ganglia circuits

The basal ganglia consist of several structures including the striatum (caudate, putamen, nucleus accumbens), globus pallidus (GP), subthalamic nucleus (STN) and the SNc. The loss of available dopamine in the SNc leads to disturbances in signal processing within the cortical-basal ganglia-thalamic-cortical loops (**Figure 2**) [31,32] that are important for selective movement control [31]. The main input structure of the basal ganglia is the striatum, where widespread signals from the motor cortex are received. The main output structures of the basal ganglia are the globus pallidus interna (GPi) and substantia nigra pars reticulata (SNr) sending inhibitory projections to the thalamus. During rest the GPi and SNr can be seen as the brake for movement execution, which requires striatal innervation to be released and allow for movement to occur. The main function of the basal ganglia is to inhibit unwanted motor commands allowing for the execution of selective motor behavior.

From the striatum two routes depart, namely (1) the direct pathway and (2) indirect pathway. The direct pathway is pro-kinetic and runs directly from the striatum via the GPi/SNr to the thalamus. When the motor cortex sends a movement signal, the striatum sends inhibitory signals to the GPi and SNr, thereby silencing the movement inhibitors and allowing for the motor command to pass through to the thalamus and brainstem. The indirect pathway is anti-kinetic and runs from the striatum to the globus pallidus externa (GPe) and the STN, which (indirectly) stimulates the GPi and SNr further increasing their inhibitory output that suppresses

movement execution. The indirect pathway is particularly important for adapting gait to external demands, which requires a greater deal of selective motor control, whereas the direct pathway is important for more stereotypical unperturbed gait [33]. Lastly, there is a hyper-direct pathway with projections from the neocortex to the STN and brainstem locomotor regions, allowing for responses to be quickly suppressed during moments of processing conflict [34,35].

Dopamine is essential for modulating these pathways by increasing direct and decreasing indirect medium spiny neurons (MSN) activity. In PD, due to dopamine loss the MSN do not get sufficient input causing an imbalance between the two pathways. As the indirect pathway has higher affinity for dopamine, than the direct pathway, [36] the net result is an over-inhibition of motor output in PD. This exaggerated inhibition is at least partially responsible for many of

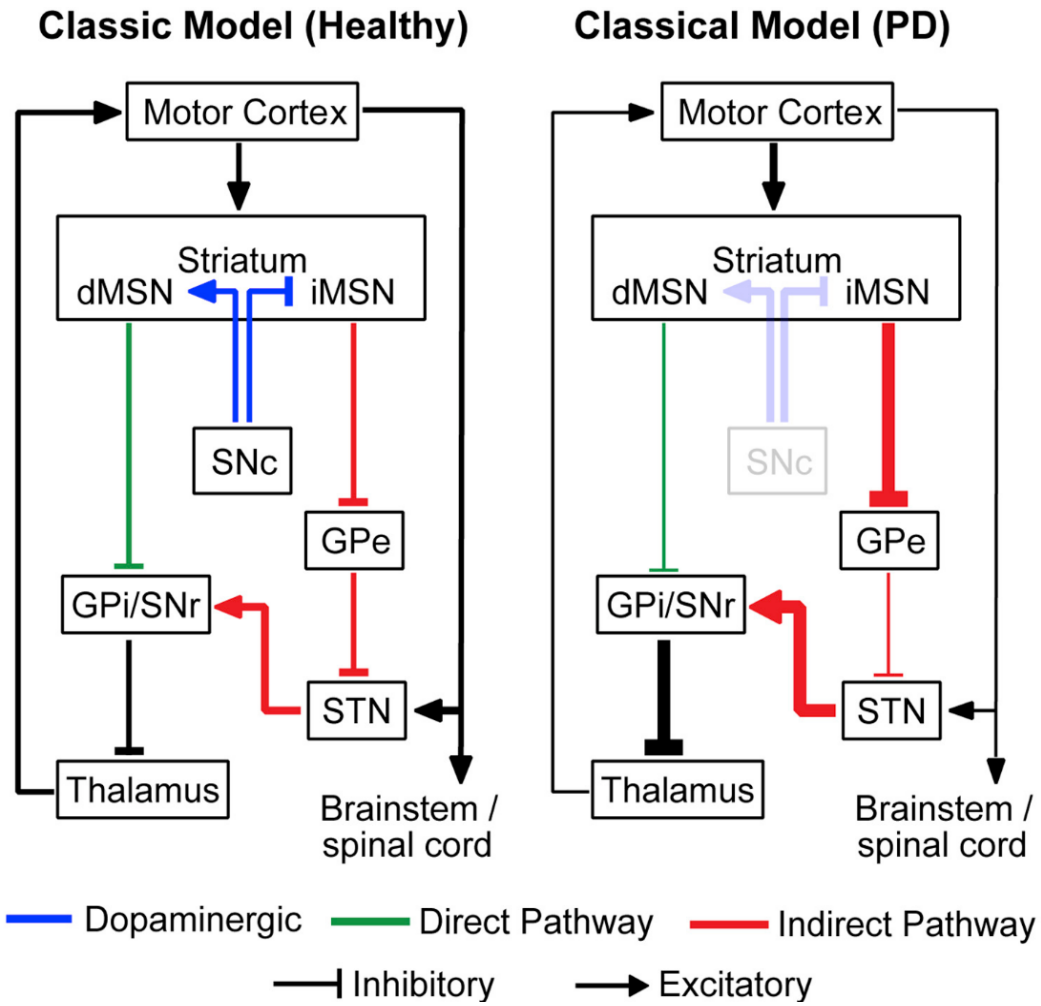


Figure 2. Classical Model of the Basal Ganglia (here only represented for the motor loops). In the healthy condition, dopamine (blue) from the SNc to the striatum activates the direct pathway (green) and inhibits the indirect pathway (red) medium spiny neurons (dMSN or iMSN). The hyper-direct pathway is represented by the arrow from the motor cortex to the STN. Inhibitory pathways are displayed as ⊣ and excitatory pathways as →. This effect decreases GPi output, releasing inhibition on the thalamus and cortex and promoting movement. In the PD condition (right), loss of SNc dopamine causes hypoactivity of the direct pathway and hyperactivity of the indirect pathway that leads to excessive GPi output (thick black line). As a result, over-inhibition of the thalamus and cortex leads to a suppression of movement. Adapted from McGregor and Nelson [32].

the symptoms in PD, including bradykinesia [37], coinciding with the subjective reports of PwPD that they feel as if they are moving with the brake on.

The dopaminergic denervation does not affect the entire striatum evenly. The SNc neurons projecting to the posterior striatum are affected first and more severely than the neurons projecting to the anterior striatum [38]. This has a differential effect on the principles of motor learning that rely on striatal functioning and resulting behavior [38,39]. The affected posterior striatum is implicated in the late stage of motor learning, namely the automatization of learned behaviors [38]. As a result, people with PD experience problems with motor automaticity that affects much of their behavior. The anterior striatum is connected to the prefrontal cortex and involved in the early stages of motor learning stage requiring a high degree of top-down motor control [39]. As a result, people with PD inherently switch from an automatic- to a goal-directed motor control even for otherwise habitual tasks such as gait [40]. This reliance on attentional resources makes them highly susceptible to dual-task interference and an increased risk of falls [38,41–43]. Due to their posterior striatal deficits PwPD also have difficulty consolidating newly learned motor tasks, which reduces the long-term effect of motor training [39,44].

Treatment

There is no cure for PD so currently only symptomatic treatments are available. The mainstay treatment is dopamine replacement therapy [45], where the shortage of dopamine in the basal ganglia is partially compensated for by intake of a synthetic equivalent. The mainstay agent is Levodopa, which can cross the blood-brain barrier to be synthesized as dopamine in the striatum. Levodopa is typically effective in improving the cardinal motor symptoms, as well as, several gait features such as gait speed, step length and arm swing [46]. However, several complex motor problems are less responsive or can even worsen after Levodopa intake, such as postural control [46]. Unfortunately, the efficacy of Levodopa declines over time, requiring an increase in dosage that eventually induces debilitating side effects, such as unpredictable symptom fluctuations and troublesome involuntary movements (dyskinesia) [47]. Another treatment option is deep brain stimulation (DBS), which is typically targeted at the STN to allow for a suppression of the inhibitory basal ganglia output in PD [45,48]. STN-DBS typically is effective for patients who are Levodopa-responsive, allowing similar symptomatic relief [45,49] with less risk of medication-induced side-effects [48,50]. However, DBS itself is also associated with side effects, such as dysarthria [48]. Finally, similar to Levodopa, DBS is less effective for complex motor problems such as gait and balance [48] and is clearly an invasive treatment choice with strict eligibility criteria that make it not applicable to all patients.

Since medication and surgery do not adequately alleviate complex motor symptoms and are associated with debilitating side effects, neurorehabilitation has emerged as an important add-on therapy in PD management [51]. Several forms of rehabilitation showed promising effects on both motor and non-motor symptoms and quality of life [52–55]. Based on the viewpoint of Ellis & Rochester (2018), physical therapy can be divided in several functional layers (**Figure 3**), each having its own main goal and requiring a different approach to achieve it [56]. At the base is general physical activity, where the main aim is to increase the volume of physical capacity and maintain physical function important for general wellbeing [56]. Next, there is the layer of exercise, which contains targeted and repetitive activities to achieve certain goals, such as treadmill training to increase gait speed or high intensity power training to increase fitness. Lastly, there is motor learning, whereby training is focused on (re)learning specific movement tasks in their entirety, such as gait compensation strategies or fine motor skills such as writing [56]. It is of importance to note, that the presented layers are not hierarchical but rather have a complementary function. For instance, motor learning may benefit from specific exercises, and vice versa. The aim of this figure is to point out that physical therapy is not one entity, but rather consists of different components which to be considered conjunctly [56]. Ideally, every person with PD should get a tailored treatment program based on a unique combination of physical training addressing the different layers of this 'pyramid' which matches their personal needs, and this in combination with pharmacological treatment [7,51,56,57].

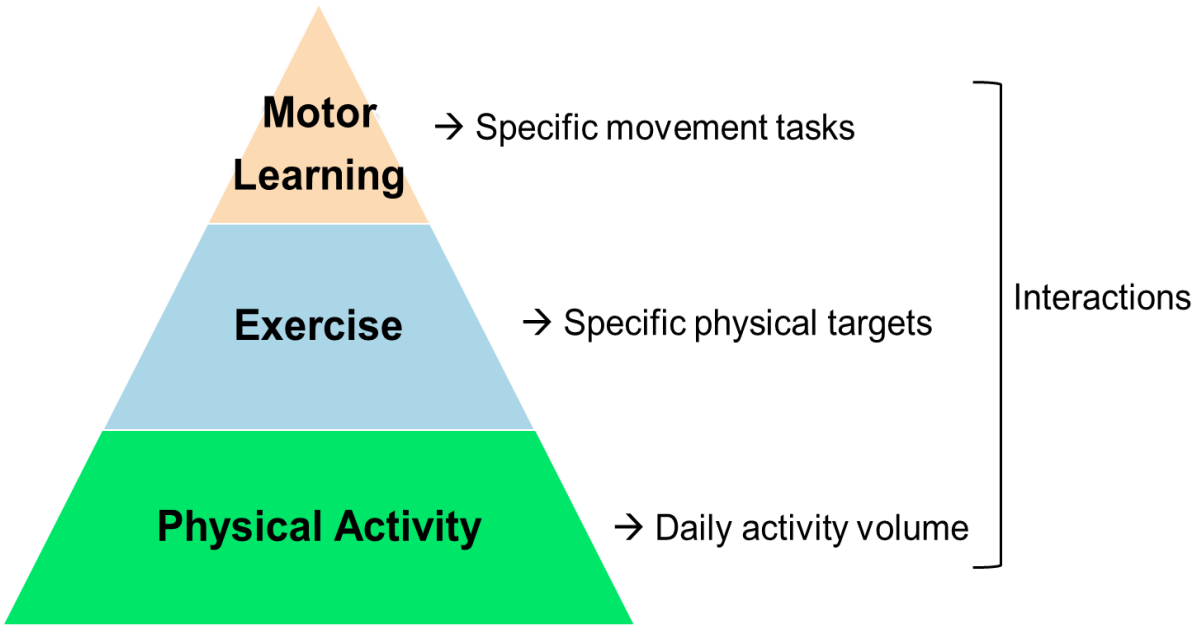


Figure 3. The different layers of physical training.
Physical therapy can be divided in several functional layers, each having their own main goal and grade of specificity. Ideally, every person with PD should get a tailored treatment program based on a unique combination of exercises from the layers of this 'pyramid of physical training' matching personal needs. Of note, the presented layers are not hierarchical in nature but rather reflect complementary components of training.

Adaptive gait deficits and clinical interventions in PD – PART I

In this thesis, we define adaptive gait as the gait feature to produce a quick initial response and adjustment of the gait pattern to withstand a sudden change in the environment [58,59], for instance when avoiding a target or adjusting gait speed. Gait adaptation, on the other hand, is defined as a slower process where a new gait pattern is learned due to prolonged exposure to a new walking condition resulting in a newly adopted gait pattern with sustained after effects [58]. As these two concepts are central to this thesis and are often interchanged in the literature, we added these definitions to the **Glossary** of this thesis. Lastly, as to our knowledge there is no standard definition of gait flexibility, we define it as the result of several components including the ability to achieve adaptive gait and gait adaptation, as well as other contributing factors such as having the cognitive and visual skills to perform gait flexibly while navigating in the daily life context (see **Glossary**).

Gait problems are one of the most common symptoms of PD and have a major impact on quality of life and are a substantial contributor to PD related health care costs [22,60,61]. Several studies have shown that next to the typical reduction in gait speed and step length, PwPD show difficulties with suddenly adapting their gait pattern to challenging and asymmetrical situations (i.e. adaptive gait), for instance when making a turn [62–64] and having to make changes to deal with environmental challenges [65]. For instance, during obstacle negotiation PwPD showed reduced gait speed, step length and balance control before, during and after the crossing, as well as a reduced foot clearance compared to controls [66–69]. During targeted stepping while walking, PwPD demonstrated reduced foot placement accuracy [59,70] and when avoiding unexpected targets they displayed lower success rates [59]. After obstacle avoidance, PwPD had more difficulty returning to their original gait pattern [71]. When turning promptly, PwPD showed a reduction in turning speed as well as an increase in number of steps needed to complete a turn as compared to controls [72]. All these adaptive gait features are essential during daily life ambulation but actual data to characterize these difficulties in daily life, collected with wearable sensors in both PwPD and HC, are currently lacking.

Adaptive gait problems are also a trigger for another gait symptom: freezing of gait (FOG). Based on the ‘threshold model’ to explain the occurrence of FOG [73], subtle gait asymmetry and impaired step coordination were proposed to reinforce each other, reaching a certain threshold of abnormality after which FOG would emerge [73]. FOG can be defined as ‘a brief episodic absence or marked reduction of forward progression of the feet despite the intention to walk’ [14,74]. PwPD often describe FOG as the feeling of the feet suddenly being glued to the floor. FOG is a very debilitating and prevalent symptom (38%-65%) [75], which becomes more common as the disease progresses and is a main contributor to increased fall risk [76–

78]. Since gait becomes less automatic due to the progressive degeneration of the basal ganglia motor loops (**Figure 2**), PwPD become more and more dependent on compensatory cognitive control [33,38,79]. This makes gait less flexible and thus vulnerable to symptoms like FOG and falls, especially during complex and unexpected environmental situations demanding a sudden adjustment of walking. Indeed, Mancini et al. (2018) found that freezers were turning less and with altered patterns than non-freezers during community ambulation, as measured with wearable sensors [80].

There are several experimental methods to assess adaptive gait, including obstacle negotiation protocols either overground or on a treadmill, virtual reality paradigms and using a split-belt (SB) treadmill [81]. The SB treadmill consists of two belts of which the speed of each belt can be controlled separately (**Figure 4**). A change in speed of one belt can therefore induce an unexpected bout of asymmetrical walking, demanding a sudden adaptation of the gait pattern [81]. In contrast to overground adaptive gait protocols, the SB-treadmill also allows practicing adaptive gait in a safe and a standardized environment. Participants can wear a safety harness and the amount and especially the scale of the perturbations can be individually programmed giving the opportunity to carefully study gait responses [81]. On the down-side, this training condition remains artificial and lab-based and transfer to daily life is not guaranteed [82,83].

Originally the SB-paradigm was most often used to study gait adaptation, which, as mentioned above in contrast to adaptive gait, is a slow process of updating gait [58]. During the classic SB-adaptation studies people were exposed to long trials of asymmetrical walking (e.g. 20 minutes) where participants needed to reorganize their standard locomotor pattern to produce a newly learned and efficient gait pattern [58,84–86]. After prolonged SB-exposure the belts were typically returned to a tied (TB) configuration, in which the newly learned gait pattern needed to be unlearned [87]. These studies gave insight into the capability of the central nervous system to adapt and learn new gait patterns. In this thesis and in analogy with studies into the training of skills to resist trips and slips in older adults [88], the focus of training will lie on repeated exposure to gait adjustments to SB-perturbations. We based this choice on the notion that such ‘reactive training’ would possibly enhance the capacity to respond to unpredictive sudden changes in the environment demanding the prompt adjustment of gait rather than learning a completely new motor program. This concept stems from the literature identifying the cerebellum as an important hub involved in gait adjustments [89,90]. Based on the prediction of movement outcomes and the subsequent error-based feedback processing, the cerebellum is thought to steer towards the most optimal motor performance and economy, possibly without extra cognitive involvement [89].

The quality of SB-treadmill gait is often expressed as the amount of step length asymmetry with less asymmetry being interpreted as better performance [81]. Previous work showed that people with mild PD were still able to adapt their gait to SB-perturbations [91,92], although at a slower rate compared to healthy controls [91]. Especially PwPD with FOG presented with impaired adaptive gait on the SB-treadmill, remaining more asymmetrical at the end of the SB-walking phase [63,93]. Additionally, Fasano et al. (2016) reported an association between subjective FOG severity and decreased SB step length, suggesting that people with more severe FOG show a larger step length reduction [93].

Interventions for improving adaptive gait in Parkinson's disease

Several training modalities exist to improve general ambulation in PwPD. Regular treadmill training is a very effective rehabilitation option, capable of improving several gait parameters such as gait speed and stride length [53,94]. However, it is not effective in improving adaptive gait such as turning [94]. Currently, the traditional rehabilitation programs lack this specific focus, which is unfortunate as intact adaptive gait ability is necessary to successfully navigate through ever changing natural environments. Task specific training is particularly recommended in PwPD [95], due to their motor learning difficulties. Therefore, repeated gait perturbation training (RGPT) might be an interesting option as it is based on repeated exposure to deliberately induced gait changes, such as turning and changing directions [96]. The principle of RGPT is based on the fact that participants receive repeated exposure, thereby targeting the ability to modulate gait. RGPT can also be implemented through a push/pull cable system, translations of the walking surface, accelerations and decelerations of the treadmill or changes in gait asymmetry by a SB-treadmill. However, an overview of the current literature on RGPT-interventions and their training effects on gait in older adults, but especially in PwPD is lacking.

An important aspect of training is the transfer of effects to daily life situations. There are some training modalities such as curved treadmill walking, which showed better adaptive gait compared to unspecific exercise, but the impact on overground turning and other adaptive tasks was not investigated [97]. As explained above, apart from studying gait adaptation over a longer period of time, a SB-treadmill can also be used to train adaptive gait by repeated short exposures to asymmetry by frequently changing belt speed ratios as a form of RGPT. A previous proof-of-concept study from our group showed that one single session of SB- training in healthy controls and PwPD with FOG led to improved adaptive gait on the treadmill when compared with traditional treadmill training.

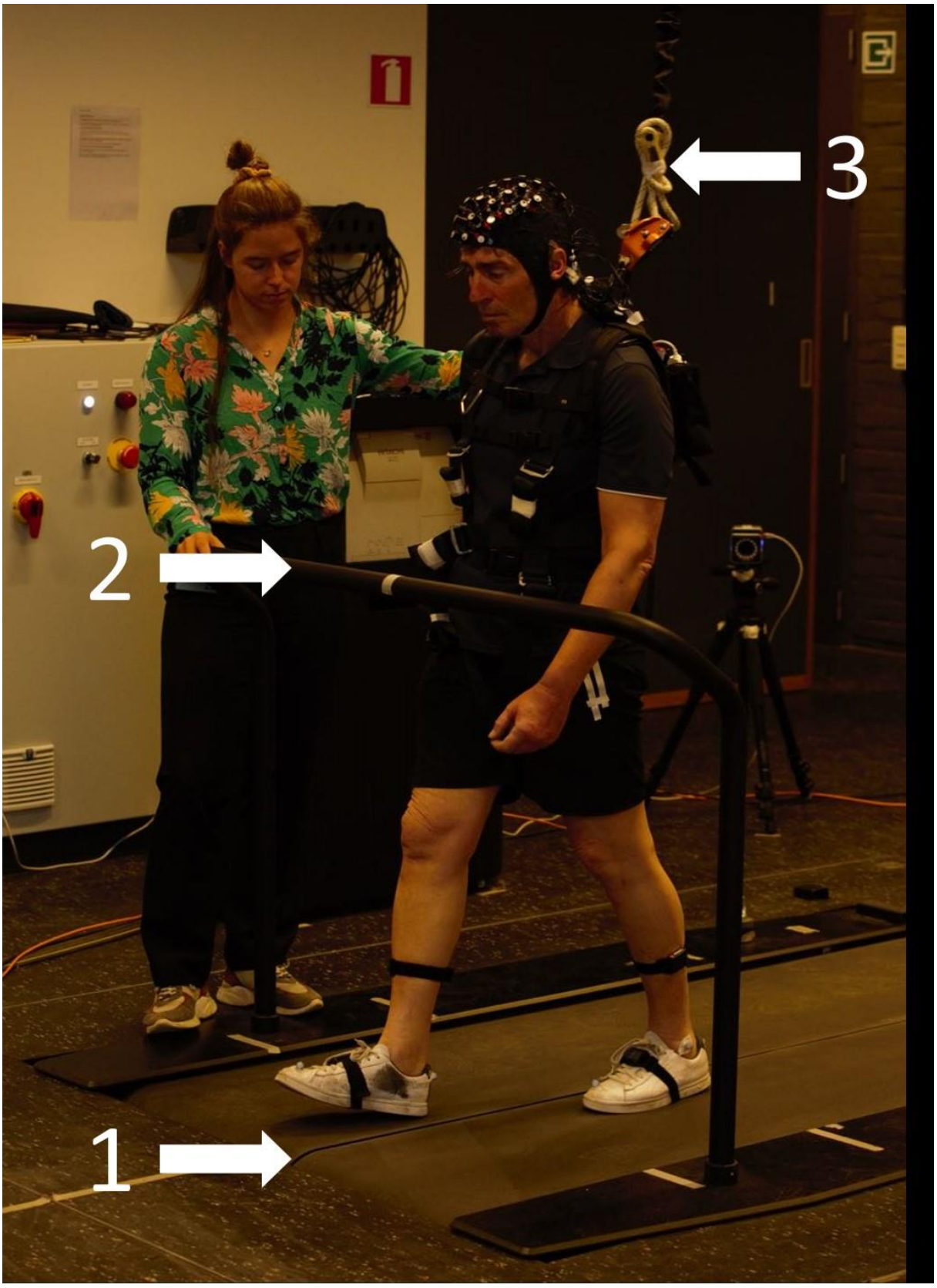


Figure 4. Split-belt treadmill set-up.
The two belts of the treadmill (1) can run at different speeds inducing a bout of asymmetrical walking to which subjects need to adapt their gait pattern. Participants are able to hold the handrails (2) If necessary (discouraged in our studies) and wear a falls harness (3) for safety.
Participant consented for identifiable publication of the picture.

As well, transfer-effects to overground turning speed and FOG were observed [82,83]. More importantly these gains were retained for 24 hours, albeit more prominently in the controls [82,83]. Additionally, the effect of the speed differences between the two belts, also known as contrasts, and the frequency of switches between contrasts were tested. Here, larger contrasts and more frequent switches between contrasts were associated with greater retention of adaptive gait improvements [82,83]. Therefore, SB-training holds promise when offered at optimal dosage and could lead to long-term retention and possibly transfer of effects to adaptive gait tasks relevant for daily life. However, currently it is unknown whether a progressive multi-session training has long-term positive effects in PwPD, when compared with regular treadmill without exposure to perturbations.

Gaps in the literature to be addressed in part 1

1. Currently, it is unclear whether repeated gait perturbation training could improve adaptive gait in both people with Parkinson's disease and older adults **(chapter 2)**.

2. Split-belt treadmill training shows promising short-term effects on adaptive gait parameters both on and off treadmill in PD. However, it is not clear whether a progressive multiple session SB-training can lead to robust and long-term effects when compared with an active control training and whether training effects transfer to overground adaptive tasks such as turning **(chapter 3)**.

Underlying mechanisms of adaptive gait in PD - PART II

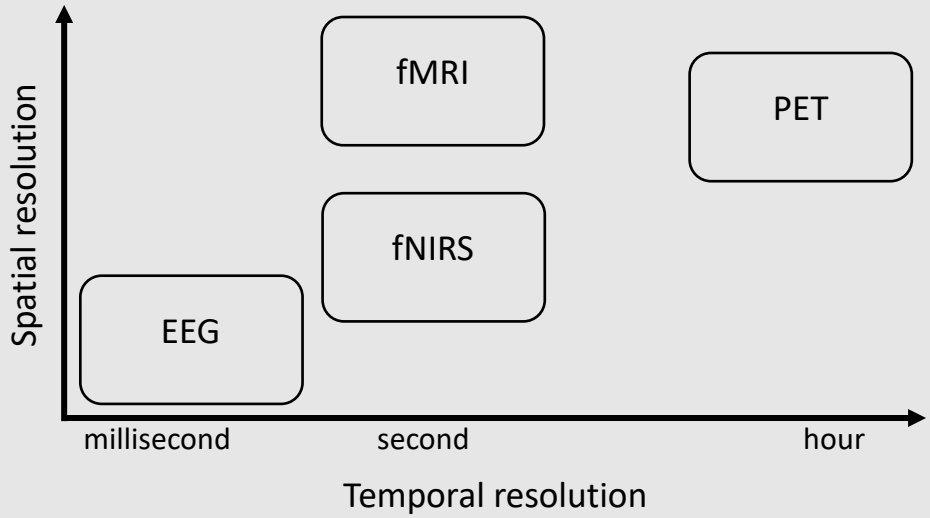
Neural control of adaptive gait

Brain activity can be measured using different modalities. The current gold standard for neuroimaging is (functional) magnetic resonance imaging (fMRI). fMRI is a safe technique offering whole brain imaging including subcortical structures with excellent spatial resolution (see **Box 1A**). It is particularly important when studying the neural control during complex behavior. A recent meta-analysis summarized all fMRI literature regarding gait control in PwPD and compared this to healthy controls [98]. The most robust finding was that a consistent under-activation of the supplementary motor area (SMA) was found during 'gait-simulation' in PwPD as compared to controls [98]. The SMA is an area involved in movement selection and is highly connected to the dorsal putamen in the basal ganglia [99]. Since, the dorsal putamen is affected by the dopaminergic deficit, it produces abnormal input to the indirect and direct pathway, changing the output to the SMA and other motor cortices as well [98]. Another prominent finding was the increased activity reported in the cerebellar locomotor region in PwPD versus healthy controls [98]. The cerebellum is an important hub for adaptive gait as it

integrates sensory information to adjust the motor programs to meet the needs of the environment [100]. It has been suggested that increased cerebellar activity could be compensatory for the lack of automatic movement control [98]. Only one recent MRI study investigated the impact of brain structure in healthy adults and its associations with SB-walking performance [101]. They found that several clusters in the sensorimotor, cerebellar and striatal regions were positively associated with SB-walking performance in both young and older adults [101]. However, as stated by the authors, interpretation of structural results with regards to behavior is difficult and ideally future research would also include functional (fMRI) measures. Adaptive gait has not yet been studied with fMRI, possibly due to the fact that it requires participants to lie absolutely still within a scanner, which hinders studying real gait, and it would require developing a supine task mimicking gait adjustments.

Other neuroimaging modalities such as positron emission tomography (PET) allows for gait tasks to be performed prior to the brain scan. During PET imaging participants are injected with a radioactive tracer which binds to the parts of the brain that are active. Typically, after injection people perform the task of interest after which they are rapidly transferred to a PET scanner. Based on the tracer bindings on these PET images, clinicians can draw conclusions about which brain areas were important for the task performed. Hinton and colleagues (2019) were the first to study brain activity with PET after adaptive SB-walking as compared to regular (tied-belt, TB) treadmill walking in young adults [102]. **Figure 5** illustrates their findings, showing increased activity during SB-walking in the SMA, posterior parietal cortex (PPC) and cerebellum in green colors, and decreased activity in the prefrontal cortex (PFC) and the posterior cingulate cortex in blue. This suggests that together with the cerebellum, the PPC is important for the integration of multisensory information during SB-walking. This coincides with the notion that these brain areas are involved in adapting the original motor program to peripheral sensory feedback until the motor performance is as intended [100,103,104]. Hinton et al. (2019) also ascribed the decrease in activity in the PFC and the posterior cingulate cortex to a switch to automated motor control required for SB-walking [102]. This study is important as it was the first to identify the regions involved in SB-walking. However, as it was only conducted in young adults the impact of ageing and neurological diseases such as PD remained unclear. Although useful, PET imaging is quite invasive and similar to fMRI unable to measure brain activity *during* the actual task (see **Box 1A**). Measuring brain activity during upright walking is essential to increase our understanding of adaptive gait control. **Box 1B** provides an overview of the most important brain regions involved in SB-walking based on the studies of Hinton et al. (2019) and Fettrow et al. (2023) [101,102]. Of note, it is important to acknowledge that these brain regions have diverse functions and roles in different contexts. Therefore, only the general functions relevant for (adaptive) gait are presented in **Box 1B**.

Box 1A. Neuroimaging modalities and their specifications.



Box 1B. Brain regions specifically involved during adaptive split-belt gait based on Hinton et al. (2019) and Fettrow et al. (2023).

Region of interest	Abbreviation	Function during gait
Supplementary motor area	SMA	Needed for motor planning and movement coordination.
Posterior parietal cortex	PPC	Integration of multisensory information from different brain areas to adjust the ongoing motor plan in close collaboration with the CBM.
Premotor cortex	PMC	Responsible for sensory guidance of movement control, releasing motor program initiated by SMA.
Prefrontal cortex	PFC	Involved in attention and cognition and is responsible for decision making, planning, anticipation both before and during movement.
Cerebellum	CBM	Integration of multisensory information from different brain areas and the periphery to adjust the ongoing motor plan to context changes.
Basal ganglia	BG	Important for automatic movement control via movement selection and motor learning.

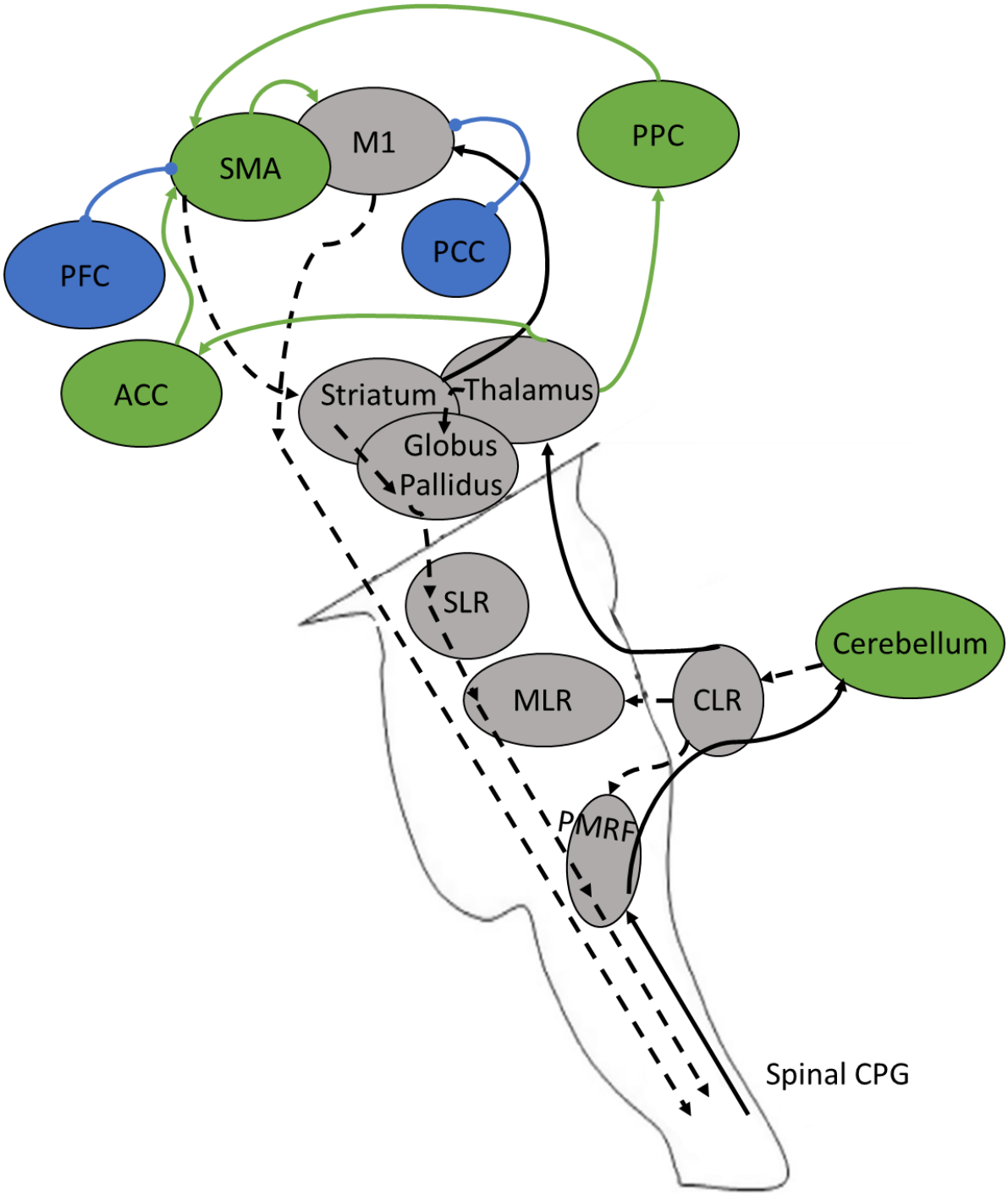


Figure 5. The "fine-tuning" network of locomotion by Hinton and colleagues (2019).
The adaptive gait network identified by Hinton and colleagues based on PET imaging after SB-walking in young adults. Green areas indicate the need for increased activity whereas the blue areas presented decreased activity during SB-walking as compared to the general areas needed for normal gait which are indicated in grey. mPFC, medial prefrontal cortex; M1, primary motor cortex; SMA, supplementary motor area; ACC, anterior cingulate; PCC, posterior cingulate cortex; PPC, posterior parietal cortex; SLR, subthalamic locomotor region; MLR, mesencephalic locomotor region; CLR, cerebellar locomotor region; PMRF, pontine and medullary reticular formations; CPG, central pattern generators.
Figure adjusted from Hinton and colleagues, Neuroimage 2019 [102].

Currently two techniques allow mobile brain imaging: electroencephalography (EEG) offers a direct measurement of neural activity, whereas functional near-infrared spectroscopy (fNIRS) comprises an indirect method [105]. **Box 1A** illustrates that both methods have their limitations on the spatial (EEG) and temporal (fNIRS) resolution spectrum. Both techniques are limited to capturing the superficial cortical areas [105,106]. Since several cortical areas are crucially implicated in adaptive gait, both EEG and fNIRS are useful in identifying the neural control of SB-walking during mobile conditions.

For this doctoral thesis fNIRS was applied as it is a particularly suitable technique for clinical research. It is easy to apply and more resilient against movement artefacts compared to EEG [106]. Similar to the gold standard fMRI [107,108], fNIRS captures the change in hemoglobin concentrations called the BOLD-response which was validated against fMRI derived BOLD-values [109]. Nowadays, fNIRS systems allow for the measurement of multiple regions of interest (ROI), albeit still limited compared to fMRI. For detailed discussion, see Supplementary materials of **Chapter 4**.

Several fNIRS studies have investigated overground gait and turning in PwPD and healthy controls [79,110–114]. However, most of these studies only measured the PFC, most likely due to ease of set-up and system constraints. Regarding PFC activity in PD, inconsistent results were reported, possibly due to the different tasks and protocols used [79,110–114]. One study by Pelicioni and colleagues (2022) included several ROI during adaptive overground gait tasks in PwPD and controls [114]. Here, brain activity was compared between regular overground walking (simple task) and stepping on or avoiding targets while walking (adaptive task). Overall, both groups showed an increase in activity during the more complex task, although this was mostly driven by the control group. This result points towards the inability of PwPD to increase SMA activity as reported by earlier MRI studies [98]. Interestingly, PwPD showed increased activity in the premotor area (PMC) during both the simple and adaptive task as compared to the controls. As the PMC is important for movement selection and execution in response to external input (see **Box 1B**) the authors suggested that the increased cortical activity found might be compensatory [114]. In addition, controls increased PFC activity (see **Box 1B**) with task complexity, whereas PwPD did not, indicating that PwPD might have used all their cognitive reserve during the simple gait task, precluding an increase during the complex task [114]. Although PwPD had worse gait performance compared to the controls, it is important to note that the authors did not correlate gait performance with brain activity outcomes thereby limiting its clinical interpretation [114]. As only a handful of studies included multiple ROIs, while using mobile brain imaging during adaptive gait protocols, our knowledge about the impact of PD on the neural control of adaptive gait is limited, pointing to the need for this study.

Neuromuscular control of adaptive gait

A recent review by Islam and colleagues (2019) provided a systematic overview of 16 studies on how muscle activity, as measured with electromyography (EMG), influences walking deficits in PD [115]. The studies that could be included were still in the proof-of-concept phase, involving small samples, a low number of muscles and weak methodological quality [85]. The authors also noted that the EMG results were not sufficiently related to clinical measures of gait, hampering the translation of the results to clinical practice [85]. The authors observed that in general PwPD present decreased activity in the distal musculature including the calf muscles (gastrocnemius lateralis/medialis) important for push-off and the shin muscle (tibialis anterior) important for lifting the foot at toe-off and before heel strike [116–118]. In addition, the tibialis muscle became prematurely active just before the onset of freezing [119]. This abnormal muscle activity translated to decreased gait speed [115] and an increased risk of falls due to foot drop [120]. In contrast, the proximal muscles (quadriceps, biceps femoris) were more active in PwPD [116,121,122], possibly to compensate for the diminished activity in the distal musculature [115]. Medication [116,117] seemed to promote distal muscle activity, where DBS [123,124] also improved proximal muscle activity. There is one study by Dietz and colleagues (1995) in which muscle activity of the tibialis anterior and gastrocnemius lateralis was captured during SB-walking in PwPD and controls [125]. The authors reported that in general PwPD showed reduced modulation and increased co-activation of the two muscles during SB-walking as compared to controls [125]. As this study only analyzed two muscles, it precludes interpretations regarding the possible difference in coordination of neuromuscular control in PD during adaptive walking.

During adaptive gait, muscle coordination needs to accommodate a sudden change in the environment. However, there are more muscles available than necessarily needed to produce movement patterns such as gait. This ‘redundancy phenomenon’ was first described in the sixties by Nikolai Bernstein [126]. How the central nervous system deals with the vast amount of movement possibilities is still up for debate, since the precise identification of this mechanism has not been established yet [127,128]. One well-known hypothesis proposes that to reduce the degrees of freedom in the motor system, the central nervous system adopts so-called muscle synergies, meaning that muscles are clustered in certain functional groups (synergies). As such, movement control is being governed by a smaller number of parameters reducing complexity [126,127]. Speculatively, therefore, muscle synergies may allow more efficient and simplified neuromuscular control by activating groups of muscles with a similar function and/or timing within a certain task [129,130]. In a review by Tresch and Jarc (2009), the evidence for and against the concept of synergies was summarized. As a final consensus was not reached, careful interpretation about the exact meaning of synergies is still warranted

[127,128]. However, as mentioned by Mulla and Keir (2023), although the exact neural origin and working mechanisms of muscle synergies are unknown, “it appears that the neural circuitry provides at least some level of constraint on the voluntary control of muscles” [128], making synergy analysis an interesting approach with which to explore motor control. Indeed, various studies on gait showed that a small number of synergies could explain a substantial portion of the overall EMG signals [129,131–133]. Studying muscle synergies, compared to individual muscle patterns, gives the opportunity to investigate the complexity of movement coordination within the neuromuscular system at large [134]. Previous studies showed that neuromuscular complexity is lower in clinical compared to healthy populations, including stroke survivors, people with cerebral palsy and PD [134–137]. Reduced neuromuscular control has been associated with worse task performance, pointing towards a less flexible movement strategy compared to healthy peers [134–137].

During overground walking, healthy young adults generally use five synergies, which accounts for 95% of the original EMG signal variance (%VAF) [132]. During SB-walking, young and middle-aged adults use three to four synergies [131,138], suggesting a limited effect of early ageing [138]. In general, these studies report that the synergies showed more activity during the initial adaptation to SB-walking compared to the later stages [131,138], and some activity peaks occurring earlier during SB- compared to TB-walking [131]. It is important to note that the precise number of synergies identified depends on several factors such as the %VAF criterion and the number and location of the muscles included [139,140]. Currently, no studies have been conducted investigating the response of PwPD to adaptive gait tasks such as SB-walking using an EMG-analysis. Two studies reporting simplified neuromuscular control in PwPD during regular treadmill walking, implying that they used less synergies compared to controls [134,135]. The simplified control was related to slower gait speed as well as worse PD symptoms, but was not affected by medication state [134,135]. Targeting neuromuscular control in PwPD through Tango dancing classes was effective in improving the consistency of muscle synergy recruitment, which was suggested to be related to better gait and balance performance [141]. This suggests that muscle synergies and therefore neuromuscular complexity can be a target for rehabilitation.

Gaps in literature to be addressed in part 2

3. Studies investigating the neural control of adaptive gait have been scarce, pointing to the need to obtain an in-depth understanding of the impact of adapting to split-belt perturbation on various brain regions in PD versus controls **(chapter 4)**.

4. It is largely unknown whether impaired neuromuscular control could be one of the contributing factors to the adaptive gait deficits in PwPD and specifically to the difficulties with responding to a SB-walking task. Knowledge about the muscle synergies involved could help the precise targeting of gait interventions **(chapter 5)**.

Outline of the thesis

Unlike previous studies in which SB-perturbations were used to investigate postural control, this PhD thesis probed the application of SB-perturbations as a paradigm to study gait adaptation in PD. It consists of two parts. In part I the current clinical evidence regarding adaptive gait interventions in PD will be discussed together with the results of a randomized controlled trial testing a newly developed adaptive gait training using SB-treadmill perturbations. Part II will elaborate on the underlying working mechanisms behind SB-training by investigating how the neural and neuromuscular control during adaptive gait is affected by PD.

Part 1: Adaptive gait deficits and clinical interventions in PD

Traditional gait rehabilitation programs do not directly address the adaptive gait deficits experienced by PwPD. Repeated gait perturbation training (RGPT) is a relatively new training modality with the possibility to target adaptive gait via repeated unexpected perturbations during walking. However, the effect of RGPT on gait remains largely unknown.

Therefore, in **Chapter 2** we conducted a systematic review with meta-analysis with the objective to determine whether RGPT could be more effective in improving gait and balance performance in the short and longer term as compared to traditional non-perturbed gait training in PwPD and controls. *We hypothesized that due to its specificity RGPT would be more effective in improving gait performance compared to non-RGPT gait training in PwPD and healthy older adults* [39,142].

One mode of RGPT is SB-treadmill training, in which the walking speed of each leg can be manipulated individually thereby offering the possibility to induce asymmetrical perturbations. Based on earlier pilot work in our lab, showing promising results, we report in **Chapter 3** on a randomized controlled trial with the aim to investigate the short and long-term effects of a progressive 4-week SB-training program as compared to an equally progressive TB-training program in PwPD. *We hypothesized that people in the SB-training group would show greater improvement of adaptive gait performance on the treadmill, as well as greater transfer of those effects to overground turning speed as compared to the TB-training group* [82,83].

Part 2: Underlying mechanisms of adaptive gait in PD

Obtaining an in-depth understanding of the mechanisms underlying adaptive gait deficits in PwPD is essential to optimize training settings and determine important training targets.

As in PwPD and older adults the cortical response to adaptive SB-walking is largely unknown, the aim of **Chapter 4** was to investigate the effect of SB- versus TB-walking on cortical activity

in PwPD and controls. More precisely, we included several ROIs previously implicated to be important in adaptive gait in controls and PwPD, being the PFC, SMA, PMC and PPC.

We hypothesized that an overall increase of activity in all ROIs would be apparent during SB- compared to TB-walking because of the increased adaptive challenge demanded by SB-walking. Additionally, we expected that PwPD would show increased activity in the PMC and PPC compared to controls during SB-walking. Due to the loss of gait automaticity in PD, we predicted less activity changes in the SMA. Because of the reactive and implicit nature of SB-walking, we anticipated no change in the PFC modulation in PwPD [90,143,144].

The impact of SB-perturbations on muscle activity is also largely illusive and could give important insights into how the neuromuscular coordination of adaptive gait in PwPD is disturbed. Therefore, the aim of **Chapter 5** was to study the neuromuscular response to SB-walking in PwPD and controls using electromyography (EMG). We included several muscles that were previously associated with adaptive walking in young and older healthy controls including the tibialis anterior, gastrocnemius lateralis, vastus lateralis, rectus femoris, biceps femoris, tensor fasciae latae and the erector spinae [133,138].

We hypothesized that PwPD would show simplified neuromuscular control during the different phases of TB- and SB-walking as compared to controls [135]*. Secondly, we expected that overall PwPD would show less pronounced activity and would produce a later onset of activity peaks in the different adaptive phases compared to controls in line with earlier work* [135].

Patient participation across the different chapters

Figure 6 provides an overview of the participants that were included across the studies of this PhD. For **Chapter 3**, we recruited 52 PwPD in a two-center RCT, of which 28 were tested in the PRO-lab in Leuven. Important to note is that the study of **Chapter 3** took part during the COVID pandemic, which resulted in 11 participants dropping out as the study needed to be halted due to governmental restrictions. For **Chapter 4**, 42 PwPD and 42 HC were included without overlap with the population of **Chapter 3** to ensure naivety to SB-walking. A sub-group of this cohort, 31 PwPD and 38 HC, opted to participate in the EMG add-on assessment after the fNIRS protocol (**Chapter 5**). During this study we experienced technical hardware problems with the EMG system, leading to data loss of 18 participants and a final population of 24 PwPD and 27 HC.

In all chapters the detailed methodology is reported in each methods section. However, since novel methods for the fNIRS-analysis were used, a more extensive elaboration of this approach is given in the Supplementary Materials of **Chapter 4**.

Overview of participants per thesis chapter

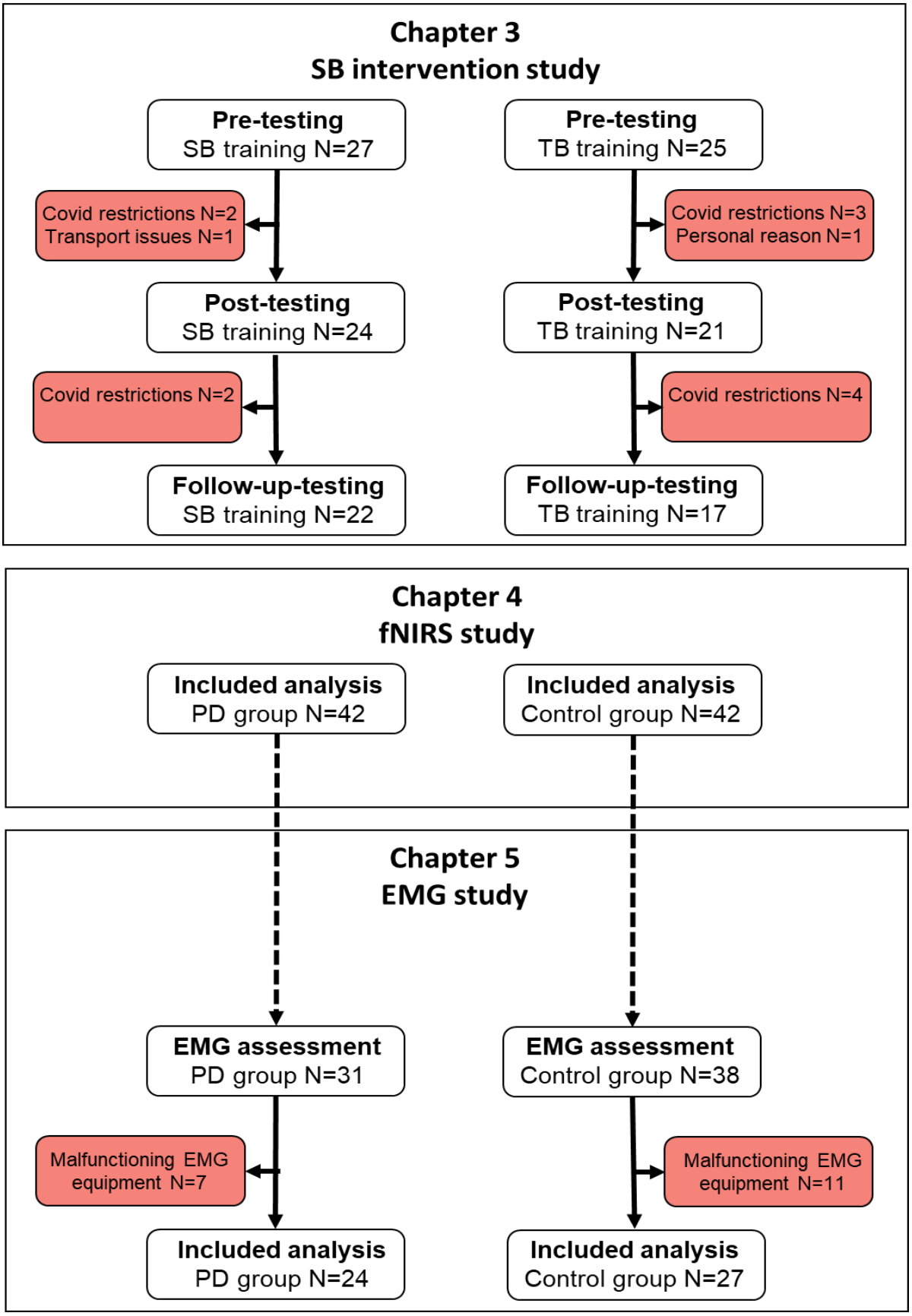


Figure 6. Overview of participants per study across the thesis chapters.
Participants from chapter 3 were not reincluded in chapters 4 and 5. Red boxes indicate the drop-out (chapter 3) and exclusion (chapter 5) of participants with the reasons why.

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Part I

Adaptive Gait Deficits and Clinical Interventions in Parkinson's Disease

Chapter 2

Repeated Gait Perturbation Training in Parkinson's Disease and Healthy Older Adults: A Systematic Review and Meta-Analysis

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Abstract

Background: Gait impairments are common in healthy older adults (HOA) and people with Parkinson's disease (PwPD), especially when adaptations to the environment are required. Traditional rehabilitation programs do not typically address these adaptive gait demands in contrast to repeated gait perturbation training (RGPT). RGPT is a novel reactive form of gait training with potential for both short and long-term consolidation in HOA and PwPD. The aim of this systematic review with meta-analysis is to determine whether RGPT is more effective than non-RGPT gait training in improving gait and balance in HOA and PwPD in the short and longer term.

Methods: This review was conducted according to the PRISMA-guidelines and pre-registered in the PROSPERO database (CRD42020183273). Included studies tested the effects of any form of repeated perturbations during gait in HOA and PwPD on gait speed, step- or stride length. Studies using balance scales or sway measures as outcomes were included in a secondary analysis. Effects of randomized controlled trials (RCT) on RGPT were pooled using a meta-analysis of final measures.

Results: Of the 4421 studies, eight studies were deemed eligible for review, of which six could be included in the meta-analysis, totaling 209 participants (159 PwPD and 50 HOA). The studies were all of moderate quality. The meta-analysis revealed no significant effects of RGPT over non-RGPT training on gait performance (SMD=0.16; 95%CI=-0.18, 0.49; Z=0.92; P=0.21). Yet, in some individual studies, favorable effects on gait speed, step length and stride length were observed immediately after the intervention as well as after a retention period. Gait variability and asymmetry, signifying more direct outcomes of gait adaptation, also indicated favorable RGPT effects in some individual studies.

Conclusion: Despite some promising results, the pooled effects of RGPT on gait and balance were not significantly greater as compared to non-RGPT gait training in PwPD and HOA. However, these findings could have been driven by low statistical power. Therefore, the present review points to the imperative to conduct sufficiently powered RCT's to verify the true effects of RGPT on gait and balance in HOA and PwPD.

Introduction

Gait impairments in the ageing population are related to falls and have other serious repercussions, such as that they are associated with reduced physical activity levels [1,2]. Gait impairments and their negative consequences are further exacerbated in people with Parkinson's disease (PwPD) [3]. The neuropathology of PD progressively affects the locomotor network, particularly the striatal circuitry and alters the cerebellar involvement in adapting gait to environmental changes [4–6]. Gait adaptation is required when the gait pattern needs to be adjusted, for instance, during the transition from straight walking to a turn. Adjusting one's gait to unexpected environmental changes additionally involves reactive postural control, which is also compromised in older adults and especially in PwPD [7,8]. Impairments to adjust gait become more and more apparent in PwPD with disease progression. These impairments also frequent trigger episodes of freezing of gait (FOG) in PwPD [9], thereby further increasing the risk of falling [10,11]. Falls are a major burden for the ageing population and more so in PwPD, where approximately 60% experience repeated falls [12,13]. This poses one of the most important hurdles for clinical management of PwPD, as falls and FOG are largely refractory to medication [14,15]. All of the above stresses the need for training interventions to safely improve adaptive mobility in PwPD and healthy older adults (HOA).

Several training modalities can improve ambulation and thereby decrease the risk of falling [16,17]. Regular treadmill training has been shown to be effective in improving gait parameters, such as speed and stride length in both HOA and PwPD [18,19], especially when a cognitive challenge is added to the motor training [20]. Moreover, combining balance and strength training has shown to bring benefits for mobility and falls [21]. However, these traditional rehabilitation programs do not directly address the typical demands of ambulation in natural environments, particularly with regard to adapting to asymmetrical demands induced by the need to make turns and directional changes [18].

Repeated gait perturbation training (RGPT) is a relatively novel training concept that addresses gait adaptation and reactive balance. RGPT consists of unexpected perturbations, such as push and pulls, applied by a trainer or a cable system during walking. Additionally, novel concepts of treadmill training have emerged under the impetus of technological advances. These modalities include the ability to offer translations of the walking surface [22], acceleration and deceleration of the treadmill or changes in gait asymmetry imposed by split-belt-treadmills, whereby the gait speed of each leg can be controlled independently [23]. Repeated exposure to such perturbations may have lasting effects on the ability to modulate walking and reduce falls [24]. Encouragingly, our group recently evaluated a single session of split-belt training in HOA and PwPD, showing beneficial effects on gait adaptation and turning performance that were retained for at least 24-hours [25,26].

Apart from mimicking daily life mobility, RGPT may prove beneficial by tapping into a more reactive and subconscious way of motor learning. Indeed, PwPD and HOA to a lesser degree, rely heavily on attentional strategies during gait performance as a result of reduced motor automaticity [27,28]. Consequently, they become less able to deal with consecutive attention-requiring environmental demands [29]. A reactive training strategy, such as RGPT, whereby participants need to adapt their gait to sudden perturbations without prior awareness of the precise timing of perturbations is thought to modulate gait automatically via cerebellar locomotor circuits, rather than overloading the cortical frontal and anterior-BG attentional reserves [29,30]. In line with this notion, Marinelli et al. (2017) proposed that training, which is not relying on attentional strategies or conscious awareness of the learning process, may still be preserved in PwPD (in some paradigms) due to cerebellar compensatory contributions [31]. As such, RGPT training may boost the compensatory cerebellar circuits, reducing attentional demand during adaptive gait in PwPD and HOA.

Following the initial reactive response to the perturbation, conscious awareness of the perturbation likely becomes involved to some degree in the control of the subsequent gait cycles. This goal-directed aspect of RGPT likely taps into the anterior BG circuits that are relatively spared in PwPD and may assist in the acquisition of new adaptive gait strategies [31]. Unfortunately, consolidation of new motor engrams is rather impaired in PwPD due to altered processing in the posterior BG circuits [31], and it therefore remains to be determined how well PwPD can retain the beneficial effects of RGPT training. All in all, the training of both reactive and goal-directed processing of gait via RGPT has potential to herald larger effects on gait in PwPD than non-RGPT types of gait training. In the present review, we therefore reviewed the literature to explore the notion that RGPT may lead to improved gait and retention in PwPD and HOA by boosting 'adaptive learning' pathways [31,32].

Previous reviews summarizing the effects of perturbation training focused mainly on young and older healthy adults and a combination of neurological populations (e.g. stroke, Parkinson's disease) [24,33,34]. The meta-analysis conducted by Mansfield et al. (2015), including both older adults and patients with varying neurological disorders, showed that perturbation training could significantly reduce falls when compared to control interventions without perturbation training (RR=0.54; 95%CI=0.34,0.85; P=0.007) [33]. These results were corroborated by Gerards et al. (2017) who concluded in their review that perturbation training is effective in reducing falls, and that treadmill-based systems and therapist-applied perturbations are likely the most feasible approaches for perturbation training [24].

The meta-analysis by Papadimitriou and Perry (2017) further showed that perturbation training reduced falls in the laboratory for both older and younger adults six-fold compared to the non-

perturbation control groups [34]. Taken together, prior reviews indicated beneficial effects of perturbation training for reducing falls. However, no review has focused specifically on gait perturbation training in PwPD. Given that PD is a complex multi-system disorder affecting both motor and non-motor symptoms that respond variably to therapy, generalizability of findings from prior reviews on healthy adults or other neurological disorders to PD is limited. As such, in this systematic review we set out to evaluate the evidence for immediate and long-term effects of repeated gait perturbation training (i.e. RGPT) on gait outcomes in PwPD and healthy older adults. RGPT. Therefore, the aim of this systematic review with meta-analysis is to ascertain whether RGPT is more effective in improving gait performance, expressed here as improvements in gait speed, step or stride length, in HOA and PwPD, as compared to other non-perturbation-based gait interventions. In addition, we aim to assess the impact of RGPT on balance performance as a secondary endpoint. We hypothesized that, via the modulation of 'adaptive learning' circuits and eventually reduced needs for attentional processing, RGPT would prove more efficacious for improving gait performance (i.e. gait speed, step length or stride length), and balance (i.e. MiniBESTest, Berg Balance Scale or postural sway) than non-RGPT gait training in both HOA and PwPD.

Methods

Search strategy and included databases

A systematic search of the literature was conducted on 27 April 2020 in the PubMed, Embase, Medline, Web of Science and Google Scholar databases without date restriction [35]. A final screening for eligible studies was performed on 10 February 2021. The following search syntax was used (Google Scholar example): *(gait OR locomotion OR walk OR walking)* AND *(split-belt OR split belt OR splitbelt OR balance loss OR dynamic balance OR dynamic stability OR surface translation OR trip OR tripping OR slip OR slipping OR pull OR push OR perturbation OR perturbations OR perturbed OR perturb)* AND *(rehabilitation OR repeated OR repetition OR training OR program)* AND *(Parkinson Disease OR ageing OR elderly OR older adults)*. The exact search syntax used for each of the other databases is presented in the supplementary materials. As no consensus exists in the current literature on how to describe 'perturbation' and 'training', we used a broad range of terms in our search syntax to avoid missing eligible studies [36]. In addition to the database search, unpublished data from a study within our research group fulfilling the eligibility criteria, was also added to the analysis [25]. The review protocol was prospectively registered in PROSPERO (CRD42020183273) and the review conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [37].

Study selection

The inclusion criteria for the selected studies were: (1) written in the English or Dutch language; (2) intervention study (RCT and non-RCT) assessing the effect of any type of repeated, and unexpected, perturbations during walking, hereafter called RGPT; (3) presenting outcomes on HOA (mean age ≥65 years) and/or PwPD; (4) measurement of effects right after the last training session and/or retention of effects (≥24hrs after the last training session); and (5) gait speed, step length or stride length obtained as either the primary or secondary outcome. Exclusion criteria were: (1) not peer-reviewed; (2) conference abstracts; (3) reviews of the literature, with or without meta-analysis or commentaries without original data; (4) perturbations not given during gait (e.g. static or optical); (5) gait only assessed during the baseline assessment without a retest after the last training session; (6) effect of training on gait speed, step length or stride length only measured during the intervention, not in separate assessment after the last training session. Since this review focuses on the effects of perturbations during gait, all other forms of perturbation training, such as perturbations in a static context or optical instead of mechanical perturbations were excluded. Studies without a randomized controlled design (RCT) were excluded from the meta-analysis. However, because of the novelty of this field, studies with repeated measures designs with or without a control group but without randomization were included in the qualitative (i.e. descriptive) analysis. This enables the evaluation of promising paradigms not yet tested in a RCT. Two reviewers independently and sequentially screened titles and abstracts (FH, BV) and full texts (FH, VR) for eligibility. Any disagreements regarding eligibility were discussed amongst the reviewers after screening, until mutual agreement was achieved and verified by a third independent reviewer (MG).

Data extraction and quality assessment

A standardized form for data extraction was used (Microsoft Excel, version 2019, Microsoft Corp. Redmond, WA) to record information about: the study population, participant demographics, details of the intervention and control conditions, study design, primary outcome measures (e.g. gait speed, step length, stride length), secondary outcome measures (e.g. balance or postural sway), and main conclusions by the study authors. In addition, information was collated to assess the studies' internal validity using the NIH National Heart, Lung, and Blood Institute's Quality Assessment Tool for Controlled Intervention Studies. This tool uses 14 criteria for assessing internal validity and potential risk of bias [38]. Two reviewers independently scored the internal validity (FH, VR).

Data synthesis and analysis

The primary outcome measure was gait performance, expressed as the pooled outcomes of the following gait measures: gait speed, step length or stride length. When several of the

outcome measures were present, only one was included in the meta-analysis based on the following predefined prioritization: (1) gait speed, (2) step length and (3) stride length. Secondary outcomes included other gait measures (i.e. asymmetry, variability), Center of Mass (CoM) measures (i.e. sway speed) and balance performance (i.e. Mini-BESTest and the Berg Balance Scale (BBS)). If included studies had these data available in secondary analyses, these were also included in the analysis. Three secondary analyses were performed: (1) including only studies that used regular treadmill training as an active comparator to RGPT; (2) including only studies that assessed PwPD; and finally (3) including only balance outcomes. Additional data were requested from the corresponding authors if not reported in the original publication. Authors were given at least 2 weeks' time to respond to this request before the data was labeled as missing. A meta-analysis of final measures was conducted for the post and retention scores separately, in which the means and standard deviations of the scores were used to compare the pooled effects of the different interventions in the pooled population (PwPD and HOA). If only change scores were reported and the corresponding authors did not respond to the data request, the mean was determined based on the baseline mean added to the change score of the post and/or retention timepoint. This was the case for one study [39]. Here, the standard deviation at baseline was entered as the estimate of the standard deviation at post and/or retention.

The standardized mean difference (SMD) between the intervention and control group was calculated with a random effects model using Reference Manager (RevMan, v5.4), which accounts for inter-study variance in the methods and outcome measures. Based on the SMD corresponding 95% confidence intervals, two-sided P-values and the main effect sizes (Z-scores) were calculated. P-values < 0.05 were considered statistically significant. Heterogeneity between study effects was assessed using both the χ^2 test and I^2 statistic [40]. I^2 values $< 25\%$, between 50% and 75%, and $> 75\%$ were considered as low, moderate or large heterogeneity, respectively [40]. The results were displayed in forest plots. Possible publication and selection bias were assessed using funnel plots. Where possible, effect sizes (ES) were calculated using Cohen's d.

Results

Study selection

The search and selection procedure are outlined in **Figure 1**. The systematic search identified 4421 potential records. After duplicate removal, 3076 titles were screened and 2916 records excluded. A total of 160 abstracts were screened for eligibility, resulting in the exclusion of 109 records. Of the remaining 51 full-text records, eleven met the inclusion criteria [39,41–49], of which two were not included as we received no response to our data requests [48,49]. Consequently, eight studies were included in the qualitative review and six could be included in the meta-analysis for having applied an RCT design and providing use-able data [39,41,42,44,46,47]. Reasons for exclusions are described in **Figure 1**. Two out of the eight included studies [42,43] had additional data available on balance outcomes in other secondary analyses papers, which were also considered in this review [50,51]. A summary of characteristics of the included studies can be found in **Table 1**.

Summary of study characteristics

A total of eight studies were considered eligible for qualitative review [39,41–47], of which six could be included in the meta-analysis [39,41,42,44,46,47]. Information about participants, modes of RGPT, training design, control groups, retention periods and main gait outcomes are presented in **Table 1**. **Table 1** also illustrates the different forms of perturbations delivered while walking, including 3D tilting [44,46] or sudden translations of the treadmill [42,46], sudden acceleration or deceleration of the treadmill [39,41,43,47], manual perturbations by the trainer [39], and push and pulls from a cable system during treadmill walking [45]. Six out of the nine studies compared RGPT with an active control intervention in an RCT design [39,42–44,47,52]. Five studies used regular treadmill training [42–44,47,52] and one used strength training [39] as comparison. The study of Cakit et al. (2007) did not specify their control intervention [41].

Duration of the training sessions differed between studies. Three studies consisted of a single session [44,45,47], two studies had 16 sessions [41,46], and the other studies provided 18 [43], 24 [42] and 44 sessions respectively [39], with an average of 2-3 sessions per week. The total length of the intervention period ranged from 1 day to 4 months. Retention of training effects was acquired in three studies. These studies measured retention after 1 week [47] and 3 months [43,46]. The study of Shen et al. (2015) measured retention effects at 3 different time points: 3, 6 and 12 months after training [39]. To reduce heterogeneity, we included the data obtained at the three-month time point into the retention, as this matches the retention period in two of the four other studies. Five studies included PwPD [39,41,43,44,46], two studies included HOA [42,47], and one study compared PwPD and HOA [45].

Table 1. Characteristics of included studies.

Study	Participants Age (SD)	Perturbation training	Control intervention	Retention	Gait outcome	Balance outcome
Cakit et al. 2007	N = 31 PwPD 71.8 (6.4) HY: 2-3 UPDRSII: 18.14 (9.32)	16 sessions, 30 min for 8 wks speed dependent treadmill training with unexpected speed increments or decrements (0.6 km/h)	Control group mentioned but content not specified	None	Max gait speed on treadmill	Berg Balance Scale
Gimmon et al. 2018	N = 53 HOA IG: 78.2 (5.6) CG: 81.4(4.3)	24 sessions, 20 min for 3 mo of treadmill at comfortable speed with unexpected perturbations of platform in random directions	24 sessions, 20 min for 3 mo of treadmill walking at comfortable speed	None	Stride length on treadmill (1.9 mph speed)	Postural sway (EC)
Klamroth et al. 2016	N = 39 PwPD IG: 64.8 (10.3) HY: 2.4 (0.6) UPDRSIII: 16.7 (5.5) CG: 64.2 (8.5) HY: 2.2(0.9) UPDRSIII: 17.7(8.7)	1 session, 20 min of treadmill walking with small 3D tilting movements of the walking surface	1 session, 20 min of regular treadmill walking at comfortable speed	10min	Comfortable over-ground gait speed, *CoV and asymm of stride length*	Postural sway (EO)
Rieger et al. 2020	N = 30 HOA 72.6 (5.4)	1 session, 16 perturbations of treadmill walking with sudden acceleration or decelerations	1 session, 8 min of conventional treadmill walking	1 week	Step length on treadmill	None
Shen et al. 2015	N = 45 PwPD IG: 63.3 (8.0) HY: 2.43 (0.47) UPDRSIII: 24.0 (8.3) CG: 65.3 (8.5) HY: 2.48(0.49) UPDRSIII: 23.2(6.5)	44 sessions, 20-60 min, 12 wks total, 2x4wks 60min of lab technology-assisted gait and balance training with volitional stepping, leaning, unexpected treadmill deceleration or manual perturbations and 1wk 20min self-supervised training.	44 sessions, 20-60 min of strength training of the lower extremity with dynamometers/leg press machines, rowing, cuff weight	3 mo (6mo/12 mo)*	Comfortable over-ground gait speed	None

Steib et al. 2017	**N = 38 PwPD IG: 67.6 (8.2) HY: 2.43(0.47) UDRSIII: 24.0(8.3) CG: 62.5(7.9) HY: 2.48(0.49) UPDRSIII: 23.2(6.5)**	**16 sessions, 30min for 8 wks of treadmill walking with 3D movements of tilting platform**	**16 sessions, 30 min for 8 wks of conventional treadmill walking**	**3 months**	**Comfortable and fast over-ground gait speed**	**Mini-BESTest, Postural sway (EO and EC)**
Paradigms without RCT design or control group						
Harro et al. 2014**	N = 20 PwPD IG: 67.3(11.47) HY: 1.9(0.57) UPDRSIII: / CG: 64(9.58) HY: 2.0(0.67) UPDRSIII: /	18 sessions, 30 min for 6 wks of rhythmic auditory cued over-ground walking, walking to the beat of music with incremental BPM to increase gait speed	18 sessions, 30 min for 6 wks of speed dependent treadmill training, unexpected increase of speed every +/- 2.5-5 min and decreases to comfortable speed	3 months	Comfortable and fast overground gait speed	Berg Balance Scale
Martelli et al. 2017	N = 18, 9 PwPD + 9 HOA PwPD: 64,3 (7,4) HY: 1.78(0.44) UPDRSIII: 14.44(6.44) HOA: 64,7 (7,3)	1 session, 30 min, 9 blocks of 8 AP or ML pull or push perturbations by external cables during walking on a treadmill	None	None	Step length of walking on treadmill	None

RCT: Randomized Controlled Trial, CC: Case Control Study, IG: Intervention Group, CG: Control Group, PD: People with Parkinson's Disease, HOA: Healthy Older Adults, HY: Hoehn and Yahr stage, UPDRSIII: Movement Disorder Society Unified Disease Rating Scale part III, Mo: months, Wks: weeks, CoM: Center of Mass, EO: Eyes Open, EC: Eyes Closed, BPM: Beats Per Minute, AP: Anterior-Posterior, ML: Medio-Lateral, *CoV: Coefficient of Variance, Asymm: asymmetry.* * these timepoints were also collected but not used in this meta-analysis, ** only control group was suitable for inclusion in qualitative analysis.

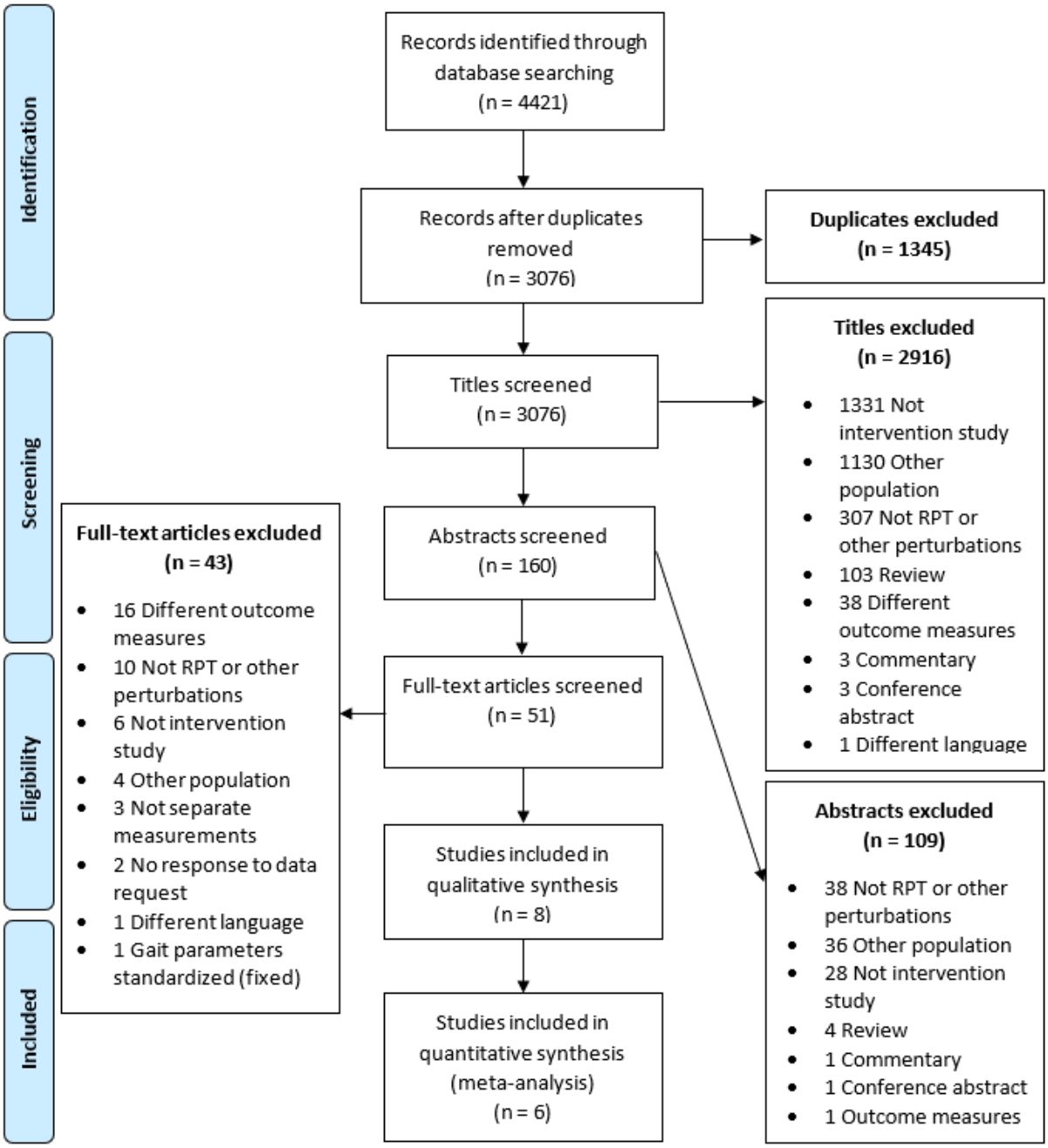

Figure 1. Flowchart of systematic search. Including the in- and exclusion and reasons per phase of the screening process. RPT: Repeated Gait Perturbation Training.

Data of both groups were pooled in the current primary meta-analysis including a total of 209 participants (159 PwPD and 50 HOA). The mean ages of PwPD and HOA differed significantly across studies (PwPD 66.4 (3.6) and HOA 76.2 (5.1), t = -2.807, P=0.048). The gait parameters most frequently used as an outcome were comfortable gait speed in four studies [39,43,44,46], followed by fast gait speed in three studies [41,43,46], step length in two studies [45,47], and stride length in one study [42]. The study of Klamroth et al. (2016) also included measures of gait variability and asymmetry (see **Table 1**) [44].

Results of the individual studies – qualitative review

Cakit et al. (2007) found a significant improvement of 0.20m/s in maximum tolerated walking speed on the treadmill in PwPD compared to the control group (unspecified), immediately after a training with sudden accelerations and decelerations (ES = 2.15, $p < 0.01$). In addition, an improvement in balance, measured with the Dynamic Balance Scale (ES = 6.21, $p < 0.01$) and the Berg Balance scale (ES = 9.32, $p < 0.01$), and a reduction in fear of falling ($p<0.01$) were observed [41]. Shen et al. (2015) did not find an improvement in over-ground gait speed, but did find a significant increase in stride length in PwPD immediately (ES = 0.968, $p = 0.003$), 6 months (ES = 0.643, $p = 0.038$) and 12 months (ES = 0.783, $p = 0.013$) after their technology-assisted balance and gait training including sudden decelerations during treadmill walking [39]. Moreover, they reported a significantly lower number of fallers after RGPT compared to the control intervention, which was retained for 12 months ($p = 0.047$) [39]. Klamroth et al. (2016) compared 3D tilting perturbations of the treadmill platform during treadmill walking to regular treadmill walking and found a group (RGPT vs control) by time (pre, post, retention) effect for over-ground walking speed in PwPD (ES = 0.41, $p = 0.014$) [44]. In addition, they reported a decrease in gait variability in the intervention group (ES = -0.34, $p = 0.048$), suggesting a more stable gait pattern [44]. The studies of Steib et al. (2017), Gimmon et al. (2018), and Rieger et al. (2020) found no improvement in gait (i.e. gait speed, stride length and step length respectively) after RGPT compared to regular treadmill walking [42,46,47].

Two studies were not suitable for inclusion in the meta-analyses. One did not have a RCT design [45], and the other compared two interventions of which only the control intervention matched our RGPT criteria. Here, the intervention group was focused on cueing and therefore not suitable as comparison in the meta-analysis [43]. Regardless, both studies showed a positive effect of RGPT on gait outcomes when comparing pre-post results (see **Table 2**). Harro et al. (2014) found an improvement in fast gait speed directly after a training with sudden accelerations and decelerations, compared to the pre-measurement (ES = 0.46, $p = 0.01$), which was retained after three months (ES = 0.36, $p = 0.05$) [43]. Martelli et al. (2017) found an effect over time in step length after training participants with a push/pull cable system (ES = 0.17, $p = 0.003$), but no difference in effects between PwPD and HOA groups was demonstrated (ES = 0.33, $p = 0.497$) [45]. Retention of these effects was not measured in this study.

Table 2. Qualitative description of studies not included in meta-analysis.

Study	Main finding on gait outcome pre-post	Main finding on gait outcome pre-ret
Harro et al. 2014	Comfortable gait speed (m/s) did improve with 4.53% after training 1.30(0.19) vs 1.36(0.21), however non-significantly (p = 0.13). Fast gait speed (m/s) did significantly improve by 7.45% after training 1.69 (0.27) vs 1.82 (0.30), p = 0.01.	Comfortable gait speed (m/s) remained increased at retention, 1.39 (0.24) vs 1.30 (0.19), however these improvements were non-significant (p=0.12). Improvements in fast gait speed retained after 3 months, 1.69 (0.27) vs 1.80 (0.33), p = 0.05.
Martelli et al. 2017	Step length (mm) increased over time in the pooled groups (HOA 14.59 (23.70), PwPD 21.78 (17.09)) (p = 0.003) after 30min of perturbation training, but no group x time effect was observed (p = 0.497)	Not measured

HOA: Healthy Older Adults, PwPD: People with Parkinson's Disease.

Effects of RGPT on Gait

Immediate effects – meta-analysis

Figure 2A shows the outcomes of the meta-analysis of the standardized mean difference (SMD), from six studies demonstrating no improvement of gait after RGPT training compared to the control training (SMD=0.16; 95%CI=-0.18, 0.49; Z=0.92; P=0.21). When pooling the data of the four studies that compared RGPT with regular treadmill training (**Figure 2B**), the SMD and significance level did not change considerably (SMD=0.10; 95% CI=-0.25, 0.45; Z=0.55; P=0.58), although heterogeneity measures decreased (from I^2= 18% to I^2= 0%). When pooling the four studies including only PwPD, the effect size and SMD remained the same (SMD=0.17; 95%CI=-0.31, 0.64; Z=0.69; P=0.49) (**Figure 2C**). Heterogeneity increased from 18% to 54%, reducing the robustness of these results.

Retention effects – meta-analysis

Three studies reported the retention effects of RGPT on gait. Overall, no gait improvements were retained (SMD=0.22; 95%CI=-0.42, 0.85; Z=0.67; P=0.50) (**Figure 3**).

Effects of RGPT on balance and postural sway

Immediate effects on balance scales

Two studies reported the effect of RGPT on balance assessments. Cakit et al. (2007) assessed the Berg Balance Scale and Steib et al. (2017) the Mini-BESTest [41,46]. Overall, RGPT showed a non-significant effect on balance assessments immediately after the training (SMD=0.09; 95%CI=-0.40, 0.58; Z=0.36; P=0.72, see supplementary materials for figures).

These results are in line with the study of Harro et al. (2014a, b)[43,51] that could not be included in this meta-analysis, while also showing no significant improvement on the Berg Balance Scale after RGPT at the individual study level [43,51].

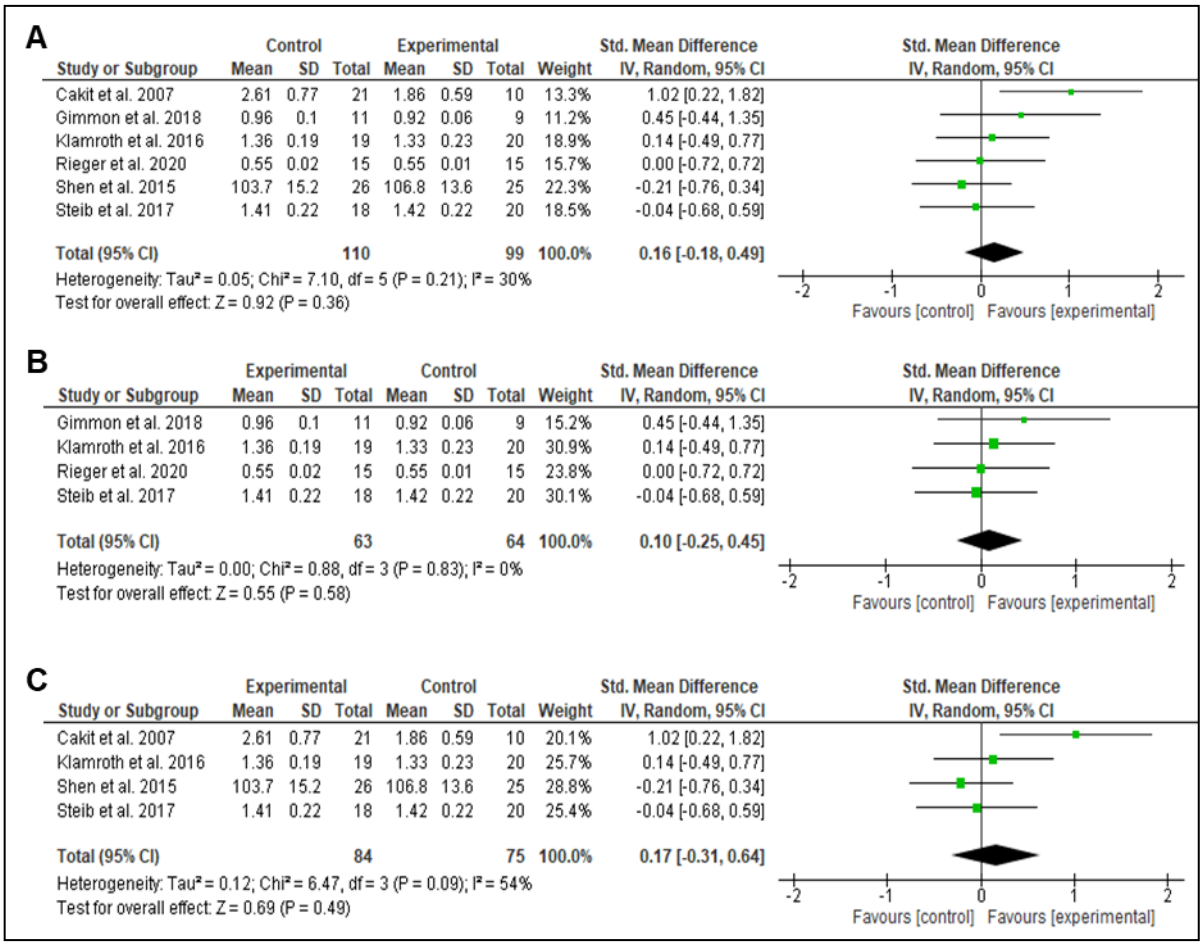

Figure 2. (A) Meta-analysis of the effect of RGPT (experimental) versus non-RGPT (control) training on gait outcomes (i.e. gait speed, step length, or stride length) in PwPW and HOA immediately after the intervention. **(B)** Meta-analysis of the effect of RGPT (experimental) versus regular treadmill training (control) on gait outcomes (i.e. gait speed, step length, or stride length) in PwPD and HOA immediately after training. **(C)** Meta-analysis of the effect of RGPT (experimental) versus non-RGPT (control) training on gait outcomes (i.e. gait speed, step length, or stride length) immediately after training in PwPD only.

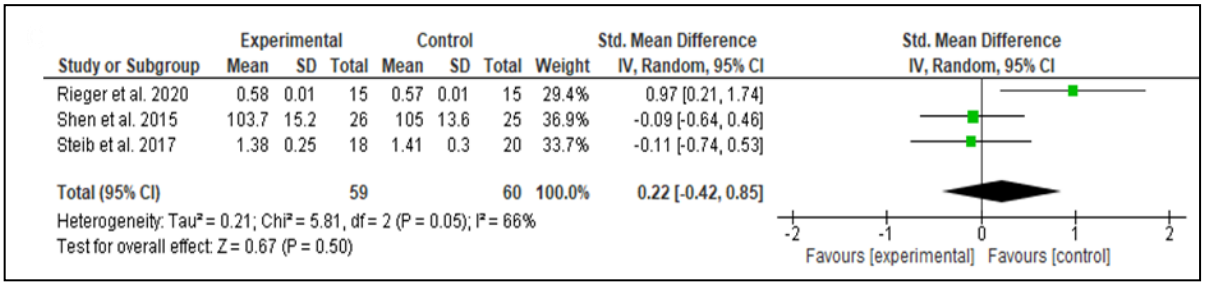

Figure 3. Meta-analysis of the effects of RPT (experimental) versus non-RPT (control) training on gait outcomes (i.e. gait speed, step length, or stride length) in PwPD and HOA at retention.

Immediate effects on postural sway

Three studies assessed velocity of postural sway during quiet stance [42,44,46]. Sway was assessed with eyes open and eyes closed in one study [46] and two either assessed with eyes open [44] or closed [42]. A decrease in postural sway velocity points towards better postural control. RGPT significantly decreased postural sway with eyes open (MD=-1.74; 95%CI=-3.18, -0.29; Z=2.35; P=0.02). Sway with eyes closed did not reduce in both studies resulting in a non-significant effect of RGPT (SMD=-1.43; 95%CI=-5.33, 2.48; Z= 0.72; P=0.47, see supplementary materials for figures).

Retention effects on balance scales and postural sway

Only Steib et al. (2017) assessed 3-month retention of balance scales and postural sway [46]. They found a slight, but statistically non-significant, decrease in Mini-BESTest scores after both the RGPT interventions in PwPD (mean difference (post-pre) =-0.1) and non-RGPT control (mean difference (post-pre) =-0.9), with lower scores indicating better performance (range from 0-28). The decrease in scores appears larger for the control group over the RGPT group, but no significant group by time interaction effect was found (P=0.441), nor a main effect of time (P=0.340). In addition, no significant improvements in postural sway with either eyes open (within group P=0.862, between group P=0.626), or eyes closed (within group P=0.446, between group P=0.626) were observed after RGPT.

Risk of bias in included studies

Selection bias

Funnel plots were generated for the primary outcomes of this review immediately after the intervention and at retention and are presented in the supplementary materials. Both plots showed balanced heterogeneous results, pointing towards a low risk of selection bias. However, the low number of studies included may have clouded interpretation of the funnel plots.

Within-study bias

Table 3 presents the internal validity of the included studies. All studies had some risk of bias. In particular, blinding of treatment allocation (Q4) was insufficient or not reported in all studies. Four of the eight studies did blind the assessors (Q5). Nearly half of the studies reached 20% or more dropout rates (Q7), though it should be noted that these rates were often similar between intervention arms (Q8). Most importantly, only few studies justified the sample size using an a-priori power calculation (Q12). Several studies preregistered their protocols (Q13), but for most studies this was not reported. All studies assessed their outcomes using valid and reliable measures (Q11).

Table 3. Quality assessment of included studies.

Item / Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14
Klamroth et al. 2016	1	1	1	0	0	1	1	1	1	?	1	0	1	1
Shen et al. 2015	1	1	1	0	1	1	0	1	1	?	1	1	?	0
Cakit et al. 2016	1	?	?	?	1	1	0	0	1	?	1	0	?	0
Gimmon et al. 2018	1	1	?	?	?	0	0	1	?	?	1	1	1	?
Steib et al. 2017	1	1	1	0	1	1	0	1	1	1	1	0	1	0
Rieger et al. 2020	1	?	?	?	?	1	1	1	?	?	1	0	?	1
Harro et al. 2014	1	?	?	0	1	1	1	1	1	?	1	1	?	1
Martelli et al. 2017				?	?	1			1	?	1	0	?	1
Sum score	8/7	4/7	3/7	0/8	4/8	7/8	3/7	6/7	6/8	1/8	8/8	3/8	3/8	4/8

Green color: low risk of bias, Red color: high risk of bias, Orange color: not reported, Dark grey color: not applicable. Q1: RCT, no/RCT, Q2: randomization quality, Q3: concealed allocation, Q4: participant blinding, Q5: blinded testers, Q6: similar groups, Q7: drop-out <20%, Q8: differential drop-out rate <15%, Q9: adherence >75%, Q10: similar background treatments, Q11: valid outcome measures, Q12: power >80%, Q13: preregistration, Q14: intention-to-treat.

Discussion

The aim of the present systematic review was to investigate the short and long-term effects of repeated gait perturbation training (RGPT) versus non-RGPT training on gait performance in healthy older adults and people with Parkinson's disease. Overall, no significant additional beneficial effects of RGPT on gait performance were found when contrasted to regular treadmill training, especially for retention. Some individual studies did show favorable pre-post and between-group effects for gait speed, step length, stride length, gait variability and asymmetry measures, immediately after RGPT and after a retention period [44].

Our findings are in contrast to our hypothesis and diverge from previous reviews showing a beneficial effect of repeated balance and/or gait perturbations in HOA and PwPD on reducing fall risk and increasing reactive recovery [33,36], though these prior reviews did not assess gait performance. We propose three complementary explanations for the lack of significant results in the present review.

First, most of the included studies in this review contrasted RGPT with regular treadmill training [42,44,46,47]. Treadmill training alone has shown strong positive effects on gait speed and stride length in several populations, including frail older adults and PwPD [18,53–55]. The Cochrane review conducted by Mehrholz et al. (2016) showed substantial effects of treadmill training in PwPD when compared to other interventions (e.g. stretching, dancing, resistance training, conventional therapy) on both gait speed (Mean difference (post-pre)=0.09m/s; 95% CI=0.03, 0.14; p=0.001) and stride length (Mean difference (post-pre)=0.05m; 95% CI=0.01, 0.09; p = 0.01) without increased drop-out rates or adverse events [18]. Ni et al. (2018) found similar results on exercise interventions including treadmill for PwPD [55]. Given the positive effects of regular treadmill training, it would require large sample sizes to detect a modest effect on gait outcomes in response to RGPT when contrasted to regular treadmill training. This is supported by the results of the individual studies included in this review, showing improvements on gait, however often less than the regular treadmill control group. As a result, most included studies were probably underpowered to detect between-group differences on gait outcomes. Moreover, only 3 out of 8 included studies determined their sample sizes based on a-priori power calculation. The outcomes of the present review might help researchers to perform a power-based sample size calculation for future intervention trials on RGPT.

Second, the effectiveness of RGPT, as for most training-based interventions, is likely dependent on the dosage and task-specificity of the training. Since the dosage of the training paradigms included in this review varied greatly, this may have influenced the results and clouded the potential of some specific perturbation paradigms. Work from Karamanidis et al. (2020) showed that RGPT could improve balance recovery responses in HOA with and without

neuropathology, as long as the amount of perturbations reached a certain critical threshold [56]. This threshold theory implies that even in neurological populations such as PwPD, retention and transfer can be achieved as long as there is sufficient training exposure to reach the optimal dose-response relationship. Future studies should conduct a meta-regression analysis to delineate the impact of intervention dosage on RGPT effectiveness, once the body of work in this domain has grown. With regard to task-specificity of RGPT, the type of perturbations may have contributed to the effectiveness of RGPT on some gait measures. Optimally, the mode of perturbation should resemble complex gait as performed in daily life, such as turns and other maneuvers. Moreover, translation should be tested in over-ground as well as experimental conditions. Seuthe et al. (2020) compared several split-belt to regular treadmill walking speeds (i.e. contrasts) in HOA and PwPD gait to see which split-belt contrasts elicited the largest improvements in step length asymmetry, which were relevant for turning [25]. They found that changing the speed ratios between the belts during one session repeatedly led to a quicker adaptation back to symmetry in step length, compared to static ratios (i.e. a constant speed reduction of one treadmill belt with either 25% or 50%). D'Cruz et al. (2020) also found that specific split-belt perturbations, especially the 'changing ratios' and steady reduction by 50%, led to improved dual-task gait speed during over-ground walking and turning in place, when compared to regular treadmill training in PwPD and HOA [26]. In addition, in both the studies of D'Cruz et al. (2020) and Seuthe et al. (2020) these improvements in turning and asymmetry were retained for 24 hours [25,26]. These results suggest that split-belt training with changing ratio's, when offered at optimal dosage and form, could lead to retention and transfer effects to daily gait challenges. Furthermore, the speed or contrast at which the perturbation is introduced, appears to play a role in how people learn to cope with the perturbation, and how well they can retain learning effects [25,26].

Third, valid outcomes that are responsive to gait adaptation, need to be adopted to capture the potential of RGPT on gait function. Gait speed, step and stride length are gait outcome measures that also improve with regular treadmill walking. One study included in this review reported additional outcomes, that could be more indicative of gait adaptation and flexibility. Klamroth et al. (2016) reported on the coefficient of variation and asymmetry of several gait parameters, including stride length and step time [44]. Significant reductions in stride length variability and significant increases of step time symmetry were observed compared to the control group, who received regular treadmill training [44]. These results are corroborated by another study not included in this review from Seuthe et al. (2020), who reported a significant reduction in asymmetry following split-belt perturbations after RGPT compared to regular treadmill training, whereas no improvements in gait speed were observed [25]. These results suggest that to quantify gait adaptation, future studies should consider incorporating gait

adaptation tasks and testing the validity of asymmetry and other variability/adaptation measures of gait as outcomes of interest. In addition, validity studies are needed to test whether outcomes, which capture change immediately after imposing perturbations and after retention, are correlated with ecological gait measures.

Finally, previous results of RGPT on balance are also in sharp contradiction to our secondary analysis on balance outcomes, in which we overall found non-significant results for both sway and balance scales. However, this is likely caused by the limited number of studies, as we only included papers that primarily focused on gait perturbation and outcomes. Careful interpretation of the secondary analyses is also warranted, given that these included some of the same study participants.

The present results challenged our hypothesis that exposing people to RGPT would lead to additional gait improvements that were better retained compared to non-RGPT. Gait adaptation is likely governed by cerebellum-motor cortex connectivity [32,57]. When a discrepancy occurs between the expected and experienced situation (i.e. sensory prediction error), sensory integration is facilitated by the cerebellum, allowing adaptation of motor control [58]. Because the cerebellum is intact in HOA and not severely affected in the early disease stages of PwPD [59], the functional circuits related to this structure may still have some capacity to induce learning effects relevant for gait adaptation training [60]. A recent ALE meta-analysis on fMRI findings showed that PwPD consistently activate the cerebellar locomotor region more than HOA during gait, supporting the view that the cerebellum plays an important compensatory role in gait processing for PwPD [60]. In patients with cerebellar lesions, Morton and Bastian (2006) showed that an intact cerebellum is essential for adaptive gait control during split-belt walking [61]. Moreover, a recent PET imaging study showed increased lateral cerebellar activity while adjusting gait during split-belt walking in healthy young adults [6]. These imaging studies further endorse that PwPD may still be able to train gait adaptation through RGPT, constituting promising gait rehabilitation strategies for these fall-prone patients.

Clinical implications

Although, the results of this review were negative, it is interesting to see that six RGPT paradigms resulted in significant within-group, and sometimes between-group improvements in gait, albeit in different outcomes. Of these five interventions, three consisted of sudden accelerations or decelerations of the treadmill, either at once or in a split-belt context, requiring an immediate response [39,41,51]. The similarities in these programs suggest, that also in regular clinical practice, even without specific instrumentation, it may be useful to offer training conditions that require speed changes to improve steady gait and gait flexibility. In addition, the only study that tested long-term effects showed retention of up to 12 months following

RGPT [39] and split-belt training [25,26] demonstrated transfer to an over-ground adaptive task, namely turning [25,26]. However, the experiments included were still at the proof-of-concept stage, as our quality assessment indicated largely underpowered samples and other potential risks of bias. Inherently, the present review could thus only include few studies and with small samples, limiting the statistical power of our meta-analyses. In addition, for the main meta-analysis, data of PwPD and HOA were pooled although there was a difference in mean age, which could have biased the results. This methodology was based on an a-priory decision (see pre-registration) to not arbitrarily restrict age for PwPD and allow for optimal power in the meta-analysis. Taken together, the present review points to the need for more well-designed, adequately powered RCTs, as well as, to gap in knowledge on the impact of RGPT on daily-life ambulation, before wide implementation in the clinical field can be recommended. Future studies should also elucidate the specific type of perturbations and dosage for use in rehabilitation, to improve flexibility of gait and balance performance in older and neurological populations.

Conclusion

This systematic review with meta-analysis on RGPT showed that despite the promising effects reported in individual studies, their pooled effects were not helpful in improving gait outcomes when compared to other training interventions. The limited number of studies, methodological heterogeneity in the type and dosage of training and the varying outcome measures further clouded possible intervention effects. However, this review also revealed the potential of RGPT, providing a rationale for conducting future effect studies in this training concept in HOA and in PwPD.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

1. Research project: A. Conception, B. Organization, C. Execution; 2. Data: A. Acquisition, B. Processing; 3. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 4. Manuscript: A. Writing of the first draft, B. Review and Critique

FH: 1A, 1B, 1C, 2A, 2B, 3A, 3B, 4A

VR: 1C, 2A, 2B, 4B

BV: 1C, 2A, 2B, 4B

MG: 1C, 2B, 3C, 4B

PG: 1C, 3C, 4B

ND: 1C, 4B

CS: 1C, 4B

AN: 1A, 1B, 1C, 3A, 3C, 4B

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Supplementary materials

Search strategies used per database

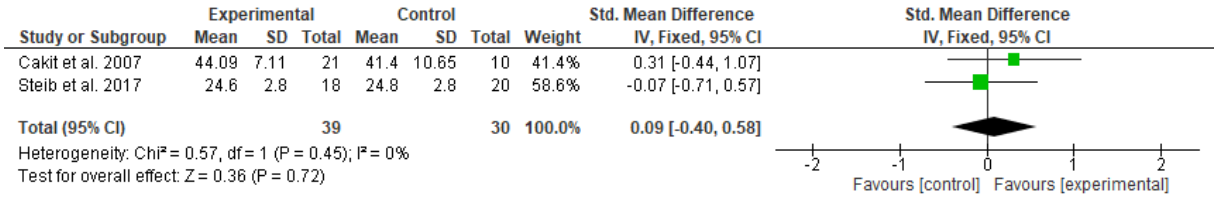
Pubmed: ("gait"[MeSH Terms] OR "gait"[All Fields] OR "locomotion"[All Fields] OR "walking"[All Fields] OR "walk"[All Fields]) AND ("split-belt" [All Fields] OR "split belt" [All Fields] OR splitbelt [All Fields] OR "balance loss" [All Fields] OR "dynamic balance" [All Fields] OR "dynamic stability" [All Fields] OR "surface translation"[All Fields] OR "trip" [All Fields] OR tripping [All Fields] OR slip [All Fields] OR slipping [All Fields] OR slips (All Fields) OR pull [All Fields] OR push [All Fields] OR perturbation [All Fields] OR perturbations [All Fields] OR perturbed [All Fields] OR perturb [All Fields]) AND ("Parkinson disease" [MeSH Terms] OR Parkinson [All Fields] OR aging [All Fields] OR ageing [All Fields] OR aged [All Fields] OR elderly [All Fields] OR "older adults"[All Fields]) AND (rehabilitation [All Fields] OR repeated [All Fields] OR repetition [All Fields] OR education [All Fields] OR training [All Fields] OR program[All Fields])

Embase: ('gait' OR 'gait'/exp OR gait OR 'locomotion' OR 'locomotion'/exp OR locomotion OR walk OR 'walking' OR 'walking'/exp OR walking) AND ('split-belt' OR 'split belt' OR splitbelt OR 'balance loss' OR 'dynamic balance'/exp OR 'dynamic balance' OR 'dynamic stability'/exp OR 'dynamic stability' OR 'surface translation' OR trip OR 'tripping' OR 'tripping'/exp OR tripping OR 'slip' OR 'slip'/exp OR slip OR 'slipping' OR 'slipping'/exp OR slipping OR pull OR push OR perturbation OR perturbations OR perturbed OR perturb) AND (parkinson* OR 'aging' OR 'aging'/exp OR aging OR 'ageing' OR 'ageing'/exp OR ageing OR 'elderly' OR 'elderly'/exp OR elderly OR 'older adults'/exp OR 'older adults') AND ('rehabilitation' OR 'rehabilitation'/exp OR rehabilitation OR repeated OR 'repetition' OR 'repetition'/exp OR repetition OR 'training' OR 'training'/exp OR training OR 'program' OR 'program'/exp OR program) AND ([article]/lim OR [article in press]/lim OR [conference paper]/lim OR [review]/lim) AND ([embase]/lim OR [medline]/lim)

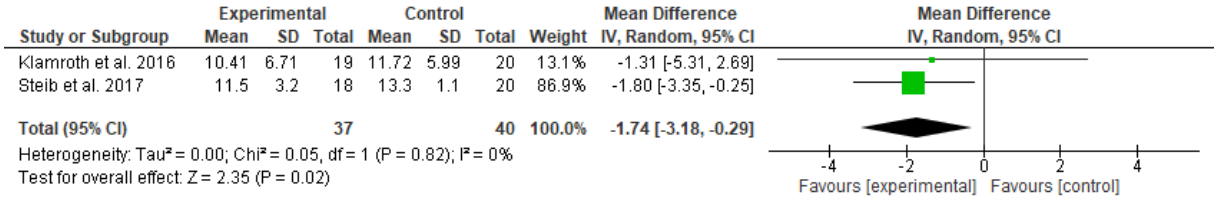
Web of Science: (ALL=((((gait OR locomotion OR walk OR walking) AND ("split-belt" OR "split belt" OR splitbelt OR "balance loss" OR "dynamic balance" OR "dynamic stability" OR "surface translation" OR trip OR tripping OR slip OR slipping OR pull OR push OR perturbation OR perturbations OR perturbed OR perturb) AND (parkinson* OR aging OR ageing OR elderly OR "older adults") AND (rehabilitation OR repeated OR repetition OR training OR program))))) AND DOCUMENT TYPES: (Article OR Review)

Google Scholar: (gait OR locomotion OR walk OR walking) AND ("split-belt" OR "split belt" OR splitbelt OR "balance loss" OR "dynamic balance" OR "dynamic stability" OR "surface translation" OR trip OR tripping OR slip OR slipping OR pull OR push OR perturbation OR perturbations OR perturbed OR perturb) AND (Parkinson OR ageing OR elderly OR "older adults") AND (rehabilitation OR repeated OR repetition OR training OR program)

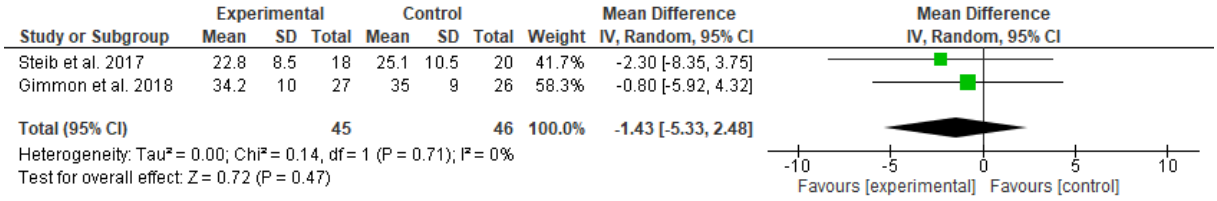
Effect of RGPT on balance and postural sway



Supplementary Figure 1. Meta-analysis of the effect of RGPT (experimental) versus non-RGPT (control) training on balance scales immediately after training.

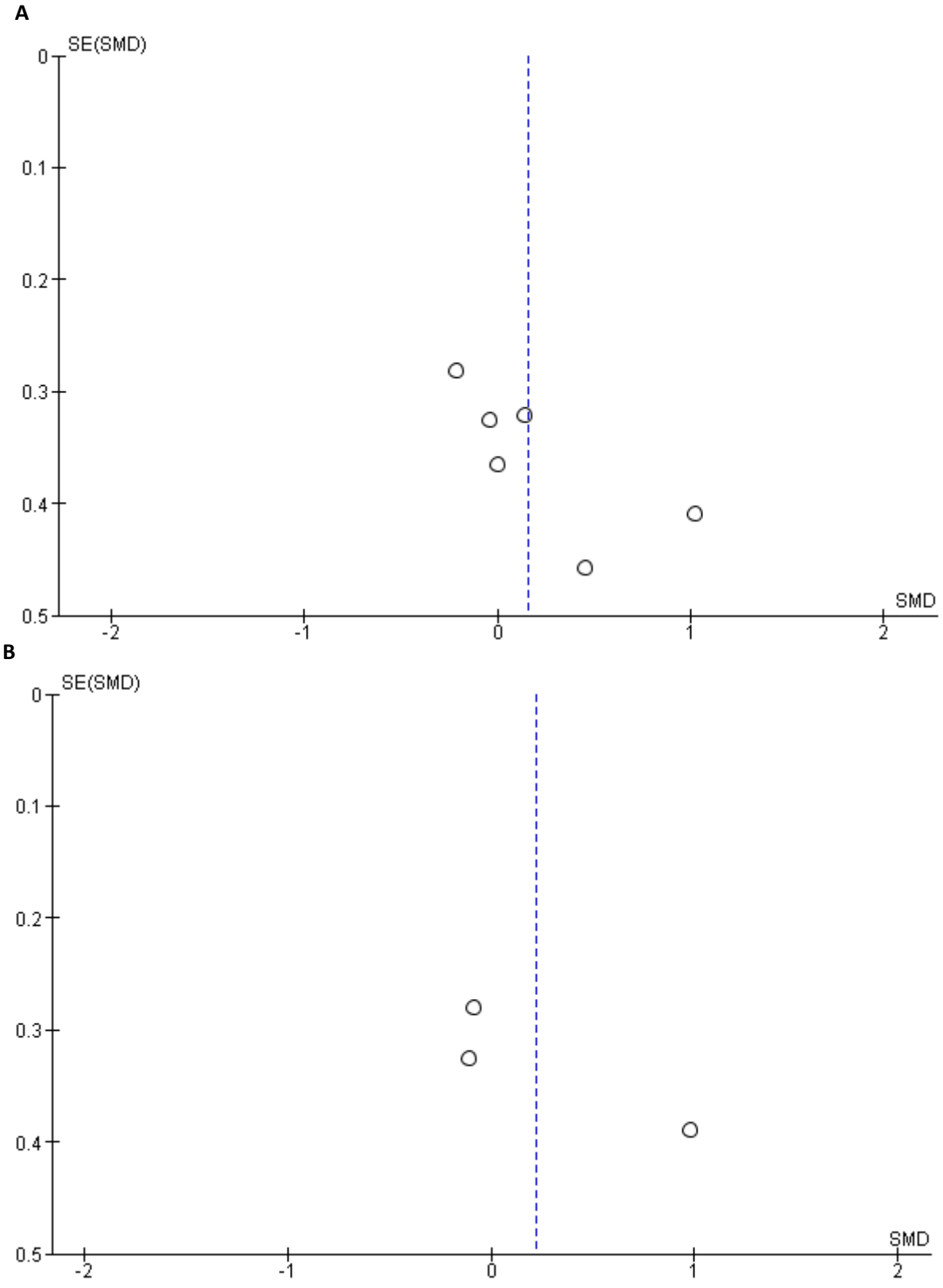


Supplementary Figure 2. Meta-analysis of the effect of RGPT (experimental) versus non-RGPT (control) training on postural sway with eyes open immediately after training.



Supplementary Figure 3. Meta-analysis of the effect of RGPT (experimental) versus non-RGPT (control) training on postural sway with eyes closed immediately after training.

Funnel plots for the primary outcome immediately after the intervention and at retention



Supplementary Figure 4. Funnel plot primary outcome (gait performance) immediately after intervention **(A)** and after retention period **(B)**.

Chapter 3

Split-Belt Treadmill Training to Improve Gait Adaptation in Parkinson's Disease

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Abstract

Background: Gait deficits in people with Parkinson's Disease (PD) are triggered by circumstances requiring gait adaptation. The effects of gait adaptation training on a split-belt treadmill (SBT) are unknown in PD.

Objective: We investigated the effects of repeated SBT versus tied-belt treadmill (TBT) training on retention and automaticity of gait adaptation and its transfer to over-ground walking and turning.

Methods: We recruited 52 individuals with PD, of whom 22 were freezers, in a multi-center randomized single-blind controlled study. Training consisted of four weeks of supervised treadmill training delivered three times per week. Tests were conducted pre- and post-training and at 4-weeks follow-up. Turning (primary outcome) and gait were assessed over-ground and during a gait adaptation protocol on the treadmill. All tasks were performed with and without a cognitive task.

Results: We found that SBT-training improved gait adaptation with moderate to large effects sizes ($p<0.02$) compared to TBT, effects which were sustained at follow-up and during dual tasking. However, better gait adaptation did not transfer to over-ground turning speed. In both SBT- and TBT-arms, over-ground walking and MDS-UPDRS III scores were improved, the latter of which reached clinically meaningful effects in the SBT-group only. No impact was found on freezing of gait.

Conclusion: People with PD are able to learn and retain the ability to overcome asymmetric gait-speed perturbations on a treadmill remarkably well, but seem unable to generalize these skills to asymmetric gait off-treadmill. Future study is warranted into gait adaptation training to boost the transfer of complex walking skills.

Introduction

People with Parkinson's disease (PD) have well-known difficulties with automatically adjusting their gait to challenging and asymmetrical conditions such as turning [1–3]. Turning often triggers freezing of gait (FOG), which is highly associated with falls [4], immobility and reduced quality of life [5–7]. Levodopa treatment is only partially effective in ameliorating gait difficulties in PD [8], pointing to the complex interactions between dopaminergic, noradrenergic and cholinergic neurotransmitters involved in gait control [9]. Various modes of gait training have also demonstrated only moderate effect sizes [10–13] and it is still unknown whether these effects actually hold during functional gait in the daily context. Recently, motor-cognitive treadmill training was studied offering practice in an enriched environment, demonstrating surplus benefits on reducing falls and negotiating obstacles [13]. However, people with PD display a wide heterogeneity in cognitive profiles [9,14,15] and therefore such multimodal training may not be suitable for all individuals. Hence, the current study investigated a paradigm for tackling gait adaptation without adding cognitive load, thereby widening possible gait training options for PD.

Split-belt treadmill (SBT) training offers safe and controlled practice of asymmetric and changing gait patterns in a progressive and individualized manner. A SBT has two belts and allows control of the speed of each belt separately. A switch of speed in one belt prompts a bout of sudden asymmetrical walking, producing a sensory prediction error and a subsequent slow adaptive response to regain symmetric motor control and gait stability [16]. Similar to turning, the body center of mass needs to be controlled and adjusted in multiple directions when the stance phases are manipulated by belt speed switches. Thus, SBT-perturbations elicit largely automatic responses without demanding explicit attention [16], which parallel the daily-life adaptations made after a sharp turn or a sudden switch in walking direction. Previous studies found that particularly patients with FOG showed impaired adaptation to SBT-conditions [2,17]. However, when comparing mild PD to healthy controls a very similar adaptive ability was found [18]. This largely preserved response may be explained by the fact that the so-called 'fine-tuning' locomotor network, including the frontoparietal cortex and the cerebellum, is relatively spared in PD taking on a compensatory role [16,19,20]. Yet, whether repeated exposure to SBT will enhance long-term acquisition of gait adaptation skills and whether this will improve turning in a wider PD population is currently unknown.

Therefore, we first conducted a pilot trial in a cohort of 45 freezers and 36 healthy controls (HC) to examine if a single session of SBT led to 24-hour retention [21,22]. We also compared various doses of speed contrasts with regular (tied-belt) treadmill (TBT) to be able to underpin the design of a prolonged SBT-program. We found that gait adaptation gains were retained, albeit less so than in HC, and that this positively modified turning and percentage time frozen

[21,22]. When freezers and healthy controls were analyzed together, the largest gains pertained to turning speed on the spot (360°). Given its association with FOG and falls [23,24], turning speed was chosen as the primary outcome allowing to include both freezers and non-freezers in the trial. This not only provided a pragmatic advantage for recruitment, but also permitted exploring the differential impact of training on the cohorts. Consequently, we set out to investigate whether 4 weeks of progressive SBT versus TBT training would lead to robust gait adaptation as manifest during [1] untrained turning over-ground, [2] after a 4-week follow-up and [3] during dual tasking.

Methods

This multi-center randomized single-blind controlled study was conducted at the Christian-Albrechts-University (CAU), Kiel, Germany and the KU Leuven, Belgium. The trial was preregistered at ClinicalTrial.gov (ID: NCT04176263).

Participants

Participants were included between August 2019 and May 2021, including the period of the Covid-19 pandemic. Inclusion criteria were: 1) diagnosis of idiopathic PD as confirmed by a neurologist according to the Brain Bank criteria [25]; 2) Hoehn and Yahr (H&Y) stage 2 or 3; 3) freezers and non-freezers who could walk without an aid for at least five minutes and 4) stable medication. Exclusion criteria were: 1) presence of other neurological diseases; 2) cognitive impairment (Mini mental State Examination <=24); 3) orthopedic or other health conditions that might influence gait or balance; 4) enrollment in another clinical study; 5) any other gait training one month prior to participation and cardiovascular exercise risk factors as diagnosed by a doctor (established via questionnaire). Participants were classified as freezers based on subjective reporting of FOG (New Freezing of Gait Questionnaire (NFOG-Q) item 1=1) or after visual observation of FOG during the baseline assessments. Both ethical committees approved the study protocol (CAU Kiel: D 454/13; KU Leuven: S62825) and participants gave written informed consent prior to participation.

Study Design and Procedures

Participants were randomly allocated to either SBT or TBT in blocks of 4 and stratified per center for H&Y stage and FOG status using the Research Randomizer software (https://www.randomizer.org). Concealment of randomization was performed by a dedicated person in each center, not involved in data acquisition or training. Allocation was communicated via a secured e-mail system. Assessments and the statistical analysis were conducted while blinded to group allocation. Due to the nature of the trial, participants and training staff were unblinded, but participants were kept blind to whether their treadmill program represented the experimental or the control intervention.

Data collection was conducted at three time points: one week before the start of training (Pre), one week after completion (Post) and after the 4-week follow-up (see **Supplementary Figure 1**). Training and testing were conducted in the ON-state, the latter with strict adherence to the time of medication intake and with close monitoring (approx. every hour) of the consistency of patients' ON-status. Change in the Levodopa equivalent daily dosage (LEDD) over time was documented. Participants wore a safety-harness when walking on the treadmill and were encouraged to walk without the handrails. Only eight participants needed the handrails and usage was kept constant throughout the study. To ensure comparability between centers, in-person meetings and regular online fidelity checks of the study procedures were held between assessors and therapists.

Primary Outcomes and Other Over-ground Mobility Measures

Based on our pilot work and our interest in investigating transfer of SBT-training to a complex gait task off-treadmill [21], we chose turning speed as our primary outcome. Turning speed was assessed by asking participants to turn as quickly as possible on the spot (360°) for one minute in alternating directions in single task (ST) offering a degree of resemblance to the asymmetrical perturbations experienced during SBT. Turning was also performed under dual-task (DT) conditions as this was found to be particularly sensitive for assessing FOG [26]. The secondary task involved an Auditory Stroop task (the words "high" and "low" in German or Dutch) were presented congruently or incongruently in a high or low pitch. Participants had to respond to the pitch of the word as quickly as possible [27]. Turning kinematics were quantified with APDM Mobility Lab accelerometers placed on the lower back and on the shins. A custom Matlab script was used to compute turning speed, the turning speed coefficient of variation (CV) and the number of turns [28]. Trials with FOG were disregarded (Pre: 24%, Post: 19%, Follow-up 20%).

For over-ground gait analysis, participants were asked to walk 10 times along a 10-meter distance (4 meter capturing area) at a comfortable pace with and without the auditory Stroop task. Gait kinematics were calculated using 3D motion capture technology (CAU: Qualisys Motion Capture Systems; KUL: Vicon Motion Systems Ltd) with six passive reflective markers placed on the feet (lateral malleolus, heel and tip of the shoe on each foot).

Gait Adaptation Outcomes on the Split-Belt Treadmill

Gait adaptation was assessed on the treadmill according to a previously developed protocol [22]. This required 3 consecutive walking cycles of one minute during which subjects were walking in 1) TBT (baseline); 2) 50% reduction on one side (to assess early and late SBT-adaptation) and 3) TBT (to assess early and late de-adaptation) (see **Supplementary Figure 2**). Testing was conducted with and without the Auditory Stroop task to probe whether training

gains were held when a secondary task was added. The ST-condition was performed before the DT to avoid order effects across repeated measures. Treadmill velocity was personalized to the individuals' comfortable over-ground walking speed. The adaptation protocol was repeated for the left and right body side in a random order. Gait characteristics were calculated using custom Matlab scripts. The outcome of interest was step length asymmetry averaged over the three-minute protocol (total adaptation) and over the different adaptation phases separately (baseline, early-split, late-split, early-tied, late-tied using 10 second segments) [22]. Step length asymmetry was calculated as a ratio value: (fast leg parameter – slow leg parameter)/(fast leg parameter + slow leg parameter). Values closer to zero represented better gait adaptation [22]. The statistical analysis was conducted on the averaged and normalized value of the total adaptation and the late-split belt phase (see Supporting Data).

Freezing of Gait

FOG-severity was evaluated as a secondary outcome in the group of freezers only by calculating: 1) the FOG-score during walking in a FOG-provoking protocol [29], 2) the percentage of time frozen [26] and 3) the FOG-ratio (mediolateral) [30] during the 360° turning test.

Clinical Measures

We also collected the Movement Disorder Society Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS III), the Mini-Balance Evaluation Systems Test (Mini-BESTest), the Fullerton Advanced Balance Scale (FAB-S) and the Falls Efficacy Scale-International (FES-I). The new Freezing of gait questionnaire (NFOGQ), the Montreal cognitive assessment (MoCA), the Frontal assessment battery (FAB), and the Trail making test (TMT) were calculated at baseline only.

Training

Both interventions consisted of four weeks of supervised treadmill training delivered three times per week with a rest day in between sessions. Training progression was standardized and increased from 30 to 45 minutes per session with five-minute increments per week. In addition, 16 progression levels were developed to achieve equal training motivation in each arm (**Table 1-4, Supplementary**). In the SBT-group, the 16 progression levels were determined by varying the magnitude and frequency of the speed contrasts and by including one or two body sides. Belt-ratio reductions between 25% and 50% of comfortable over-ground gait speed were implemented, shown in previous study to elicit the best results [21,22]. The frequency of speed changes increased from 4 to 16 switches per 5 minutes. The 16 progression levels in the TBT group were characterized by increasing the walking speed from 80% to 110% of comfortable walking speed and by increasing the duration of bouts from 5 to

20 minutes of continuous walking. Therapists only personalized the training progression if needed by omitting or adding a challenge according to the participants' level of exertion as assessed by heart rate monitors and the Borg scale. However, training duration was always kept in accordance with the required duration for each training week. Details of the training protocols are summarized in **Appendix I** and explained through the TIDieR checklist [29].

Sample Size Calculation

Sample size was determined a-priori based on our pilot data, demonstrating an average turning speed of 74.32 (±27.22) at baseline. We assumed that the SBT-group would improve 14% compared to the TBT-group [21]. We calculated a sample size of n=50, including 20% dropout rate to detect a significant time*training-group interaction with alpha=0.05 and a power of 80%.

Statistical Analysis

Mann-Whitney-U-Tests were used to compare between-group differences at baseline. Linear mixed models were calculated for the primary and secondary outcomes. The model included time (3 levels) and training-group (2 levels) as independent variables to investigate the time*training group interaction as well as center (2 levels) as a covariate. The model included a random effects term with a correlated random intercept and slope for time and subject. A Satterthwaite approximation of the denominator degrees of freedom was used. Histograms and Q-Q plots were used to visually check for normality of the residuals. Post-hoc tests were conducted with Tukey adjustment for multiple comparisons. In line with the intention-to-treat approach, all randomized participants were included in the mixed linear models to accommodate missing data. We explored the impact of SBT versus TBT on FOG-related outcomes in freezers as a sub-analysis. To investigate whether freezers and non-freezers improved similarly, an additional linear mixed model was run with time and FOG-status as independent variables to investigate the time*FOG-status interaction (for the total sample and for the two training-groups separately). Additionally, in an extra analysis including the factor 'task' (single vs. dual task) to the model we investigated whether training effects were similar in these conditions. Effect sizes (ES: Cohen's D) [31] were calculated between groups for the differences from pre to post or pre to follow-up, respectively and reported in **Table 2**. ES [31] and their confidence intervals [32] are also visually depicted in a metaview of the pre- to follow-up differences only. We interpreted effect size as small (≥0.2), medium (≥0.5), or large (≥0.8) [33]. The statistical analysis was conducted with SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA) with a preset level of significance of $p<0.05$.

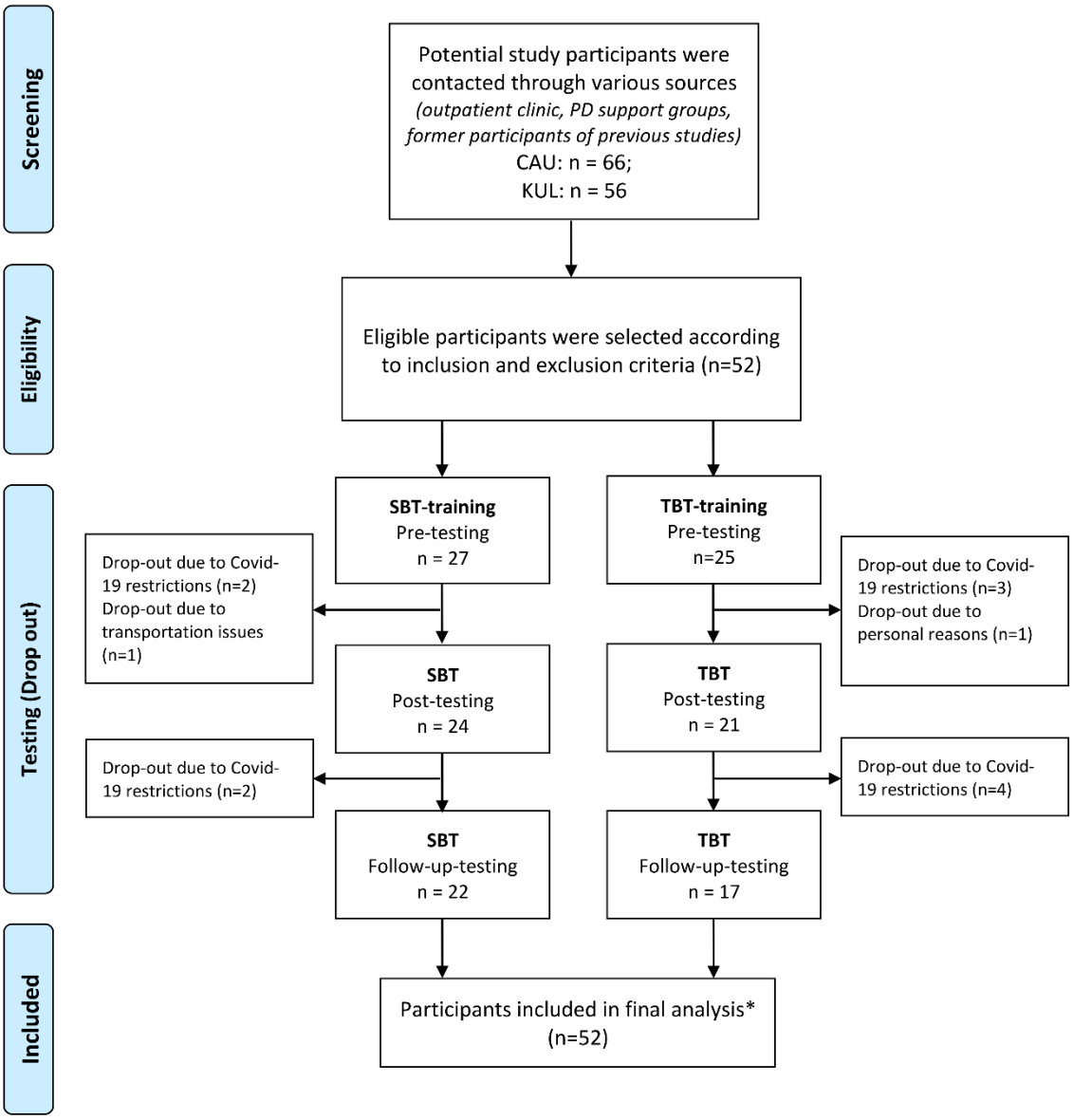

Figure 1. Flow diagram of recruitment process and study conduction; n=number of participants; CAU= Christian-Albrechts-University Kiel, KUL= KU Leuven, SBT=Split-Belt treadmill, TBT= Tied-Belt treadmill, *intention-to-treat based analysis, incomplete data for 13 participants.

Results

Participants' Characteristics

Fifty-two individuals with PD were included (see **Figure 1**) of whom n=22 were freezers (SBT: n=12; TBT: n=10). Two (4%) additional participants were recruited to compensate for the dropout risk due to the Covid-19-pandemic. Overall, compliance and protocol deviations were similar in each group (**Table 5 & 6, Supplementary**). **Table 1** shows that participant characteristics were similar for both groups. There was no significant difference in LEDD between groups (p = 0.408) and between time points (p=0.840).

Table 1. Participant characteristics.

	SBT (n=27)	**TBT** (n=25)	**p-value**
Age (years)	66.41 (7.87), 51-85	64.24 (11.49), 42-90	0.539
Duration since Diagnosis (years)	7.61 (5.30), 1-18	6.94 (4.36), 1-16	0.713
H&Y (1/2/3/4)	0/22/5/0	0/21/4/0	0.810
MDS-UPDRS-III (0-132)	35.07 (12.76), 9-59	31.20 (13.53), 12-69	0.190
LEDD (mg)	645.79 (338.31), 100-1745	563.94 (318.24), 0-1378	0.408
Mini-BESTest (0-28)	23.93 (3.12), 17-28	22.40 (4.66), 9-27	0.243
FAB-Scale (0-40)	33.15 (5.17), 17-39	30.44 (7.45), 14-40	0.295
MoCA (0-30)	26.41 (2.39), 20-30	25.56 (2.63), 20-29	0.304
TMT-A (s)	42.39 (14.70), 21.74-85	48.95 (43.71), 23.31-239	0.481
TMT-B (s)	92.46 (46.94), 35-213	122.66 (109.22), 44.37-582	0.089
FES-I (16-64)	25.63 (9.56), 16-47	24.13 (8.72), 16-50	0.629
NFOG-Q (1-28)	14.33 (5.41), 6-23	16.30 (5.87), 6-26	0.485

Values represent mean (standard deviation), range, except for H&Y. Abbreviations: MDS-UPDRS-III, Movement Disorders Society-Unified Parkinson's Disease rating Scale Part III; H&Y, Hoehn and Yahr stage; LEDD, levodopa equivalent daily dose; FES-I, Falls Efficacy Scale International; NFOG-Q, New Freezing of Gait Questionnaire.

Effects on Turning

No significant time by training group interaction was found for turning speed (ST: p=0.55, DT: p=0.95) and for any of the other turning outcomes (**Table 2**). However, there was a significant time effect for the number of turns. (ST: p=0.020, DT: p=0.020). Exploratory within-group analysis showed significant increases in the number of turns performed in ST and DT in the SBT-group from pre to follow-up (ST: p=0.030, DT: p=0.004) not present in the TBT-group. **Figure 3** displays the pre to follow-up effect sizes in a metaview, showing effects in favor of SBT for the number of turns, whereas turning speed variability showed larger changes in the TBT group. Effect sizes were largely similar in ST and DT, which was confirmed by the fact the factor task was not significant in the model (p=0.42).

Effects on Gait Adaptation

A significant time by training-group interaction was found for the gait adaptation outcomes with significantly greater improvements of step length asymmetry in the SBT- compared to the TBT-group (total: p=0.014 (ST); late-split: p=0.005 (ST), p=0.004 (DT)) (**Table 2**). **Figure 2** illustrates that compared to pre, the SBT-group was able to achieve greater reduction in their step length asymmetry at late-split adaptation (ST: p<0.001, DT: p<0.001) and for total adaptation (ST: p=0.020) at post. Furthermore, these improvements were also retained at the

4-week follow-up for late-split (ST: p<0.001, DT: p=0.020) and total adaptation (ST: p<0.001) in both ST and DT. When adding the factor task (ST vs DT) to the model, no significant interactions were found (p=0.19), indicating that improvements were similar in both conditions.



Figure 2. Step length asymmetry (mean(standard error)) during the gait adaptation task on the split-belt treadmill (non-normalized data). Values of zero represent perfect symmetry. SBT, split-belt training group; TBT, tied-belt training group; ST, single task condition; DT, dual task condition.

Effects on Over-Ground Gait

For over-ground gait no significant time by training-group interactions were found for gait speed (ST: p=0.143, DT: p=0.154), step length (ST: p=0.137, DT: p=0.301) and gait asymmetry (ST: p=0.236, DT: p=0.744) (**Table 2**). Significant time effects showed that both training groups increased their gait speed and step length under ST and DT conditions (ST: p=0.026; DT: p<0.001 and ST: p=0.028; DT: p=0.006, respectively). **Figure 3** shows a metaview of the effect sizes of the change scores from pre to 4-weeks follow-up between the SBT- and TBT-group, whereby values greater than zero indicate larger effects for the SBT-group. Between-group analyses shows a tendency for larger effects in the SBT- compared to the TBT-group, suggesting that these time effects were mostly driven by the improvements in the SBT-group. Furthermore, there was a significant time effect for gait asymmetry (ST: p=0.011), showing decreased asymmetry from pre to follow-up in both training groups.

Effects on FOG

We found no significant time by training-group interactions for the freezing-related outcomes, i.e., the medio-lateral FOG ratio, the Ziegler score and the percentage time frozen (**Table 8, Supplementary**). When adding FOG status as a factor in the statistical model, it had no significant impact on the results, indicating that freezers and non-freezers benefitted similarly from the training. The sub-analysis for freezers showed no significant time*group interactions for the FOG-related outcomes. When running models for each group separately, including FOG status and time as fixed factors, it was shown that non-freezers benefited less from TBT than freezers for DT gait speed (p=0.040) and DT step length (p=0.013) (**Table 9, Supplementary**).

Clinical Measures

A significant time effect was found for MDS-UPDRS III (p=0.002) showing a clinically meaningful improvement of -5.1 points from pre to post and -5.8 points from pre to follow-up for the SBT-group, in contrast to the TBT-group (-2.9 points from pre to post; -3.5 points from pre to follow-up). No significant interaction or time effects were apparent on the clinical balance and fear of falling scales (see **Supplementary Table 7**). Self-perceived fatigue (BORG-scale) during training was similar across both groups, 11.76 (±2.4) in the SBT group and 11.73 (±2.2) in the TBT group (p=0.963).

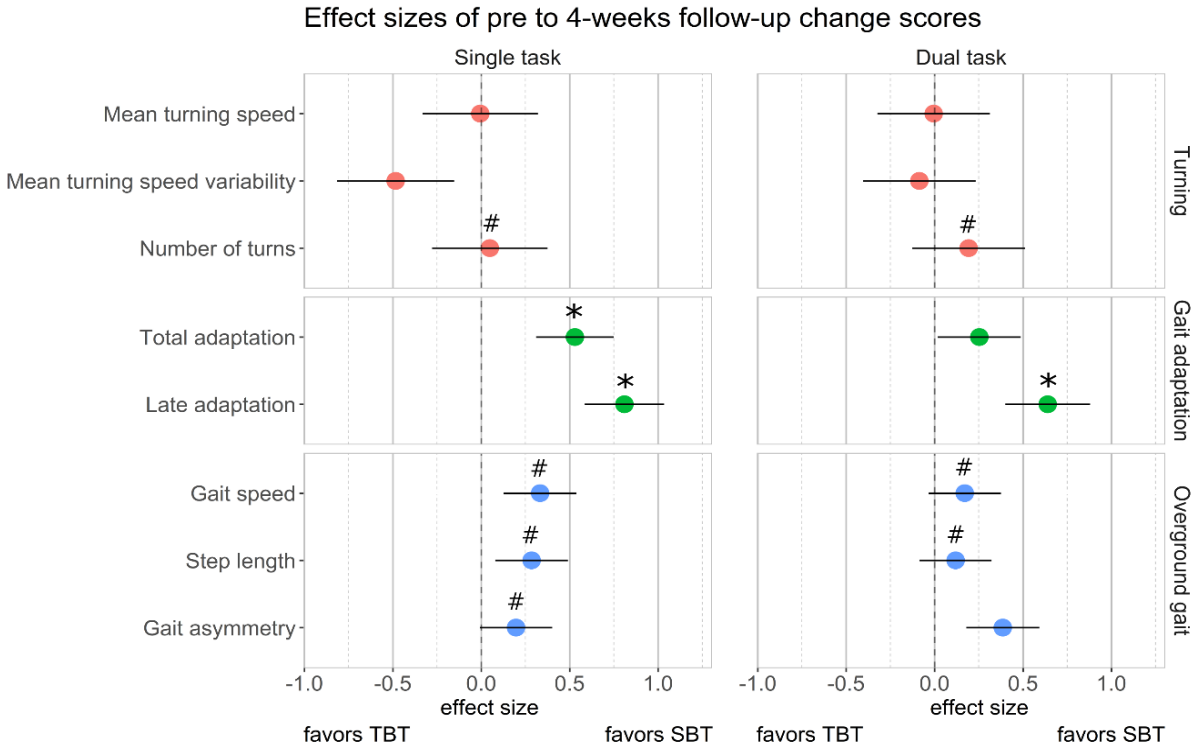

Figure 3. Meta-view of the relative effect sizes from pre to 4-weeks follow-up comparing SBT vs TBT. The width of the line indicates the 95% confidence intervals. Single and dual task conditions are depicted separately in the right and left panel. The zero line means that SBT and TBT effect sizes were equal. Values above zero means that the effect sizes are favorable for SBT versus TBT. The * indicates the significant (group*time) interactions and # illustrates significant time effects.

Table 2 (continues on next pages). Main outcomes for overground turning, treadmill adaptation and overground gait.

Outcome	ST or DT	Time	SBT (n=27) Mean (SD)	SBT (n=27) Median (Q1-Q3)	SBT (n=27) Post hoc *p*-value	TBT (n=25) Mean (SD)	TBT (n=25) Median (Q1-Q3)	TBT (n=25) Post hoc *p*-value	Effect-size	Between group Pre (*p*-value)	Between group (*p*-value)	Time-effect / group*time (*p*-values)
Overground turning												
Turning speed (°/s)	ST	Pre	110.5(30.9)	100.7(90.3-136.6)	-	99.4(24.7)	96.4(81.4-118.6)	-	-	0.22		0.05/0.55
		Pre-Post	3.8(13.7)	7.6(-6.5-13.3)	0.34	9.2(16.9)	7.4(1.9-14.3)	0.04*	-0.19		0.32	
		Pre-Ret	4.9(15.5)	6.7(-8.2-18.6)	0.09	5.1(11.9)	-1.2(-1.7-7.6)	0.15	-0.01		0.93	
	DT	Pre	111.1(30.3)	106.4(84.8-131.5)	-	96.8(34.4)	90.9(71.9-128.5)	-	-	0.18		0.21/0.95
		Pre-Post	3.4(13.4)	3.2(-6.1-15.5)	0.31	4.2(14.4)	3.4(-4.2-7.7)	0.21	-0.03		0.82	
		Pre-Ret	4.0(20.7)	-3.9(-8.9-19.7)	0.23	4.1(10.3)	1.4(-2.6-12.4)	0.30	0.00		0.97	
Turning speed CV	ST	Pre	0.08 (0.03)	0.07(0.06-0.09)	-	0.08(0.04)	0.06(0.05-0.11)	-	-	0.75		0.61/0.25
		Pre-Post	-0.01(0.03)	-0.01(-0.02-0.01)	0.58	0.00(0.07)	-0.00(-0.04-0.02)	0.82	-0.22		0.60	
		Pre-Ret	-0.00(0.03)	0.00(-0.01-0.02)	0.85	-0.02(0.05)	-0.00(-0.01-0.00)	0.44	-0.48		0.63	
	DT	Pre	0.08(0.02)	0.08(0.07-0.09)	-	0.08(0.04)	0.07(0.05-0.11)	-	-	0.75		0.39/0.76
		Pre-Post	-0.01(0.03)	-0.00(-0.02-0.00)	0.63	0.00(0.06)	-0.01(-0.03-0.01)	0.95	0.37		0.78	
		Pre-Ret	-0.01(0.03)	-0.01(-0.02-0.01)	0.18	-0.01(0.03)	-0.00(-0.03-0.02)	0.73	0.08		0.50	
Number of Turns	ST	Pre	17.1(4.8)	16.0(13.5-20.0)	-	15.0(4.3)	14.0(11.3-18.0)	-	-	0.19		0.02*/0.76
		Pre-Post	0.2(2.4)	0.0(-1.0-1.0)	0.80	0.6(2.4)	0.5(0.0-1.5)	0.31	-0.08		0.54	
		Pre-Ret	1.2(2.6)	1.0(-1.0-4.0)	0.03*	1.0(2.4)	1.0(0.0-2.0)	0.09	0.05		0.99	
	DT	Pre	17.0(4.9)	16.0(13.0-19.5)	-	15.1(6.2)	14.0(11.0-19.0)	-	-	0.20		0.02*/0.36
		Pre-Post	1.1(1.9)	1.0(0.0-2.0)	0.09	0.1(2.4)	0.0(-1.0-1.0)	0.85	0.18		0.30	
		Pre-Ret	1.7(2.8)	1.0(0.0-3.0)	<0.01*	0.6(1.2)	1.0(0.0-1.0)	0.29	0.19		0.20	

Outcome	ST or DT	Time	SBT (n=27) Mean (SD)	Median (Q1-Q3)	Post hoc *p*-value	TBT (n=25) Mean (SD)	Median (Q1-Q3)	Post hoc *p*-value	Effect-size	Between group Pre (*p*-value)	Between group (*p*-value)	Time-effect / group*time (*p*-values)
Treadmill adaptation												
Total step length asymmetry	ST	Pre	0.11(0.07)	0.09(0.07-0.11)	-	0.09(0.05)	0.08(0.06-0.10)	-	-	0.32		<0.01*/0.01*
		Pre-Post	-0.05(0.05)	-0.04(-0.06- -0.03)	<0.01*	-0.02 (0.04)	-0.01(-0.03-0.00)	0.12	0.51		0.02*	
		Pre-Ret	-0.05 (0.04)	-0.05(-0.06- -0.03)	<0.01*	-0.01 (0.02)	-0.02(-0.03-0.00)	0.09	0.53		<0.01*	
	DT	Pre	0.11(0.10)	0.08(0.06-0.11)	-	0.08(0.05)	0.06(0.05-0.08)	-	-	0.09		<0.01*/0.17
		Pre-Post	-0.04(0.06)	-0.04(-0.05- 0.00)	<0.01*	0.01(0.05)	-0.01(-0.02-0.00)	0.57	0.41		0.07	
		Pre-Ret	-0.04(0.05)	-0.04(-0.05- -0.02)	<0.01*	-0.02(0.04)	-0.02(-0.03-0.00)	0.07	0.25		0.25	
Step length asymmetry at late-split	ST	Pre	0.17(0.15)	0.13(0.07-0.19)	-	0.13(0.08)	0.12(0.07-0.18)	-	-	0.18		<0.01*/<0.01*
		Pre-Post	-0.10(0.11)	-0.10(-0.11- -0.05)	<0.01*	-0.02(0.06)	-0.01(-0.05-0.01)	0.46	0.79		<0.01*	
		Pre-Ret	-0.09(0.10)	-0.09(-0.10- -0.07)	<0.01*	0.01(0.04)	-0.02(-0.04-0.03)	0.73	0.81		<0.01*	
	DT	Pre	0.17(0.14)	0.13(0.09-0.21)	-	0.11(0.06)	0.11(0.07-0.16)	-	-	0.03*		<0.01*/<0.01*
		Pre-Post	-0.07(0.07)	-0.07(-0.11- -0.03)	<0.01*	-0.01(0.06)	-0.02(-0.05-0.01)	0.47	0.75		<0.01*	
		Pre-Ret	-0.07(0.07)	-0.08(-0.13- -0.03)	<0.01*	-0.02(0.05)	-0.03(-0.05-0.03)	0.28	0.64		0.02*	
Overground gait												
Gait speed (m/s)	ST	Pre	1.22(0.21)	1.20(1.09-1.38)	-	1.26(0.21)	1.31(1.17-1.37)	-	-	0.56		0.03*/0.14
		Pre-Post	0.08(0.15)	0.04(-0.02-0.18)	<0.01*	0.01(0.07)	-0.02(-0.04-0.03)	0.73	0.32		0.07	
		Pre-Ret	0.09(0.16)	0.06(0.01-0.21)	<0.01*	0.03(0.09)	0.01(-0.03-0.08)	0.58	0.33		0.05	

Outcome	ST or DT	Time	SBT (n=27)			TBT (n=25)			Effect-size	Between group Pre (*p*-value)	Between group (*p*-value)	Time-effect / group*time (*p*-values)
			Mean (SD)	Median (Q1-Q3)	Post hoc *p*-value	Mean (SD)	Median (Q1-Q3)	Post hoc *p*-value				
Overground gait												
Gait speed (m/s)	DT	Pre	1.13(0.26)	1.06(0.97-1.33)	-	1.13(0.26)	1.20(0.95-1.30)	-	-	0.98		<0.01*/0.15
		Pre-Post	0.09(0.13)	0.08(-0.01-0.14)	<0.01*	0.03(0.07)	0.02(-0.01-0.07)	0.22	0.24		0.06	
		Pre-Ret	0.09(0.13)	0.07(0.00-0.20)	<0.01*	0.05(0.10)	0.03(-0.02-0.12)	0.10	0.17		0.10	
Step length (mm)	ST	Pre	658.2(88.2)	664.4(624.1-724.1)	-	672.6(95.5)	698.3(627.1-736.5)	-	-	0.58		0.03*/0.14
		Pre-Post	25.8(51.3)	20.8(-4.6-48.8)	<0.01*	8.3(23.0)	1.1(-8.3-21.4)	0.38	0.20		0.15	
		Pre-Ret	32.5(61.5)	29.4(0.5-65.7)	<0.01*	6.4(29.4)	-2.3(-11.9-27.7)	0.73	0.28		0.05	
	DT	Pre	613.7(108.2)	617.4(544.5-686.7)	-	623.1(114)	653.0(549.9-696.8)	-	-	0.76		<0.01*/0.30
		Pre-Post	32.1(54.9)	27.0(1.5-53.2)	<0.01*	10.1(31.2)	11.2(-10.7-25.5)	0.27	0.20		0.13	
		Pre-Ret	30.0(60.6)	28.6(-0.3-51.6)	<0.01*	16.8(34.1)	14.4(-3.7-45.3)	0.19	0.12		0.19	
Gait asymmetry	ST	Pre	3.8(1.5)	3.3(2.8-4.1)	-	3.6(1.3)	3.2(2.7-4.0)	-	-	0.60		<0.01*/0.24
		Pre-Post	-0.2(1.0)	-0.1(-0.7-0.4)	0.31	-0.2(0.8)	-0.4(-0.6-0.4)	0.19	0.00		0.80	
		Pre-Ret	-0.5(1.0)	-0.3(-1.0-0.1)	<0.01*	-0.3(0.6)	-0.3(-0.7-0.3)	0.19	0.20		0.25	
	DT	Pre	4.3(1.6)	3.9(3.3-4.7)	-	4.2(1.8)	4.1(2.9-4.7)	-	-	0.84		0.97/0.74
		Pre-Post	0.1(2.3)	-0.3(-1.0-0.4)	0.75	0.1(1.3)	0.4(-1.0-0.8)	0.996	-0.03		0.83	
		Pre-Ret	-0.2(1.3)	-0.4(-0.7-0.3)	0.71	0.4(4.4)	-0.4(-1.0-0.1)	0.587	0.39		0.51	

Abbreviations: SBT, Split-belt treadmill training; TBT, Tied-belt treadmill training; ST, single task; DT, dual-task; SD, standard deviation; Q1, 1st quantile, Q3, 3rd quantile; CV, coefficient of variation; * significant p-value (<0.05).

Discussion

This is the first study investigating the effects of prolonged SBT-training in a mid-stage population of people with PD. We found that SBT was superior to TBT in improving gait adaptation on the treadmill with effect sizes ranging from medium to large. Furthermore, these effects were sustained at 4-weeks follow-up and still present during dual task conditions. Gaining consolidated improvements of gait adaptation through an implicit learning paradigm is remarkable in a condition such as PD, which is notorious for its automaticity and motor learning deficits [34]. However, and crucially, the training effects did not transfer to over-ground turning speed, the primary outcome of this trial. Both treadmill groups achieved significant gains for over-ground step length and gait speed, exceeding the minimal clinically important difference [35]. Additionally, the MDS-UPDRS III scores were improved and retained for 4 weeks in both groups, but this only reached clinically meaningful effects in the SBT-group. These results indicate that SBT improved over-ground gait and symptom severity similarly or better than the well-established traditional treadmill training [10,36]. Additionally, it had surplus and specific value for gaining gait adaptation capacity to asymmetric treadmill perturbations. Balance outcomes and falls efficacy were not improved in either group.

The lack of transfer effects to over-ground turning is an important finding, even though at the within-group level the number of turns increased significantly in the SBT- and not in the TBT-group. Of note, this was not supported by significant interaction effects. This unexpected result was in contrast to our pilot data indicating that one session of SBT-training not only improved gait adaptation but also DT turning speed in a population of freezers [21]. We propose four explanations for this lack of transfer. First, as our pilot trial included freezers only, the baseline values of turning speed were worse than in the present mixed cohort. In previous rehabilitation studies [37,38], worse baseline scores predicted better training effects probably due the larger scope for improvement. The sample size calculation was also based on these pilot data. Hence, our study was probably underpowered. Indeed, although the effects on the turning outcomes were consistently better in SBT than in TBT (recall **Figure 3**), they never reached statistical significance.

Second, as SBT-perturbations require multidimensional stepping and postural responses, the choice of the transfer task may have been suboptimal. Transfer of motor learning implies that after the training of one specific task, benefits can be expected in task variants or in novel conditions [39]. Depending on the level of resemblance with the trained task, ‘near’ or ‘far’ transfer is inferred. We used 1 minute of over-ground turning (360°), while switching directions, as the transfer task. However, turning is deemed a complex task requiring visual guidance, extensive sensory integration and head-pelvis dissociation [30]. As turning on the spot does

not capture a shift from straight-line walking to asymmetrical gait, it may have been too dissimilar as a near or far marker of transfer.

Third, the lack of transfer could be specific to PD [34,40]. Compared to healthy controls, people with PD showed overall intact motor learning capacity of goal-directed upper limb tasks, but a clear lack of transfer to different task conditions and different contexts [41]. Also, during an adaptive visuomotor reaching task, people with PD had a lower capacity for inter-limb transfer of the acquired adaptation skills. Interestingly, this deficit proved correlated to reduced DAT-binding in the right striatum [42].

Fourth, the training conditions could have been responsible for the lack of transfer [40]. Motor learning theory stipulates that offering a wide range of task variations will induce better transfer as it builds a global representation of a motor skill, thereby facilitating independence from the learning context [43]. This principle has been confirmed by a recent study showing better transfer to overground gait after treadmill training when practicing under varying conditions [44]. Work on SBT-training in healthy people confirms this notion, as it was found that introducing switches every 20 seconds rather than exposure to constant asymmetry, induced better transfer to a novel SBT-task [45]. Similarly, we found in our pilot trial that changing the ratios of the speed perturbations led to better transfer than keeping the contrasts constant. Consequently, this principle was adhered to during this study, yet without the desired transfer. It is possible that for some subjects too much variety was offered over a 4-week training, so that due to their compromised brain capacity predictions could not be made to off-treadmill performance. Certainly, transfer of SBT-training in stroke patients was enhanced by introducing perturbations gradually over a longer period rather than abruptly [46]. Recently, it was proposed that learning a repertoire of motor memories via contextual inference is key to growing transferable sensorimotor repertoires [47]. This would point to the need for adding off-treadmill learning conditions to SBT-training in order to develop the flexibility to cope with the transitions inherent to daily life [47].

We included both freezers and non-freezers in this study and found that both groups benefitted equally from SBT-training in line with our earlier work. However, the finding that SBT had no effect on FOG-outcomes was not in agreement with our previous data [21] and may partly be attributable to the inability to capture FOG-phenomena during laboratory testing in ON. Motor-cognitive treadmill training also showed no effect on self-reported FOG [48]. Interestingly, within-group analyses showed that freezers benefited more from TBT-training than non-freezers, especially for DT gait outcomes. This may be explained by the fact that TBT, especially when provided at the high and progressive dose that was administered in our study, enhanced gait capacity in freezers, which is commonly more depleted than in non-freezers

[38]. Overall, both treadmill programs showed significant time effects on over-ground gait and motor symptom severity, which were consistently larger in the SBT-group yet training effort was the same. Effects sizes for the MDS-UPDRS-III in the SBT-group resembled those of other walking endurance studies [11,36], supporting the notion that challenging treadmill training constitutes another viable rehabilitation option to alleviate motor symptoms in PD.

The current findings suggest that SBT-training is a promising therapeutic avenue to modify gait adaptation in PD in addition to the similar and sometimes even larger improvements in over-ground gait and disease severity when compared to traditional treadmill training. As perturbation training relies on wearing a harness during practice, it has potential for a wide segment of the PD population [49], including those with cognitive decline and compromised balance. However, before wide clinical implementation is recommended, further study is needed to address whether an add-on off-treadmill training can facilitate the transfer of adaptive skills, and whether SBT is useful for more cognitively impaired patients. Furthermore, transfer to daily-life ambulation and unsupervised contexts is yet to be demonstrated [50].

The present results need to be interpreted with some caution as participants and trainers were not blinded and the same trainers were involved in delivering SBT and TBT. However, we minimized performance bias by using structured progression levels in each training arm to ensure equal compliance. Also, participants were unaware of whether they were receiving the experimental treadmill training or not. Finally, the sub-analysis in freezers was not preregistered and due to the small sample (N=22) inadequately powered for the primary outcome.

Conclusion

This study has shown that people with PD learn and retain the gait adaptation skills to overcome asymmetric gait-speed perturbations on a treadmill remarkably well, but seem unable to generalize these skills to turning off-treadmill. The current findings warrant further investigation into whether an enriched SBT-training targeted to enhance transfer to over-ground turning, will eventually translate into better mobility in daily life.

Acknowledgements

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Data Availability Statement

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

Authors' Roles

(1) Research project: A. Conception, B. Organization, C. Execution;

(2) Statistical analysis: A. Design, B. Execution, C. Review and critique;

(3) Manuscript: A. Writing of the first draft, B. Review and critique.

FH: 1C, 2A, 2B, 3A

JS: 1C, 2A, 2B, 3A

ND: 1B, 2A, 2C, 3B

PG: 1B, 2C, 3B

AN: 1A, 1B, 2C, 3B

CS: 1A, 1B, 2C, 3B

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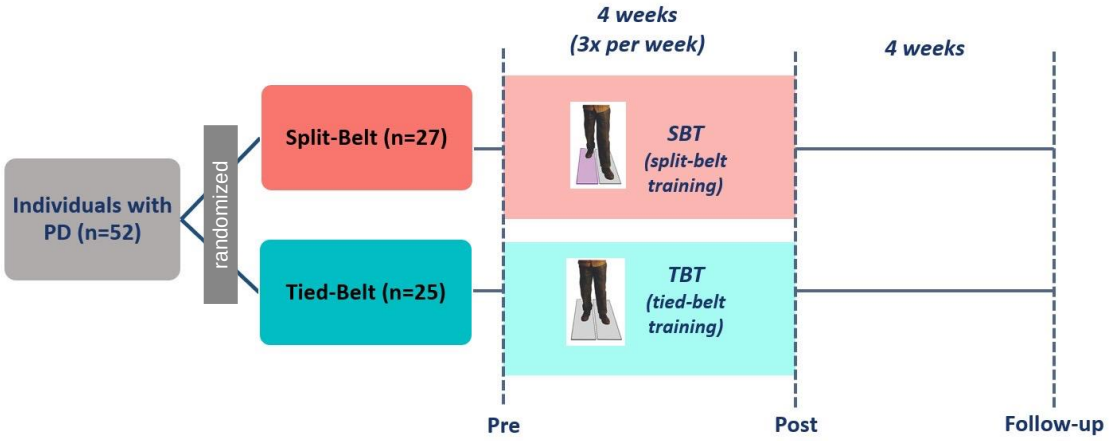
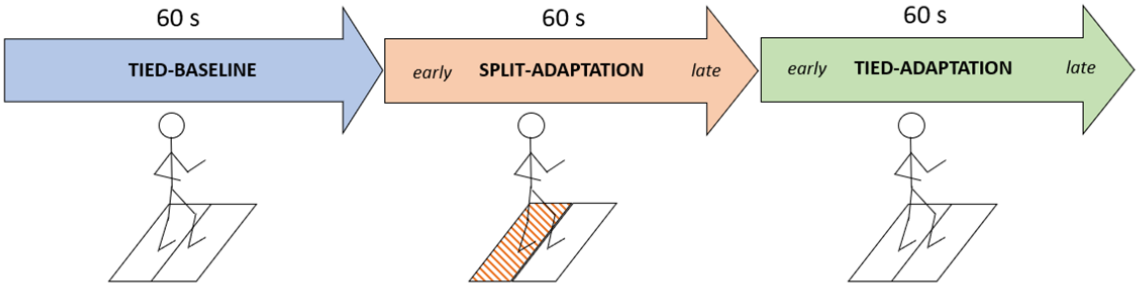
Supplementary materials



Supplementary Figure 1. Study design.

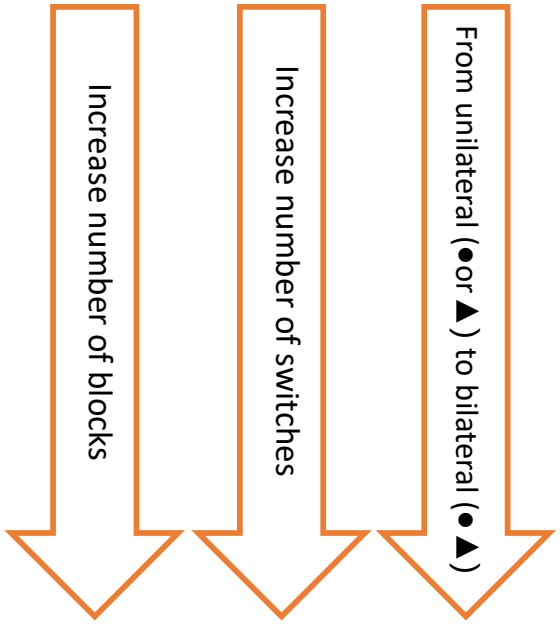


Supplementary Figure 2. Gait adaptation task: step length asymmetry during the gait adaptation task was calculated as follows: (fast leg parameter - slow leg parameter)/(fast leg parameter + slow leg parameter). This calculation allows step length asymmetry to be represented in the direction of both body sides. Due to the nature of the task, asymmetry will increase into the negative direction as upon first exposure of the leg on the fast belt, this limb will take shorter steps. Subsequently, asymmetry will usually decrease from early-split to late-split. When switching back to tied-belts, asymmetry will then present in the direction of positive values as an aftereffect of the adaptation (tied-adaptation) at early-tied, which will diminish at late-tied. Through SBT this spatial asymmetry during the split-phase can be reduced, hence values close to '0' represent symmetry and better gait adaptation.

Split-belt training protocol

In our pilot work, we tested the training principle of slowing down the best side (defined as the side with the initially longer step length), a notion which was recommended previously (Fasano et al., 2016). We found that the side of perturbation was not critical, but that the best retention was gained by having larger speed contrasts between belts and by changing the speed manipulations more often (D'Cruz et al. 2020, Seuthe et al. 2020). This led to a design of the training program which allowed a gradual build up from unilateral to bilateral gait speed switches.

Supplementary Table 1. Split-belt training progression.



	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	Block 9	
Level 1	A ●	A ●	A ●	A ▲	A ▲	A ▲	COOL▲			
Level 2	A ●	A ▲	B ●	B ●	B ▲	B ▲	COOL●			
Level 3	B ●	B ▲	C ●	C ●	C ▲	C ▲	COOL▲			
Level 4	C ●	C ▲	D ●	D ●	D ▲	D ▲	COOL●			
Level 5	D ●	D ▲	E ●	E ▲	E ●	E ▲	E ●	COOL▲		
Level 6	E ●	E ▲	F ●	F ▲	F ●	F ▲	F ▲	COOL●		
Level 7	F ●	F ▲	G ●	G ▲	G ●	G ▲	G ●	COOL▲		
Level 8	G ●	G ▲	H ●	H ▲	H ●	H ▲	H ▲	COOL●		
Level 9	H ●	H ▲	I ●▲	I ●▲	I ●▲	I ●▲	I ●▲	I●▲	COOL▲	
Level 10	I ●▲	I ●▲	J ●▲	J ●▲	J ●▲	J ●▲	J ●▲	J●▲	COOL●	
Level 11	J ●▲	J ●▲	K ●▲	K ●▲	K ●▲	K ●▲	K ●▲	K●▲	COOL▲	
Level 12	K ●▲	K ●▲	L ●▲	L ●▲	L ●▲	L ●▲	L ●▲	L●▲	COOL●	
Level 13	L ●▲	L ●▲	M ●▲	M ●▲	M ●▲	M ●▲	M ●▲	M●▲	M●▲	COOL▲
Level 14	M ●▲	M ●▲	N ●▲	N ●▲	N ●▲	N ●▲	N ●▲	N●▲	N●▲	COOL●
Level 15	N ●▲	N ●▲	O ●▲	O ●▲	O ●▲	O ●▲	O ●▲	O●▲	O●▲	COOL▲
Level 16	O ●▲	O ●▲	O ●▲	O ●▲	O ●▲	O ●▲	O ●▲	O●▲	O●▲	COOL●

● = Left side, ▲ = Right side, ●▲ = Bilateral (both sides)

Abbreviations: Side=side of the split-belt treadmill that was manipulated, ● = Left side, ▲ = Right side; ●▲ bilateral (both sides); color saturation represents the amount of ratio switches within one block (warmer = more switches); duration of each block: 5 minutes; for belt-speeds ratios in each block please see **Table 2**, sec=seconds.

Supplementary Table 2 (continues on next page). Split-belt level training details.

Unilateral	# switches	protocol																	
A	4	ratio	3:4	1:2	3:4	1:2	3:4												
		sec	50	80	55	70	45												
B	6	ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4										
		sec	35	50	40	45	30	55	45										
C	7	ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2									
		sec	35	40	30	35	45	30	40	45									
D	8	ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4								
		sec	25	45	30	35	30	40	35	30	30								
E	10	ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2					
		sec	20	30	25	20	30	25	20	30	25	20	30	25					
F	12	ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	3:4	1:2	3:4	1:2	3:4	1:2	3:4			
		sec	15	20	25	30	20	25	15	15	20	25	30	20	25	15			
G	14	ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	
		sec	20	25	15	15	25	15	15	20	20	25	15	15	25	15	15	20	
H	16	ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4
		sec	15	25	15	15	10	15	20	20	15	15	25	15	15	10	15	20	20	15
Bilateral	# switches	protocol																	
I	6	side	●	●	▲	▲	▲	●	●										
		ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4										
		sec	50	30	25	75	50	45	25										
J	7	side	●	●	▲	▲	●	●	▲	▲									
		ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2									
		sec	40	45	30	35	30	35	40	45									

K	8	side	●	●	▲	▲	●	●	▲	▲	▲									
		ratio	3:4	1:2	3:4	1:2	3:4	1:2	3:4	1:2	3:4									
		sec	30	45	30	35	35	45	30	25	30									
L	10	side	●	▲	●	▲	●	▲	●	▲	●	▲	●	▲						
		ratio	3:4	1:2	1:2	3:4	3:4	1:2	3:4	1:2	1:2	3:4	3:4	1:2						
		sec	20	30	25	20	30	25	20	30	25	20	30	25						
M	12	side	●	▲	●	▲	●	▲	●	●	▲	●	▲	●	▲	●				
		ratio	3:4	1:2	1:2	3:4	3:4	1:2	1:2	3:4	1:2	1:2	3:4	3:4	1:2	1:2				
		sec	15	20	25	30	20	25	15	15	20	25	30	20	25	15				
N	14	side	●	▲	●	▲	●	▲	●	▲	●	▲	●	▲	●	▲	●	▲		
		ratio	3:4	1:2	1:2	3:4	1:2	1:2	3:4	1:2	3:4	1:2	1:2	3:4	1:2	1:2	3:4	1:2		
		sec	20	25	15	15	25	15	15	20	20	25	15	15	25	15	15	20		
O	16	side	●	▲	●	▲	●	▲	●	▲	●	●	▲	●	▲	●	▲	●	▲	●
		ratio	1:2	1:2	1:2	3:4	3:4	1:2	1:2	1:2	3:4	1:2	1:2	1:2	3:4	3:4	1:2	1:2	1:2	3:4
		sec	15	25	15	15	10	15	20	20	15	15	25	15	15	10	15	20	20	15
COOL	Cool down	2min	3:4																	

Abbreviations: Side=side of the split-belt treadmill that was manipulated, ● = Left side, ▲ = Right side; for the orange levels within one block only one side was manipulated (unilateral switches); for the blue levels within one block both sides were manipulated (bilateral switches); color saturation represents the amount of ratio switches within one block (warmer = more switches); duration of each block: 5 minutes; ratio= ratio between the two belts; 1:2= one belt is reduced by 50% of the individual gait speed; 3:4= one belt is reduced by 25% of the individual gait speed, sec=seconds.

Tied-belt training protocol

The TBT-training program was aimed to keep patients as motivated for this program as for the SBT-training group. Progression was built following the example of previous treadmill interventions in PD (Mirelman et al., 2013). A design of a gradual build up using levels was done similarly to the SBT protocol see **Table 3**.

Supplementary Table 3. Tied-belt level training progression.

	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	Block 9		Max length
Level 1	80	85	85	85	85	80	COOL				5’
Level 2	80	85	90	90	85	80	COOL				5’
Level 3	85	90	90	90	90	85	COOL				5’
Level 4	85	90	95	95	90	85	COOL				5’
Level 5	90	95		95		90		COOL			10’
Level 6	90	95			95		90	COOL			15’
Level 7	95	95	100		100		95	COOL			10’
Level 8	95	100			100		95	COOL			15’
Level 9	95	100			100			95	COOL		15’
Level 10	95	100	105		105		100	95	COOL		10’
Level 11	100	105			105			100	COOL		15’
Level 12	100	105			105			100	COOL		20’
Level 13	105	105	110		110		110		105	COOL	10’
Level 14	105	110			110			105		COOL	15’
Level 15	105	110			110			105		COOL	20’
Level 16	105	110			110			105		COOL	20’

Abbreviations: Blue colored blocks are the training blocks; Grey colored blocks are the cooling-down blocks, Max length: maximal length of the longest walking block within one session in minutes; color saturation represents the length and speed of the walking block (darker = longer, faster).

KEY RULES FOR THE PROGRESSION LEVELS FOR SBT AND TBT TRAINING

The participant started at level 1. For each consecutive training session, the participant moved one level up. Subjective perception of training load/exertion was determined after each session. In case the participant was insufficiently challenged, a level was skipped (max. 1 level per week). If a participant was challenged too much, the same level was done in the next session. However, the duration of training was standardized (week 1: 30 min, week 2: 35 min, week 3: 40 min, week 4: 45 min). To ensure the same duration, a level was extended to correct for the duration of the training week by repeating the first block(s) at the end of the original level (e.g. if level 3 needed to be redone; this was repeated in week 2).

Supplementary Table 4. Tied-belt level training details.

Condition	% walking speed	Duration (min)	Condition	% walking speed	Duration (min)	Condition	% walking speed	Duration (min)
A	80	5	H	95	10	O	110	10
B	85	5	I	95	15	P	110	15
C	90	5	J	100	10	Q	110	20
D	95	5	K	100	15	R	105	2.5
E	100	5	L	105	10	COOL	80	2
F	105	5	M	105	15			
G	90	10	N	105	20			

Abbreviations: %Walking speed: the % of the comfortable walking speed used; min: minutes; color saturation represents the length and speed of the walking block (darker = longer, faster).

Protocol deviations

Supplementary Table 5. Attended training sessions of complete cases.

	SBT	TBT
Number of training sessions completed	Number of participants	Number of participants
12	14	11
11	5	3
10	2	3
9	1	1

Supplementary Table 6. Attended training sessions of drop-outs.

	SBT	TBT
Number of training sessions completed	Number of drop-outs	Number of drop-outs
12	1	3
11	1	
10		1
9	1	
6		
5		1
4		2
3	1	1

Change in medication: n=1 patient started Levodopa therapy during the training period, n=2 patients changed medication during training period

Adverse events: n=1 patient experienced a fall during the assessment protocol; was re-recruited and successfully completed the training after consultation with both patient and doctors

Re-recruitment: n=4 patients were re-recruited after drop-out (n=2 due to Covid, n=1 due to health issues, n=1 due to fall). Wash-out period was ≥ 6 months. All assessments were re-administered

Supplementary Table 7. Clinical measures.

Outcome	Time	SBT (n=27)			TBT (n=25)			Effect-size	Between group Pre *p*-value	Between group *p*-value	Time-effect / group*time *p*-values
		Mean (SD)	Median (Q1-Q3)	Post hoc *p*-value	Mean (SD)	Median (Q1-Q3)	Post hoc *p*-value				
MDS-UPDRS-III (0-132)	Pre	35.1(12.8)	35.0(26.5-43.0)		31.2(13.5)	29.0(23.0-39.0)			0.19		<0.01*(0.0003)/0.35
	Pre-Post	-5.1(8.0)	-4.5(-9.3- -0.8)	0.02	-1.8(6.8)	-2.0(-8.0-5.0)	0.79	-0.1		0.16	
	Pre-Ret	-5.8(14.8)	-6.5(-13- -1.25)	0.25	-2.9(6.8)	-3.5(-6.5- -1.3)	0.80	0.0		0.50	
Mini-BESTest (0-28)	Pre	23.9 (3.1)	25.0(22.0-26.0)		22.4(4.7)	25.0(21.0-26.0)			0.24		0.30/0.11
	Pre-Post	-0.3(2.1)	0.0(-2.0-1.0)	0.50	1.0(2.6)	0.0(-1.0-2.0)	0.03	0.3		0.04*	
	Pre-Ret	0.4(2.3)	0.5(-1.0-2.0)	0.45	0.7(2.5)	0.5(-1.0-2.0)	0.0.19	0.1		0.70	
FAB-Scale (0-40)	Pre	33.1(5.2)	34.0(32.0-36.5)		30.4(7.5)	33.0(24-0-36.0)			0.30		0.44/0.54
	Pre-Post	-0.3(2.3)	-0.5(-1.5-1.0)	0.48	0.3(2.6)	0.0(-1.0-1.0)	0.40	0.1		0.27	
	Pre-Ret	0.2(3.8)	0.0(-1.0-2.0)	0.70	0.9(3.5)	1.5(-2.0-3.0)	0.0.23	0.1		0.58	
FES-I (16-64)	Pre	25.6(9.6)	22.0 (18.5-32)		24.1(8.7)	20.5(17.8-29.3)			0.63		0.07/0.36
	Pre-Post	-0.8(5.0)	0.0(-2.0-1.0)	0.47	-2.2(6.7)	0.0(-2.0-2.0)	0.09	-0.1		0.56	
	Pre-Ret	-1.2(7.8)	-0.5(-3.5-1.5)	0.55	-5.2(10.8)	0.0(-11.0-3.0)	0.01	0.0		0.52	

Abbreviations: SBT=Split-belt treadmill training; TBT=Tied-belt treadmill training; SD=standard deviation; CV=covariance; Q1=1st quantile; Q3=3rd quantile; MDS-UPDRS-III=Movement Disorder Society – Unified Parkinson's Disease rating Scale Part III; Mini-BESTest=Mini Balance Evaluation Systems Test; FAB-Scale=Fullerton Advanced Balance Scale; FES-I=Falls Efficacy Scale – International; *significant p-value (<0.05).

Supplementary Table 8. Freezing related outcomes.

Outcome	ST or DT	Time	SBT (n=27)			TBT (n=25)			Effect-size	Between group Pre (*p*-value)	Between group (*p*-value)	Time-effect / group*time (*p*-values)
			Mean (SD)	Median (Q1-Q3)	Post hoc (*p*-value)	Mean (SD)	Median (Q1-Q3)	Post hoc (*p*-value)				
FOG ratio ML	ST	Pre	3.24(3.77)	1.74(1.13-4.15)	-	2.99(3.01)	2.12(1.20-3.10)	-	-	0.80		0.55/0.11
		Pre-Post	-0.65(2.85)	-0.45(-0.94-0.41)	0.49	2.03(5.72)	0.15(-0.20-0.80)	0.03	-0.79		0.05	
		Pre-Ret	-0.84(4.36)	-0.36(-1.08-0.32)	0.62	1.72(8.72)	-0.19(-0.56-0.05)	0.12	-0.75		0.14	
	DT	Pre	3.35(5.19)	1.76(1.11-3.01)	-	4.59(8.38)	2.03(1.18-3.72)	-	-	0.52		0.41/0.56
		Pre-Post	-0.69(3.88)	-0.04(-0.57-0.48)	0.59	-0.42(6.92)	0.05(-0.59-0.54)	0.77	-0.04		0.88	
		Pre-Ret	-0.53(6.06)	0.05(-0.45-1.00)	0.76	-0.33(1.70)	0.10(-0.80-0.65)	0.34	-0.03		0.40	
Ziegler	ST	Pre	0.90(1.37)	0.00(0.00-2.00)	-	5.50(5.80)	3.50(1.50-8.50)	-	-	0.04		0.10/0.42
		Pre-Post	0.89(1.69)	0.00(0.00-2.00)	0.34	2.57(6.95)	0.00(-1.00-3.00)	0.11	-0.40		0.50	
		Pre-Ret	0.67(1.80)	1.00(0.00-2.00)	0.47	-1.29(9.62)	0.00(-3.00-0.00)	0.70	0.46		0.86	
%FOG	ST	Pre	0.74(1.71)	0.00(0.00-0.56)	-	10.27(25.23	0.31(0.00-6.82)	-	-	0.29		0.88/0.32
		Pre-Post	0.35(2.76)	0.00(0.00-1.92)	0.92	-4.36(23.00)	0.00(-0.62-1.62)	0.67	0.26		0.70	
		Pre-Ret	-0.45(2.00)	0.00(-1.08-0.00)	0.92	-3.45(29.39)	0.00(-0.61-0.60)	0.91	0.17		0.98	
	DT	Pre	1.51(2.22)	0.00(0.00-2.88)	-	18.47(36.40	1.19(0.00-22.69)	-	-	0.22		0.31/0.49
		Pre-Post	-0.97(1.82)	0.00(-2.27-0.00)	0.77	-9.04(18.41)	0.00(-25.64-0.10)	0.08	0.31		0.25	
		Pre-Ret	-0.81(5.00)	0.00(-2.27-1.75)	0.80	-10.67(21.07	0.00(-24.85-1.00)	0.08	0.38		0.24	

Abbreviations: SBT=Split-belt treadmill training; TBT=Tied-belt treadmill training; ST=single task, DT=dual task; SD=standard deviation; CV=coefficient of variation, Q1=1st quantile, Q3=3rd quantile, FOG=Freezing of gait; ML=medio-lateral, *significant p-value (<0.05).

Supplementary Table 9. Linear mixed model results for FOG by time interactions.

			FOG*time	
	variable	**condition**	**F-value**	**p-value**
		SBT-group		
Overground gait	gait speed	ST	0.50	0.61
		DT	0.07	0.94
	step length	ST	0.81	0.46
		DT	0.17	0.84
	gait asymmetry	ST	0.23	0.79
		DT	1.58	0.23
Gait adaptation	total	ST	0.63	0.54
		DT	0.04	0.96
	early-split	ST	1.56	0.22
		DT	0.53	0.59
	late-split	ST	1.42	0.25
		DT	0.17	0.84
Turning	turning speed	ST	1.16	0.33
		DT	1.97	0.32
	turning speed CV	ST	0.14	0.87
		DT	0.12	0.89
	No. of turns	ST	1.12	0.34
		DT	1.70	0.21
	FOG ratio ML	ST	0.76	0.48
		DT	0.44	0.65
	Ziegler	ST	1.38	0.27
		TBT-group		
Overground gait	gait speed	ST	1.24	0.31
		DT	3.84	0.04*
	step length	ST	1.10	0.35
		DT	5.58	0.01*
	gait asymmetry	ST	2.90	0.08
		DT	1.57	0.24
Gait adaptation	total	ST	0.15	0.87
		DT	0.00	0.99
	early-split	ST	0.37	0.69
		DT	0.25	0.78
	late-split	ST	0.07	0.93
		DT	0.27	0.76
Turning	turning speed	ST	2.44	0.12
		DT	1.77	0.20
	turning speed CV	ST	1.67	0.22
		DT	0.94	0.41
	No. of turns	ST	1.52	0.25
		DT	1.09	0.36
	FOG ratio ML	ST	1.05	0.38
		DT	1.40	0.51
	Ziegler	ST	0.98	0.39

Abbreviations: SBT=Split-belt treadmill training; TBT=Tied-belt treadmill training; CV, coefficient of variation; ML, Medio-lateral

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Part II

Underlying Mechanisms of Adaptive Gait in Parkinson's Disease

Chapter 4

Cortical Activation during Split-belt Treadmill Walking in People with Parkinson's Disease and Healthy Controls

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Abstract

People with Parkinson's disease (PwPD) have difficulty adapting their gait to asymmetrical conditions. To better understand this deficit, we compared cortical activity between 42 PwPD and 42 healthy controls using functional near-infrared spectroscopy during tied-belt (TB) and split-belt (SB) treadmill walking. Oxygenated hemoglobin (HbO_2) was measured in the prefrontal cortex, supplementary motor area (SMA), premotor cortex (PMC) and posterior parietal cortex (PPC). Gait adaptation, assessed by step length asymmetry, was worse during the last five steps of SB versus TB in PwPD compared to controls. PwPD showed higher HbO2 in the PMC ($p=0.005$) and PPC ($p=0.004$) than controls, regardless of condition. However, an increase in HbO_2 in the SMA during SB was shown relative to TB in PwPD but not in controls (group*condition interaction $p=0.048$; pairwise post-hoc $p=0.032$). Interestingly, increased PPC activity in PwPD was associated with less well-adapted gait. We infer that both regular and adaptive gait required increased cortical processing in PwPD. Increases in cortical activity seemed not to be associated with normalized asymmetry. Instead, the extent of cortical recruitment in the premotor and posterior parietal regions may be a marker of impaired adaptive gait in PD.

Introduction

Parkinson's disease (PD) results in reduced transmission of the neurotransmitter dopamine in the basal ganglia with severe repercussions for related motor and non-motor circuitries [1]. Particularly, the control of automatic movements, such as gait, is disrupted [2]. As a result, people with PD (PwPD) have difficulty adapting their stepping pattern to challenging and asymmetrical conditions, including turning [3–5], often provoking gait instability and freezing of gait. These adaptive deficits worsen as the disease progresses, and are associated with an increased risk of falls [6,7]. In this study, we used a split-belt (SB) treadmill as a paradigm for studying adaptation difficulties in PD for three reasons. First, a SB treadmill can induce highly standardized asymmetrical gait perturbations by controlling the walking speed of both legs separately. Second, our previous work on SB-perturbations showed that a 4-week SB training program resulted in substantial improvements of SB-walking in PD [8]. Third, Hinton et al. (2019) used Positron Emission Tomography (PET) imaging to highlight that the supplementary motor area (SMA) and the posterior parietal cortex (PPC) are central hubs in the so-called adaptive gait network studied during SB-walking in healthy subjects [9]. However, the neural mechanisms underpinning problems with gait adjustments in PD are still unclear.

Functional near-infrared spectroscopy (fNIRS) is able to capture cortical brain activity, as derived from oxygenated (HbO_2) and deoxygenated (HHb) hemoglobin levels, and has been used to study upright walking in PwPD and healthy controls (HC) [10–15]. Most previous studies only analyzed the prefrontal cortex (PFC) and found inconsistent results. These discrepancies might be explained by the heterogeneity of the protocols used as well as by the varying task requirements [10–14,16]. Recently, Pelicioni et al. (2022) investigated the premotor cortex (PMC) , the SMA, and the PFC in PwPD and HC using fNIRS during a motor planning task. They examined overground walking (simple task) and compared it to stepping onto or avoiding a target (complex tasks) [15]. They found that PwPD increased PMC activity during both simple and complex walking tasks compared to HC [15]. Moreover, HC showed increased PFC activity when the task became more complex, while no difference was seen in PwPD. The authors suggested that the PFC in PwPD may have already been maximally engaged in compensatory executive control during simple walking [15]. Overall, SMA activity was increased during complex walking, but this effect was mostly driven by HC. Until now, it is unknown whether PwPD modulate brain activity differently to HC when adapting to asymmetric gait on a SB treadmill. This question is relevant as SB-perturbations resemble to some degree the asymmetrical gait adjustments during turning, which are closely related to freezing and falling [6,7].

The present study aimed to reveal the brain activity changes during adaptation to asymmetric walking with fNIRS in PwPD and HC. To do this, participants performed three blocks of

treadmill walking – first with the belts moving at the same speed (tied-belt, TB), followed by two blocks where the speed of one side was reduced by 50% (SB). The ability to adjust their gait to asymmetric conditions was quantified by step length asymmetry and its variability.

We selected four cortical regions of interest (ROIs), earlier shown to be active during adaptive gait in healthy subjects, i.e. the PFC, SMA, PMC and PPC [9,15]. We expected:

1. An overall increase in cortical activity in all ROIs during SB- compared to TB-walking (condition effect) given the increased adaptive challenge imposed by SB-walking.
2. General increased activity in all ROIs in PwPD versus HC during TB-walking (group effect), reflecting compensation for the loss of gait automaticity in PD [15], except for the SMA due to the hypoactivity often observed in this region in PD [17].
3. That PwPD would increase their cortical activity during SB-walking in the PMC and PPC compared to TB-walking, more so than HC (group*condition interaction), as these regions were previously associated with motor compensation [15]. Less involvement of the SMA and PFC in coping with SB-walking was also expected due to alterations in SMA-function in PwPD [17] and the implicit nature of SB-perturbations [18].

To test our hypotheses, we first explored whether there were carry-over effects between the consecutive blocks of SB-walking (SB1 and SB2), the possible influence of leg and disease side dominance, as well as of outliers and treadmill speed to see if we could pool SB-conditions and whether covariates were necessary for the TB versus SB comparison. Additionally, a channel to channel analysis was conducted to visualize the overall distribution of activity changes regardless of pre-assigned ROI. Lastly, exploratory correlation analyses were performed to understand the variance in the hemodynamic responses by analyzing the relationship with adaptative behavior.

Methods

Participants

We included 84 participants in the study (42 PwPD and 42 HC) based on an a-priori power calculation (see ‘statistical analysis’), who were assessed between October 2021 and September 2022. For both PwPD and HC, inclusion criteria were: (1) subjective confirmation of ability to walk five minutes without assistance or assistive device, and (2) no probable dementia (Mini-Mental State Examination (MMSE) ≥24). For PwPD, additional inclusion criteria were: (3) clinical diagnosis of idiopathic PD by a neurologist based on the criteria of the International Movement Disorders Society, (4) Hoehn & Yahr stage II or III in the ON medication state [19], (5) steady antiparkinsonian medication schedule for the month before enrollment, and (6) stable medication state to allow testing for at least 4 hours. Exclusion criteria comprised of: (1) participation in another clinical study that could influence study

outcomes, (2) acute orthopedic injuries or musculoskeletal problems that could affect balance and gait, (3) neurological or significant psychiatric impairments (other than idiopathic PD and psychiatric conditions associated with PD), (4) cardiovascular exercise risk factors based on self-report, (5) previous exposure to SB-walking, and (6) presence of a deep brain stimulator. This study was approved by the Ethical Committee Research UZ/KU Leuven (study ID: S64280), and all participants gave written informed consent before participation. The trial was registered at the Open Science Framework (https://osf.io/gkeq3/).

Assessment of fNIRS and gait on the treadmill

Participants visited our laboratory for a single 3.5 to 4-hour assessment. PwPD were assessed in their typical "ON" state of medication, which was subjectively confirmed by the participants during the assessment. The fNIRS assessment involved a blocked design, ensuring reliable signal acquisition. Data was collected during three blocks, including one TB- and two SB-walking blocks offered in a fixed order, each consisting of seven trials (see **Figure 1**). TB-walking was always performed first to avoid carry-over effects of the SB-walking conditions. The two SB-blocks (SB1 and SB2) involved 50% reduced belt speed for either the right or left leg, the order of which was randomized for leg dominance based on the question: 'With which leg would you kick a ball?' [20].

In **Figure 1,** the protocol **(A)** and within-trial timing **(B)** are displayed. A trial started with 15 seconds of quiet stance to acquire baseline cortical activity. Next, the treadmill (Motek Medical, Amsterdam, The Netherlands) accelerated to the designated speed, personalized for each subject, in 7.5 seconds. Subsequently, the belts would run in TB or SB for 25 seconds, after

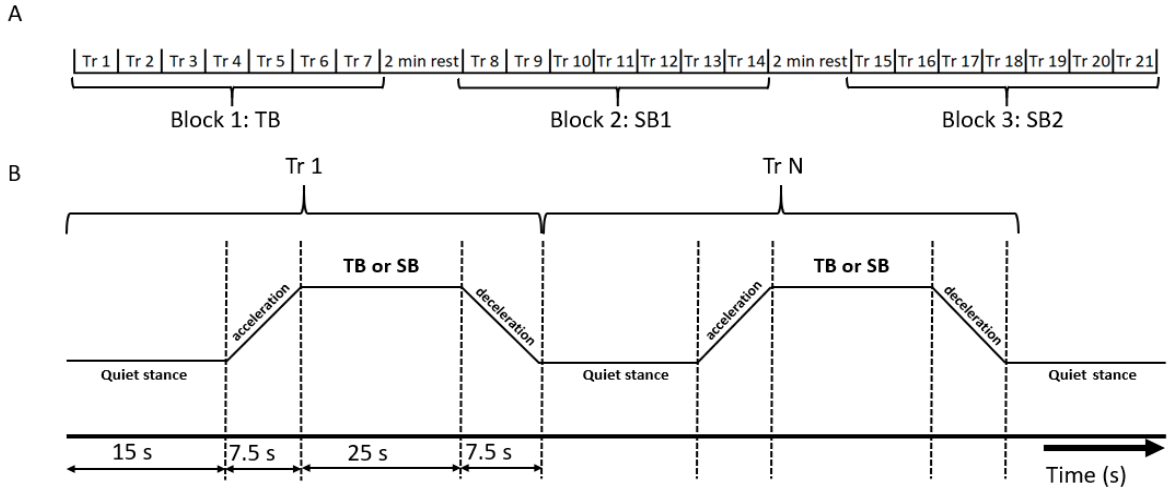

Figure 1. Protocol and within-trial timing of the fNIRS assessment. (A) Displays the overall protocol. Each of the 3 blocks (TB, SB1, SB2) consisted of 7 trials. (B) Each trial consisted of 15 seconds of quiet stance followed by an acceleration phase (7.5 sec) to reach comfortable walking speed. Next, participants underwent 25 seconds of TB- or SB-walking followed by a deceleration phase. Abbreviations: Tr, trial; min, minutes; TB, tied-belt; SB, split-belt; SB1, first SB-trial; SB2, second SB-trial; s, seconds

which the treadmill decelerated to 0 m/s. As such, the calculation of the hemodynamic change between baseline (quiet stance) and gait could be calculated.

Gait adaptation was quantified as the mean step length asymmetry, standard deviation (SD) and coefficient of variation (CV) of asymmetry over the total trial and the first and last five steps of each trial. These outcomes were captured with VICON (Vicon Motion Systems Ltd, Oxford, UK) with six passive reflective markers placed on the feet (lateral malleolus, heel and tip of the shoe on each foot). As described in previous work [21], step length asymmetry was calculated as a ratio value: (fast leg parameter – slow leg parameter)/(fast leg parameter + slow leg parameter). Values closer to zero represented more symmetrical gait. Negative values represented asymmetry whereby the fast leg would make smaller steps than the slow leg [21].

Treadmill speed was based on the participant's comfortable overground gait speed (see 'Overground gait assessment' for details). Before the protocol commenced, participants had a 2-minute TB familiarization trial to verify their comfortable walking speed. If overground gait speed was perceived as too fast on the TB, this was adjusted in a standardized fashion by reducing the speed by steps of 5% until the participant confirmed the speed to be comfortable. Then, participants were familiarized with the above-mentioned blocked design, where they experienced one trial of each condition (TB, SB1, SB2). Participants were asked to let go of the handrails by verbal cue as soon as they reached the desired speed. However, if participants were not able to walk safely without holding the handrails, they were allowed to hold the handrail during the entire assessment for consistency (N=8, in PD only). Participants wore a non-weight-bearing harness that also ensured their safety.

Overground gait assessment

Prior to the treadmill assessment, overground gait speed was assessed during ten trials of six-meter walking at a steady comfortable walking speed. Participants also performed a 360° turning-in-place task to measure overground adaptation capacity. Here, they were asked to turn for one minute, in alternating directions as quickly as possible on the spot . Gait and turning kinematics were captured using three inertial measurement units on the feet and lower back (APDM Inc., Portland, OR, USA). Turning speed and number of turns were calculated using a custom MATLAB script (Matlab R2019a, MathWorks, Natick, USA) [22].

Clinical and cognitive assessment

The following balance and cognitive assessments were obtained from all participants: (1) the Mini-Balance Evaluations System Test (MiniBEST) [23], (2) the Falls Efficacy Scale (FES-I) [24], (3) Trail-Making-Test Part A and B [25], (4) Montreal Cognitive Assessment (MoCA) [26] and (5) Frontal Assessment Battery (FAB) [27]. From the PwPD, we also collected: (1) the Movement Disorders Society - Unified Parkinson's Disease Rating Scale Part III (MDS-

UPDRS-III) to assess their motor symptoms [28], (2) Levodopa Equivalent Daily Dosage (LEDD) [29] and (3) the New Freezing of Gait Questionnaire (NFOGQ) [30] to determine the patients' self-reported freezing status.

fNIRS data acquisition and analysis

Brain oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (HHb) levels were recorded with the Aurora software (version 2021.4.1.1) using a continuous wave fNIRS system (NIRSport2, NIRx, Berlin, Germany) including 16 LED sources and 16 detectors. Wavelengths were preset at 760 nm and 850 nm with a sampling frequency of 7.81 Hz. The 32 optodes were placed approximately 3 cm apart on a cap, corresponding with the head circumference of the participants (5 available cap sizes). Additionally, the cap included 16 short channels, one for each source (8 mm distance), to correct for possible extracerebral physiological noise [31], and two accelerometers on the back of the cap to correct for artefacts due to head movement [32]. An opaque cap then covered the optodes on the fNIRS cap to reduce possible external light interference. The reference for cap placement was Cz, determined by the locations of the nasion, inion and preauricular points. Before testing, signal quality was checked, and optodes were adjusted if needed.

Brain ROIs and corresponding optode locations were determined according to Zimeo-Morais et al. (2018) using the fOLD toolbox and Brodmann areas (see **Figure 2**) [33]. ROIs were selected based on their known involvement in adaptive gait [9]. The specificity of the ROI was set at ≥ 50% as per previous work [15,34,35]. ROIs included the prefrontal cortex (PFC, BA9, 10 and 46), supplementary motor area (SMA, BA8 or frontal eye fields and BA6 medial), premotor cortex (PMC, BA6 lateral) and the posterior parietal cortex (PPC, BA5 and BA7) [35]. Previous work showed that PFC, PPC and PMC had excellent test-retest reliability in healthy older adults when performing a balance task (intra-class correlations ≥ 0.78 without cap removal). However, this value was moderate for the SMA (ICC=0.48) [35]. The midline channels were excluded from the analysis (see **Figure 2**) to avoid noise due to cerebral spinal fluid in the superior sagittal sinus [35,36].

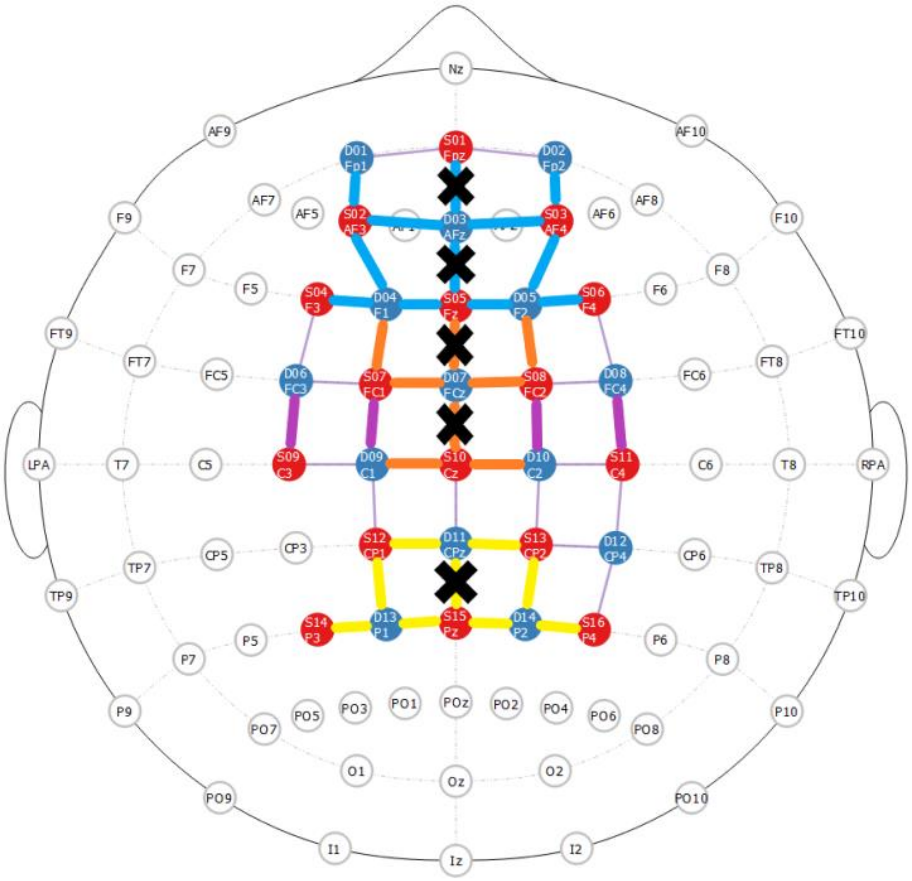

Figure 2. Optode montage as determined by the fOLD-toolbox with a specificity ≥50%. The colored lines represent the channels included in the analysis per ROI. Blue: PFC (BA9, BA10), orange: SMA (BA8 and medial BA6), purple: PMC (lateral BA6), yellow: PPC (BA5, BA7). Red optodes are sources. Blue optodes are detectors. Black crosses are the excluded channels on the midline to prevent interference from the superior sagittal sinus. General lay-out adapted from de Rond et al. (2023).

The fNIRS data were exported in .nirs format and preprocessed in NIRStoolbox in MATLAB version R2019a (MathWorks, Natick, USA) [37]. First, the data was resampled to 5 Hz and converted to optical density data using the Beer-Lambert Law with a partial path length correction factor of 0.1. Second, the change in hemodynamic response between rest and walking (TB or SB) was estimated utilizing a General Linear Model (GLM) with autoregressive pre-whitening, using the iteratively reweighted least squares (AR-IRLS) method. Boxcar stimuli for the duration of the acceleration, steady-walking, and deceleration phases were created and convolved with the canonical hemodynamic response function before entering them into the model. The GLM model also included the short channels and accelerometry data as noise regressors, which is recommended to accommodate physiological noise and movement artefacts [37–39]. The beta for the steady-state walking within each channel were averaged according to the previously specified ROIs. Mean relative HbO_2 (µmol/L) in the left and right PFC, SMA, PMC and PPC was chosen as the primary fNIRS outcome. Secondary fNIRS outcomes included the mean relative HHb (µmol/L) values due to the lower signal-to-noise ratio in this signal [16,40,41].

Statistical analysis

The preregistered analysis plan can be found at https://osf.io/gkeq3/. The group (PwPD versus HC) *condition (SB versus TB) interaction was the main interest of this study and we hypothesized that PwPD would show more increases in PMC and PPC activity from TB to SB than HC. We calculated the sample size with G*Power (v3.1), using a previous fNIRS study comparing PD and HC during simple and more complex overground walking, showing an effect size (Cohen's D) of 0.31 for HbO_2 changes in the PFC [14]. This resulted in 42 participants per group, assuming a repeated measures analysis of variance (ANOVA) with a within-between factorial design, $\alpha = 0.05$ and $\beta = 0.20$.

To analyze between-group differences in the continuous demographic and overground gait data, Independent Student T-tests were used or Mann-Whitney-U-Tests in case of an abnormal data distribution. For categorical data, Chi-squared tests were performed. Linear Mixed Models with the factors group (HC, PwPD) and condition (TB, SB) and age and treadmill speed as covariates were used to compare treadmill hemodynamic responses (mean relative HbO_2 and mean relative HHb) and step length asymmetry outcomes (mean, coefficient of variation (CV) and standard deviation (SD)). In line with previous studies [8,21,42], we used the CV as an outcome of variability (preregistered), but decided in this study to focus instead on the SD for ease of interpretability (not preregistered). CV values become very large during the TB condition as the mean asymmetry is very small and the SD relatively large. This makes the CV values hard to interpret when compared with the SB condition. For completeness, we do report the CV values in the **Supplementary Materials** (**Table S1, S2, S10**). We also investigated in similar models as described above, the possible differences between SB1 and SB2 exposures to see if we could pool both SB-conditions, the effect of outliers based on the 1.5 * inter quartile range (IQR) criterion and the influence of handrail use on our main model (TB vs. SB1). In addition, the influence of leg and disease side dominance were explored in separate models by adding them as covariates. Post-hoc tests were Bonferroni corrected. To perform an anatomically fine-graded analysis, we also conducted an exploratory channel-to-channel analysis rather than being confined to the predetermined ROIs with the NIRStoolbox [37]. All other statistical analyses were performed in SPSS v.28 (IBM Corp., Armonk, NY). Statistical significance was set at $p<0.05$.

To explore whether the hemodynamic changes between TB- and SB-walking (ΔHbO_2) were related to adaptation performance and clinical characteristics, we performed an exploratory correlation analysis including the ROIs and the separate channels which were statistically significant after Bonferroni correction, and with significant Group (not preregistered) or Group*Condition (preregistered) effects for HbO_2. Greater changes of asymmetry (Δ

asymmetry) and variability (Δ SD) between TB- and SB-walking were interpreted as worse adaptative performance. These analyses were Holm-Bonferroni corrected.

Results

Participants' characteristics

Forty-two PwPD and 42 HC were included in this study. Participant characteristics are displayed in **Table 1**. Overall, the age and sex distributions between PwPD and HC groups were similar. Comfortable treadmill walking speed was lower in PwPD compared to HC ($p<0.001$). PwPD also showed worse balance, more impaired cognitive performance, higher fear of falling, and slower and fewer turns during the turning-in-place task.

Table 1. Participant characteristics.

	HC (N=42)	**PD (N=42)**	**p-value**
Sex (M/F)^	22/20	23/19	0.661
Age (yrs)	65.62 (5.8)	63.95 (6.9)	0.236
Cognitive scales			
Education (years)	15.26 (2.69)	14.48 (2.38)	0.160
MoCA (0-30)	26.74 (1.73)	25.88 (2.59)	0.078
FAB* (0-18)	16.95 (1.25)	15.69 (2.08)	**0.002**
TMT part A* (s)	32.84 (10.67)	43.16 (13.40)	**<0.001**
TMT part B* (s)	72.84 (22.72)	96.91 (49.02)	**0.005**
TMT B-A* (s)	40.00 (20.37)	53.74 (45.37)	0.142
Clinical scales			
FES-I* (16-64)	18.83 (2.54)	26.36 (8.24)	**<0.001**
MiniBEST* (0-28)	25.19 (4.34)	22.28 (5.51)	**<0.001**
PD-specific scales			
Duration since diagnosis (years)	-	8.24 (5.02)	-
LEDD (mg)	-	681.89 (401.47)	-
HY-stage (2/3)	-	36/6	-
MDS-UPDRS-III (0-132)	-	30.52 (11.73)	-
Participants with/without FOG#	-	10/32	-
Gait outcomes			
Personalized treadmill speed (m/s)	1.13 (0.11)	1.02 (0.16)	**<0.001**
Turning speed* (deg/s)	145.26 (31.63)	100.52 (33.47)	**<0.001**
Total 360° turns in 60 s*	22.5 (4.74)	15.33 (5.25)	**<0.001**

Significant values ($p<0.05$) are marked in bold. Values represent mean (standard deviation), except for HY-stage and FOG-status. All comparisons made with independent student's t test except: ^ Chi-squared test; * Mann-Whitney U test. Abbreviations: HC, healthy controls; PD, people with Parkinson's disease; M, Male; F, Female; MoCA, Montreal Cognitive Assessment; FAB, Frontal Assessment Battery; TMT, Trail-making-test; FES-I, Falls Efficacy Scale International; MiniBEST, Mini Balance Evaluations Systems Test; LEDD, Levodopa Equivalent Daily Dose; mg, milligrams; HY-stage, Hoehn and Yahr stage; MDS-UPDRS-III, Movement Disorders Society-Unified Parkinson's Disease Rating Scale Part III; FOG, Freezing of Gait; # determined with question 1 of the New Freezing of Gait Questionnaire.

Gait outcomes

When comparing the first block of SB (SB1) with the second block (SB2) in the two groups, we found no difference in mean step length asymmetry between SB1 and SB2 (Condition effect: p = 0.135, **Supplementary (S) Table S1**). Therefore, behavioral results were pooled for SB1 and SB2. Both groups showed more asymmetry (all p<0.001) and higher variability of asymmetry (all p<0.001) during the SB-walking compared to TB (**Table 2**). Additionally, we explored the initial and prolonged effects of the SB-perturbation by comparing the first and last five steps of each trial. This analysis showed a group*condition interaction effect for the last five steps of each trial ($F_{(1,80)}$=4.80, p=0.031) indicating more step length asymmetry in PwPD than HC ($F_{(1,80)}$=5.21, p=0.025). For the first five steps, no such interaction was present. The separate results of SB1 versus SB2 and TB versus SB1 are presented in supplementary **Tables S1-S2**.

Table 2. Treadmill behavioral results TB vs. SB pooled.

		HC (N= 42)		PD (N=42)		Group	Condition	Interaction
		TB	SB	TB	SB	p-value	p-value	p-value
Total trial								
Step length asymmetry	**mean**	0.01 (<0.01)	-0.12 (0.01)	0.01 (0.01)	-0.14 (0.01)	0.160	**<0.001**	**0.037**
	SD	0.02 (<0.01)	0.04 (<0.01)	0.03 (<0.01)	0.04 (<0.01)	0.153	**<0.001**	0.127
First 5 steps								
Step length asymmetry	**mean**	0.01 (<0.01)	-0.13 (0.01)	0.01 (0.01)	-0.15 (0.01)	0.234	**<0.001**	0.074
	SD	0.02 (<0.01)	0.04 (<0.01)	0.02 (<0.01)	0.04 (<0.01)	0.291	**<0.001**	0.067
Last 5 steps								
Step length asymmetry	**mean**	0.01 (<0.01)	-0.12 (0.01)	0.01 (0.01)	-0.14 (0.01)	0.103	**<0.001**	**0.031**
	SD	0.02 (<0.01)	0.04 (<0.01)	0.02 (<0.01)	0.04 (<0.01)	0.141	**<0.001**	0.417

Significant values (p<0.05) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's disease; TB, Tied-belt; SB, Split-belt; SD, standard deviation.

Cortical brain activity changes

When comparing the fNIRS results of SB1 versus SB2 (**Table S3**), no differences were found in hemodynamic responses in both the HC and PwPD. Therefore, we pooled the two blocks. We also found that disease side, leg dominance, outliers and handrail use did not substantially affect these results (**Table S3, S4 and S5**) and thus were not further considered in the models. The distribution of outliers did not show a different pattern for PD or HC or condition (median exclusion per ROI 3.36%). When comparing TB versus SB-pooled with TB versus SB1 (**Table 3 and Table S6**) we found no substantial differences between the model outcomes, justifying the approach taken.

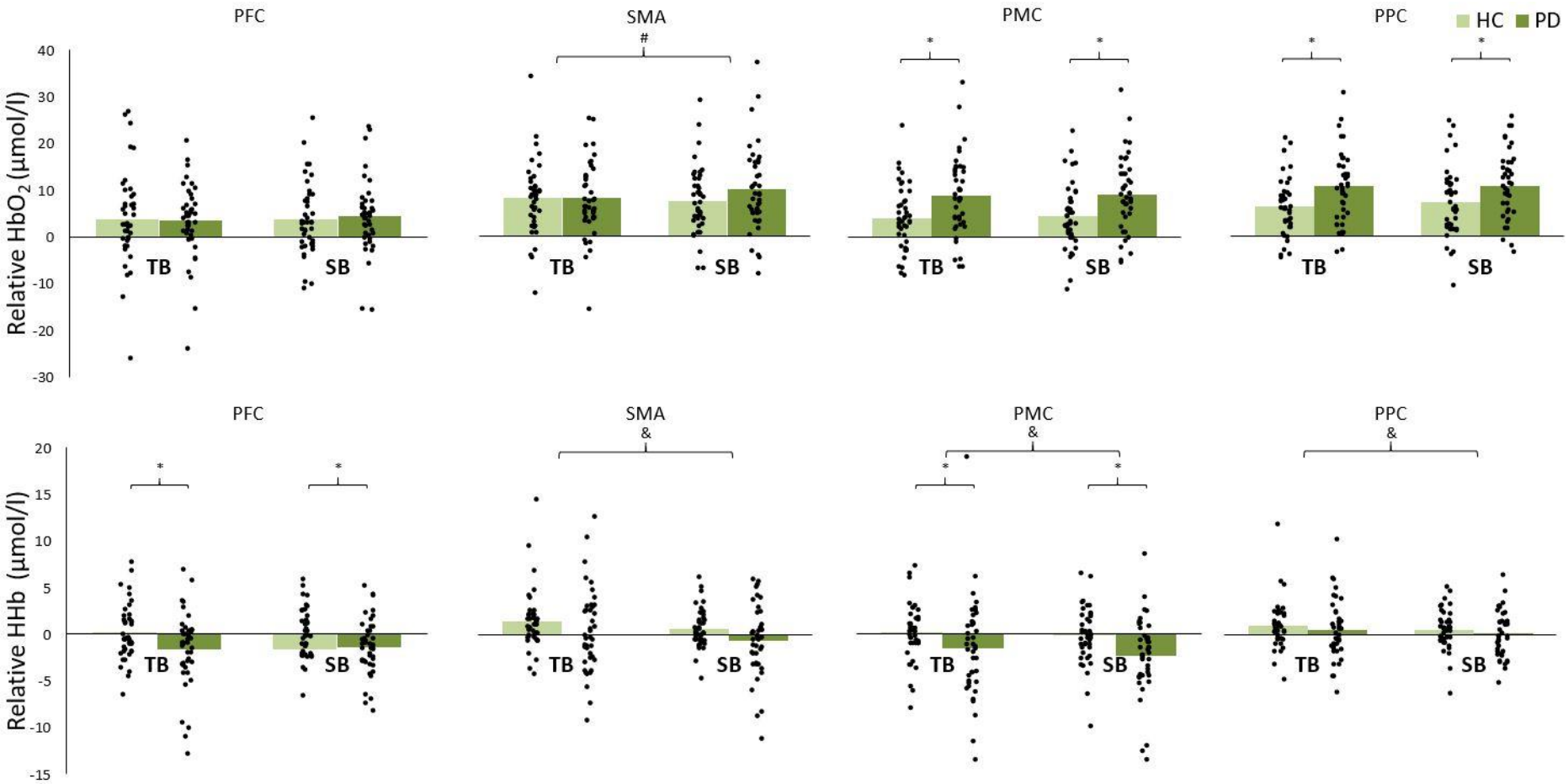

Figure 3. Mean relative activations in total PFC, SMA, PMC and PPC. Significant ($p<0.05$) interaction effects are marked with #, group effects by an * and condition effects by &. Abbreviations: PFC, prefrontal cortex; SMA, supplementary motor area; PMC, premotor cortex; PPC, posterior parietal cortex; TB, tied-belt; SB, split-belt; HC, healthy controls; PD, people with Parkinson's Disease; HbO2, oxygenated hemoglobin; HHb, deoxygenated hemoglobin.

Table 3. Relative oxygenated and deoxygenated hemoglobin concentrations (HbO2/HHb) TB vs. SB pooled in the total ROI.

		HC (N=42)		PD (N=42)		Group	Condition	Interaction
		TB	SB	TB	SB	p-value	p-value	p-value
HbO2	**Total**							
(µmol/l)	PFC	3.75(1.50)	3.72(1.12)	3.35(1.29)	4.33(1.28)	0.953	0.336	0.310
	SMA	8.08(1.13)	7.78(1.07)	8.08(1.33)	9.94(1.48)	0.532	0.150	**0.048**
	PMC	3.93(1.02)	4.33(1.02)	8.71(1.43)	9.11(1.32)	**0.005**	0.451	0.999
	PPC	6.45(0.940	7.24(1.11)	10.80(1.16)	11.76(1.14)	**0.004**	0.076	0.861
HHb	**Total**							
(µmol/l)	PFC	0.20(0.46)	-0.02(0.40)	-1.60(0.63)	-1.35(0.47)	**0.025**	0.920	0.215
	SMA	1.33(0.54)	0.59(0.36)	-0.14(0.76)	-0.67(0.56)	0.075	**0.036**	0.712
	PMC	0.11(0.46)	-0.19(0.45)	-1.49(0.80)	-2.35(0.63)	**0.023**	**0.045**	0.324
	PPC	0.97(0.42)	0.40(0.35)	0.54(0.46)	0.03(0.36)	0.436	**0.036**	0.899

Significant values (p<0.05) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; TB, Tied-belt; SB, Split-belt; HbO2, oxygenated hemoglobin; HHb, deoxygenated hemoglobin.

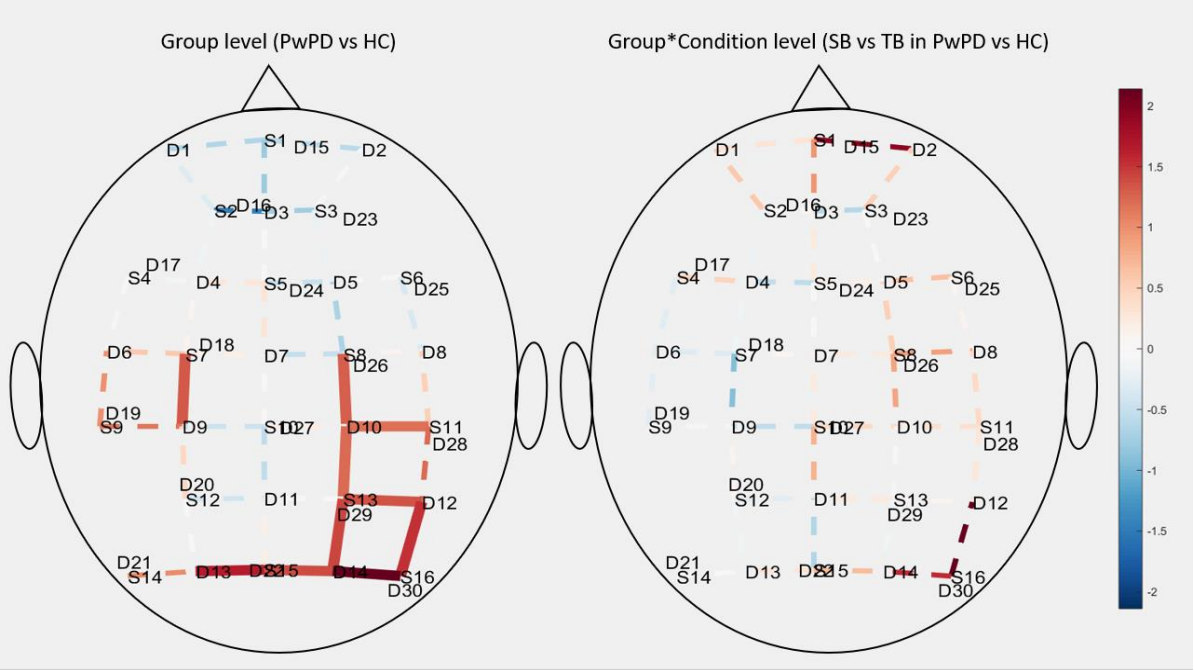

Figure 4. Channel to channel analysis for HbO2. This figure shows the group differences and the interaction between Group and Condition using a T-statistic heat map. Solid lines represent significant channels and dashes lines non-significant channels. The displayed channel t-statistics were corrected for multiple testing with Bonferroni correction.

Table 3 and **Figure 3** show the main and interaction effects for the final models on HbO_2 and HHb for the total ROI. The lateralized activity changes are reported in **Table S7**. As for HbO_2, a significant group*condition effect was found in the SMA (total, $F_{(1,79)}$=4.05, p=0.048), signifying increased activity during SB-walking in PD only ($F_{(1,52)}$=4.84, p=0.032). In addition, main group effects were present in the PMC (total, $F_{(1,74)}$=8.58, p=0.005) and PPC (total,

$F_{(1,80)}$=9.04, p=0.004), whereby PwPD had higher HbO_2 levels compared to HC. No significant differences in HbO_2 were observed for the PFC. Channel-to-channel analysis of HbO_2 showed similar results regarding the group differences (**Figure 4**, left panel). PwPD showed elevated HbO_2 concentrations compared to HC in the PMC and PPC areas. However, in contrast to the ROI analysis, the channel-to-channel analysis did not show a significant interaction effect in the right SMA (**Figure 4**, right panel).

The HHb results corroborated the group effects found for HbO_2 (**Table 3**) in the PMC (total, $F_{(1,73)}$=5.38, p=0.023); however not for the PPC (total, $F_{(1,81)}$=0.61, p=0.436). HHb did not confirm the group*condition interaction effect found in the SMA for HbO_2 (total, $F_{(1,67)}$=0.14, p=0.712). However, a condition effect appeared for HHb in the SMA (total, $F_{(1,67)}$=4.59, p=0.036) and also for the PMC (total, $F_{(1,69)}$=4.18, p=0.045) and PPC (total, $F_{(1,80)}$=4.56, p=0.036), whereby both HC and PwPD presented lower HHb values (i.e. increased activity) during SB- compared to TB-walking. Additionally, a significant group effect was present in the PFC (total, $F_{(1,78)}$=5.24, p=0.025) where HHb was lower (i.e. increased activity) in PwPD compared to HC, not mirrored by the HbO_2 results. Of note, the HbO_2 SMA interaction and the HHb results did not surpass Bonferroni corrections.

Correlations between behavioral, clinical and fNIRS outcomes

We performed a correlation analysis with the significantly involved ROIs and separate channels to better understand the cortical activity changes and their relationship with gait adjustments from TB to SB within each group. **Figure 5A** shows a significant positive correlation in PwPD indicating that a larger increase in activity in channel S15-D14 (higher ΔHbO_2), representing a superiorly located channel of the right PPC, was associated with higher variability in asymmetry (worse adaptation to SB, ΔSD, r=0.425, p=0.005). A similar result was apparent when considering the total PPC-ROI (ΔHbO_2, r=0.35, p=0.025), not shown in **Figure 5**. **Figure 5B** displays that larger increases in activity in the SMA-ROI (higher ΔHbO_2) was also associated with higher variability in asymmetry, but this time in the HC group (ΔSD, r=0.32, p=0.042). Finally, in contrast to the other regions, **Figure 5C** shows that larger increases in activity in channel S13-D12, representing a more inferiorly located channel of the right PPC, was associated with lower asymmetry in PwPD but not in HC (better adaptation to SB, Δasymmetry, r=-0.324, p=0.036). See supplementary **Table S8** for detailed results. Of note, these correlations were performed to look into the within-group relationships between the respective outcomes. After performing an exploratory regression analysis, it appeared that only the correlation in **panel 5C** showed a significant between-group difference between slopes (**panel 5C:** interaction effect group*Δasymmetry, $F_{(1)}$=5.00, p=0.028, **panel 5A:** group*ΔSD, $F_{(1)}$=0.50, p=0.484, **panel 5B:** group*ΔSD, $F_{(1)}$=0.29, p=0.594).

Associations between turning performance, clinical scales and HbO_2 during SB-walking showed that in HC increased PPC-ROI activity was associated with worse turning performance (number of turns, r=-0.34, p=0.030), executive functioning (TMT part-A, r=0.36, p=0.019), set-shifting skills (TMT part-B, r=0.39, p=0.010), and worse balance (MiniBEST, r=-0.39, p=0.012). No such associations were found in the PwPD and no other clinical correlations proved significant. Of note, none of these correlations passed Holm-Bonferroni correction and therefore no multiple regression analysis was undertaken. See supplementary **Table S9** for detailed outcomes.

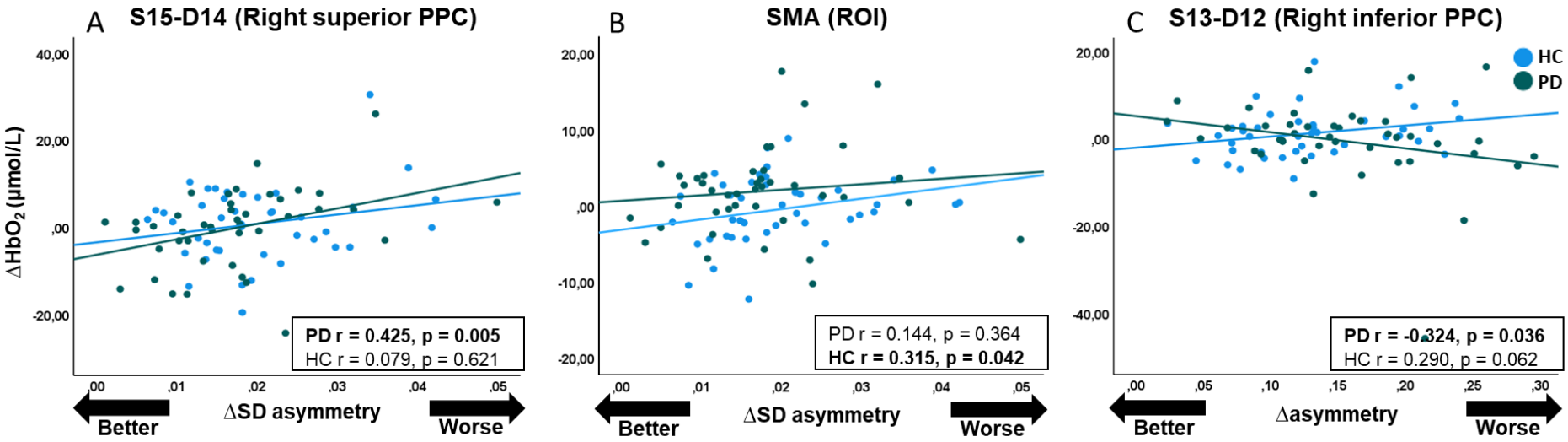

Figure 5. Spearman correlations between ΔHbO2 (SB-TB HbO2) and adaptation performance (Δasymmetry and ΔSD asymmetry). Panel A and B show the correlation between ΔHbO2 and ΔSD asymmetry, Panel C shows the correlation between ΔHbO2 and Δasymmetry. Panel A and C include the ΔHbO2 of individual channels and panel B shows the ΔHbO2 of the averaged ROI. Correlations were performed to ascertain the within-group relationships between the respective outcomes. Only the correlation in panel C showed a significant between-group difference between slopes (interaction effect group*ΔHbO_2, $F_{(1)}$=5.00, p=0.028). Abbreviations: ΔHbO2, change in HbO2 between TB and SB-condition; ΔSD, change in standard deviation of asymmetry between TB and SB-condition; PPC, posterior parietal cortex; SMA, supplementary motor area; PD, Parkinson's Disease; HC, healthy controls.

Discussion

This is the first study investigating the effect of SB-walking on brain activity in HC and PwPD. Behavioral results showed no difference in treadmill adaptation between PwPD and HC, except for the last five steps of the SB-trials where PwPD remained more asymmetrical. We hypothesized increased cortical activity in four cortical ROIs, formerly shown to be implicated in the adaptive gait network during SB- compared to TB-walking (condition effect). Additionally, we expected that PwPD would show increased activity regardless of condition, except for the SMA (group effect). Lastly, we anticipated an interaction effect for the PMC and PPC, where PwPD would show increased activity from SB- to TB-walking as compared to HC. Unexpectedly, we found no increased HbO_2 of ROIs during SB walking, although HHb did suggest increased activity in the PMC and PPC. Partly in line with our second hypothesis, we found a group effect for the PMC and PPC showing overall increased HbO_2, except for the SMA and the PFC. In contrast to our third hypothesis, there were no group*condition interactions for the PMC and PPC. Yet, there was a significant interaction for the SMA, which indicated that PwPD increased activity during SB-walking not present in HC.

The overall hyperactivation found in the PMC in PD relative to HC is in line with previous work on fNIRS involving complex overground gait tasks [15]. As automated control of gait becomes impaired, PwPD may rely more on secondary cortical motor areas such as the PMC even during normal treadmill walking [43,44]. The PMC is essential for selecting appropriate motor programs, which in turn depend on the sensory input from the PPC [45]. In coherence, we also found increased activity in the PPC in PD (see interpretation below). In contrast to our hypothesis, we did not find an interaction effect, indicating that activity in the PMC was exaggerated already during TB-walking in PwPD. Such increased brain activity has previously been interpreted in the context of ageing and neuropathology as either an expression of compensation or as non-specific and unhelpful neural dedifferentiation [46]. In contrast, the HHb values did suggest increased PMC involvement during SB-walking in both groups, although not surpassing Bonferroni correction. In this study, we found no correlations between changes in PMC-related channels and gait performance, suggesting that the hyperactivity signifies cortical dedifferentiation rather than compensatory recruitment [47,48].

Our results also revealed abnormal hyperactivity in the PPC in PD relative to HC, irrespective of the SB-perturbations (no interaction effect). Similar to the PMC, the HbO_2 values did not show an effect of condition, whereas the HHb values did, suggesting that both groups increased their activity in response to the higher task demands of SB- versus TB-walking. Of note, the group and condition effects were not congruent in both chromophores and, therefore, may be less robust. In addition, the HHb values did not withstand multiple comparison correction. A recent neurostimulation study in young adults showed that suppression of the

PPC during SB-walking with tDCS resulted in more steps needed for successful adaptation and longer-lasting after-effect, corroborating that the PPC plays an important role in fine-tuning gait to SB-perturbations due to its sensory-integration function[9,49,50]. In line with this, MRI studies indicated that the PPC was involved in controlling more complex bimanual hand in- and anti-phase movements in PD [51]. As we used the Brodmann map in the fOLD toolbox to determine the anatomical location of our ROIs, we did not make an a-priori distinction between superior and inferior segments of the PPC. However, exploratory correlation analyses between activity changes in the PPC-channels and behavioral changes revealed a differentiated pattern in PD for more superiorly and inferiorly located channels as seen from the midline, not observed in HC. In PwPD, increased PPC HbO_2-changes (between TB and SB) were associated with worse adaptive gait (more variability) in the full ROI as well as in the more superiorly located channel. In contrast, increased PPC activity in the more inferior segment was associated with improved adaptive gait (less asymmetry). The above results point towards different functions for various portions of the PPC. In line with these findings, several fMRI studies reported that the superior PPC might be more involved in acute movement planning using feedback-based updating [52–54]. In contrast, the inferior PPC appears to be more involved in extensive motor planning beyond the situation at hand utilizing feedforward predictions [52–54]. The abnormally high recruitment of the superior PPC when reacting to the immediate asymmetrical situation could be in line with the Compensation-Related Utilization of Neural Circuits (CRUNCH) hypothesis [55], which presumes that increased cortical activity can function as compensation for the loss of neural efficiency, but only up to a certain task level after which behavior deteriorates. Indeed, in the present study a negative correlation was found, denoting that increased superior PPC activity predicted worse behavioral performance. Similarly, the idea of 'compensatory overload' was previously found with respect to greater PFC activity during a toe-tapping task in PD [56]. While the possible 'compensatory overload' in the superior PPC was present in PD, it is interesting to note that we also found a correlation in HC in whom worse baseline scores on cognition, balance and turning were associated with higher PPC-ROI activation, suggesting compensatory inefficiency in healthy aging [57].

For the inferior PPC, a different result, namely a positive brain-behavior association was observed, which aligns with beneficial compensatory recruitment. Here, PwPD who recruited this brain area more were better able to cope with the perturbations, possibly by utilizing anticipatory motor planning. However, these correlations should be interpreted with caution as they were only observed in two single channels and were not significant after correction for multiple comparison.

Previous fMRI studies reported an overall hypoactivity of the SMA during various motor tasks in PD versus HC [17,58,59]. Contrary to our hypothesis, we found that PwPD increased HbO_2

in the SMA during the SB-walking condition more so than HC (significant group*condition interaction). As the SMA is important for bilateral movement control and gait initiation [9,60], it is unsurprising that this area is involved during an asymmetrical gait task such as SB-walking. However, two recent studies on a stationary stepping task and on adaptive walking did not show substantial increases in SMA activity in PD, unlike in HC [15,34]. The authors attributed this to the close link between the SMA and the PD-related degradation of the basal ganglia, preventing modulation of SMA activity with an ensuing decline in behavioral performance [15,34]. In contrast, PwPD in our study were still able to react to SB-perturbations to some extent but failed to completely correct their asymmetry by the last five steps of the SB-condition. Failure to fully correct asymmetry in response to SB-perturbations was found previously in PwPD with but not without freezing [4]. The abnormal surplus SMA-recruitment in this study probably signified an increased effort or a higher engagement of compensatory reserve in PD [51] to manage the higher demands of SB-walking although this result would not remain after multiple comparison correction. Additionally, no association was found between the change in SMA activity and the quality of adaptive behavior. The exploratory correlation analysis did show that an increase in SMA-activity in HC was associated with worse adaptive performance (more variability) in line with the concept of 'compensational overload'.

To our surprise, we did not find differences in HbO_2 between TB- or SB-walking in the PFC in both groups. The HHb results did indicate that PwPD had increased PFC activity compared to HC. However, this was not influenced by the treadmill conditions nor mirrored by the more robust HbO_2 outcomes. Most fNIRS studies, to date, reported PFC-modulations during complex overground walking in older adults and PwPD [10–14,61]. Also, traditional treadmill walking reduced PFC activity compared to overground walking, ascribed to the implicit prompting of the belts to maintain gait activity [10]. A previous PET SB-walking study in young adults reported decreased PFC activity, interpreted as a possible shift from PFC resources to motor planning regions [9]. Additionally, a randomized controlled study from our group investigating the long-term effects of a 4-week SB-training program in PwPD compared to regular TB-training, showed that SB-training improved adaptive gait on the treadmill, effects which were unaffected by dual-tasking [8]. These results suggest that SB-walking relies more on reactive rather than on cognitive control, possibly driven by cerebellar input [9,18] rather than PFC-engagement.

This study included an a-priori powered sample size, and analyses were pre-registered. Despite this sample estimation, there is risk that some comparisons were underpowered since estimations were based on dual-task changes in the PFC [14]. We preregistered several of the secondary analyses, including a channel-to-channel analysis, as averaging channels into ROIs could have concealed focal activations. The correlation analyses mostly showed associations

with specific channels and not with the full ROIs. Furthermore, none of the HHb results or exploratory analyses surpassed Holm-Bonferroni correction and should thus be interpreted with caution. However, reporting channel by channel changes allowed interpretation of the results in a more data-driven and anatomically specific fashion than is common in the fNIRS-field [16,62]. Although fNIRS allows for measuring cortical activity with greater spatial resolution as compared to EEG, its temporal resolution and the maximal depth of measurement are limited [63]. As a result of these limitations, we could not analyze brief sub-sections of the data in an event-related manner, such as by focusing on the initial response to the perturbation. Even though fNIRS is quite resilient to movement artefacts, we included accelerometry and short separation channels as covariates. Yet, despite these precautions we do not exclude the possibility that some residual noise could have contaminated the signals. For instance, HbO_2-results were not always mirrored in the HHb outcomes and vice versa. Therefore, a cautious interpretation remains indicated, but this is inherent to the current state of the art of this technique. Finally, we always started with the TB-walking condition to prevent carry-over effects and thus only randomized the SB-walking conditions. Therefore, bias resulting from order effects cannot be discounted. Future studies could consider a fully randomized protocol with sufficient wash-out periods in between tasks to mitigate such confounding, although this would imply longer experimental protocols compromising feasibility for patients.

This study showed that PwPD have difficulty adapting to SB-perturbations even while ON-medication, further indicating the possible use of SB-training to improve the capacity to adjust gait in PD [8,21,42]. The present findings showed no consistent increases in PFC activity in response to SB-perturbations suggesting that SB-walking does not induce extra cognitive load but involves a feedforward mode of learning. Future studies need to determine whether SB-training is also effective and safe for more cognitively impaired PwPD. Lastly, more research is required in order to investigate whether prolonged SB-training promotes neural plasticity and can reverse abnormal cortical recruitment.

In conclusion, PwPD showed more cortical activity than HC during treadmill walking in the sensorimotor regions previously associated with adaptive gait [9], and even further increased in the SMA during SB- versus TB-walking. The upsurges in cortical activity were largely non-functional as they did not normalize asymmetry. Therefore, greater difficulty with reaching a symmetrical gait during SB-walking may be explained by abnormal recruitment of these adaptive gait areas.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

F.H.: experimental design, data acquisition, data analysis, interpretation, manuscript writing. P.S. and N.D.: experimental design, data analysis, interpretation, manuscript review and critique. V.R.: experimental design, data acquisition, manuscript review and critique, C.M.: data acquisition, manuscript review and critique, P.G. and M.G.: experimental design, manuscript review and critique, A.N.: experimental design, data analysis, interpretation, manuscript review and critique. All authors read and approved the final manuscript.

Competing interests

All authors declare no financial or non-financial competing interests.

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Supplementary materials

Table S1. Asymmetry measures SB1 vs SB2.

		HC (N= 42)		PD (N=42)		Group	Condition	Interaction
		SB1	SB2	SB1	SB2	p-value	p-value	p-value
Total trial								
Step length asymmetry	**mean**	-0.12 (0.10)	-0.13 (0.01)	-0.13 (0.01)	-0.16 (0.01)	0.083	0.135	0.510
	SD	0.04 (<0.01)	0.04 (<0.01)	0.04 (<0.01)	0.04 (<0.01)	0.520	**0.010**	0.292
	CV	63.79 (9.35)	67.89 (14.27)	87.75 (31.79)	45.8 (16.62)	0.961	0.350	0.256
First 5 steps								
Step length asymmetry	**mean**	-0.12 (0.10)	-0.13 (0.01)	-0.13 (0.01)	-0.16 (0.01)	0.185	0.153	0.560
	SD	0.04 (<0.01)	0.04 (<0.01)	0.04 (<0.01)	0.04 (<0.01)	0.889	**0.021**	0.288
	CV	80.59 (18.59)	52.23 (8.77)	87.04 (23.06)	32.74 (6.47)	0.685	**0.010**	0.407
Last 5 steps								
Step length asymmetry	**mean**	-0.11 (0.01)	-0.12 (0.01)	-0.13 (0.01)	-0.15 (0.02)	**0.047**	0.155	0.476
	SD	0.04 (<0.01)	0.03 (<0.01)	0.04 (<0.01)	0.04 (<0.01)	0.318	0.730	0.558
	CV	97.63 (27.72)	64.50 (15.65)	206.79 (126.77)	36.23 (7.31)	0.380	0.128	0.302

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; SB1/2, first/second split-belt exposure; mm, millimeters; CV, coefficient of variation.

Table S2. Asymmetry measures TB vs SB1.

		HC (N= 42)		PD (N=42)		Group	Condition	Interaction
		TB	SB1	TB	SB1	p-value	p-value	p-value
Total trial								
Step length asymmetry	**mean**	0.01 (<0.01)	-0.11 (0.01)	0.01 (0.01)	-0.12 (0.01)	0.715	**<0.001**	0.276
	SD	0.02 (<0.01)	0.04 (<0.01)	0.03 (<0.01)	0.04 (<0.01)	**0.023**	**<0.001**	0.410
	CV	550.39 (253.18)	70.25 (12.19)	261.29 (56.98)	85.02 (22.82)	0.302	**0.015**	0.248
First 5 steps								
Step length asymmetry	**mean**	0.01 (<0.01)	-0.11 (0.01)	0.01 (0.01)	-0.12 (0.01)	0.757	**<0.001**	0.338
	SD	0.02 (<0.01)	0.04 (<0.01)	0.02 (<0.01)	0.04 (<0.01)	**0.017**	**<0.001**	0.281
	CV	442.36 (176.86)	70.37 (16.37)	182.66 (27.82)	79.86 (20.81)	0.179	**0.012**	0.143
Last 5 steps								
Step length asymmetry	**mean**	0.01 (<0.01)	-0.11 (0.01)	0.01 (0.01)	-0.12 (0.01)	0.689	**<0.001**	0.297
	SD	0.02 (<0.01)	0.04 (0.00)	0.02 (0.00)	0.04 (0.00)	0.101	**<0.001**	0.246
	CV	485.54 (146.83)	93.29 (28.09)	492.25 (209.74)	87.14 (34.83)	0.998	**0.003**	0.961

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; TB, Tied-belt; SB, Split-belt; SB1, first split-belt exposure; SD, standard deviation; CV, coefficient of variation.

Table S3. fNIRS results SB1 vs. SB2.

		HC (N=42)		PD (N=42)		Covariates		Group	Condition	Interaction
		SB1	SB2	SB1	SB2	legdom	diseaseside	p-value	p-value	p-value
HbO_2 (µmol/l)	**Right**									
	PFC	3.62 (1.60)	3.65 (1.47)	4.29 (1.76)	3.69 (1.53)	0.303	0.122	0.873	0.576	0.536
	SMA	8.97 (1.28)	9.56 (1.34)	10.96 (2.01)	11.35 (1.67)	0.148	0.191	0.387	0.463	0.885
	PMC	5.60 (1.20)	5.77 (1.45)	8.61 (1.47)	9.58 (1.43)	0.492	0.717	0.059	0.342	0.500
	PPC	7.75 (1.31)	9.10 (1.64)	14.08 (1.58)	12.81 (1.77)	0.623	0.236	**0.018**	0.962	0.128
	Left									
	PFC	3.65 (1.13)	4.00 (1.07)	4.63 (1.78)	4.37 (1.32)	0.917	0.895	0.715	0.936	0.602
	SMA	5.78 (1.08)	6.02 (1.13)	8.56 (1.69)	9.36 (1.53)	0.920	0.949	0.096	0.477	0.714
	PMC	2.73 (1.47)	3.45 (1.51)	8.90 (1.61)	9.21 (1.61)	0.840	0.204	**0.005**	0.459	0.771
	PPC	6.17 (1.02)	6.28 (1.05)	9.50 (1.25)	10.44 (1.46)	0.256	0.296	**0.022**	0.394	0.500
	Total									
	PFC	3.64 (1.15)	3.83 (1.05)	4.46 (1.56)	4.03 (1.26)	0.544	0.326	0.772	0.781	0.480
	SMA	7.36 (1.08)	7.78 (1.11)	9.77 (1.72)	10.37 (1.46)	0.371	0.451	0.175	0.442	0.893
	PMC	4.18 (1.20)	4.63 (1.16)	8.74 (1.29)	9.38 (1.22)	0.813	0.528	**0.006**	0.292	0.845
	PPC	6.96 (1.02)	7.69 (1.20)	11.78 (1.19)	11.62 (1.43)	0.377	0.830	**0.008**	0.670	0.502
HHb (µmol/l)	**Right**									
	PFC	0.12 (0.52)	0.18 (0.50)	-1.02 (0.62)	-1.07 (0.52)	0.902	0.059	0.110	0.972	0.813
	SMA	0.43 (0.43)	0.36 (0.38)	-0.74 (0.84)	-0.62 (0.68)	0.660	**0.041**	0.167	0.952	0.817
	PMC	0.17 (0.46)	0.14 (0.47)	-1.77 (0.76)	-1.46 (0.71)	0.457	0.562	**0.036**	0.633	0.552
	PPC	0.54 (0.63)	0.09 (0.73)	0.45 (0.51)	-0.21 (0.46)	0.720	0.638	0.769	0.285	0.837
	Left									
	PFC	-0.22 (0.49)	-0.08 (0.49)	-1.67 (0.65)	-1.72 (0.54)	0.193	0.179	**0.032**	0.884	0.757
	SMA	0.94 (0.47)	0.55 (0.44)	-0.53 (0.57)	-0.76 (0.59)	0.326	0.664	0.052	0.373	0.831
	PMC	-0.35 (0.71)	-0.58 (0.64)	-2.83 (0.77)	-3.41 (0.68)	0.278	0.827	**0.006**	0.238	0.619
	PPC	0.52 (0.31)	0.60 (0.34)	-0.13 (0.48)	-0.05 (0.51)	0.638	0.257	0.232	0.756	0.994
	Total									
	PFC	-0.05 (0.43)	0.04 (0.41)	-1.34 (0.57)	-1.39 (0.47)	0.506	0.075	**0.035**	0.925	0.768
	SMA	0.68 (0.42)	0.44 (0.36)	-0.63 (0.69)	-0.69 (0.59)	0.439	0.155	0.069	0.678	0.802
	PMC	-0.09 (0.52)	-0.22 (0.47)	-2.30 (0.68)	-2.43 (0.61)	0.807	0.821	**0.006**	0.609	0.994
	PPC	0.53 (0.40)	0.34 (0.48)	0.17 (0.41)	-0.13 (0.42)	0.981	0.762	0.418	0.482	0.879

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; TB, Tied-belt; SB, Split-belt; HbO2, oxygenated hemoglobin; HHb, deoxygenated hemoglobin; SB1/2, first/second split-belt exposure.

Table S4. fNIRS results TB vs. SB1 outliers excluded.

		HC (N=42)		PD (N=42)		Group	Condition	Interaction
		TB	SB1	TB	SB1	p-value	p-value	p-value
HbO_2 (µmol/l)	***Right***							
	PFC	1.69 (1.18)	1.95 (1.25)	4.49 (1.01)	4.93 (1.08)	0.066	0.428	0.837
	SMA	8.28 (1.15)	8.28 (1.06)	10.05 (1.29)	11.51 (1.69)	0.198	0.501	0.170
	PMC	4.66 (1.03)	5.48 (0.96)	7.42 (1.25)	7.86 (1.22)	0.087	0.280	0.745
	PPC	6.06 (1.12)	7.81 (1.38)	12.05 (1.36)	12.64 (1.50)	**0.002**	0.051	0.330
	Left							
	PFC	4.48 (1.50)	3.57 (1.16)	4.92 (1.26)	5.79 (1.29)	0.450	0.976	0.166
	SMA	6.54 (1.05)	5.85 (1.00)	6.25 (1.15)	7.41 (1.48)	0.686	0.695	0.124
	PMC	2.33 (1.0)	1.51 (1.08)	8.67 (1.41)	7.99 (1.51)	**<0.001**	0.280	0.921
	PPC	5.57 (0.87)	6.17 (1.07)	7.95 (1.10)	9.54 (1.17)	**0.044**	**0.045**	0.365
	Total							
	PFC	2.90 (1.09)	2.64 (1.01)	4.19 (0.96)	4.71 (0.94)	0.218	0.759	0.368
	SMA	8.10 (0.97)	7.41 (1.02)	8.10 (0.97)	7.41 (1.02)	0.356	0.808	0.156
	PMC	3.41 (0.92)	3.37 (0.92)	8.13 (1.13)	8.19 (1.29)	**0.002**	0.990	0.932
	PPC	6.44 (1.00)	6.97 (1.12)	10.77 (1.10)	11.81 (1.08)	**0.002**	0.094	0.578
HHb (µmol/l)	***Right***							
	PFC	0.40 (0.49)	0.21 (0.44)	-1.34 (0.59)	-0.95 (0.57)	**0.047**	0.625	0.148
	SMA	0.87 (0.37)	0.49 (0.29)	-0.19 (0.61)	-0.49 (0.60)	0.098	0.306	0.903
	PMC	0.77 (0.33)	0.32 (0.40)	-1.77 (0.75)	-1.64 (0.49)	**<0.001**	0.646	0.410
	PPC	0.36 (0.35)	-0.18 (0.39)	0.23 (0.44)	-0.09 (0.42)	0.973	**0.022**	0.565
	Left							
	PFC	0.20 (0.47)	-0.21 (0.46)	-0.88 (0.44)	-0.83 (0.48)	0.165	0.480	0.366
	SMA	0.85 (0.28)	0.55 (0.29)	-0.24 (0.59)	-0.97 (0.55)	**0.031**	**0.034**	0.379
	PMC	-0.22 (0.37)	-0.53 (0.37)	-2.17 (0.71)	-2.36 (0.48)	**0.003**	0.475	0.868
	PPC	0.83 (0.35)	0.61 (0.27)	0.19 (0.36)	-0.35 (0.38)	0.072	0.081	0.457
	Total							
	PFC	0.27 (0.46)	-0.07 (0.43)	-1.23 (0.52)	-1.04 (0.51)	0.064	0.680	0.151
	SMA	0.96 (0.32)	0.71 (0.35)	-0.32 (0.66)	-0.95 (0.60)	**0.034**	0.053	0.405
	PMC	0.03 (0.30)	-0.22 (0.35)	-2.00 (0.63)	-2.11 (0.48)	**0.001**	0.501	0.844
	PPC	0.84 (0.29)	0.53 (0.32)	0.30 (0.41)	0.15 (0.41)	0.337	0.232	0.665

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; TB, Tied-belt; SB, Split-belt; SB1, first split-belt exposure; HbO2, oxygenated hemoglobin; HHb, deoxygenated hemoglobin.

Table S5. fNIRS results TB vs. SB1 excluding handrail users.

		HC (N=42)		PD (N=34)		Group	Condition	Interaction
		TB	SB1	TB	SB1	p-value	p-value	p-value
HbO_2	**Right**							
(µmol/l)	PFC	3.03(1.84)	3.52(1.63)	2.53(1.73)	3.87(2.12)	0.975	0.145	0.498
	SMA	9.40(1.37)	8.77(1.23)	9.57(1.81)	12.17(2.18)	0.426	0.279	0.079
	PMC	4.91(1.02)	5.60(1.18)	8.54(1.84)	8.79(1.85)	0.092	0.579	0.791
	PPC	14.12(1.73)	14.72(1.51)	7.04(1.64)	7.48(1.45)	**0.001**	0.432	0.903
	Left							
	PFC	4.43(1.56)	3.52(1.21)	4.06(1.54)	5.26(1.63)	0.739	0.829	0.116
	SMA	6.47(1.09)	5.77(1.04)	8.47(1.62)	8.76(1.98)	0.218	0.765	0.462
	PMC	2.96(1.42)	2.76(1.38)	8.99(2.07)	9.04(1.98)	**0.011**	0.876	0.925
	PPC	5.42(0.86)	6.02(1.06)	7.73(1.08)	9.85(1.30)	**0.036**	0.022	0.198
	Total							
	PFC	3.72(1.50)	3.51(1.21)	3.31(1.49)	4.58(1.66)	0.872	0.285	0.137
	SMA	7.93(1.07)	7.27(1.03)	9.02(1.54)	10.46(1.95)	0.273	0.585	0.147
	PMC	3.91(1.03)	4.16(1.12)	8.82(1.68)	8.92(1.70)	**0.014**	0.791	0.909
	PPC	6.23(0.98)	6.76(1.10)	10.92(1.16)	12.27(1.12)	**<0.001**	0.058	0.394
HHb	**Right**							
(**µmol**/l)	PFC	0.21(0.58)	0.12(0.55)	-1.35(0.70)	-0.94(0.66)	0.136	0.465	0.247
	SMA	0.96(0.49)	0.39(0.41)	0.49(0.94)	-0.04(0.79)	0.623	0.121	0.955
	PMC	0.52(0.50)	0.21(0.48)	-1.10(0.99)	-1.65(0.82)	0.073	0.281	0.763
	PPC	1.14(0.70)	0.57(0.60)	0.93(0.70)	0.63(0.62)	0.938	**0.048**	0.536
	Left							
	PFC	0.17(0.51)	-0.24(0.50)	-1.93(0.79)	-1.66(0.58)	**0.035**	0.808	0.234
	SMA	1.65(0.57)	0.91(0.44)	0.45(0.89)	-0.36(0.82)	0.197	**0.026**	0.918
	PMC	-0.26(0.61)	-0.36(0.72)	-1.96(0.98)	-2.67(0.82)	0.068	0.223	0.361
	PPC	0.83(0.35)	0.52(0.33)	-0.01(0.48)	0.02(0.53)	0.236	0.560	0.487
	Total							
	PFC	-1.63(0.38)	-1.29(0.53)	-0.07(0.45)	0.18(0.47)	**0.044**	0.833	0.165
	SMA	1.30(0.47)	0.64(0.38)	0.48(0.88)	-0.19(0.77)	0.353	**0.038**	0.98
	PMC	0.12(0.50)	-0.90(0.54)	-1.51(0.88)	-2.15(0.69)	**0.045**	0.176	0.491
	PPC	0.96(0.41)	0.52(0.40)	0.50(0.53)	0.36(0.48)	0.621	0.139	0.433

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; TB, Tied-belt; SB, Split-belt; SB1, first split-belt exposure; HbO2, oxygenated hemoglobin; HHb, deoxygenated hemoglobin.

Table S6. fNIRS results TB vs. SB1.

		HC (N=42)		PD (N=42)		Group	Condition	Interaction
		TB	SB1	TB	SB1	p-value	p-value	p-value
HbO_2	**Right**							
(µmol/l)	PFC	3.11 (1.80)	3.51 (1.59)	3.01 (8.48)	4.47 (1.80)	0.837	0.135	0.405
	SMA	9.49 (1.41)	9.01 (1.27)	8.72 (1.68)	11.06 (2.01)	0.767	0.256	0.087
	PMC	4.90 (1.02)	5.62 (1.17)	7.82 (1.68)	8.64 (1.53)	0.099	0.341	0.952
	PPC	7.46 (1.63)	7.76 (1.46)	13.52 (1.54)	14.02 (1.43)	**0.004**	0.529	0.879
	Left							
	PFC	4.35 (1.61)	3.58 (1.21)	3.76 (1.48)	4.76 (1.65)	0.884	0.860	0.179
	SMA	6.22 (1.00)	5.86 (1.00)	7.82 (1.47)	8.60 (1.85)	0.245	0.734	0.361
	PMC	2.97 (1.41)	2.76 (1.35)	9.54 (1.64)	8.90 (1.75)	**0.003**	0.555	0.762
	PPC	5.67 (0.87)	6.16 (1.08)	9.50 (1.17)	7.91 (1.10)	0.050	0.058	0.314
	Total							
	PFC	3.70 (1.49)	3.54 (1.18)	3.45 (1.32)	4.63 (1.50)	0.825	0.293	0.166
	SMA	7.86 (1.05)	7.44 (1.04)	8.26 (1.39)	9.83 (1.80)	0.444	0.380	0.131
	PMC	3.92 (1.02)	4.17 (1.09)	8.70 (1.44)	8.78 (1.43)	**0.007**	0.784	0.890
	PPC	6.59 (0.97)	6.96 (1.12)	1.71 (1.10)	11.75 (1.08)	**0.003**	0.134	0.470
HHb	**Right**							
(**µmol**/l)	PFC	0.24 (0.59)	0.11 (0.54)	-1.34 (0.63)	-1.00 (0.59)	0.102	0.614	0.245
	SMA	0.95 (0.51)	0.43 (0.44)	-0.35 (0.98)	-0.69 (0.83)	0.218	0.191	0.773
	PMC	0.45 (0.48)	0.17 (0.47)	-1.20 (0.88)	-1.74 (0.76)	**0.044**	0.297	0.734
	PPC	1.18 (0.70)	0.57 (0.59)	0.38 (0.54)	0.87 (0.62)	0.769	**0.011**	0.801
	Left							
	PFC	0.11 (0.50)	-0.23 (0.50)	-1.85 (0.72)	-1.63 (0.64)	**0.039**	0.825	0.299
	SMA	1.37 (0.41)	0.93 (0.43)	0.27 (0.75)	-0.39 (0.73)	0.135	0.068	0.721
	PMC	-0.37 (0.57)	-0.37 (0.71)	-1.75 (0.85)	-2.79 (0.76)	0.056	0.116	0.118
	PPC	0.83 (0.34)	0.51 (0.32)	0.08 (0.45)	-0.14 (0.45)	0.171	0.254	0.832
	Total							
	PFC	0.17 (0.47)	-0.06 (0.45)	-1.59 (0.62)	-1.31 (0.54)	**0.040**	0.906	0.204
	SMA	1.16 (0.41)	0.68 (0.40)	-0.04 (0.82)	-0.54 (0.71)	0.146	0.081	0.982
	PMC	0.04 (0.46)	-0.10 (0.53)	-1.47 (0.77)	-2.26 (0.67)	**0.029**	0.131	0.294
	PPC	0.99 (0.41)	0.53 (0.39)	0.49 (0.48)	0.13 (0.43)	0.444	**0.034**	0.783

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; TB, Tied-belt; SB, Split-belt; SB1, first split-belt exposure; HbO2, oxygenated hemoglobin; HHb, deoxygenated hemoglobin.

Table S7. Relative oxygenated and deoxygenated hemoglobin concentrations (HbO2/HHb) TB vs. SB pooled for the left and right hemisphere.

		HC (N=42)		PD (N=42)		Group	Condition	Interaction
		TB	SB	TB	SB	p-value	p-value	p-value
HbO2	**Right**							
(µmol/l)	PFC	3.03(1.80)	3.51(1.50)	3.08(1.46)	4.16(1.61)	0.872	0.185	0.606
	SMA	9.63(1.44)	9.35(1.29)	8.59(1.65)	11.13(1.71)	0.860	0.094	**0.037**
	PMC	4.95(1.02)	5.66(1.11)	7.80(1.65)	9.10(1.38)	0.074	0.144	0.670
	PPC	7.32(1.58)	8.39(1.50)	13.61(1.59)	13.47(1.42)	**0.007**	0.492	0.370
	Left							
	PFC	4.47(1.61)	3.93(1.13)	3.62(1.44)	4.49(1.33)	0.936	0.798	0.283
	SMA	6.49(1.10)	6.16(1.02)	7.61(1.41)	8.79(1.55)	0.287	0.448	0.181
	PMC	2.94(1.38)	3.02(1.27)	9.59(1.66)	9.10(1.68)	**0.003**	0.747	0.646
	PPC	5.57(0.86)	6.15(1.03)	7.96(1.12)	10.02(1.19)	**0.030**	**0.011**	0.151
HHb	**Right**							
(µmol/l)	PFC	0.22(0.57)	0.08(0.49)	-1.31(0.63)	-1.00(0.51)	0.090	0.684	0.281
	SMA	1.00(0.550	0.37(0.36)	-0.38(0.95)	-0.65(0.66)	0.174	0.206	0.620
	PMC	0.49(0.47)	0.12(0.42)	-1.21(0.89)	-1.60(0.72)	**0.050**	0.281	0.976
	PPC	1.12(0.79)	0.30(0.45)	0.94(0.55)	0.11(0.42)	0.799	**0.036**	0.988
	Left							
	PFC	0.18(0.50)	-0.13(0.45)	-1.90(0.73)	-1.70(0.55)	**0.020**	0.829	0.295
	SMA	1.167(0.61)	0.80(0.43)	0.10(0.71)	-0.68(0.58)	0.053	**0.010**	0.888
	PMC	-0.27(0.58)	-0.48(0.62)	-1.78(0.87)	-3.11(0.70)	**0.032**	**0.015**	0.078
	PPC	0.82(0.33)	0.51(0.30)	0.12(0.46)	-0.07(0.42)	0.203	0.252	0.753

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; TB, Tied-belt; SB, Split-belt; HbO2, oxygenated hemoglobin; HHb, deoxygenated hemoglobin.

Table S8. Spearman correlations between ΔHbO2 concentrations in significant ROIs and channels and Δasymmetry and ΔSD.

	HC (N=42)		PD (N=42)	
ΔHbO2	**Δasymmetry**	**ΔSD**	**ΔAsymmetry**	**ΔSD**
PFC total	0.09 (0.560)	0.05 (0.763)	0.26 (0.097)	0.02 (0.889)
SMA total	-0.17 (0.296)	**0.32 (0.042)**	0.26 (0.095)	0.14 (0.364)
PMC total	-0.14 (0.373)	0.10 (0.541)	-0.10 (0.517)	0.08 (0.604)
PPC total	0.27 (0.087)	0.05 (0.763)	0.01 (0.965)	**0.35 (0.025)**
S7_D9 - left PMC	-0.12 (0.434)	0.18 (0.255)	0.05 (0.750)	0.09 (0.555)
S15_D13 - left sPPC	0.17 (0.286)	0.19 (0.236)	0.04 (0.783)	0.10 (0.536)
S8_D10 - right PMC	-0.30 (0.052)	0.21 (0.189)	-0.06 (0.685)	0.08 (0.630)
S11_D10 - right PMC*	0.20 (0.201)	0.03 (0.832)	-0.02 (0.910)	0.07 (0.630)
S13_D10 – M1/S1*	0.22 (0.155)	0.17 (0.290)	-0.04 (0.815)	-0.24 (0.132)
S13_D12 - right iPPC*	0.29 (0.062)	-0.01 (0.952)	**-0.32 (0.036)**	0.24 (0.133)
S13_D14 - right sPPC	0.05 (0.734)	-0.04 (0.787)	-0.08 (0.599)	0.28 (0.077)
S15_D14 - right sPPC	0.23 (0.151)	0.08 (0.621)	-0.13 (0.410)	**0.43 (0.005)**
S16_D14 - right sPPC	0.28 (0.076)	0.08 (0.595)	0.17 (0.276)	0.17 (0.276)
S16_D12 - right iPPC	0.11 (0.474)	-0.11 (0.472)	0.10 (0.543)	0.12 (0.495)
right PFC (ROI)	0.12 (0.446)	0.02 (0.909)	0.05 (0.768)	0.16 (0.326)
right SMA (ROI)	-0.23 (0.136)	**0.42 (0.007)**	0.18 (0.261)	0.10 (0.536)
right PMC (ROI)	-0.29 (0.145)	0.22 (0.157)	-0.22 (0.154)	0.08 (0.626)
right PPC (ROI)	0.25 (0.114)	0.05 (0.770)	-0.08 (0.598)	**0.34 (0.027)**
left PFC (ROI)	-0.01 (0.938)	-0.05 (0.748)	0.30 (0.052)	-0.07 (0.679)
left SMA (ROI)	-0.17 (0.289)	0.21 (0.173)	0.26 (0.097)	0.19 (0.226)
left PMC (ROI)	-0.03 (0.860)	-0.16 (0.319)	-0.02 (0.889)	0.07 (0.650)
left PPC (ROI)	0.17 (0.277)	0.13 (0.413)	0.08 (0.602)	0.25 (0.113)

Significant values ($p<0.05$) are marked in bold, * channel not included in ROI configuration, landmark based on fOLD toolbox Brodmann mapping without pre-set specificity levels. Values represent uncorrected Spearman correlation coefficient r (p-value). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; SMA, supplementary motor area; PMC, premotor cortex; s/iPPC, superior/inferior posterior parietal cortex; ΔHbO2, change in HbO2 from TB- to SB-walking; Δasymmetry, change in step length asymmetry from TB- to SB-walking; ΔSD, change in the standard deviation of the step length asymmetry from TB- to SB-walking.

Table S9. Pearson correlations between SB-walking HbO2 concentrations in significant ROIs (total, right and left values) and treadmill step length asymmetry, turning performance and clinical scales.

	HC (N=42)									PD (N=42)								
Treadmill	**SMA**	**PMC**	**PPC**	**rSMA**	**rPMC**	**rPPC**	**lSMA**	**lPMC**	**lPPC**	**SMA**	**PMC**	**PPC**	**rSMA**	**rPMC**	**rPPC**	**lSMA**	**lPMC**	**lPPC**
Mean asymmetry (last 5 steps)	-0.14 (0.381)	-0.04 (0.789)	-0.23 (0.136)	-0.16 (0.381)	0.01 (0.976)	-0.17 (0.276)	-0.08 (0.601)	-0.07 (0.645)	-0.26 (0.092)	-0.24 (0.129)	0.03 (0.846)	-0.52 (0.744)	-0.16 (0.306)	-0.20 (0.197)	-0.14 (0.367)	-0.28 (0.073)	0.21 (0.174)	0.08 (0.625)
CV asymmetry (last 5 steps)	0.035 (0.828)	0.29 (0.063)	-0.11 (0.486)	-0.04 (0.805)	0.31 (0.043)	-0.17 (0.289)	0.13 (0.414)	0.20 (0.213)	0.00 (0.991)	-0.15 (0.359)	-0.24 (0.131)	0.09 (0.583)	-0.12 (0.465)	-0.29 (0.061)	0.03 (0.850)	-0.15 (0.336)	-0.13 (0.417)	0.13 (0.412)
Overground turning																		
Turning speed (deg/s)	-0.14 (0.370)	-0.13 (0.427)	-0.30 (0.055)	-0.20 (0.209)	-0.10 (0.527)	-0.31 (0.048)	-0.04 (0.819)	-0.12 (0.460)	-0.21 (0.184)	0.13 (0.419)	0.01 (0.967)	-0.29 (0.069)	0.14 (0.392)	-0.00 (0.991)	-0.33 (0.041)	0.10 (0.530)	0.01 (0.941)	-0.16 (0.340)
#Turns	-0.16 (0.306)	-0.14 (0.372)	-0.34 (0.030)	-0.21 (0.184)	-0.04 (0.781)	-0.30 (0.053)	-0.07 (0.684)	-0.19 (0.225)	-0.30 (0.054)	0.13 (0.417)	0.03 (0.878)	-0.25 (0.120)	0.15 (0.373)	0.02 (0.911)	-0.27 (0.091)	0.10 (0.552)	0.02 (0.884)	-0.15 (0.362)
Clinical scales																		
MoCA (0-30)	0.07 (0.670)	0.07 (0.662)	-0.18 (0.251)	0.09 (0.579)	0.17 (0.295)	-0.28 (0.076)	0.03 (0.870)	-0.03 (0.844)	0.01 (0.974)	-0.07 (0.645)	0.14 (0.373)	-0.07 (0.673)	-0.16 (0.325)	0.19 (0.231)	-0.00 (0.989)	0.03 (0.874)	0.06 (0.687)	-0.13 (0.426)
FAB (0-18)	0.05 (0.760)	-0.07 (0.649)	-0.05 (0.771)	0.08 (0.618)	0.07 (0.656)	-0.08 (0.596)	-0.00 (0.983)	-0.18 (0.265)	0.02 (0.895)	0.11 (0.487)	0.03 (0.870)	0.06 (0.685)	0.10 (0.532)	0.19 (0.241)	0.12 (0.460)	0.10 (0.513)	-0.11 (0.485)	-0.02 (0.891)
TMT part A (s)	0.25 (0.117)	0.05 (0.773)	0.36 (0.019)	0.30 (0.054)	0.05 (0.762)	0.34 (0.012)	0.12 (0.444)	0.03 (0.839)	0.23 (0.138)	0.03 (0.868)	-0.11 (0.741)	0.02 (0.921)	0.05 (0.779)	-0.10 (0.544)	0.06 (0.713)	0.00 (0.985)	-0.10 (0.536)	-0.04 (0.791)
TMT part B (s)	0.10 (0.543)	-0.13 (0.406)	0.39 (0.010)	0.15 (0.329)	-0.13 (0.418)	0.45 (0.003)	-0.00 (0.988)	-0.10 (0.520)	0.21 (0.177)	-0.06 (0.687)	0.00 (1.000)	0.01 (0.936)	-0.01 (0.933)	-0.10 (0.539)	-0.04 (0.817)	-0.11 (0.500)	0.08 (0.615)	0.07 (0.658)
TMT B-A (s)	-0.02 (0.895)	-0.17 (0.279)	0.25 (0.114)	0.02 (0.924)	-0.17 (0.286)	0.30 (0.058)	-0.07 (0.676)	-0.13 (0.409)	0.12 (0.467)	-0.08 (0.628)	0.03 (0.832)	0.01 (0.954)	-0.03 (0.862)	-0.08 (0.628)	-0.06 (0.720)	-0.12 (0.463)	0.12 (0.467)	0.09 (0.578)
FES-I (16-64)	-0.13 (0.397)	0.01 (0.970)	0.23 (0.153)	-0.09 (0.564)	-0.11 (0.471)	0.18 (0.258)	-0.17 (0.295)	0.11 (0.490)	0.23 (0.137)	-0.25 (0.107)	-0.27 (0.090)	0.10 (0.532)	-0.31 (0.047)	-0.20 (0.208)	0.00 (0.996)	-0.15 (0.335)	-0.25 (0.114)	0.19 (0.230)
MiniBEST (0-28)	-0.11 (0.491)	-0.114 (0.477)	-0.39 (0.012)	-0.14 (0.381)	-0.05 (0.762)	-0.36 (0.020)	-0.07 (0.774)	-0.14 (0.375)	-0.34 (0.031)	0.19 (0.250)	0.29 (0.079)	-0.10 (0.566)	0.23 (0.171)	0.22 (0.185)	0.05 (0.757)	0.11 (0.498)	0.27 (0.106)	-0.25 (0.136)
PD-specific																		
Duration since diagnosis (yrs)	-	-	-	-	-	-	-	-	-	-0.06 (0.725)	-0.03 (0.877)	-0.05 (0.751)	-0.03 (0.847)	-0.05 (0.740)	0.01 (0.937)	-0.07 (0.645)	0.01 (0.974)	-0.11 (0.510)
LEDD (mg)	-	-	-	-	-	-	-	-	-	0.14 (0.366)	0.04 (0.809)	-0.05 (0.751)	0.17 (0.279)	0.09 (0.589)	0.01 (0.932)	0.09 (0.569)	-0.01 (0.947)	-0.11 (0.474)
MDS-UPDRS-III (0-132)	-	-	-	-	-	-	-	-	-	-0.02 (0.889)	0.20 (0.215)	0.01 (0.931)	-0.04 (0.812)	0.06 (0.691)	0.03 (0.854)	-0.00 (0.988)	0.25 (0.109)	-0.01 (0.950)

Significant values ($p<0.05$) are marked in bold. Values represent uncorrected Pearson correlation coefficient r (p-value). Abbreviations: HC, healthy controls; PD, people with Parkinson's Disease; r/lSMA, right/left supplementary motor area; PMC, premotor cortex; PPC, posterior parietal cortex; CV, coefficient of variation; deg, degrees; MoCA, Montreal Cognitive Assessment; FAB, Frontal Assessment Battery; TMT, Trail-making-test; s, seconds; FES-I, Falls Efficacy Scale International; MiniBEST, Mini Balance Evaluations Systems Test; yrs, years; LEDD, Levodopa Equivalent Daily Dose; mg, milligrams; MDS-UPDRS-III, Movement Disorders Society-Unified Parkinson's Disease Rating Scale Part.

Table S10. Treadmill behavioral results TB vs. SB.

		HC (N= 42)		PD (N=42)		Group	Condition	Interaction
Step length asymmetry		**TB**	**SB**	**TB**	**SB**	**p-value**	**p-value**	**p-value**
Total trial	**CV**	564.67 (252.47)	64.25 (8.11)	254.09 (55.86)	68.87 (17.47)	0.244	**0.011**	0.213
First 5 steps	**CV**	456.89 (173.87)	65.36 (9.78)	175.03 (26.47)	61.35 (11.76)	0.113	**0.007**	0.123
Last 5 steps	**CV**	464.43 (144.39)	78.97 (15.69)	502.28 (209.81)	124.38 (62.60)	0.752	**0.005**	0.977

Significant values ($p<0.05$) are marked in bold. Values are presented as mean (standard deviation). Abbreviations: HC, healthy controls; PD, people with Parkinson's disease; TB, Tied-belt; SB, Split-belt; CV, coefficient of variation; SD, standard deviation.

fNIRS a-priori methodological considerations regarding study design

Functional near-infrared spectroscopy (fNIRS) allows for the measurement of cortical activity during actual movement and is based, similar to fMRI, on the concept of neurovascular coupling [64–68]. Neurovascular coupling implies that when neurons are more active a vascular response is triggered to deliver extra oxygen to the cells. Enhanced blood flow to these active areas is also known as the hemodynamic response [67,69,70]. fNIRS uses near-infrared light to capture this indirect measure of cortical activity by emitting distinct light wavelengths into the brain. The two wavelengths emitted are able to differentiate between oxygenated and deoxygenated hemoglobin (HbO_2 and HHb) as both molecules absorb different wavelengths of light [71]. The refracted light is captured again by detectors on the scalp giving an indication of the concentrations of HbO_2 and HHb (see **Figure 6**). Based on neurovascular coupling, increased brain activity would be reflected by an increase in HbO_2 and a decrease in HHb, making the two outcome measures anticorrelated. Therefore, a positive correlation between HbO_2 and HHb signals is currently assumed to be a marker of the presence of artefacts affecting the signal [16,72–76].

The fNIRS field is rapidly evolving in the domain of gait and balance research. For instance, before more sophisticated filtering techniques were available, anticorrelations were forced upon the signals based on the HbO_2 concentrations as a form of artefact removal [72–76]. However, the assumption of anticorrelated signals only holds under certain assumptions which are not always met or checked. For example, the ratio between HbO_2 and HHb is assumed to be constant during the task [71–73] meaning that the regional blood volume should be constant, which during a whole body movement such as a gait is likely not the case because of the redistribution of blood volume across the body. Additionally, the systemic changes could be subject to changes in alveolar ventilation, which can also be influenced by aerobic activities such as treadmill walking [71]. Menant and colleagues pointed out in a recent review on fNIRS methodology that neurological and older populations often have vascular disease and asymmetrical pathologies, which also might affect the normally anti-correlated hemodynamics [16].

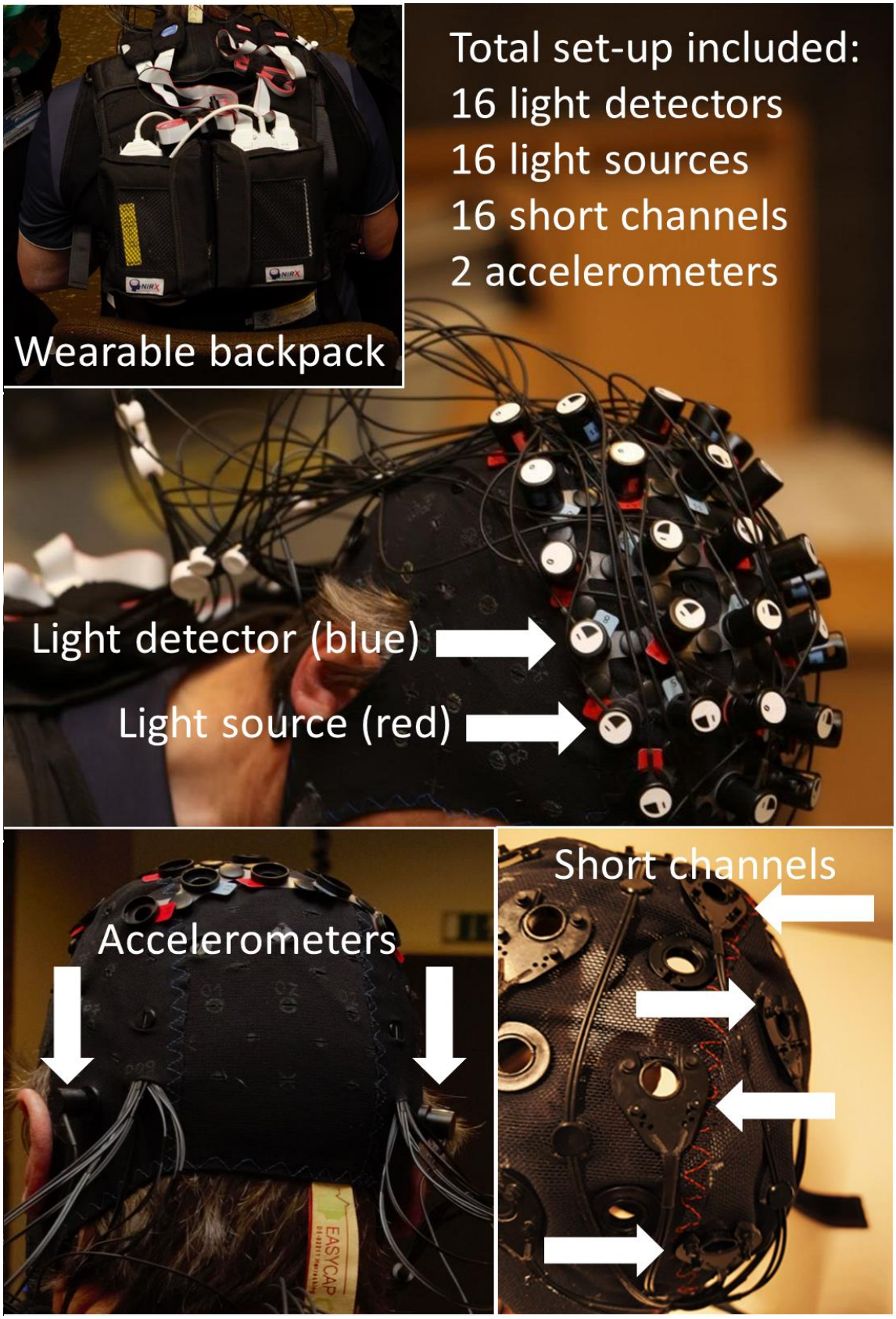

Figure 6. The fNIRS cap set-up.
The fNIRS set-up included 16 light detectors, 16 light sources, 16 short channels (1 around each source) for physiological noise detection and 2 accelerometers on the back of the cap to capture head movements. Sources and detectors were placed in alternating order to form channels where oxygenated and deoxygenated hemoglobin concentrations could be detected from the cortex.

Nowadays, more sophisticated artefact removal techniques are being developed including the simultaneous measurement of extracerebral hemodynamics with the use of short distance channels. Short distance channels work in a similar manner as the regular fNIRS channels albeit that these channels only measure extracerebral blood flow during the task. Signals arising from short channels could thus be used to filter out physiological noise such as changes in heart rate and blood pressure [31]. The fNIRS system used in the current doctorate was the NIRSport2 (NIRx, Berlin, Germany) and included 16 LED sources and 16 detectors, two accelerometers on the head for picking up motor noise together with 16 short distance channels (see **Figure 6**), which is currently considered the best artefact removal approach [32]. Here, the fNIRS data are processed employing a General Linear Model (GLM) with the accelerometry and short channel data as additional regressors, thereby filtering out physiological noise and movement artefacts. To ensure reliable signal acquisition and proper condition comparators, data were captured using a block design alternating 15 seconds of quiet stance with 40 seconds of either SB or TB walking. This allowed for the calculation of the change in hemodynamics between stance and the TB and SB conditions.

To further ensure valid signal acquisition, we compared several block designs in a healthy pilot subject and analyzed the amount of signal variability based on receiver operating curves considering specificity and sensitivity of the signal. We compared blocks with three, five, seven and ten trials, as the current fNIRS recommendations promote the use of repeated trials to ensure valid signals [16]. The receiver operating curves indeed showed that the true positive rates increased with more trials and a subsequent reduction in false positive rates is observed (**Figure 7**). Between three, five and seven trials the reduction in false positive rates was substantial. Whereas the difference between seven and ten trials in comparison with the other number of trials was minimal, although it should be noted that it would have resulted in a further 50% reduction in false positive rates. However, since including ten instead of seven trials would have resulted in an additional +/- 3 minutes of upright stance and walking per trial block we decided to include seven trials to have a good compromise between reliability of signals and feasibility of the protocol with regards to length and comfort for our participants.

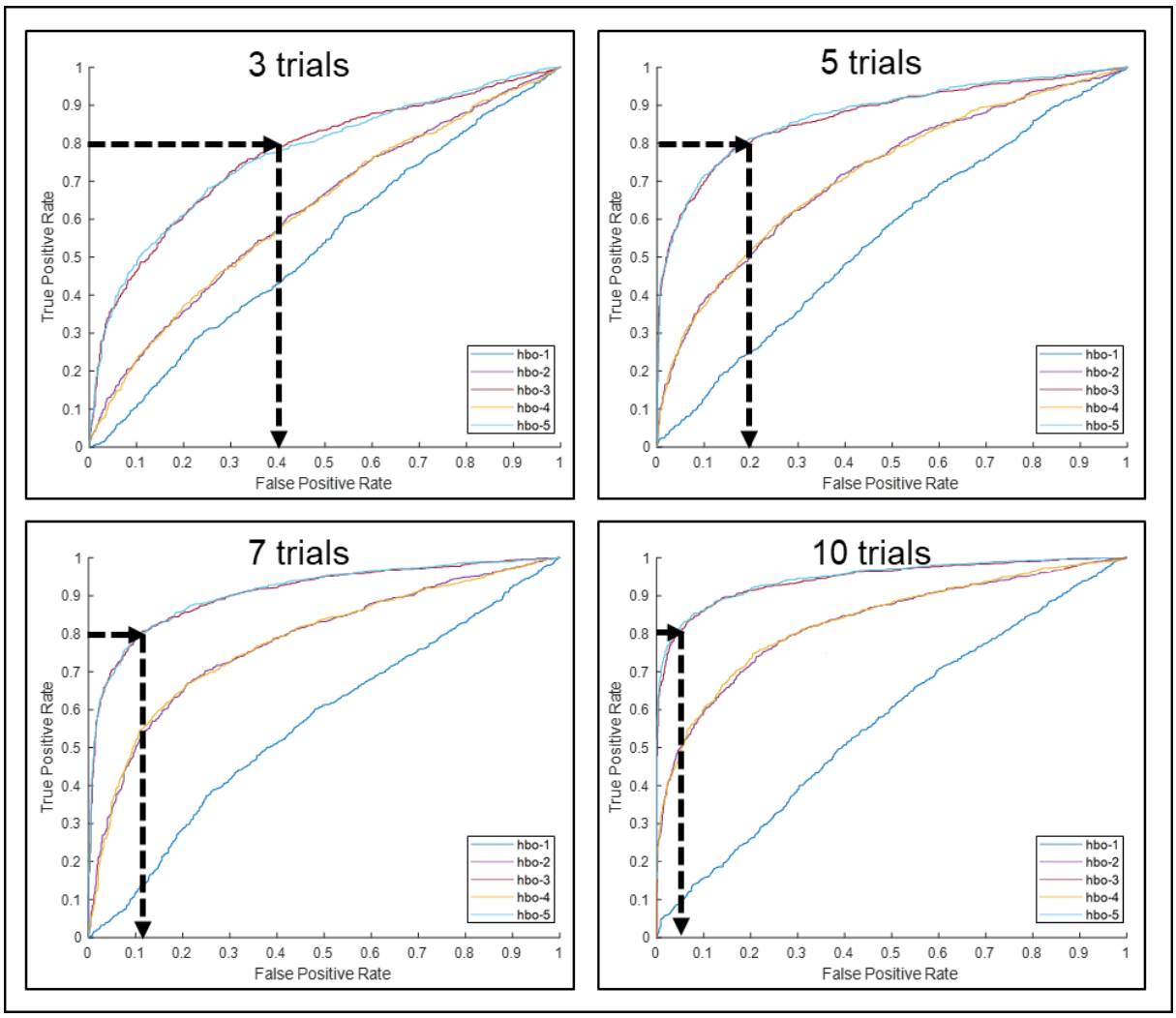

Figure 7. Comparison of signal reliability of HbO_2 with increasing number of trials.
The true positive rate drastically increases with more included trials, with the most substantial improvements between three, five and seven trials. The different colored curves represent different model characteristics. Hbo-1, OLS model with no motion correction; Hbo-2, AR-IRLS model without motion correction; Hbo-3, short channels only; HbO-4, AR-IRLS model with accelerometry; Hbo-5 AR-IRLS model with short channels + accelerometry. The AR-IRLS model with short channels and accelerometry (Hbo-5) performed best in all cases and was therefore used as analysis pipeline. HbO-n, oxygenated hemoglobin levels using model number n; OLS, ordinary least squares regression model; AR-IRLS, iterative autoregressive least-squares regression model.

Chapter 5

The Impact of Parkinson's Disease on Adaptive Split-belt Walking: A Muscle Synergy Analysis

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Abstract

People with Parkinson's disease (PwPD) have difficulty adapting gait to asymmetrical environmental demands such as when making a rapid turn, often resulting in gait abnormalities. In this study, we investigated the neuromuscular control underlying these difficulties using electromyography (EMG) and muscle synergy analysis. We compared PwPD with age-matched controls (HC) during asymmetrical split-belt (SB) walking and regular tied-belt (TB) walking (2 min TB, 2 min SB, 2 min TB). Both groups decreased from four to three synergies during TB- versus SB-walking. However, when only considering the transition back to TB-walking in a post-hoc exploratory analysis, PwPD were unable to increase their number of synergies as quickly as HC. Assuming four synergies, we compared synergy timing across treadmill phases and groups with statistical parametric mapping (SPM) analysis. We observed consistent phase effects in both legs, albeit most strongly in the slowed leg, but no group-by-phase interactions. Interestingly, the synergy encompassing the tibialis anterior showed abnormal timing and activation in PwPD in the slowed leg during the baseline-TB and SB-walking phases (post-hoc $p<0.001$). Correlation analysis revealed that simplified neuromuscular control, based on the % variance accounted for in the original EMG-signal, between baseline-TB and early SB was related to worse adaptive performance (i.e. more step length asymmetry) in PwPD ($r=-0.52$, $p=0.009$). We conclude that although the number of synergies during adaptive walking was similar between PwPD and HC, subtler within-synergy changes (i.e., reduced neuromuscular complexity) were apparent in PwPD, possibly underlying their adaptive gait difficulties.

New & Noteworthy

We identified that the muscle synergies underlying split-belt (SB) walking in people with PD and controls were largely intact. Both groups adopted four synergies during regular tied-belt (TB) walking and three during SB-walking, signifying simplified neuromuscular control during asymmetrical gait perturbations. In PD, extra abnormal timing and activation were observed in the tibialis anterior synergy in the slowed leg. Additionally, simplified neuromuscular control was related to worse adaptive gait performance in PD.

Keywords: adaptive gait; muscle synergies; motor modules; Parkinson's Disease; Split-belt treadmill

Introduction

People with Parkinson's disease (PwPD) typically experience difficulty with adapting their gait to rapidly changing conditions, especially in asymmetrical circumstances. For instance, making a sharp turn often provokes gait instability [1–3]. Problems with adaptation get worse with disease progression and can trigger freezing of gait, which in turn leads to an increased falls risk [4,5]. A split-belt (SB) treadmill offers the possibility to create asymmetrical perturbations by varying the walking speed of each leg individually. Therefore, interventions including repeated perturbations, such as SB-training, are currently being considered to target adaptive gait and balance difficulties in PwPD [6–9]. As the impact of SB-walking on muscle activity in PwPD is largely unknown, we set out to investigate the neuromuscular control underlying adaptative deficits in PD using electromyography (EMG).

In order for the central nervous system to control the wide range of muscles and their numerous degrees of freedom, locomotion as well as other habitual motor tasks are hypothesized to be governed by the precise utilization of muscle synergies that activate functional muscle groups simultaneously [10]. It has been proposed that through synergies the neuromuscular control of movement becomes more efficient as well as more adaptable [10–13], albeit that the concept of synergies is still subject to debate [14]. As such, investigating synergies, instead of individual muscles, may provide a novel approach to study how the nervous system controls complex gait [15]. Most studies suggest that higher neuromuscular complexity (i.e. recruiting more synergies) is associated with better gait performance [16–18]. However, reduced motor complexity is often observed in populations with impaired mobility, such as people with stroke, cerebral palsy and PD, who recruited less synergies compared to healthy age-matched controls. This was attributed to the need to simplify top-down movement control and overcome the degrees of freedom when the nervous system itself is compromised [15,17–19].

Previous research reported that during walking in young healthy controls (HC), four to five synergies can account for 95% of the variability (percentage variance accounted for, %VAF) in the overall EMG signal [20,21]. During SB-walking, Vervoort et al. (2020) showed that both healthy young and middle-aged adults need approximately three synergies to explain ~77 %VAF [22]. The lack of difference in the number of synergies between young and middle-aged HC suggests a limited effect of early ageing on the control of adaptive gait [22]. These results were in line with another SB-walking study in young adults identifying four synergies needed to explain ~90 %VAF [23]. This also indicates that the %VAF criterion affects the number of synergies, constituting a limitation of this approach. In the same SB-walking study, the timing of the synergy patterns showed that certain synergies occurred earlier in the gait cycle compared to regular 'tied-belt' (TB) walking and overall activation was lower in the later stage of SB-walking compared to the early stage [23]. To our knowledge, no such studies have been

performed to investigate synergies in PwPD during SB-walking. Dietz et al. (1995) found less modulated EMG activity and increased coactivation when studying only two agonist leg muscles (tibialis anterior and gastrocnemius medialis) in PwPD versus HC [24], but without including a synergy analysis. Two other studies compared PwPD with HC during regular TB treadmill walking and found simplified neuromuscular control in PwPD, which was negatively associated with gait speed, but not impacted by dopaminergic medication [15,19].

As the neuromuscular control underlying SB-walking in PD is largely unknown, the aim of the present study was threefold. First, we wanted to identify the number of synergies needed in PwPD and HC to reach 95 %VAF, comparing SB- and TB-walking. Our primary phase of interest was the early SB phase to capture the initial adaptation response, as previous study from our own group highlighted most of the difficulties to occur immediately after the perturbation in PD [2]. In line with previous work, we hypothesized that PwPD would present simplified neuromuscular control, thus less synergies to reach 95 %VAF compared to HC [15,19]. Second, we compared the timing and activation profiles of the synergies during the different SB-walking phases between PwPD and HC. We anticipated that PwPD would show lower and delayed onset of activity peaks of the respective synergies compared to HC [15,19], particularly during the early SB adaptation phase. Lastly, we wanted to explore whether neuromuscular complexity was related to 1) adaptive gait performance during SB (i.e. step length asymmetry and variability of asymmetry (SD)), 2) gait speed and 3) disease severity and dopaminergic medication. We expected that simplified neuromuscular control would be related to increased step length asymmetry and variability (worse adaptation), negatively correlated with gait speed (both groups) and positively correlated with disease severity and dopaminergic medication (PD only).

Materials and methods

Participants

This is an add-on analysis of a previous study including 84 participants (42 PwPD and 42 HC), investigating the effects of SB-walking with functional near-infrared spectroscopy (fNIRS). After the fNIRS protocol (± 2 hours) and a resting period a convenience sample of 31 PwPD and 38 HC underwent the EMG-experiment (lasting ± 30 minutes). As this was a secondary analysis, no *a priori* sample size calculation was performed. Due to technical failure of the EMG equipment, the data of 24 PwPD and 27 HC were included in the final analysis.

Participants were recruited between October 2021 and September 2022. For both PwPD and HC inclusion criteria were: Self-reported ability to walk 5 minutes without assistance or assistive device, and; No probable dementia (Mini-Mental State Examination (MMSE) ≥24). For PwPD additional inclusion criteria were: clinical diagnosis of idiopathic PD made by a

neurologist according to the most recent diagnostic criteria of the international Movement Disorders Society, Hoehn & Yahr stage II or III in the ON medication state, steady antiparkinsonian medication schedule for the month prior to enrollment and stable medication state to allow testing for at least 4 hours. Exclusion criteria were: acute orthopedic injuries or musculoskeletal problems which could affect balance and gait, other neurological or significant psychiatric impairments, cardiovascular exercise risk factors based on self-report, previous exposure to SB-walking except the SB-walking study they were currently enrolled in, and presence of a deep brain stimulator. This study was approved by the Ethical Committee Research UZ/KU Leuven (study ID: S64280). All participants gave written informed consent prior to participation. The analysis was preregistered at the Open Science Framework (https://osf.io/sd8pk/).

Clinical and cognitive assessment

All participants performed several balance and cognitive assessments, including: the (1) Mini-Balance Evaluations System Test (MiniBEST) [25], (2) Falls Efficacy Scale (FES-I) [26], (3) Trail-Making-Test Part A and B [27], (4) Montreal Cognitive Assessment (MoCA) [28] and (5) Frontal Assessment Battery (FAB) [29]. For PwPD we additionally assessed: (1) the Movement Disorders Society - Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS-III) to rate motor symptoms [30] and (2) the Levodopa Equivalent Daily Dosage (LEDD) [31]. Patients were indicated as being a freezer or a non-freezer based on item 1 of the New Freezing of Gait Questionnaire (NFOGQ, 0 = no FOG and 1 = FOG in the past month)[32].

Treadmill protocol and behavioral outcomes

The treadmill speed employed during the experimental protocol was based on the participants' comfortable overground gait speed assessed at the study onset during 10 trials of 10-meter walking with three inertial measurement units (APDM Inc., Portland, OR, USA). In **Figure 1**, the study protocol is displayed. The baseline condition started with participants walking on the treadmill for two minutes at their comfortable gait speed with both belts at equal speed (tied-belt condition, TB) (Motek Medical, Amsterdam, The Netherlands). After two minutes, one belt was decelerated in speed by 50% with 0.2m/s^2, while the other belt remained at the original speed (split-belt condition, SB) for two minutes. This was called the adaptive phase. The belts then returned to TB to capture the post-adaptation response for another two minutes. The side of the reduction was randomized 1:1 based on subjective leg dominance determined by the question: 'with which leg would you kick a ball?' [22,33].

Adaptive gait performance was captured by two outcomes: 1) the mean step length asymmetry and 2) the variability of step length asymmetry (SD). For each treadmill phase five strides were included in the analysis [22]: the first five strides after perturbation were termed early

adaptation, (EA) and the first five after return to TB were named early post-adaptation (EP) [22]. We also identified 3 more phases based on the last five strides: 1) baseline (BL); 2) late adaptation (LA); and 3) late post-adaptation (LP) [22]. The start and end of each gait cycle was defined by the first heel strike to the consecutive heel strike. Heel strike was defined as the ground reaction force reaching the threshold of 20N, using the treadmill force plate data captured at 1000Hz. As described in previous work [6], step length asymmetry was calculated as a ratio value: (fast leg parameter – slow leg parameter)/(fast leg parameter + slow leg parameter). Values closer to zero represented less step length asymmetry and thus more adaptive gait. Negative values indicated that the fast leg took smaller steps compared to the slow leg [6]. Increased step length asymmetry variability was interpreted as less adaptive performance.

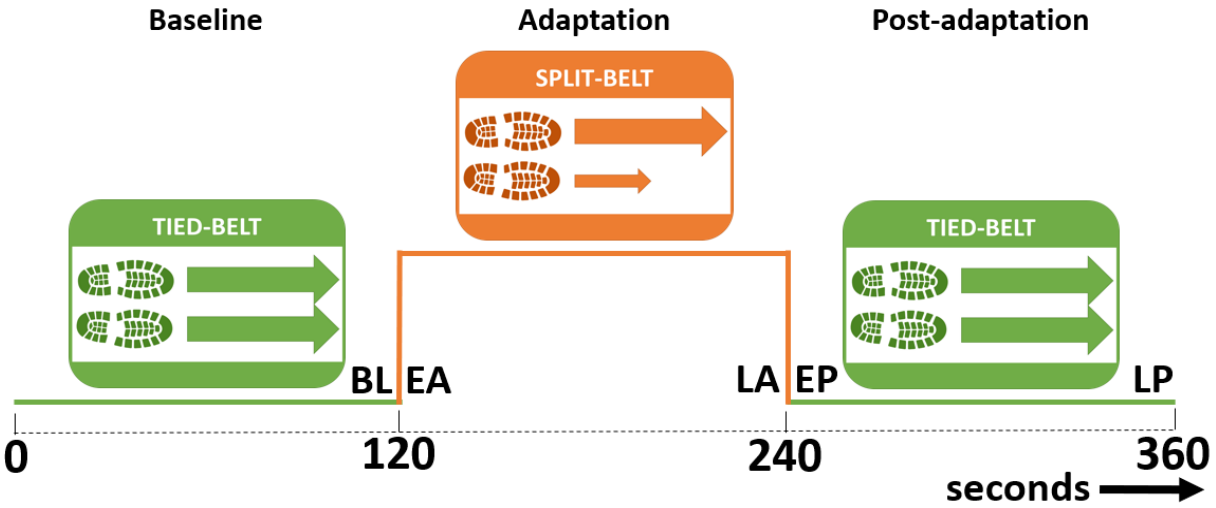

Figure 1. Study protocol. Participants started with two minutes of TB-walking followed by two minutes of SB-walking with 50% speed reduction of one leg called the adaptation phase. The belts then returned back to TB-walking to capture the post-adaptation. During the adaptation phase the side of speed reduction was randomized per participant.
Abbreviations: BL, late baseline; EA, early adaptation; LA, late adaptation; EP, early post-adaptation; LP, late post-adaptation; TB, tied-belt; SB, split-belt.

EMG data acquisition and analysis

EMG was recorded at 1000 Hz (Cometa, Italy) in seven muscles bilaterally deemed important for gait control based on earlier work [22,34]. The muscles included were: Tibialis Anterior (TA), Gastrocnemius Lateralis (GL), Vastus Lateralis (VL), Rectus Femoris (RF), Biceps Femoris (BF), Tensor Fasciae Latae (TFL) and the Erector Spinae (ES). The placement and preparations of the electrodes were done in accordance with the SENIAM guidelines [35]. The skin was shaved if needed and cleaned with alcohol. Electrodes (Blue sensor N, AMBU, Denmark) were placed on the muscle belly with 20-millimeter inter-electrode distance.

The EMG data were analyzed using custom Matlab scripts (R2019a, Mathworks, MA, USA). Raw data were bandpass filtered (20-450Hz, Butterworth, 2nd order), rectified, and low-pass filtered (6Hz, Butterworth, 4th order) [36,37]. The EMG data included were time normalized per gait cycle, including a total of 100 data points, 60 data points for stance and 40 for swing phase, starting at heel strike [38]. EMG amplitude normalization was based on the maximum amplitude during the baseline TB condition for each individual muscle [22]. Similar to the behavioral data, the EMG data were averaged for the first (EA, EP) or last five strides (BL, LA, LP) of each phase [22].

Synergy analysis – Non-negative matrix factorization

Muscle synergies were determined using non-negative matrix factorization (NNMF) for each participant, calculated for each phase and leg separately using the following formula: $EMG_0 \approx EMG_r = W \times C + residual$. The input for the NNMF was a matrix including the preprocessed EMG data, EMG_0 (7 muscles x 100 data points). The output of the NNMF were two matrices containing the contribution (the weight, W) of each muscle per synergy and the temporal projections (C). W is a *m* x *n* matrix, where *m* is the number of muscles included and *n* the preselected number of synergies to be constructed. C is a *n* x *t* matrix containing the timing profiles or activation coefficients, with *t* being the number of data points over time (100 per phase). To reconstruct the EMG signal (EMG_r) matrix W and C need to be multiplied. The NNMF was performed using the built-in Matlab function *nnmf* and utilized an alternating least-squares (als) algorithm with 1000 replicates for each participant to ensure the best fit of the results [39]. The appropriate number of synergies (*n-synergies*) was determined based on the percentage variance accounted for (%VAF) using the following formula: $\%VAF = (EMG_0 - EMG_r)^2/EMG_0^2 * 100$ [40]. For our primary research question, *n-synergies* was iteratively increased (between 1 and 7) until it surpassed the 95 %VAF criterion for all muscles combined [15,19] per phase and per participant. This means that 95% of the variability in the original EMG signal could be reconstructed by the respective number of synergies. The 95% VAF for each individual muscle was also calculated, but this was not used as a cut-off criterion.

For our secondary research question, we compared the timing profiles of the synergies between PwPD with HC across phases. To be able to compare the data between groups, we determined *n-synergies* – based on the number that HC needed to reach 95 %VAF during TB-walking (group median) and re-ran the NNMF analysis. This approach was validated in previous work, and served to equalize the composition of each synergy in terms of timing and weights across participants and groups [19]. Next to comparing timing, this approach allows comparing the neuromuscular complexity between groups by inspecting the %VAF. A higher %VAF with a fixed *n-synergies* can be interpreted as lower neuromuscular complexity (i.e. simplified control), since more variability of the original signal can be explained with the same

number of synergies. Since synergies with similar characteristics were not always extracted in the same order with NNMF for every participant, the structures of the synergies were sorted per participant based on the muscle weights (W matrix) within each phase and group using the Matlab function *kmeans*. As input parameters we used: *n-synergies* as the number of k-means clusters, maximum of 1000 iterations, 15 replicates and the cosine as a distance metric [41]. In case two synergies of a participant were assigned to the same cluster, we assigned the double synergy with the largest distance from the original cluster centroid to the second nearest cluster if vacant. If this approach was not possible, then this double synergy was rejected as to capture the most common patterns available in the dataset [34]. Because of this grouping procedure, the number of synergies per cluster could vary [34].

Statistical analysis

Missing data analysis encompassed the calculation of the probability that a value is missing at time *i*, given that it is observed at time *i* – 1, and given its value at time *i* – 1, for all participants with incomplete data. This indicated that the data were missing completely at random (MCAR). Therefore, a complete case analysis would most likely be unbiased and imputation was not performed.

To analyze between-group differences in the continuous demographic data, Independent Student T-tests or Mann-Whitney-U-Tests in case of an abnormal data distribution were used. For categorical data, Chi-squared test were performed. Since comfortable treadmill walking speed was significantly lower in the PD group, we used this as a covariate in the subsequent analyses. A Linear Mixed Model with the factors group (HC, PwPD) and phase, including five levels: BL, EA, EP, LA, LP were used to analyze adaptive treadmill performance. We report mean step length asymmetry as the primary adaptive gait outcome and asymmetry variability (step length asymmetry SD) as the secondary one.

For our first research question, we performed a Negative Binomial Generalized Linear Mixed Model with logarithmic link function comparing the %VAF including all phases and the two groups, because this provided the best model fit. Additionally, we explored the post-adaptation effects in more detail (LA vs EP) in a separate model. The above analyses were performed in SPSS v.28 (IBM Corp., Armonk, NY). Statistical significance was set at $p<0.05$ and post-hoc tests were Holm-Bonferroni corrected.

For our second research question, we compared PwPD and HC with a fixed number of synergies based on the median number of synergies needed by HC during baseline. Of these synergies, we compared their timing profiles over the different phases and across groups. For this purpose, we used Statistical Parametric Mapping (SPM) [42–44]. SPM first calculates a test statistic for each time point separately to reconstruct a statistical parametric map. Next,

the temporal smoothness of the SPM trajectory was determined and a critical significance threshold (CT) set, which only 5% of the curve was expected to surpass. Above this threshold, a supra-threshold cluster was defined, corresponding with a statistically significant difference. Small supra-threshold clusters (<5% of gait cycle), which in the context of the gait cycle are most likely to be less informative, were disregarded [22], but are reported in the results table in non-bold font (p-values). The timing profiles were analyzed with a non-parametric repeated measures SPM ANOVA, with the factors group (HC, PwPD) and phase (BL, EA, LA, EP, LP). If significant clusters were observed, non-parametric T-tests with Bonferroni correction were performed. The primary endpoint of this analysis was the group by phase interaction. Secondary endpoints were the main effects of group and phase and subsequent post-hoc tests. Within SPM, it is not possible to include covariates or use unbalanced samples. Therefore, we balanced our sample first by random exclusion of cases, as well as by gait speed to validate the SPM results obtained. This analysis was performed in Matlab using the open-source *spm1d* code (Version M.0.4.10, www.spm1d.org).

For the third research question, we performed a Spearman correlation analysis to investigate within each group the possible associations between neuromuscular complexity during EA (%VAF) and the change (delta) in the number of synergies from BL to EA and (1) adaptive gait outcomes, (2) treadmill speed and (3) disease severity and (4) LEDD. Additionally, we performed partial correlations to investigate the mediating effects of treadmill speed, disease severity and LEDD. The correlation analyses were Bonferroni corrected and performed in SPSS v.28 (IBM Corp., Armonk, NY). Statistical significance was set at $p<0.05$. We report the results in the main text for the slow leg only, unless the fast leg presented a different picture.

Results

Participant characteristics

Twenty-four PwPD and 27 HC were included in this study. Participant characteristics are presented in **Table 1**. Age and sex distributions were similar between PwPD and HC. Comfortable treadmill walking speed was significantly lower in PwPD compared to HC ($p<0.041$). Overall, PwPD presented with worse cognitive performance and higher fear of falling as assessed with the FES (all $p<0.024$). Balance scores were lower in PwPD, although not significantly different from HC ($p=0.056$).

Adaptive gait outcomes

The results (**Figure 2, Supplementary Table S1**) showed that there was a significant phase effect ($F_{(1.93)}=13.55$, $p<0.001$), but no group ($F_{(1)}=0.31$, $p=0.583$) or interaction effect ($F_{(1.93)}=2.32$, $p=0.714$) for step length asymmetry. Compared to TB-walking at baseline (BL), both PwPD and HC showed more asymmetry during the early and late adaptive phases (EA, LA)

as well as during the early post-adaptation phase (EP). During EA there was more asymmetry compared to LA, EP and LP. Additionally, during LA there was more asymmetry compared to EP and LP. P-values of all comparisons were <0.001 after Bonferroni correction.

Similar results were found for asymmetry variability (SD) with a significant phase effect (p=0.009), but no group (p=0.867) or interaction effect (p=0.554). Variability was significantly higher during the EA and LA phases compared to BL (all p<0.002). Variability was similar during the TB-phases BL, EP and LP (all p>0.05). There was more variability during EA compared to LA (p=0.041), EP and LP (all p<0.001). Similarly, there was more variability during LA compared to EP (p=0.035).

Table 1. Participant characteristics.

	HC (N=27)	**PD (N=24)**	**p-value**
Sex (M/F)^	14/13	14/11	0.869
Age (years)*	67.78(5.55)	63.63(6.98)	0.931
Treadmill speed (m/s)	1.12(0.11)	1.04(0.17)	**0.041**
Cognitive capacity			
Education (years)	15.41(2.55)	14.71(2.74)	0.560
MoCA (0-30)	28.85(1.85)	25.25(2.59)	**0.007**
FAB (0-18)*	16.85(1.41)	15.71(2.01)	**0.024**
TMT part A (sec)	32.17(9.99)	45.18(12.87)	**<0.001**
TMT part B (sec)*	73.00(25.17)	93.41(30.52)	**0.010**
TMT B minus A*	40.83 (21.27)	48.23 (22.45)	0.187
Balance capacity			
FES-I (16-64)*	18.78(2.50)	26.08(7.33)	**<0.001**
MiniBEST (0-28)*	24.78(5.31)	21.08(8.03)	0.056
PD characteristics			
Years since diagnosis	-	8.48(4.58)	-
LEDD (mg)	-	704.97(402.89)	-
HY-stage (2 vs. 3)	-	21/3	-
UPDRS-III (0-132)	-	28.42(11.52)	-
Participants with/without FOG	-	5/19	-

Significant values (p<0.05) are marked in bold. Values represent mean(standard deviation), except for sex and HY-stage. All comparisons with independent student's t test except: ^ Chi-squared test; * Mann-Whitney U test. Abbreviations: M, male; F, female; MoCA, Montreal Cognitive Assessment; Frontal Assessment Battery; TMT, Trail Making Test; FES, Falls Efficacy Scale; MiniBEST, Mini Balance Evaluation Systems Test; LEDD, Levodopa Equivalent Daily Dosage; HY-stage, Hoehn and Yahr stage; UPDRS-III, Movement Disorders Society Unified Parkinson's Disease Rating Scale part III.

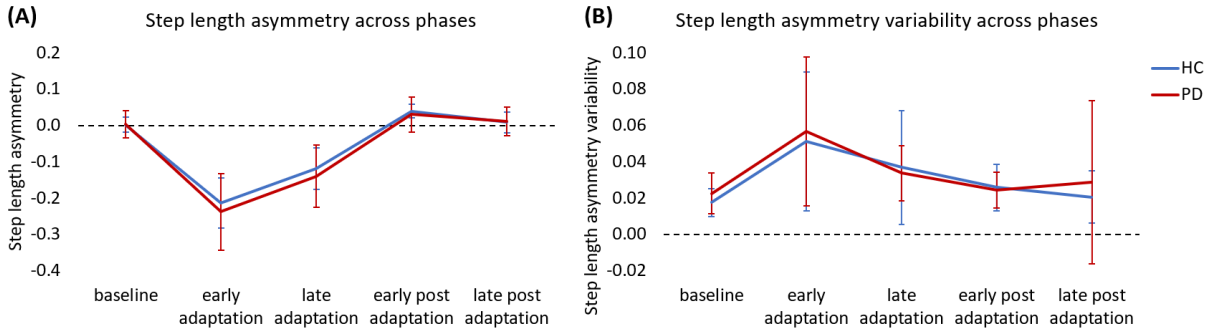

Figure 2. Gait adaptation outcomes, (A) step length asymmetry and (B) step length asymmetry variability across the different treadmill phases. The dotted zero line represents perfect symmetry (A) or no variability (B).

Neuromuscular complexity across the different phases

Figure 3A illustrates that both groups dropped from four to three synergies (which reached 95 %VAF) during the adaptive phases (EA, LA) compared to TB-walking (BL, EP, LP). There was a significant phase effect meaning that in the post-hoc tests (**Figure 3B**) both PwPD and HC showed a decrease in neuromuscular complexity (number of synergies) during the adaptive SB-walking phases (EA, LA) as compared to the TB-walking phases (phase effect p = <0.001, all post-hoc TB vs. SB phases p<0.001, **Table 2**). No group or interaction effect was present (group effect p = 0.720, interaction p = 0.105). As **Figure 3A** visually suggested less synergies in PD versus HC during post-adaptation, we explored the contrast between LA and EP phases separately. We found a significant group by phase interaction effect ($F_{(1,97)}$ = 4.37, p=0.039) indicating that PwPD were slower in increasing the number of synergies than HC during EP (post-hoc p = 0.030). Comparable but fewer statistically significant results were obtained in the fast leg (**Supplementary (S) Table S2**).

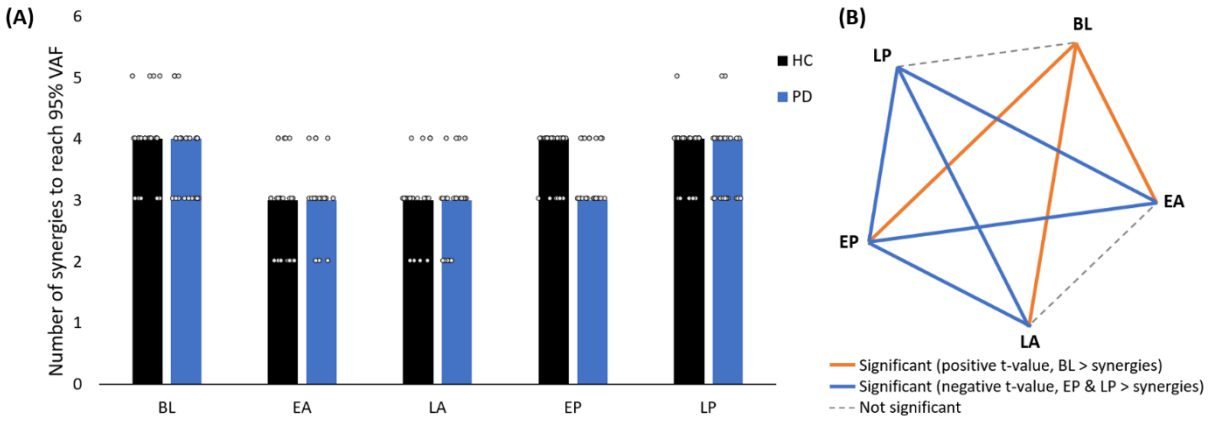

Figure 3. (A) Number of synergies in the slow leg needed to reach 95% VAF in the different treadmill phases. (B) GLMM post-hoc tests of phase effects. The orange lines display significant differences in *n-synergies* where BL had more synergies compared to the other phase the line connects with. The blue lines indicate a significant difference in *n-synergies* where EP & LP had more synergies compared to the other phases. Dotted lines were not significant comparisons. Abbreviations: BL, late baseline; EA, early adaptation; LA, late adaptation; EP, early post-adaptation; LP, late post-adaptation.

Table 2. GLMM outcomes number of synergies per phase of the slow leg.

	HC (N = 27)		PwPD (N=24)				
	Number of synergies		Number of synergies		Group	Phase	Interaction
Phase	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	p-value	p-value	p-value
BL	3.96 (0.59)	4 (0)	3.71 (0.69)	4 (1)	0.720	**<0.001**	0.105
EA	2.85 (0.72)	3 (1)	3.04 (0.55)	3 (0)			
LA	2.89 (0.64)	3 (1)	2.96 (0.62)	3 (0)			
EP	3.74 (0.45)	4 (1)	3.38 (0.50)	3 (1)			
LP	3.85 (0.46)	4 (0)	3.63 (0.65)	4 (1)			
Post hoc	**Mean difference**						
Phase	**Mean (SD)**	**t-value**	**df**	**p-value**			
BL vs. EA	0.89 (0.12)	7.43	244	**<0.001**			
BL vs. LA	0.91 (0.10)	9.62	244	**<0.001**			
BL vs. EP	0.28 (0.09)	3.28	244	**0.005**			
BL vs. LP	0.10 (0.09)	1.11	244	0.533			
EA vs. LA	0.02 (0.11)	0.20	244	0.839			
EA vs. EP	-0.61 (0.11)	-5.73	244	**<0.001**			
EA vs. LP	-0.79 (0.10)	-7.79	244	**<0.001**			
LA vs. EP	-0.63 (0.09)	-6.78	244	**<0.001**			
LA vs. LP	-0.81 (0.10)	-8.56	244	**<0.001**			
EP vs. LP	-0.18 (0.06)	-3.01	244	**0.009**			

Significant values (p<0.05) are marked in bold. HC, Healthy controls; PD, people with Parkinson's Disease; SD, standard deviation; BL, baseline; EA, early adaptation; LA, late adaptation; EP, early post adaptation, LP, late post adaptation. The covariate comfortable treadmill speed was not significant in the model (p=0.08).

Muscle synergy timing and magnitude across treadmill phases (SPM-analysis)

SPM interactions and main effects per synergy

Figure 4 displays the individual muscle weights of the four extracted synergies in the slow leg and the average timing of each synergy during the BL phase, which were similar in the slow and fast leg and reported for both groups. The main contributor to synergy 1 was the TA muscle. Synergy 2 mainly consisted of GL activity. Synergy 3 encompassed the quadriceps muscles (RF and VL). The main contributors of synergy 4 were the hamstrings (BF) and the lower back muscles (ES). See **Table 3** for the main synergy functions during normal gait as reported in the literature. The results of the SPM analysis are reported in **Tables S3** (slow leg) **and S4** (fast leg)**.** Both legs showed no group by phase interaction effects but did present with consistent main effects of phase across all four synergies (p=0.002) and a group effect (p=0.002) for synergy 1. Comparable results were obtained when not randomly balancing the populations but when this was based on treadmill speed (**Table S5 and S6)**.

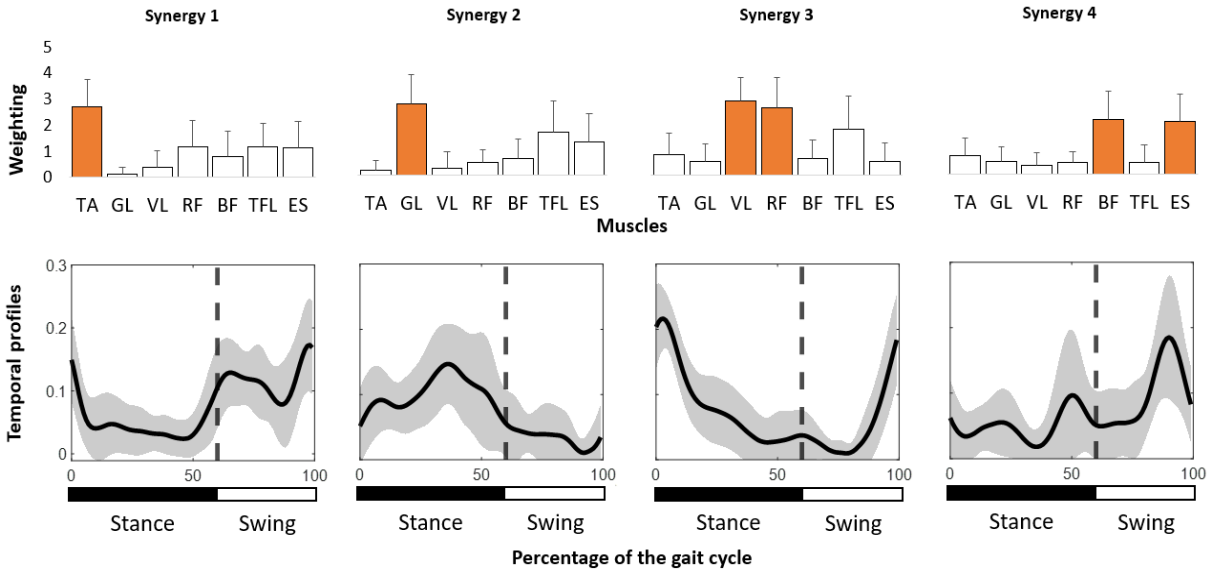

Figure 4. Top panels represent the average individual muscle weights (i.e. contribution of each muscle to the synergy for HC and PwPD per extracted synergy for the slow leg. Orange bars indicate the main muscles contributing to the synergy. Bottom panels represent the temporal profiles per synergy during baseline. Grey areas represent ± 1 standard deviation, grey dotted line indicates 60% of the gait cycle, indicating the stance (0-60%) and swing phase (60-100%). Abbreviations: TA, tibialis anterior; GL, gastrocnemius lateralis; VL, vastus lateralis; RF, rectus femoris; BF, biceps femoris; TFL, tensor fasciae latae; ES, erector spinae.

Table 3. Synergies and their main functions during the gait cycle.

	Synergy 1	**Synergy 2**	**Synergy 3**	**Synergy 4**
Main contributors	Tibialis anterior	Gastrocnemius lateralis	Vastus lateralis Rectus femoris	Biceps femoris Erector spinae
Functional group	Dorsiflexors	Plantarflexors	Quadriceps	Hamstrings Lower back
Main functions	1. Deceleration at initial contact 2. Acceleration for toe clearance during swing	1. Acceleration during stance 2. Provide push off during preswing	1. Shock absorption during initial contact 2. Knee control during initial and terminal swing	1. Acceleration during preswing 2. Deceleration during terminal swing 3. Trunk stabilization during initial contact and preswing

Main functions identified during regular walking as derived from Houglum et al. (2022) [45] and based on the data of Perry and Burnfield (2010) [46].

SPM post-hoc tests

Figure 5 illustrates the main SPM results of interest, pertaining to the slow leg in synergy 1 (m. tibialis anterior). During BL PwPD showed less activity between 78.29-86.34% (mid swing) and more activity between 93.90-99% (terminal swing) of the gait cycle compared to HC (**Figure 5**). During EA and LA, PwPD showed more activity during initial-swing (EA: 59.59-72.88%, LA: 63.54-73.21%) and less activity during terminal swing (EA: 90.64-2.82%, LA: 89.08-98.51%) compared to HC. However, no group differences were found during the post-

adaptation phases (see **Figure S1**). Phase effects are reported in **Table S3**. No group or phase effects were present in the fast leg, but a similar pattern of results could be observed visually.

For synergy 2, although a main effect of phase was present, the post-hoc tests did not show any substantial clusters (>5%) in either the slow or fast leg (see **Tables S3 and S4**). In synergy 3, phase effects were mainly present in the fast leg. Here, more and seemingly earlier muscle activity was generated during LP as compared to BL at the end of the swing phase (LP>BL: 93.67-99%, see **Figure S2**). In the slow leg, similar activation patterns were present but without robust clusters that exceeded the 5% threshold. A phase effect was present in synergy 4 of the slow leg, where both groups showed increased activity during LA as compared to EA between 41.24-51.06% of the gait cycle (see **Figure S2**). No substantial significant clusters were present in the fast leg.

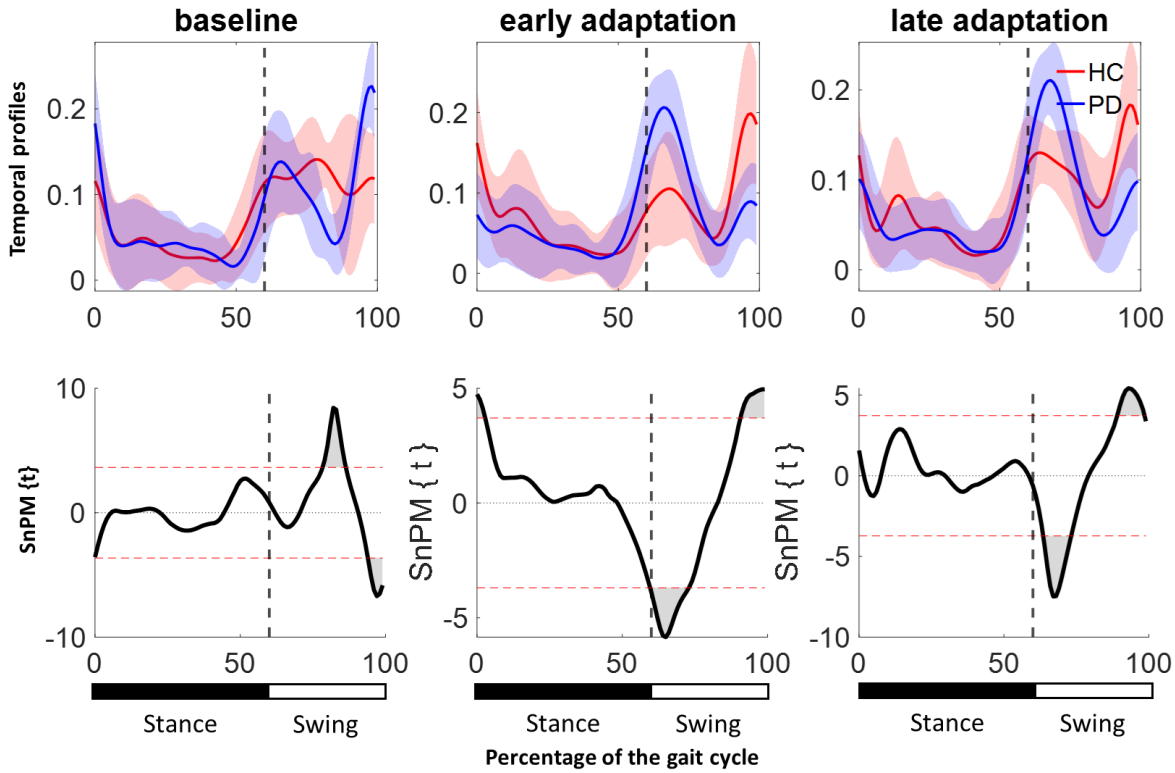

Figure 5. Significant group effects SPM post-hoc tests for synergy 1. Top panels represent the temporal profiles per synergy during the baseline, early and late adaptation phase for each group separately. Blue and red shaded areas represent ± 1 standard deviation of the PD and HC data respectively. Bottom panels represent the SPM t-test results of each phase. The red dotted lines indicate the critical significance threshold. The grey marked are the instances of significant group differences all >5% of the gait cycle (all p<0.001). The vertical grey dotted line in all graphs indicates 60% of the gait cycle, displaying the stance (0-60%) and swing phases (60-100%).

Correlations neuromuscular complexity and gait performance

To get more insight into whether neuromuscular complexity was associated with adaptive gait performance in early adaptation, we conducted a Spearman correlation analysis. **Figure 6** shows that in PwPD, more step length asymmetry was associated with less neuromuscular complexity as expressed by a higher %VAF in the fast leg with the same number of synergies, r=-0.52, p=0.009, although not surpassing the critical Bonferroni p-value of 0.005 (**Table 4**). A similar negative, but non-significant, association was observed in the slow leg (r=-0.23, p=0.282). Of note, this correlation analysis was performed to ascertain a possible within-group relationship between the respective outcomes. In an exploratory regression analysis, we found no between-group difference between the slopes, indicating that correlations between %VAF and step length asymmetry were similar (interaction effect group*%VAF fast leg, $F_{(1)}$=2.71, p=0.106, **Figure 6**). When considering the inter-phase changes in the number of synergies from BL to EA, the HC group showed that greater step length asymmetry during EA was associated with a larger decline in the number of synergies, r = 0.45, p=0.019, although not surpassing Bonferroni correction (**Table S7**). No other correlations between adaptive gait and EMG-outcomes were found. Lower gait speed, however, was associated with a larger decline in synergies from BL to EA in the fast leg in HC (r = 0.48, p=0.012). In the PD group no associations were found between disease severity, dopaminergic medication (LEDD) and neuromuscular complexity.

Partial correlation analyses correcting for between-group age and treadmill speed differences did not change the results.

Table 4. Spearman correlations between neuromuscular complexity during the early adaptation phase and treadmill speed, performance and disease severity.

	HC (N = 27)		**PD (N = 24)**	
Variable	**%VAF fast leg**	**%VAF slow leg**	**%VAF fast leg**	**%VAF slow leg**
Treadmill speed (m/s)	0.22 (0.269)	-0.22 (0.280)	-0.02 (0.940)	-0.02 (0.916)
Mean SL asymmetry	-0.24 (0.230)	-0.02 (0.909)	**-0.52 (0.009)**	-0.23 (0.282)
Mean SL asymmetry variability	-0.35 (0.078)	-0.23 (0.252)	0.39 (0.059)	0.03 (0.878)
UPDRS-III total score (0-132)	-	-	-0.21 (0.320)	-0.25 (0.240)
LEDD (mg)			<0.01 (0.995)	-0.12 (0.565)

Significant values (p<0.05) are marked in bold, critical Bonferroni p-value = 0.005 in PD and 0.008 in HC. HC, Healthy controls; PD, people with Parkinson's Disease; SL, step length; UPDRS-III, Unified Parkinson's Disease Rating Scale part III; LEDD, Levodopa Equivalent Daily Dosage.

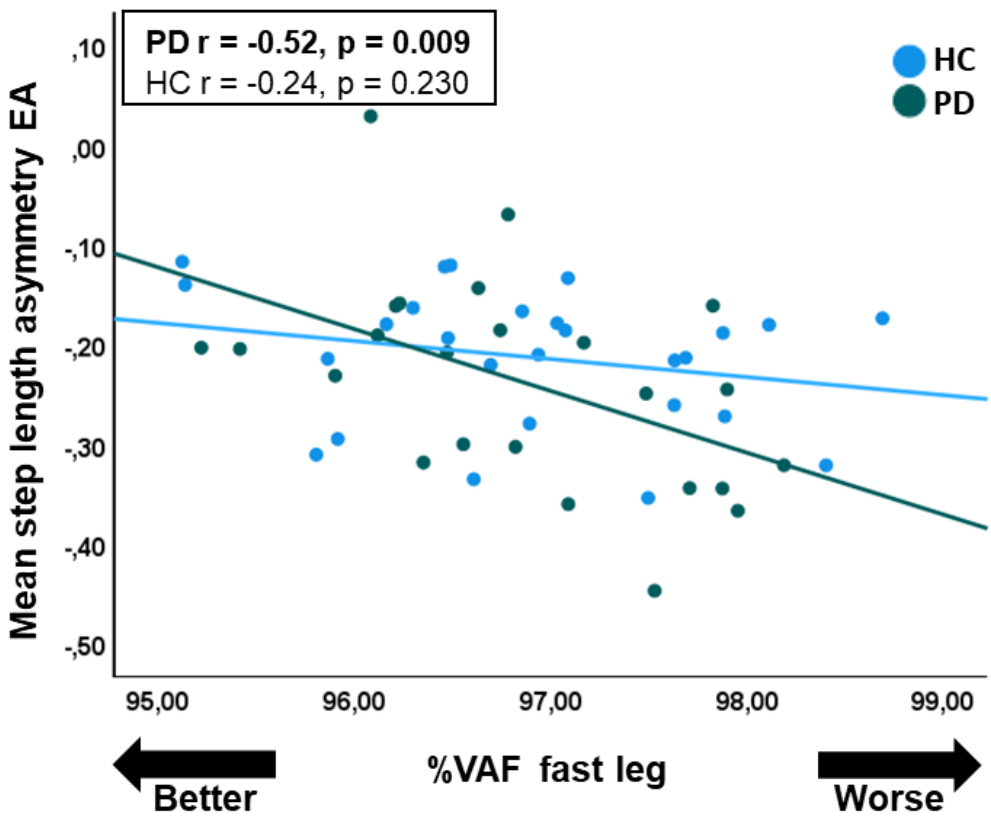

Figure 6. Scatter plot between neuromuscular complexity (%VAF) and adaptive performance (step length asymmetry) in the fast leg during EA. Here, we report the spearman correlation coefficients and corresponding p-values for PD and HC. Correlation analysis was performed within each group. No between-group difference between slopes was found (interaction effect group*%VAF fast leg, $F_{(1)}$=2.71, p=0.106). Abbreviations: PD, Parkinson's Disease; HC, Healthy controls; SL, step length; %VAF, % Variance Accounted For; EA, early adaptation.

Discussion

In this study, we investigated the impact of PD on the neuromuscular control underlying the ability to adapt to SB-walking. We hypothesized that PwPD would present simplified neuromuscular control compared to HC and that this would be related to compromised adaptive performance, lower gait speed, and worse PD-specific outcomes. We found that PwPD and HC adapted similarly to the imposed perturbations, while also demonstrating a comparable number of synergies across regular treadmill as well as asymmetrical walking (no group by phase interaction effect). Interestingly our exploratory analysis indicated that during post-adaptation, PwPD were unable to transition back to increasing their number of synergies in the same way as HC. For our second research question, we hypothesized that PwPD would present a different within-synergy pattern of muscle activity than HC and this mostly in the early adaptation phase. Indeed, we found a main group effect in synergy 1 corresponding to the activity in TA in both early and late adaptation phases, but not during post-adaptation. When responding to the perturbations, PwPD generated heightened TA activity in mid swing and

lower TA activity at foot strike compared to HC, although in contrast to our hypothesis no group by phase interactions were apparent. In the early adaptation phase, we also probed the potential meaningfulness of simplified neuromuscular control and found that it was significantly associated with worse gait asymmetry in PD.

In this study, we identified the main muscle activity patterns underlying SB-walking, showing that on average HC and PwPD adopted four synergies during the TB-walking conditions (BL, EP, LP) and three during SB-walking (EA, LA). These synergistic patterns were in line with previous studies mostly involving HC, applying regular TB-walking as well as adaptive SB-walking [15,20–23,47]. Still, it should be noted that the absolute number of synergies per study depended on the muscles chosen and %VAF cut-offs employed. To our knowledge, no study has looked at changes in the number of synergies utilized across different SB-walking phases. Here, we report the novel finding that the number of synergies observed in both groups dropped from four to three during the SB- compared to the TB-phases, signifying the tendency to simplify neuromuscular control to cope with a perturbation. In several previous studies simplified motor control was also reported during skill acquisition, whereby subjects tended to 'freeze' in order to constrain the degrees of freedom of the motor system [10,13,48,49]. As skill acquisition progressed, the degrees of freedom were gradually increased to be able to fine-tune movement execution [13,48]. Since in our study all participants were relatively naïve to SB-walking, reducing the number of synergies could represent a similar initial response. Additionally, post-hoc exploration revealed that in the post adaptation period (LA versus EP), PwPD seemed unable to increase the number of synergies immediately after the switch from SB- to TB-walking in contrast to HC, suggesting that the former had difficulty with flexibly re-adapting their coordination from SB- to TB-walking. A previous study also underscored a reduction of synergies in PwPD compared to HC but this during regular treadmill walking, pointing towards a general propensity towards stereotyped neuromuscular control even when gait is less challenged [19].

Although not apparent in the current study, problems with switching between TB and SB conditions were reported before in behavioral measures (e.g. step length asymmetry), whereby PwPD, and especially freezers, showed slow initial adaptation as well as re-adaptation [2]. During the fNIRS protocol preceding the EMG assessment, the PD group did show greater asymmetry after seven trials of 25 seconds of SB-walking. The lack of behavioral differences in this study could be explained by: (1) the possibility that the patients who volunteered to partake in the EMG assessment were better able to withstand the lighter protocol of SB-perturbations (only one switch and re-switch) or (2) the fact that only a single trial was captured, whereas the fNIRS protocol included multiple trials.

In partial agreement with our second hypothesis, PwPD did show a different pattern of within synergy activity compared to HC, and this particularly within synergy 1 which included the TA muscle as the main contributor. First, during regular TB-walking, an activity decrease in synergy 1 was demonstrated during mid swing as well as an increase during terminal swing although the post-hoc test only just exceeded the >5% threshold (5.1%). In disagreement with our work, increased TA activity during initial and mid swing was reported earlier in PwPD during regular treadmill walking and was explained as a possible compensatory response to reduced GL activity [50]. However, it should be noted that this study only included 10 PwPD, reported on individual muscles (no synergy approach), and pertained to a very slow walking speed (0.3m/s). A recent systematic review on EMG-activity in PD versus HC, mostly studied during regular walking, observed a general under-activation of the distal musculature, including the TA [51]. Impaired TA activity was also associated with an increased risk of falls in PwPD, as the TA is deemed responsible for a correct foot position during heel strike [51]. Against this background, the abnormal TA modulation, which was the main contributor to synergy 1 in this study, is not surprising.

Interestingly, during adaptive gait, the abnormal synergy pattern reversed, as PwPD presented with increased synergy 1 activity during the initial swing phase, and less activity at foot strike (terminal swing and early stance) in contrast to HC. Dietz et al. (1995), although not investigating synergies, reported increased TA activity during swing while SB-walking in both PD and HC, without making an exact distinction in which portion of the swing phase this occurred [24]. We interpret the higher synergy activity during swing as an effort to increase stride length on the slow side to bring movement execution in line with the fast leg, similar to previous work on increasing gait speed through TA-modulation [50]. The decreased synergy activity found during foot strike seems to agree with two studies which reported reduced TA-activity in PD in the off-medication state during overground walking, albeit not in a synergy context [52,53]. This decreased activity disappeared after medication intake, pointing towards dopaminergic input needed for optimal muscle recruitment [52,53]. Although on medication in this study, it is possible that due to the increased adaptive demands of SB-walking, patients were unable to recruit synergy 1 optimally for foot strike. Future study with detailed gait analysis during prolonged gait perturbations is needed to fully understand the effects of PD on synergy 1 or TA recruitment, as well as the subsequent repercussions on joint angles and foot strike function.

Both groups showed similar timing and magnitude of EMG-activity for the other three synergies and no differences were found post-adaptation. This was most surprising for synergy 2, as it consisted mainly of the GL. Lower GL activity was reported previously during regular and SB-walking in PD [24,51] and was associated with reduced push-off power and a subsequent

reduction of gait speed [51,54]. Despite the fact that PwPD walked with a lower comfortable gait speed on the treadmill, they were able to modulate synergy 2 similarly to their healthy counterparts across the gait cycles in all experimental conditions.

An intriguing result was that for the first two synergies, the strongest post-hoc effects were observed in the slow leg, whereas for synergy 3, some effects were found in the fast leg. Synergy 3 comprised of the quadriceps muscles RF and VL, mostly active during terminal and initial stance for stabilizing the knee and hip joint to prepare for initial contact and the initiation of swing. A previous SB-study reported that the relative contribution of the double support time during early SB-walking increased for the fast leg, increasing the reliance on the quadriceps for a longer period of the gait cycle [55]. In the same study, the double support duration adapted gradually during the 10-minute SB-trial and showed strong after-effects [55], similar to the increased activity seen in our LP-phase compared to earlier phases.

Lastly, synergy 4, mainly involving the BF and lower back muscles, showed more activity in LA versus EA during terminal stance (significant post-hoc for phase). Synergy 4 was described by Choi et al. (2019) during both straight walking and turning, and was mostly activated during terminal stance and early swing, with which our results are in congruence [56]. During walking, weight needs to be redistributed from the stance to the swing leg and synergy 4 facilitates both axial stabilization as well as a deceleration of the swing leg. These functions were probably found to increase in importance when simultaneously dealing with asymmetrical demands and postural control [56].

Overall, the activity of the four synergies reported in this study were in line with previous work on TB- and SB-gait, as well as on adaptive gait tasks including overground turning [19,23,34,40,56]. Our findings imply that participants used basic gait synergies to adapt to asymmetrical conditions and that this was largely irrespective of pathology. The relative similarity of synergies across diverse tasks [40,56,57] makes sense as it may facilitate motor learning and motor efficiency by utilizing a basic neuromuscular template that can be adjusted to the new task at hand [58,59]. However, we also found that, in PwPD, simplified neuromuscular control (expressed as a higher %VAF between BL and EA) was related to a less adaptive treadmill performance, with a similar trend in HC, although both correlations did not surpass Bonferroni correction warranting careful interpretation. Other studies involving several gait tasks, also found that simplified neuromuscular control was associated with worse gait performance in both HC and neurological populations [15–19,58].

In PD, no associations were found between adaptive gait performance, disease severity and LEDD, contradicting Falaki et al. (2017) who reported a positive effect of medication on %VAF during postural tasks [60]. Levodopa is thought to enhance activity modulation through

increasing movement vigor and reducing bradykinesia [61] [52,53]. Also, an optimal medication state was reported to be important for the ability to generate adaptive gait during SB-walking [62]. However, Roemmich et al. (2014) as well as Mileti et al. (2019) reported no difference in synergy organization between ON and OFF medication states during gait and balance tasks [15,63].

Limitations

This study was an add-on analysis to a previously conducted study and therefore consisted of a convenience sample that might be influenced by insufficient statistical power in some of the analyses. Additionally, participants were possibly affected by carry-over and fatigue of the preceding protocol. Still, our results did not show differences in adaptive gait outcomes in PwPD, who are prone to fatigue, when compared to HC. It is, however, possible that the fitter patients opted to partake in this add-on study, compromising the generalizability of our findings. We normalized the gait cycle based on 100 data points, assuming 60 points for the stance and 40 points for the swing phase [22]. However, the exact stance/swing distribution could have varied between participants, implying that cautious interpretation is warranted. Still, since we were interested in timing differences across the whole gait cycle (recall SPM analysis), this approach seems justified. For the synergy analysis we employed a 95% VAF criterion for all muscles combined rather than for each muscle individually, although the %VAF was monitored to validate the synergy reconstruction. Finally, previous SB-studies, mostly involving HC, included prolonged protocols [23,64,65]. The present study only included six minutes of walking with two transitions from TB- to SB-walking for pragmatic reasons, preventing inferences made to sustained adaptation effects. We also included only 5 strides per phase [22], pointing to the need for future studies to incorporate the full adaptation curves.

Perspectives and significance

Problems with movement vigor (bradykinesia), especially during transitions from stationary to dynamic tasks were previously described in PD [61] and could have impacted adaptive gait difficulties. Here, we found that decreased neuromuscular complexity during SB-walking tended to be related to worse adaptative gait performance in PD. We also demonstrated abnormal TA-modulation during adaptive gait. Future studies need to address the possible effects of repeated SB-training on improving neuromuscular complexity and whether this results in enhanced adaptive gait capacity.

Data availability

Data will be available upon reasonable request.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Author contributions

F.H.: experimental design, data acquisition, data analysis, interpretation, manuscript writing. N.D.: experimental design, data analysis, interpretation, manuscript review and critique. C.M.: data acquisition, manuscript review and critique, P.G. and M.G.: experimental design, manuscript review and critique, A.N.: experimental design, data analysis, interpretation, manuscript review and critique. All authors read and approved the final manuscript.

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Supplementary materials

Table S1. Step length asymmetry and variability (SD) during the different phases of the treadmill task.

Phase	Variable	HC (N=27) Mean (SD)	PD (N=24) Mean (SD)	Group p-value	Phase p-value	Interaction p-value	Tmspeed p-value
BL	mean	0.003 (0.020)	0.004 (0.037)	0.583	**<0.001**	0.714	0.273
	SD	0.018 (0.008)	0.023 (0.011)	0.867	**0.009**	0.554	**0.004**
EA	mean	-0.213 (0.068)	-0.237 (0.105)	**Post hoc Phase**	**Mean p-value**	**SD p-value**	
	SD	0.051 (0.038)	0.057 (0.041)				
LA	mean	-0.118 (0.057)	-0.140 (0.086)	BL vs. EA	**<0.001**	**<0.001**	
	SD	0.037 (0.031)	0.034 (0.015)	BL vs. LA	**<0.001**	**0.002**	
EP	mean	0.039 (0.019)	0.031 (0.048)	BL vs. EP	**<0.001**	0.123	
	SD	0.026 (0.013)	0.024 (0.010)	BL vs. LP	0.195	1.000	
LP	mean	0.009 (0.028)	0.012 (0.039)	EA vs. LA	**<0.001**	**0.041**	
	SD	0.021 (0.014)	0.029 (0.045)	EA vs. EP	**<0.001**	**<0.001**	
				EA vs. LP	**<0.001**	**<0.001**	
				LA vs. EP	**<0.001**	**0.035**	
				LA vs. LP	**<0.001**	0.478	
				EP vs. LP	**<0.001**	1.000	

Significant values (p<0.05) are marked in bold. HC, Healthy controls; PD, people with Parkinson's Disease; SD, standard deviation; TMspeed, comfortable treadmill speed; BL, baseline; EA, early adaptation; LA, late adaptation; EP, early post adaptation, LP, late post adaptation.

Table S2. GLMM outcomes number of synergies per phase of the fast leg.

	HC (N=27) mean (SD)	median (IQR)	PwPD (N=24) mean (SD)	median (IQR)	Group p-value	Phase p-value	Interaction p-value	Tmspeed p-value
Phase	*nr. synergies*		*nr. synergies*		0.518	**<0.001**	0.981	**0.015**
BL	3.96 (0.44)	4 (0)	3.75 (0.53)	4 (1)				
EA	3.52 (0.58)	4 (1)	3.42 (0.50)	3 (1)				
LA	3.44 (0.70)	3 (1)	3.29 (0.55)	3 (1)				
EP	3.74 (0.59)	4 (1)	3.58 (0.58)	4 (1)				
LP	3.89 (0.58)	4 (0)	3.71 (0.62)	4 (1)				
Post hoc Phase	**mean difference Mean (SD)**	**t-value**	**df**	**p-value**				
BL vs. EA	0.39 (0.08)	4.82	244	**<0.001**				
BL vs. LA	0.49 (0.10)	5.14	244	**<0.001**				
BL vs. EP	0.19 (0.08)	2.43	244	0.063				
BL vs. LP	0.06 (0.08)	0.75	244	0.453				
EA vs. LA	0.10 (0.07)	1.42	244	0.315				
EA vs. EP	-0.19 (0.08)	-2.58	244	0.052				
EA vs. LP	-0.33 (0.08)	-4.20	244	**<0.001**				
LA vs. EP	-0.23 (0.09)	-3.24	244	**0.008**				
LA vs. LP	-0.43 (0.08)	-5.31	244	**<0.001**				
EP vs. LP	-0.14 (0.06)	-2.14	244	0.100				

Significant values (p<0.05) are marked in bold. HC, Healthy controls; PD, people with Parkinson's Disease; SD, standard deviation; TMspeed, comfortable treadmill speed; BL, baseline; EA, early adaptation; LA, late adaptation; EP, early post adaptation, LP, late post adaptation.

Table S3. SPM outcomes slow leg per synergy, populations randomly balanced.

SPM{F} tests	**CT**	**Synergy 1 Supra-threshold clusters**	**p-value**	**CT**	**Synergy 2 Supra-threshold clusters**	**p-value**	**CT**	**Synergy 3 Supra-threshold clusters**	**p-value**	**CT**	**Synergy 4 Supra-threshold clusters**	**p-value**
Phase	4.4	1. 0-2.0% 2. 6.3-37.1% 3. 52.7-64.6% 4. 86.8-99%	**0.002** **0.002** **0.002** **0.002**	4.7	1. 0-5.1% 2. 9.8-44.0% 3. 48.2-61.7% 4. 71.9-99%	**0.002** **0.002** **0.002** **0.002**	4.4	1. 0-9.5% 2. 11.9-47.7% 3. 52.7-64.6% 4. 80.7-91.4% 5. 97.9-99%	**0.002** **0.002** **0.002** **0.002** **0.002**	4.3	1. 3.0-6.9% 2. 13.9-26.1% 3. 55.0-63.1% 4. 87.7-93.7%	0.002 **0.002** **0.002** **0.002**
Group	10.7	1. 64.8-71.4%	**0.002**	10.1	-	-	10.7	-	-	11.0	-	-
Interaction	4.5	-	-	4.5	-	-	4.3	-	-	4.5	-	-
Post-Hoc SPM{T} tests												
Phase effect												
BL vs. EA	4.0	1. 80.6-84.5%	<0.001	3.8	-	-	4.0	-	-	3.8	-	-
BL vs. LA	4.0	-	-	4.1	-	-	3.8	-	-	4.2	-	-
BL vs. EP	4.4	-	-	4.1	-	-	3.9	-	-	3.9	-	-
BL vs. LP	3.6	1. 0-2.9% 2. 93.0-99%	**<0.001** **<0.001**	3.7	-	-	3.9	-	-	4.0	-	-
EA vs. LA	3.7	-	-	4.3	-	-	3.9	1. 86.6-89.2%	<0.001	4.0	1. 41.2-51.1%	**<0.001**
EA vs. EP	4.1	-	-	4.1	-	-	4.1	-	-	4.2	-	-
EA vs. LP	3.9	1. 77.8-83.7%	**<0.001**	4.1	-	-	4.1	-	-	3.9	-	-
LA vs. EP	3.9	-	-	4.3	-	-	3.9	-	-	4.3	-	-
LA vs. LP	4.3	-	-	4.2	1. 63.3-67.1%	<0.001	3.8	-	-	4.3	-	-
EP vs. LP	3.9	1. 93.6-99%	**<0.001**	3.7	1. 64.3-68.0%	<0.001	3.8	-	-	3.5	-	-
Group effect												
BL	3.6	1. 78.3-86.3% 2. 93.9-99%	**<0.001** **<0.001**	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
EA	3.7	1. 59.6-72.9% 2. 90.6-99% 3. 0-2.82%	**<0.001** **<0.001** **<0.001**	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
LA	3.7	1. 63.5-73.2% 2. 89.1-98.5%	**<0.001** **<0.001**	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
EP	3.6	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
LP	3.9	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

The top part of the table includes the F tests for main (phase and group) and interaction (group by phase) effects. The bottom part of the table includes the post-hoc T-tests if main effects for phase or group were present in the F-tests, otherwise these areas are marked with n/a (not applicable). For each synergy the significant time periods of the gait cycle (supra-threshold clusters) are numbered and indicated with percentages, and their significance levels. All p-values of significant substantial clusters (>5%) are marked in **bold**. CT, is the critical threshold, all SPM test values exceeding this threshold are considered significant; BL, baseline; EA, early adaptation; LA, late adaptation; EP, early post adaptation, LP, late post adaptation.

Table S4. SPM outcomes fast leg per synergy, populations randomly balanced.

SPM{F} tests	**CT**	**Synergy 1 Supra-threshold clusters**	**p-value**	**CT**	**Synergy 2 Supra-threshold clusters**	**p-value**	**CT**	**Synergy 3 Supra-threshold clusters**	**p-value**	**CT**	**Synergy 4 Supra-threshold clusters**	**p-value**
Phase	4.2	1. 0-5.3%	**0.002**	4.4	1. 0.5-24.6%	**0.002**	4.3	1. 8.2-11.9%	0.002	4.7	1. 37.6-44.1%	**0.002**
		2. 46.2-70.3%	**0.002**		2. 37.8-45.9%	**0.002**		2. 57.1-64.1%	**0.002**		2. 50.7-55.9%	**0.002**
		3. 93.1-99%	**0.002**		3. 71.7-77.6%	**0.002**					3. 70.9-75.9%	**0.002**
Group	10.6	1. 91.0-94.2%	0.002	11.1	-	-	10.7	-	-	11.3	-	-
Interaction	4.4	-	-	4.3	-	-	4.3	-	-	4.6	-	-
Post-Hoc SPM{T} tests												
BL vs. EA	4.0	-	-	3.9	-	-	3.9	1. 70.9-72.1%	0.003	3.9	-	-
BL vs. LA	3.9	-	-	3.9	1. 0-2.7%	<0.001	4.0	-	-	4.2	-	-
BL vs. EP	4.0	-	-	3.8	-	-	3.8	1. 45-7-50.4%	<0.001	3.8	-	-
BL vs. LP	3.9	-	-	4.3	-	-	4.0	2. 6.5-11.9%	**<0.001**	4.1	-	-
								3. 93.7-99%	**<0.001**			
EA vs. LA	4.1	-	-	4.1	1. 67.3-70.8%	<0.001	4.0	-	-	3.8	-	-
EA vs. EP	4.0	-	-	3.8	-	-	4.0	-	-	4.4	-	-
EA vs. LP	3.9	-	-	3.9	-	-	3.7	1. 0-2.8%	**<0.001**	3.7	-	-
								2. 10.6-13.9%	<0.001			
								3. 95.8-99%	**<0.001**			
LA vs. EP	4.0	-	-	4.3	-	-	4.0	-	-	4.2	1. 10.6-13.1%	<0.001
LA vs. LP	4.1	-	-	4.2	-	-	3.9	1. 5.7-14.0%	**<0.001**	4.9	-	-
								2. 94.9-99%	<0.001			
EP vs. LP	4.0	-	-	4.0	-	-	4.1	1. 7.8-10.4%	<0.001	4.3	-	-
BL	3.8	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
EA	3.8	1. 91.9-99%	**<0.001**	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
LA	3.6	1. 47.6-49.5%	0.006	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
EP	3.7	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
LP	3.7	-	-	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

The top part of the table includes the F tests for main (phase and group) and interaction (group by phase) effects. The bottom part of the table includes the post-hoc T-tests if main effects for phase or group were present in the F-tests, otherwise these areas are marked with n/a (not applicable). For each synergy the significant time periods of the gait cycle (supra-threshold clusters) are indicated with percentages, and their significance levels. All p-values of significant substantial clusters (>5%) are marked in **bold**. CT, is the critical threshold, all SPM test values exceeding this threshold are considered significant; BL, baseline; EA, early adaptation; LA, late adaptation; EP, early post adaptation, LP, late post adaptation.

Table S5. SPM outcomes slow leg per synergy, populations balanced by treadmill speed.

slow leg		**Synergy 1**			**Synergy 2**			**Synergy 3**			**Synergy 4**		
SPM{F}		**Supra-threshold**			**Supra-threshold**			**Supra-threshold**			**Supra-threshold**		
tests	**CT**	**clusters**	**p-values**	**CT**	**clusters**	**p-values**	**CT**	**clusters**	**p-values**	**CT**	**clusters**	**p-values**
Phase	4.4	1. 0-2.3%	0.002	4.2	1. 0-5.3%	**0.002**	4.5	1. 0-9.9%	**0.002**	4.5	1. 15.2-24.0%	**0.002**
		2. 5.9-38.2%	**0.002**		2. 9.4-44.1%	**0.002**		2. 12.2-48.1%	**0.002**		2. 59.2-63.0%	0.002
		3. 52.1-64.7%	**0.002**		3. 48.0-62.2%	**0.002**		3. 52.8-63.7%	**0.002**		3. 88.5-91.6%	0.002
		4. 86.0-99%	**0.002**		4. 72.9-99%	**0.002**		4. 80.5-99%	**0.002**			
Group	10.5	1. 65.9-70.5%	0.002	10.5	-	-	10.0	-	-	9.7	-	-
Interaction	4.4	-	-	4.4	-	-	4.3	-	-	4.5	-	-
Post-Hoc SPM{T} tests												
BL vs. EA	4,0	1. 80.6-84.5%	<0.001	4.0	-	-	3.7	-	-	3.8	-	-
BL vs. LA	4	-	-	3.8	-	-	3.6	-	-	4.4	-	-
BL vs. EP	4.4	-	-	4.0	-	-	3.9	-	-	3.7	-	-
BL vs. LP	3.7	1. 0-0.8%	**<0.001**	4.1	-	-	3.7	-	-	3.8	-	-
		2. 93.4-99%	**<0.001**									
EA vs. LA	3.7	-	-	4.0	-	-	3.8	1. 19.9-32.6%	**<0.001**	3.7	-	-
								2. 89.4-95.8%	**<0.001**			
EA vs. EP	4.1	-	-	4.1	-	-	3.9	-	-	3.9	-	-
EA vs. LP	3.9	1. 79.8-82.1%	<0.001	4.1	-	-	4.1	-	-	4.1	-	-
LA vs. EP	3.9	-	-	3.9	-	-	3.6	-	-	4.2	-	-
LA vs. LP	3.9	-	-	4.1	-	-	3.7	-	-	4.2	-	-
EP vs. LP	3.9	1. 93.9-97.2%	<0.001	4.0	-	-	3.9	-	-	3.9	1. 6.0-7.4%	0.002
BL	3.6	1. 78.3-86.3%	**<0.001**	3.8	n/a	n/a	3.3	n/a	n/a	3.6	n/a	n/a
		2. 93.9-99%	**<0.001**									
EA	3.7	1. 59.6-72.9%	**<0.001**	3.8	n/a	n/a	3.8	n/a	n/a	3.7	n/a	n/a
		2. 90.6-99%	**<0.001**									
		3. 0-2.82%	**<0.001**									
LA	3.7	1. 63.5-73.2%	**<0.001**	3.9	n/a	n/a	3.5	n/a	n/a	4.0	n/a	n/a
		2. 89.1-98.5%	**<0.001**									
EP	3.6	-	-	3.7	n/a	n/a	3.6	n/a	n/a	3.8	n/a	n/a
LP	3.6	-	-	3.8	n/a	n/a	3.6	n/a	n/a	3.6	n/a	n/a

The top part of the table includes the F tests for main (phase and group) and interaction (group by phase) effects. The bottom part of the table includes the post-hoc T-tests if main effects for phase or group were present in the F-tests, otherwise these areas are marked with n/a (not applicable). For each synergy the significant time periods of the gait cycle (supra-threshold clusters) are numbered and indicated with percentages, and their significance levels. All p-values of significant substantial clusters (>5%) are marked in **bold**. CT, is the critical threshold, all SPM test values exceeding this threshold are considered significant; BL, baseline; EA, early adaptation; LA, late adaptation; EP, early post adaptation, LP, late post adaptation.

Table S6. SPM outcomes fast leg per synergy, populations balanced by treadmill speed.

	Synergy 1			Synergy 2			Synergy 3			Synergy 4		
SPM{F} tests	**CT**	**Supra-threshold clusters**	**p-values**	**CT**	**Supra-threshold clusters**	**p-values**	**CT**	**Supra-threshold clusters**	**p-values**	**CT**	**Supra-threshold clusters**	**p-values**
Phase	4.5	1. 0-5.6%	**0.002**	4.5	1. 0-28.8%	**0.002**	4.4	1. 7.2-12.5%	**0.002**	4.7	1. 36.6-44.8%	**0.002**
		2. 46.4-69.1%	**0.002**		2. 37.5-50.4%	**0.002**		2. 57.6-62.0%	0.002		2. 51.4-56.3%	0.002
		3. 92.9-99%	**0.002**									
Group	10.7	1. 91.5-93.5%	0.002	11.0	-	-	10.7	-	-	13.0	-	-
Interaction	4.2	-	-	4.4	-	-	4.2	-	-	4.2	-	-
Post-Hoc SPM{T} tests												
BL vs. EA	3.8	-	-	4.2	-	-	3.6	1. 13.3-20.7%	**0.003**	4.3	-	-
								2. 69.0-75.2%	**0.003**			
BL vs. LA	3.8	-	-	3.9	-	-	4.2	-	-	4.0	1. 9.8-12.3%	<0.001
BL vs. EP	3.9	-	-	3.8	-	-	4.2	1. 45.7-47.9%	<0.001	3.9	1. 56.0-59.1%	<0.001
BL vs. LP	3.9	-	-	3.7	-	-	3.6	1. 7.6-11.6%	<0.001	4.0	-	-
								2. 94.7-99%	<0.001			
EA vs. LA	4.1	-	-	3.8	-	-	4.0	-	-	3.8	-	-
EA vs. EP	3.9	-	-	3.9	-	-	4.1	-	-	4.4	-	-
EA vs. LP	3.8	-	-	4.3	-	-	3.6	1. 0-1.5%	<0.001	4.0	-	-
								2. 7.5-19.3%	**<0.001**			
								3. 92.3-99%	**<0.001**			
LA vs. EP	4.2	-	-	3.8	-	-	4.1	-	-	4.0	1. 9.9-12.0%	0.003
LA vs. LP	3.9	-	-	4.5	-	-	3.9	1. 5.3-14.0%	**<0.001**	4.2	-	-
								2. 96.3-99%	<0.001		-	-
EP vs. LP	4.0	-	-	4.1	-	-		-	-	3.9	-	-
BL	3.9	-	-	3.7	n/a	n/a	3.7	n/a	n/a	n/a	n/a	n/a
EA	3.6	1. 0-0.2%	0.005	4.0	n/a	n/a	3.4	n/a	n/a	n/a	n/a	n/a
		2. 91.9-99%	**<0.001**									
LA	3.6	-	-	3.8	n/a	n/a	3.7	n/a	n/a	n/a	n/a	n/a
EP	3.7	-	-	3.8	n/a	n/a	3.7	n/a	n/a	n/a	n/a	n/a
LP	3.7	-	-	4.0	n/a	n/a	4.0	n/a	n/a	n/a	n/a	n/a

The top part of the table includes the F tests for main (phase and group) and interaction (group by phase) effects. The bottom part of the table includes the post-hoc T-tests if main effects for phase or group were present in the F-tests, otherwise these areas are marked with n/a (not applicable). For each synergy the significant time periods of the gait cycle (supra-threshold clusters) are numbered and indicated with percentages, and their significance levels. All p-values of significant substantial clusters (>5%) are marked in **bold**. CT, is the critical threshold, all SPM test values exceeding this threshold are considered significant; BL, baseline; EA, early adaptation; LA, late adaptation; EP, early post adaptation, LP, late post adaptation.

Table S7. Spearman correlations between Δ number of synergies between baseline and early adaptation and treadmill speed, performance and disease severity.

	HC (N = 27)		PD (N = 24)	
Variable	**Δ EA-BL fast leg**	**Δ EA-BL slow leg**	**Δ EA-BL fast leg**	**Δ EA-BL slow leg**
Treadmill speed (m/s)	**0.48 (0.012)**	-0.37 (0.055)	0.06 (0.767)	-0.23 (0.284)
Mean SL asymmetry	**0.45 (0.019)**	-0.14 (0.487)	-0.06 (0.767)	-0.11 (0.617)
Mean SL asymmetry variability	-0.32 (0.100)	0.16 (0.440)	-0.04 (0.859)	0.03 (0.873)
UPDRS-III total score (0-132)	-	-	-0.18 (0.402)	0.19 (0.386)
LEDD (mg)			0.17 (0.420)	-0.39 (0.062)

Significant values (p<0.05) are marked in bold, critical Bonferroni p-value = 0.010 in PD and 0.017 in HC. HC, Healthy controls; PD, people with Parkinson's Disease; SL, step length; UPDRS-III, Unified Parkinson's Disease Rating Scale part III; LEDD, Levodopa Equivalent Daily Dosage; Δ EA-BL, change in number of synergies between EA and BL.

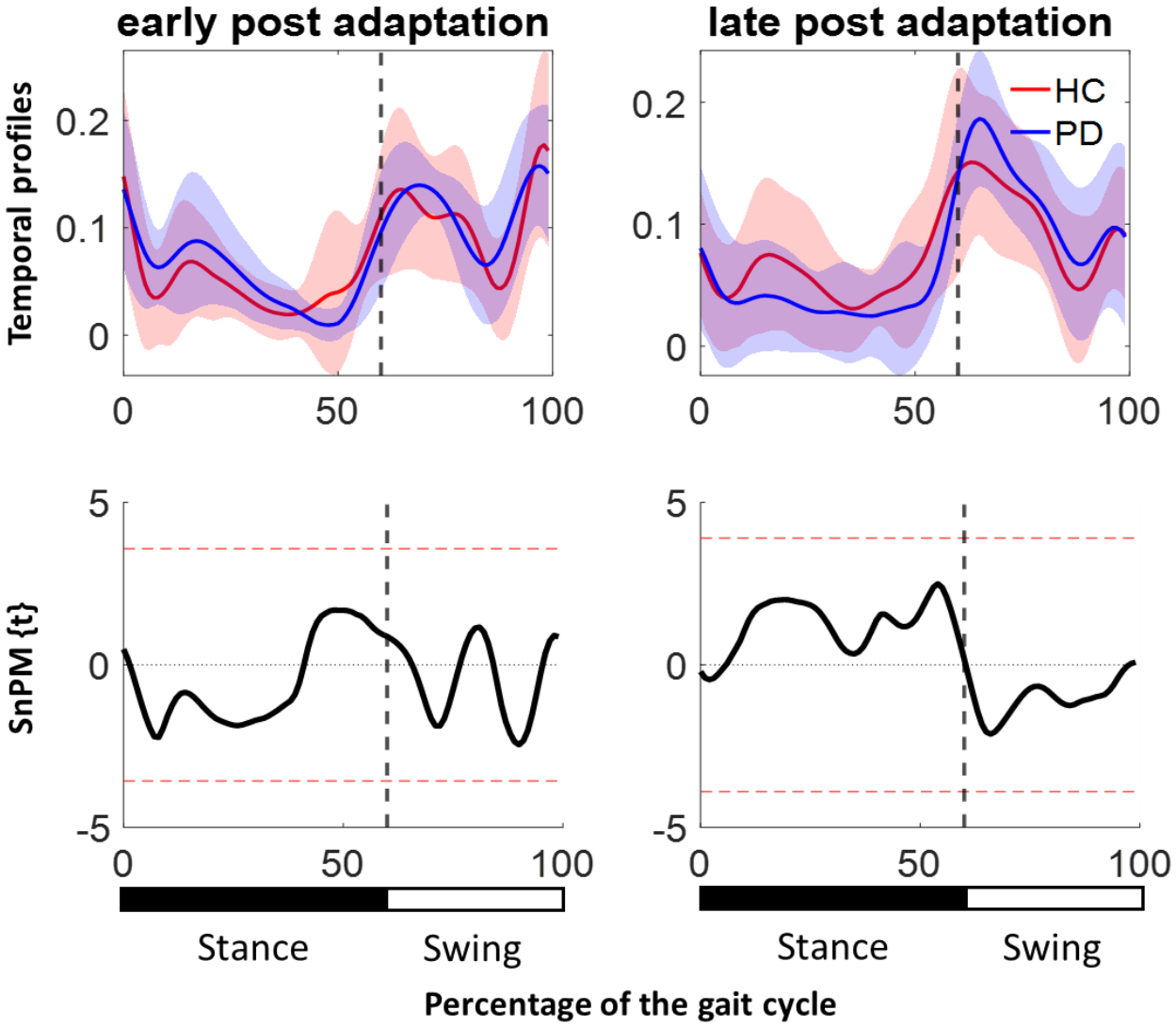

Figure S1. Non-significant group effects SPM post-hoc tests for synergy 1. Top panels represent the temporal profiles per synergy during the early and late post adaptation phase. Blue and red shaded areas represent ± 1 standard deviation of the PD and HC data respectively. Bottom panels represent the SPM t-test results of the respective phase, the red dotted lines represent the critical significance threshold. The vertical grey dotted line in all graphs indicates 60% of the gait cycle, indicating the stance (0-60%) and swing phase (60-100%).

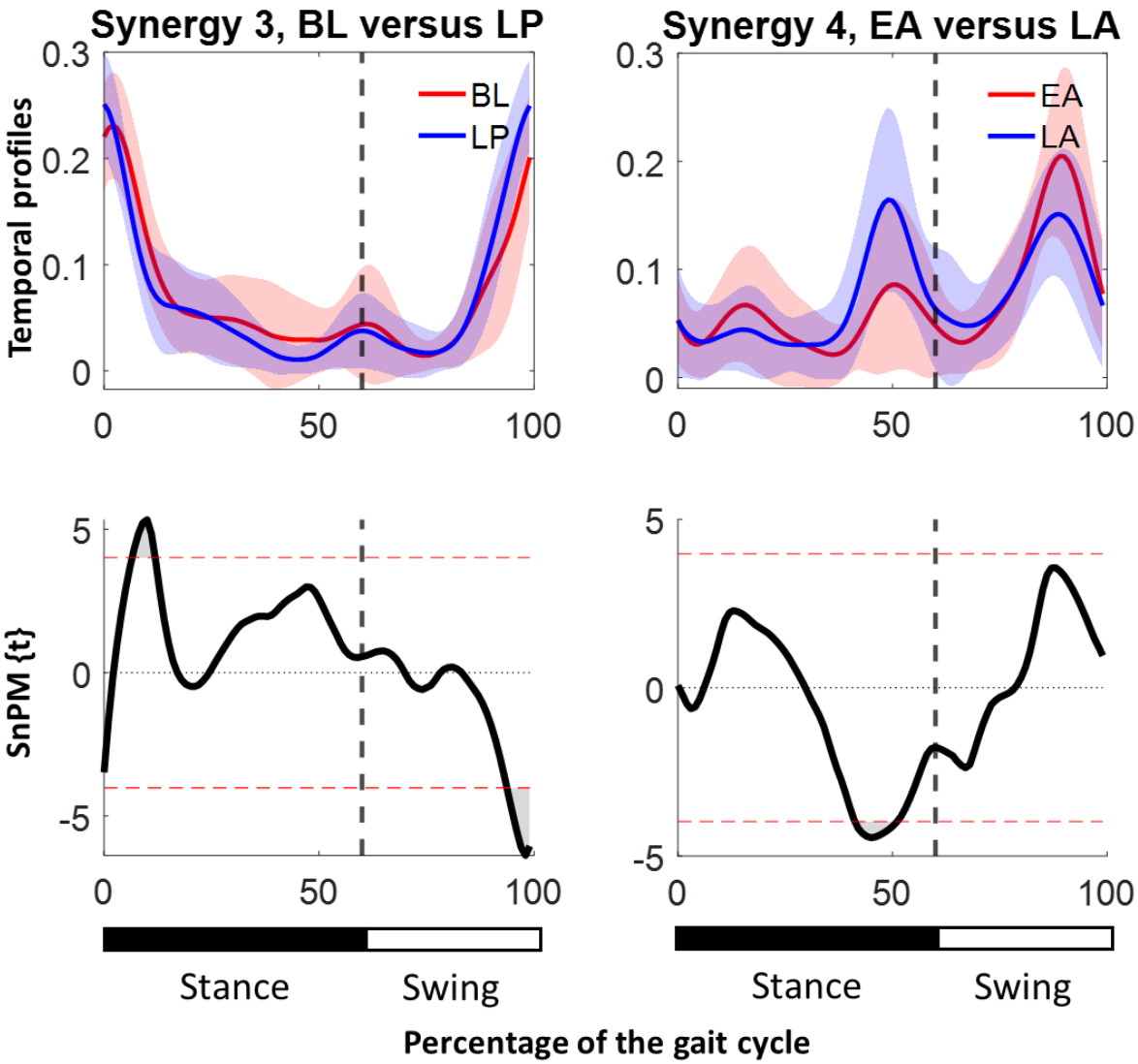

Figure S2. Significant phase effects SPM post-hoc tests for synergy 3 (fast leg) and synergy 4 (slow leg). Top panels represent the temporal profiles per synergy comparing baseline versus late post-adaptation (left panel) and the early and late adaptation phase (right panel). Blue and red shaded areas represent ± 1 standard deviation of the studied phases. Bottom panels represent the SPM t-test results of the respective phase, the red dotted lines represent the critical significance threshold. Grey areas mark the significant time periods (i.e. surpassing the critical threshold). The vertical grey dotted line in all graphs indicates 60% of the gait cycle, indicating the stance (0-60%) and swing phase (60-100%).

Chapter 6

General Discussion

This dissertation aimed to investigate the concept of repeated gait perturbation training by means of a split-belt treadmill on the one hand, and to advance our understanding of the neurophysiological deficits of adaptive gait in PD on the other. Therefore, part I included a systematic review with meta-analysis describing the current literature in people with PD and healthy controls. As well, a randomized controlled clinical trial examining the effects of a 4-week split-belt training on adaptive gait in PD. In part II of this thesis we reported on the underlying cortical and neuromuscular mechanisms of adaptive split-belt walking in both people with PD and healthy controls. An overview of participants per study across the thesis chapters can be found at the end of the general introduction in **Figure 6.** This general discussion will first provide a brief overview of the main findings of this dissertation, followed by an in-depth discussion and integration the results of part I and II. Next, a critical reflection on the methodology used will be expounded as well as the future research lookout. Last but not less important, clinical implications sprouting from this dissertation are discussed.

Summary of main findings

Part I: Adaptive gait deficits and clinical interventions in PD

Chapter 2 describes the results of a systematic review with meta-analysis investigating the short and long-term effects of repeated gait perturbation training (RGPT) on gait performance in people with PD (PwPD) and healthy controls (HC) compared to regular non-perturbation-based gait training [1]. Eight studies were eligible for review, of which six were included in the meta-analysis with a total of 209 participants (159 PwPD, 50 HC). In contrast to our hypothesis, RGPT seemed to have no surplus effect compared to regular treadmill training (mostly). Although, it should be noted that several individual RGPT studies reported immediate within- and/or between-group improvements of gait as well as retention effects, mainly consisting of sudden speed changes in both regular and split-belt (SB) treadmill contexts [1]. Importantly, limited outcome measures regarding adaptive gait were included in the studies selected and mostly feasibility studies with small samples were included suggesting potential bias. Hence, this review illustrated the need for high quality randomized controlled training studies investigating the potential of RGPT for improving adaptive gait in PD [1].

Therefore, as reported in **Chapter 3**, we conducted a power-based single blind randomized controlled study in 52 PwPD on the effects of SB-training on adaptive gait and its possible transfer to overground gait and turning as compared to tied-belt (TB) treadmill training [2]. Participants were randomized to either 4-weeks of SB- or equally intensive TB-training for three sessions a week. We found that the SB-training significantly improved adaptive gait on the treadmill compared to TB-training [2]. These effects were robust for dual-task interference and retained over a 4-week period without training. Surprisingly, and in contrasts to our pilot study

[3], these large training effects were not transferred to overground turning, but both groups improved overground gait speed and step length beyond the clinical important difference [2]. We concluded that PwPD are able to improve and retain adaptive gait performance on a SB-treadmill, but appear unable to transfer these gains to overground gait.

Part II: Underlying mechanisms of adaptive gait in PD

Chapter 4 aimed at unraveling the cortical regions involved during adaptive SB-walking in 42 PwPD and 42 HC through functional near-infrared spectroscopy (fNIRS). Behaviorally, PwPD remained more asymmetric at the end of the adaptation period after a perturbation compared to HC. In contrast to our hypothesis, we only found increased involvement of the posterior parietal cortex (PPC) during SB- compared to TB-walking in both groups (condition effect). Also, consistently increased activity in the premotor cortex (PMC) and PPC were observed in PwPD compared to HC (group effect) during both treadmill conditions. Surprisingly, an interaction effect was present in the right supplementary motor area (SMA), in which PwPD increased activity to cope with SB-walking, whereas HC did not. No differences in prefrontal cortex activity were found. Higher PPC recruitment in PwPD was associated with worse adaptive gait performance. These results imply that the adaptive gait problems in PD were related to dysfunctional hyperactivity in the sensorimotor areas.

In **Chapter 5** we studied the effect of PD on coping with SB-perturbations on the neuromuscular control of gait, as drawn from electromyography (EMG) and muscle synergy analysis. PwPD (N=24) and HC (N=27) showed similar step length asymmetry during the adaptative task. In contrast to our hypothesis, both groups utilized four synergies during TB-walking and dropped to three synergies during SB-walking, suggesting similar simplified control during the adaptive task. The four synergies observed encompassed (1) the tibialis anterior, (2) the gastrocnemius lateralis, (3) the quadriceps and (4) a combination of the biceps femoris and erector spinae. When comparing the patterns of activity within these four synergies across time, consistent phase effects were observed in both legs, albeit particularly in the slow leg. Interestingly, no group by phase interactions were present. Only synergy 1 (tibialis anterior) showed a group effect, indicating that during the adaptive SB-phases PwPD increased their activity during swing, and decreased activity during terminal swing and early stance in the slow leg. Although adaptive gait performance was largely comparable between groups, correlation analysis showed that simplified neuromuscular control related to worse adaptive gait performance in PD. Based on these results we conclude that adaptive gait changes in PD were mostly explained by within-synergy changes, rather than by the number of synergies employed.

Integrated discussion of thesis results

Why the lack of transfer?

Despite this overly active neural system and tendency to show a less flexible neuromuscular system, remarkably PwPD were able to improve their SB-performance in a robust fashion after training, even with some evidence of consolidated motor learning shown by retention effects. For that reason, the lack of transfer to overground gait is surprising. Vangheluwe et al. (2006) proposed that transfer consist of two components: (1) an effector-independent (general features of a movement) and (2) an effector-dependent (specific features of a movement) component [4]. The model proposes that although effector-dependent information is needed to learn a specific task, successful transfer is dependent on the overlap between effector-independent information of one task with the effector-independent information needed for the new (transfer) task [4,5]. Typically, PwPD can still learn a new task (effector-dependent learning), but show problems with the transfer of the effector-independent component [6]. Prior work showed that this lack of effector-independent transfer could be related to the cortical hyperactivity within the motor network (including the SMA) and between the PMC and the dorsal attention network (including the parietal cortex) [6]. The current study corroborates this model, as indeed effector-dependent learning was achieved with SB gains occurring in both limbs to an equal degree and in both adaptation and de-adaptation phases (**Chapter 3**). As such, it seems that the effector-independent component was driving the lack of a transfer effect.

There may be two explanations for why effector-independent transfer did not occur. First, turning on the spot might not have been an optimal transfer task as the effector-independent information required for turning and SB-walking is too dissimilar. We chose this task as it is posing a very challenging level of asymmetry between the two legs and also because it often provokes freezing of gait [7]. However, the turning task requires additional balance control and does not capture an abrupt change from symmetrical to asymmetrical walking and vice versa, as imposed by the SB treadmill. An alternative transfer task for future studies could be a figure of eight task, where asymmetrical turning as well as transitioning to straight walking are captured similar to SB-perturbations as well as to complex walking in daily life [8–11]. Second, our neural findings also showed abnormal activity in the PMC and PPC, which are part of the dorsal attentional network implicated in successful transfer of learning [6]. Together, our findings thus corroborate the notion that PwPD have difficulty with transfer between two different motor tasks [6]. Indeed, even in our pilot study which did show a transfer effect, the transfer effects were considerably smaller when only taking the PD participants into account [3]. Interestingly, these results were found on the same overground turning task as used in the current work. In a review about the effects of slips and trips-resisting skills training in healthy

older adults, it was emphasized that there is a need to target various perturbation contexts instead of learning to respond to a single perturbation [12]. The present results point towards the need for generalizability of training with a specific focus on effector-independent learning. We therefore propose that in addition to applying multiple changing ratios of perturbations, as already implemented in **Chapter 3**, complementary effector-independent training may be required. For example, offering additional overground adaptive gait training to facilitate the transfer from the SB treadmill to real-life gait could be indicated. At present, there are no studies which address the transfer of skill learning to actual daily circumstance with respect to gait and PD. However, we tentatively suggest that overground adaptive gait training could include gait tasks, which challenge the adjustments of step lengths, f. i. walking a slalom or figure of eight. Also, transitions from straight to asymmetrical walking [8–11] or responding to an instruction to suddenly change direction, could be incorporated. It is also deemed useful to capture both EMG and fNIRS when conducting a future SB training study as to further elucidate whether training can normalize cortical and neuromuscular activity. Adding such a neurophysiological protocol could also explain why improvements occur or not, and why retention and transfer may or may not have been achieved**.**

Can gait training normalize neuromuscular and cortical control?

Currently, studies on the effect of gait interventions on muscle activity in PD, let alone synergy metrics, are limited. To our knowledge, only one study by Allen et al. (2017) investigated the effect of training on synergies in PwPD, and reported no change in number of synergies after three weeks of daily Tango dancing classes [13]. However, after training the recruitment and organization of the muscle synergies became more consistent, and this was associated with improvements in gait and balance, pointing towards the need for more sophisticated synergy outcomes [13]. Regarding brain activity changes after training in PD, a recent meta-analysis including seven functional magnetic resonance imaging (fMRI) studies reported increased activity in the cerebellum and the frontal, parietal and occipital lobe after exercise compared to a control group without training [14]. In addition, exercise has elicited a shift of connectivity in sensorimotor networks from the posterior to anterior striatum [15].

Mobile brain imaging in training studies in PD (e.g. fNIRS), especially those which include several ROIs, are still scarce. So far, two studies report on activity in the prefrontal cortex activity (PFC), which seemed to decrease after multidisciplinary exercise [16] and treadmill training [17]. A recent study combining fNIRS with resting state fMRI at baseline, reported increased PFC activity in PwPD compared to HC based on fNIRS, which was likely governed by unidirectional connectivity from the cerebellum as predicted by the fMRI-based model [18]. After a 6-week treadmill training PFC activity in PwPD was decreased and gait performance improved. Additionally, the fMRI model indicated an increase in brain regions involved in the

gait network besides the cerebellum, which was more similar to HC group. These results suggested a normalization of the brain connectivity [18]. This points towards the added value of multi-modal brain imaging approaches and neuromuscular analysis before and after training to obtain a clear understanding of the neurophysiological mechanisms underlying movement interventions.

Revisiting the adaptive gait network in PD

In **Chapter 4** we identified the main cortical areas involved in PD during SB-walking. Previous work by Hinton et al. (2019) proposed the so called "fine-tuning network of locomotion", based on young adults during SB-walking as measured with positron emission tomography (PET) imaging [19]. Here, we present what we learned in C**hapter 4** about the fine-tuning network for locomotion in PD based on another brain imaging technique. In **Figure 1** we have added our findings (right panel) to the framework based on Hinton's work (left panel) [19]. In F**igure 1**, we indicated the hyperactivated regions found in C**hapter 4** in red, as in some regions (PPC) the incremental activity was actually related to worse adaptive behavior and was therefore interpreted as possibly dysfunctional activations. Also, the higher activations in the PMC and PPC were already apparent during TB-walking in PD, pointing towards the need for enhanced cortical movement control during treadmill walking, quite apart from dealing with perturbations [20]. Since PFC activity did not change between TB- and SB-walking we colored this area in white. Finally, based on the fNIRS measurements, we could only speculate about the contribution of the PFC, SMA, PMC and PPC and not about the, primary motor (M1), cingulate (ACC and PCC) subcortical and brainstem, areas identified by Hinton as implicated [19]. Therefore, these areas and subsequent connections were faded out in the hypothetical PD model.

In line with the healthy model of Hinton et al. (2019) [19], our fNIRS results verified that the supplementary motor area (SMA) and posterior parietal cortex (PPC) are important areas that were also activated during adaptive gait in PwPD. This is also in line with the known involvement of these areas in bilateral co-ordination and integration of sensory-motor function that are needed for coping with sudden asymmetrical gait. Not identified by Hinton et al. (2019), we found that the premotor cortex (PMC) was also involved in adaptive gait in PwPD. It is plausible that the PMC, as a secondary motor area often implicated in motor learning [21], is extra involved in PD compared to younger adults as a way to compensate for both the aging process and the dysfunctional basal ganglia [22,23]. The PMC, together with the cerebellum, is considered important for compensatory locomotor control in PD in response to the loss of sufficient basal ganglia function, particularly in PD freezers [24]. Indeed, both the PMC and

cerebellum were responsive to external cues for gait initiation [24]. Finally, this discrepancy regarding the PMC between Hinton's work and ours could be due to different ROI definitions used as the two areas are closely located to each other and PET has a higher spatial resolution as compared to fNIRS [25].

Also, Hinton et al. (2019) reported decreased activity in the PFC during SB-walking [19], while we found no change in PFC activity in PD, indicating less cognitive involvement during SB-walking in older HC and PwPD. In young adults, responding to SB-perturbations could become quickly implicit and mostly driven by the motor system [19]. In healthy older people and those with PD, it appears that cognitive involvement remained but was also not recruited at higher levels as PFC activity remained the same. Coping with perturbations thus seemed to depend more on sensory-motor control in these cohorts rather than a reliance on heightened attention. Previous studies reported an effect of cognitive impairment on the ability to adapt to SB-perturbations [26], but possibly these were SB-independent. Future work, using multimodal approaches combined with mobile techniques are needed to map out the impact of PD on the fine-tuning network in a more complete fashion.

Methodological considerations

From the literature review in **Chapter 2** we found that most of the perturbation training studies were still in the proof-of-concept phase [1], limiting the strength of conclusions drawn from the meta-analysis [1]. Against this background, we considered it a strength that in this thesis we were able to include relatively large study samples, that were a-priori powered for **Chapters 3 and 4**. Additionally, we preregistered the study protocols for all chapters and will make data available upon request in line with an open science approach.

Balancing bias against ecological validity

Performing a randomized controlled training study is difficult as many factors could potentially influence and bias the study results [27,28]. The RCT study took place in a controlled laboratory environment, which allowed for standardized testing ruling out possible confounders. Additionally, the two training schedules were developed in such a way that progression of training was mostly fixed, but could be personalized in a standardized step-wise fashion [2]. However, this choice limited the generalizability of our results to more ecological situations, as previous research showed that testing in a lab environment can significantly influence gait outcome measures [29]. Also, walking on a treadmill was earlier found to influence the regularity and quality of walking (e.g. increased speed and step length) [30], and altered the connectivity of brain regions significantly [18,31]. This could be another reason why in **Chapter 3** we did not find the transfer of the adaptive gait gains on the treadmill to the overground turning task [2]. The choice for the turning in place task was based on the data of

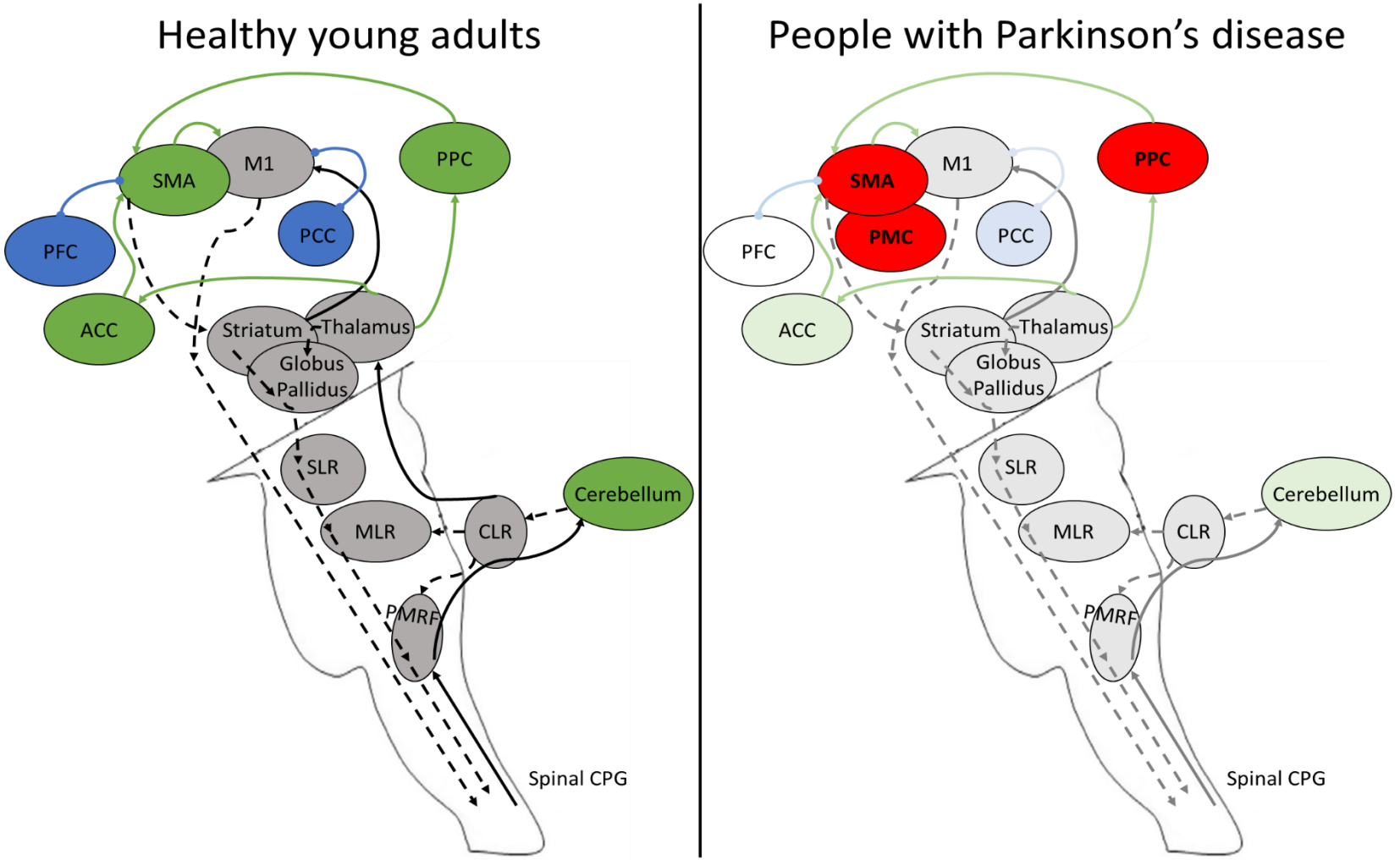

Figure 1. The "fine-tuning" network of locomotion by Hinton and colleagues (2019) and the proposed network in Parkinson's disease.
The fine-tuning network during SB-walking in young adults (left) and the network hypothesized in PwPD based on the fNIRS data of chapter 4 (right). Green areas indicate increased activity whereas the blue areas presented decreased activity during SB-walking as compared to the general areas needed for normal gait (indicated in grey). Red areas represent the overactivity found in PD compared to HC and white areas indicate no activity change from regular treadmill walking. PFC, prefrontal cortex; SMA, supplementary motor area; M1, primary motor cortex; PMC, premotor cortex; ACC, anterior cingulate; PCC, posterior cingulate cortex; PPC, posterior parietal cortex; SLR, subthalamic locomotor region; MLR, mesencephalic locomotor region; CLR, cerebellar locomotor region; PMRF, pontine and medullary reticular formations; CPG, central pattern generators. Figure adjusted from Hinton and colleagues [19].

a pilot study [3,32], but was not the most ecologically valid task to test adaptive gait capacity. These drawbacks urge for sensitive and ecological valid gait measures to be used in future studies, for instance by capturing walking and turning in daily life with inertial measurement units (IMUs) [33]. The accuracy, reliability and validity of digital mobility outcomes worn for several days in unsupervised settings is now under investigation and is likely to earn regulatory approval in the near future [34–36]. Particularly, PwPD are boosted by performance bias in a laboratory context and tend to have more pronounced walking difficulties during spontaneous walking. PwPD also suffer more than HC from problems with transfer of training to a different context [21,37–39]. All this, points to the need for bringing perturbation training to day-to-day rehabilitation (see 'clinical implications') and performing studies in the daily setting [40,41].

Reflections on the split-belt protocol and analysis procedures

For the **Chapters 4 and 5,** we randomized the two SB-conditions (left side/right side reduction), although the TB-condition was always performed first to avoid carry-over effects [42]. This could have produced bias as our protocol was not fully randomized. It is possible that since the participants did not receive a wash-out between the two SB trials, carry-over effects could have affected the second SB-trial [42]. However, when checking for order bias, in **Chapter 4** it appeared that the gait and fNIRS outcomes were not significantly different between the two SB conditions. To avoid order bias in **Chapter 5,** we decided to only include the first trial in our analysis.

Throughout this thesis, we individualized treadmill speed based on the concept of 'comfortable overground walking speed' rather than equalizing speed between the groups via the treadmill [2]. Both fixed speed [43–48] and personalized speed [26,49–53] methodologies were used in earlier SB-work and have pros and cons. We based our final choice on the assumption that finding an appropriate speed that created the same 'task load' for both groups would have been difficult. For some healthy individuals, a Parkinson-like speed could have been perceived as very slow, and could have altered the gait pattern [54]. For others, a high speed could have been too fast or even impossible to adhere to. Another way to deal with speed confounding is subject selection, whereby recruitment would be based on baseline gait speed falling within a certain speed category. Still, this method would probably have biased the sample and compromised generalizability. This is why we used the individualized method, as a consequence of which gait speed was significantly lower in the PwPD compared to the HC group in **Chapters 4 and 5**. To control for this, we added gait speed into our models as a covariate.

Determining 'comfortable gait speed' is however, also not self-evident. In this thesis, it was based on assessing overground gait speed in a standardized test with IMUs, whereby

participants walked 10 times 10 meters across the lab [2,18,19]. In addition, we adapted this speed slightly to treadmill walking if needed. Although we had a two-minute familiarization to get participants used to treadmill walking, this might not have been long enough to really determine comfortable speed as for some participants treadmill walking was a completely new experience, which might have resulted in a lower than comfortable speed. For future work, we recommend to adopt a longer familiarization on the treadmill, where in cases where the overground speed was initially perceived as too fast, speed might be gradually increased during the familiarization to avoid an underestimation of comfortable speed.

Methodological reflection on the fNIRS technique

In **Chapter 4**, we measured cortical activity with the fNIRS technique. At present, the computational methods to deal with fNIRS-data are still evolving [55,56]. Based on the concept of neurovascular coupling [57–61], it is assumed that the signal will express itself as an increase in oxygenated hemoglobin (HbO_2) and a decrease in deoxygenated hemoglobin (HHb) [62–64], as such reflecting indirect measures of increased brain activity. HbO_2 and HHb are thus expected to be anti-correlated, and positive correlations between the two chromophores is currently suggested to be indicative of artefact contamination [55,65–69].

As explained in C**hapter 1**, we used state-of-the-art fNIRS analysis methods to filter out artefacts including short channels and accelerometry [70,71]. However, when inspecting our signals, after applying the filtering techniques in **Chapter 4**, we noticed that HbO_2 and HHb were often positively correlated in both PwPD and controls (**Figure 2**). To check whether these positive correlations were gait-related or not, we calculated correlations for a control task. Before the start of the treadmill protocol, we recorded three minutes of 'resting state' fNIRS as a control condition. In this protocol, participants were standing still while swinging arms and making head movements alternatingly with standing still, using a similar block design as in the 'real experiment'. This way we were able to capture the hemodynamic changes associated with bilateral movements, which also occurred during gait, but were not the main interest. Indeed, from six randomly picked participants (3 PD, 3HC), we observed the expected anticorrelations in the control condition, suggesting that gait itself probably caused the systemic changes underlying the lack of anticorrelations in the treadmill protocol. Based on these results, we recommend to systematically report on the presence of positive correlations in fNIRS studies, as it seems that this does not necessarily mean corrupted signals. Rather, it could be task-related, as was also found in previous study in PwPD [72].

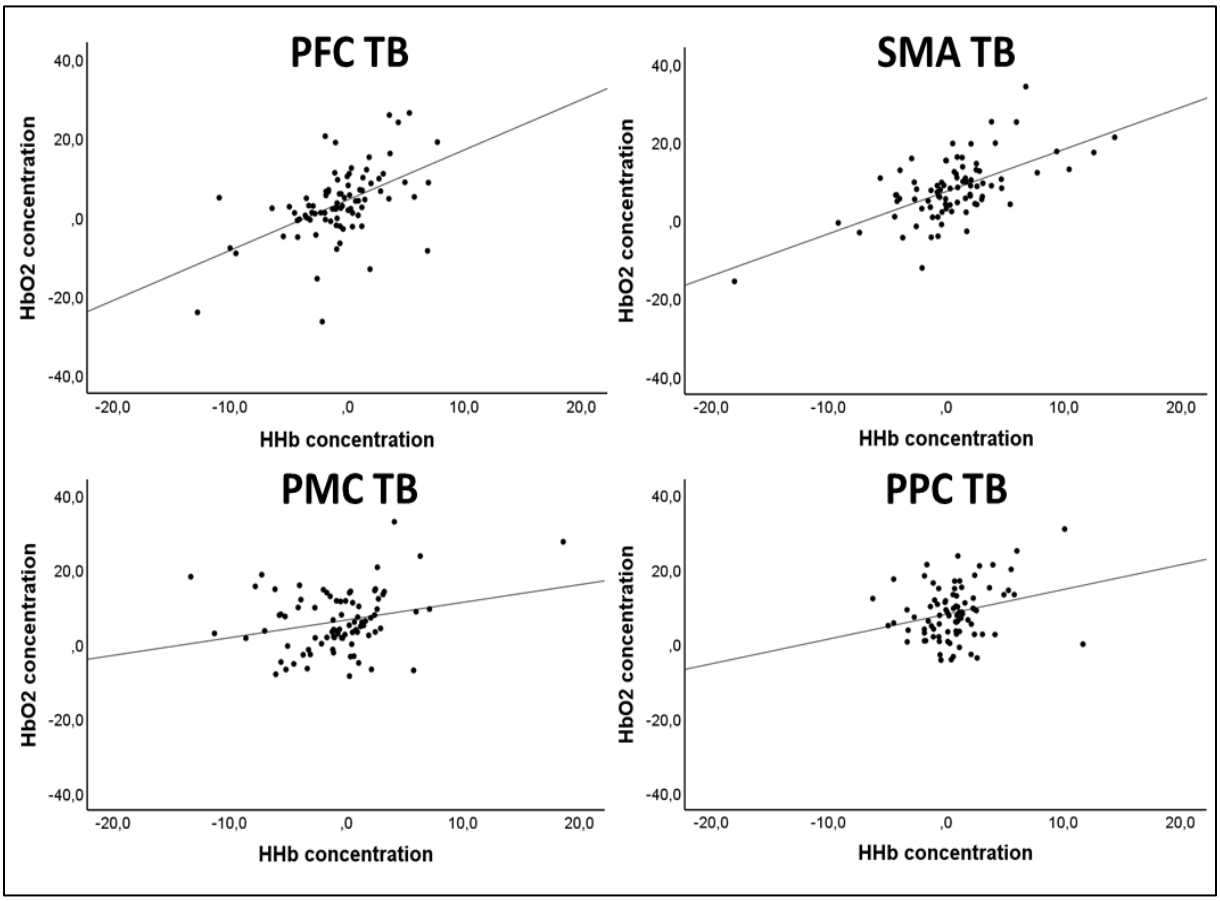

Figure 2. Correlations between HbO_2 and HHb values in the regions of interest during treadmill walking.
The TB-walking values are displayed of the complete population (42 HC and 42 PwPD). The correlations were all significant ($p<0.021$)

To date, most methodological research on fNIRS signal processing and artefact removal is based on relatively small movement tasks such as button presses, finger tapping, visual stimuli or even just cognitive tasks [66–69,71]. However, it is vital to start investigating how these techniques perform during large whole-body movements such as gait, as these tasks likely induce large systemic blood flow changes thereby violating the current anti-correlation assumptions [72]. As well, mobile imaging techniques are indispensable for gait and balance paradigms as fMRI cannot be applied in these conditions.

Based on the above described control test, the results found in **Chapter 4** are unlikely to reflect artefacts as we contrasted our task of interest (SB-walking) with a very similar comparator task (TB-walking), which shares a similar physiological noise response. Until a consensus regarding the interpretation and mitigation of positive signal correlations is established, future studies should indeed carefully consider task contrasts (comparator) as well as include a control task. Ideally, the comparator should consist of similar movements as the task of interest, thereby eliciting a similar physiological (noise) response, and the control should only differ in scale from the main movement of interest [55].

An important issue of interpretation is that fNIRS only allows for the measurement of cortical regions with limited penetration depth (up to 3 cm) [61,73]. Therefore, the involvement of subcortical regions during gait tasks cannot be picked up. Since several subcortical regions, such as the cerebellum are probably involved during adaptive gait [74,75], it is of great importance to acknowledge that in **Chapter 4** we only observed one 'piece of the puzzle'. PD is known for its abnormal subcortical manifestation [76] and the changes in cortical activity could be due to a redistribution of activity between subcortical regions as well (i.e. subregions within the basal ganglia and the cerebellum).

The test-retest reliability of fNIRS, as well as its signal sensitivity are important aspects to consider, yet study in this domain is rare. Recently, a study from our group showed that the test-retest reliability in older adults during a weight-shifting task was fair for the SMA, and excellent for the PFC, PMC, and PPC [77], suggesting validity for the use of fNIRS in repeated measures of tasks requiring balance control [77]. In another recent study, Cockx et al. (2023) investigated the sensitivity of fNIRS to pick up leg movements [78], reporting that fNIRS was able to differentiate between finger and leg movements within the primary motor cortex [78]. Subtle movements deeper within the interhemispheric fissure, such as in the leg area [78], were thus discernable. Note, that these authors also observed 'systemic fluctuations' in the leg but not in the finger movements trials [78]. Here, short channels were able to successfully correct for the fluctuations, probably because the leg activity was captured during a seated

task rather than a whole-body task. These results confirm that large bodily movements can incur substantial systemic artefacts which need to be corrected for [78].

Methodological reflections on the EMG synergy approach

In **Chapter 5**, we were able to employ extensive multi-channel EMG measurements from distal and proximal leg muscles as well as the lower back in a relatively large sample of 24 PwPD and 27 HC. Previous research mostly included the distal musculature only (tibialis anterior, gastrocnemius muscles) thereby limiting a more detailed analysis of the overarching neuromuscular control of gait [79–82]. The synergy approach was novel as was the use of statistical parametric mapping (SPM) allowing an analysis of activity within-synergies across the complete gait cycle [83,84]. In contrast to other techniques, SPM does not bin certain parts of the gait cycle into phases or determines peak locations, but rather takes the whole gait cycle into account [83,84]. Also, since the SPM toolbox is still in development, it does not accommodate for unbalanced samples or for covariate inclusion. The impact of these drawbacks was that we could not control for speed differences between cohorts and were forced to manually balance our samples. As well, SPM assumes global similarity of the cyclic signal analyzed between groups, whereas it could be that the overall contribution of the swing and stance phases was different in PwPD and HC [85], compromising a full interpretation of the functional meaning of the changes found.

Besides these restrictions, the use of a synergy analysis brings some additional limitations. Since we compared the activation curves of the synergies as a whole, representing the contributions of multiple muscles together, we were not able to precisely understand the role of each change in peak activity in each muscle. Additionally, we averaged five strides of each treadmill phase before extracting the muscle synergies, in line with Vervoort et al. (2020) [53]. It is, however, likely during an adaptive process that the between stride variability would have increased or altered over time, especially during the early adaptive phase, which could have influenced the synergy extraction process.

To investigate whether there was increased variability and whether this could have explained why we found less synergies during the early SB-phase, we performed some additional analyses (see **Appendix II**). First, we identified the between-stride variability of the main contributing muscles to the four identified synergies found in **Chapter 5,** being the TA, GL, VL and BF. We demonstrated that there was more variability in some but not in all muscles during EA as compared to BL in both the slow and fast leg irrespective of group. Interestingly, the muscles expressing more variability differed between the fast and slow leg. For the slow leg the TA and VL showed increased variability, whereas for the fast leg GL and BF had more variance (see **Appendix II**).

Second, we made scatter plots of the change in the number of synergies from BL to EA and the individual muscle EMG variability, which showed no relationship between these two outcomes. The random nature of this pattern rather suggested that although variability was larger in some muscles during EA as compared to BL, it could not have solely explained the drop in the number of synergies. Instead and as suggested in **Chapter 5,** the results could reflect a 'freeze' response to cope with the relatively novel perturbation. In line, a previous study comparing synergies in trained ballet dancers and novices during a novel balance task [86] reported that the experts recruited more synergies compared to the novices. As well, the experts used more synergies that were comparable to regular walking, suggesting that they could generalize the gait synergies to novel situations [86].

The above explained exploratory analyses hinted that the influence of variability did not substantially affect the general synergy metrics. Also, the increased variability was found in different muscles in the slow and fast leg, yet synergy extraction was similar in both legs. Furthermore, the four synergies identified in **Chapter 5** showed similarities with those reported in previous studies using stable walking paradigms [52,87]. Lastly, we found that the between-group comparisons conducted through the SPM analysis were based on similar between-group variability. Taken together, our analyses point to a great need to conduct future studies on the effect of variability on synergy extraction methods applied during adaptive gait paradigms, an issue which goes beyond the scope of the present PhD thesis.

As for the conceptual framework underlying synergy analysis and as mentioned in **Chapter 1,** the precise way in which the central nervous system mitigates the 'redundancy phenomenon' is still an area of speculation [88,89]. Although the muscle synergy hypothesis is broadly used as a basis for many studies in the neurological field, it is important to acknowledge that the working mechanisms and anatomical structures underlying the 'construct of a synergy' are still unknown [90]. For instance, other neuromuscular control theories, such as the 'uncontrolled manifold hypothesis' [89,90], does not propose 'fixed' synergies for reducing the degrees of freedom. Rather, it identifies the degrees of freedom that are relevant for the motor task and those that are not, by only controlling parameters which otherwise would have led to increased errors or variance during task performance [90]. According to this model, the central nervous system is more flexible compared to the synergy approach, since it allows the exploration of different solutions for a single task within the task context (manifold). Eventually though, the manifold model also adopts 'synergies'. Here, a synergy means the unique combination of parameters needed for a specific task. Although the notion of muscle synergies is thus broadly embraced, there is still a lot of uncertainty about their actual existence and their explanatory validity [90]. Nevertheless, in this doctoral thesis, muscle synergy analysis provided an

opportunity to study motor control within a well-defined and novel framework, albeit that careful interpretation remains warranted [89,90].

Future research lookout

This thesis provides important clues for future studies. First, as espoused before, transfer of skills is particularly difficult for PwPD [37–39,91] and could be enhanced by training adaptive aspects in different contexts [41,92]. Such enriched training could lead to a more flexible motor skill development that possibly generalizes to daily life adaptive walking. Therefore, future studies should consider combining SB-training with overground practice to promote transfer to daily life mobility. Such study is now ongoing in our lab (ClinicalTrials.gov ID NCT05511597). In line, outcome measures which address and quantify adaptive gait capacity are needed. Digital mobility outcomes are the most likely candidates for this purpose, applied to standardized home protocols, which challenge adaptive gait ability. In addition, mobility monitoring for several days in the home situation could be captured as well to validate the ecological value of such protocols [33,93].

Second, the integration of fNIRS and EMG-signals would be an important follow-up study, as this would give much needed insight in the relationship between central and peripheral control of adaptive gait. However, as the two signals are captured with a different temporal resolution, achieving a more granular analysis would pose some challenges. Still, both EMG and fNIRS are relatively cheap and easy to set-up in pathological populations [94–96]. Ideally, however, a mobile brain imaging method such as EEG would offer a better temporal resolution for combining with EMG, also allowing the calculation of EEG-EMG spectral coherence metrics. EEG-EMG coherence studies could enhance insight into the functional connection between brain and muscle [97], and are now increasingly being undertaken in the field of motor control in PD [98].

The results of **Chapter 4 and 5** provide an important rationale for employing fNIRS and EMG pre- and post-perturbation training to assess the possible impact on cortical and neuromuscular control. fNIRS and EMG measurements might not only be useful to assess the training response at different levels of the neural system, but also to predict its responsiveness. Understanding the mechanisms of perturbation training at the neural level, may help to refine the dose and target of training and inform about the remaining neural reserve to respond to training in different patients. Being able to better predict which patients will benefit, will feed into a better personalization of training.

Lastly, although adaptive gait is partially governed by the bilateral coordination of the two legs [99], the results of **Chapter 4** indicate that it is imperative to further examine the different roles of the fast and slow leg separately during SB-walking, since perturbations are specifically

applied to one side. Although we did not constrain the synergy analysis to specifically accommodate the slow leg or the fast leg, thus allowing the synergies to differ, similar synergies were extracted for both legs in analogy to other work [100]. However, the present findings revealed that some synergies showed more prominent phase effects in the slow leg and others in the fast leg. Additionally, the exploration of between-stride variability during the early SB-phase (**Appendix II**) showed increased variability in different muscles for the slow and the fast leg. Although beyond the scope of this thesis, we recommend future studies to analyze the complete adaptation curves, combining kinematic as well as EMG of individual muscles (including variability), to gain more detailed insight into the adaptive process within each leg separately. Fasano et al. (2016) explored the effects of best-side (i.e. side with the larger step length) and worst-side reduction in a group of 20 freezers. They found that best-side reduction led to an initial worsening of coordination but showed better after-effects for symmetry. However, the determination of the 'best' or 'worst' side is not straightforward and no distinction was made between the fast and the slow leg as such [101]. In addition, MacLellan et al. (2014) through synergy analysis reported four bilaterally governed synergies during SB-walking in young adults, with distinct activity within synergies for the slow and fast leg muscles. They showed similar patterns when analyzing the two legs separately, although timing differences were present indicating some lateralized differences [100].

Clinical implications

Chapter 2 and 3 indicated that training interventions that offer repeated accelerations and decelerations of treadmill belts, either in a TB or SB context, resulted in substantial training effects [1,2]. This suggests that even a low-tech training set-up, with equipment already available in most physiotherapy clinics, such as a regular treadmill, could be used to enhance adaptive gait, if used to provide perturbed gait. Although this concept should still be tested, clinicians could already implement adaptive components of training, for instance, through frequently changing the speed of the treadmill, or by providing an overground gait task with frequent turns and directional changes [8–11].

Chapter 5 showed that PwPD are surprisingly able to recruit muscle synergies as well as HC during both TB and SB walking, indicating that the overall muscle control is still intact. Though, within- synergy activation was affected by PD [13,52,102]. We found that the distal musculature (tibialis anterior) showed abnormal activity in PwPD [81], characterized by hyperactivity at swing initiation during the perturbation and hypoactivity at foot strike. This knowledge may help clinicians to refine their instructions during adaptive gait work. The tibialis anterior is particularly important for foot positioning and to initiate swing [81]. Problems with the transitioning from stance to swing have been shown to bring on gait complications such as tripping, resulting in increased fall risk [81]. Focusing patients' attention on the normalization

of foot strike, especially during gait perturbations could be an effective method to help reducing fall risk [103].

Although this dissertation mostly pertained to early to mid-stage PwPD, limiting the generalizability of results, the experience with SB-training suggested that it could also be useful for more severely affected patients for three reasons. First, the SB-training did not incur adverse events as it took place in a safe environment incorporating a fall harness [2]. Second, SB-training was applied implicitly requiring patients responses without explicit instruction and not requiring any extra cognitive involvement [19]. This would suggest that PwPD with cognitive impairment could be eligible for such training. Of critical note, a recent pilot study in PwPD with FOG reported that impaired working memory reduced adaptive SB gait performance and its after-effects during a single 20-minute training [26]. However, another pilot study found improvements in both adaptive gait and cognitive dual-task performance in PwPD with FOG with mild cognitive impairment [3]. Lastly, SB-training in this thesis was highly standardized according to 16 levels. However, the nature of training offered many opportunities to adjust the dose to personal abilities (e.g. speed, magnitude of perturbations, hand rail use). As such, SB-training opens up the possibility for progressive perturbation training for all PwPD, who are still able to walk, including fall prone and fragile PwPD [2]. Whether the same effects sizes can be gained in such populations will require further study.

Conclusion

The objective of this PhD project was to shed light on the efficacy of adaptive gait training and to understand the neural and neuromuscular correlates underlying the response to asymmetrical gait perturbations in people with PD. This thesis contributed to knowledge gains in this domain by showing, in a nutshell, that cortical and neuromuscular control during SB-perturbations showed patterns of hyperactivity in PD compared to healthy age-matched controls. These findings are consistent with a maladaptive response of the neural system to gait perturbations even in mildly affected patients. Against this background, it was the more remarkable that our findings showed that PwPD responded very well to SB-training. Patients in the SB-training group were able to implicitly learn how to adjust their gait to sudden asymmetrical changes, over and above the ability to withstand perturbations in those who received traditional treadmill training. These gains were sustained at follow-up and proved resilient to dual tasking. However, training effects were specific for SB-conditions and did not transfer to overground walking. Our findings support further study in this domain to help determine how to improve generalization of SB-training effects to daily life and personalize the dose of this rehabilitation approach.

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Summary

Parkinson's disease (PD) is the fastest growing, and second most common neurodegenerative disorder after Alzheimer's disease. If current predictions are correct, more than 12 million people worldwide will be living with PD by 2040, which is a doubling of cases since 2015. People with PD (PwPD) experience a broad range of symptoms, including prevalent gait problems such as decreases in gait speed and step length. Especially, adapting the gait pattern to sudden changes in the environment, such as when needing to make a rapid turn, lead to exaggeration of gait problems and can additionally trigger 'freezing of gait' (FOG). Here, people have the feeling that their feet are glued to the floor, despite their intention to move, which can lead to instability and falls. Although there are several interventions available focusing on improving aspects of gait such as gait speed, current rehabilitation techniques are not specifically aimed at improving the adaptive component of gait. This is important, as during daily life situations we constantly need to adjust our gait to the changing environmental context. This is why in the first part of this thesis we set out to investigate whether a training with repeated perturbations during gait could improve adaptive gait in PwPD.

In **Chapter 2** we summarized the current literature available regarding repeated gait perturbation training (RGPT) and its effect on gait in both PwPD as well as healthy older adults. Unlike we expected, when analyzing all the results of the studies together, we found no beneficial effect of RGPT on gait performance compared to regular treadmill training, even though some individual studies did report improvements and retention of effects. These studies comprised mainly of paradigms with sudden accelerations and decelerations of the treadmill. Additionally, only one study reported about the effect of training on the adaptive components of gait, where significant improvements were found for step length asymmetry and variability outcomes. The body of literature was still limited as only eight studies could be included in this review, and these were all still in the proof of concept phase including small groups and limited methodological quality. This pointed out the need for more larger scaled training studies to uncover the real potential of RPGT for improving adaptive gait in PwPD.

This is why in **Chapter 3** we performed a larger-scaled randomized controlled training study with 52 PwPD who received either 4 weeks of traditional 'tied-belt' (TB) treadmill training or adaptive 'split-belt' (SB) treadmill training, three times a week. A SB treadmill comprises of two belts, one under each foot, allowing for the speed of each leg to be regulated separately. By reducing the speed of one leg one can induce asymmetrical perturbations during gait, thereby demanding an adaptive gait response from the participant. Based on this concept, we anticipated that we could train adaptive gait, as previous work from our lab already showed improvements of adaptive gait on the treadmill and transfer to overground turning in a group of PwPD with FOG after one single session of SB training. Indeed, after 4 weeks the PwPD who received SB training improved their adaptive gait on the treadmill significantly compared

to the group who received TB training. Additionally, those improvements were unaffected by dual task interference and retained for four weeks without training. Unexpectedly these gains did not transfer to overground turning although overground gait speed and step length improved in both groups. These results indicate that SB training is very effective in improving adaptive gait on the treadmill with robust retention effects, although future work should focus on how we can enhance transfer of these improvements to overground gait situations for instance by combining SB training with an overground adaptive training.

In part II of this thesis we studied the underlying neural and neuromuscular mechanisms of adaptive gait in PwPD and compared this to healthy older adults to gain a better understanding why PwPD experience adaptive gait problems. Therefore, in **Chapter 4** we measured cortical brain activity using functional near-infrared spectroscopy (fNIRS) during TB and SB walking. We found that SB walking results in increased posterior parietal cortex (PPC) activity in both PwPD and controls, an area important for sensorimotor integration. Interestingly, PwPD increased the activity in the supplementary motor area (SMA) from TB to SB walking, whereas controls did not. The SMA is important for motor planning and coordination. Lastly, PwPD showed consistent increased activity in the PPC and premotor cortex (PMC) already during TB walking, as well as during SB walking compared to controls. The PMC is an important area for the selection of movements for the motor program and subsequent adjustments if necessary. Correlation analysis showed that these increases in activity are most likely not functional compensation since higher PPC activity during SB walking was related to worse adaptive performance in PwPD, which might partially explain their adaptive gait deficits.

In **Chapter 5** we studied the neuromuscular control of adaptive gait in PwPD and controls during TB and SB walking with the use of electromyography (EMG). Here, we investigated how muscle groups with as similar function (i.e. muscle synergies) respond to adaptive SB walking. We found that during TB walking both groups used four synergies and reduced to three synergies while SB walking, suggesting a more simplified control during the adaptive task. When comparing the activity curves of the four TB synergies across all TB and SB phases we found consistent phase effects in both legs, albeit more prominently in the leg that was slowed down during SB walking. No group by phase interactions were present, indicating a similar response over the phases in both groups. However, the synergy comprising of the tibialis anterior presented with a group effect in the slow leg, where PwPD showed more activity during swing and less activity around heel strike during the adaptive SB phase as compared to controls. Lastly, we found an association where more simplified muscle control during SB-walking was related to worse adaptive gait performance on the treadmill in both PwPD and controls. This suggests that although the overall synergy organization during TB and SB

walking appeared similar between groups, more sophisticated changes within synergies could be impacted by PD and potentially underly their adaptive gait problems.

The results of this dissertation indicate that RGPT is a promising rehabilitative tool compared to the more traditional gait rehabilitation interventions to improve adaptive gait in PwPD. Four weeks of SB training showed robust improvements in adaptive treadmill gait performance with excellent retention, although enhancing the transfer of these effects still needs to be studied in more detail. We also showed that the adaptive gait problems PwPD experience are possibly governed by altered cortical and neuromuscular control which should therefore be monitored and targeted in future rehabilitation interventions for optimal training impact.

Samenvatting

De ziekte van Parkinson (PD) is the snelst groeiende en tweede meest voorkomende neurodegeneratieve ziekte na de ziekte van Alzheimer. Als de huidige voorspellingen correct zijn, zullen in 2040 meer dan 12 miljoen mensen leven met PD, wat een verdubbeling van het aantal mensen met deze diagnose is sinds 2015. Mensen met de ziekte van Parkinson (MmPD) ervaren een breed spectrum aan symptomen, zo ook veelvoorkomende stapproblemen zoals een afname van de gangsnelheid en staplengte. Met name het aanpassen van het stappatroon tijdens een plotse verandering van de omgeving, bijvoorbeeld het moeten maken van een snelle draai, kan leiden tot een verergering van deze stapproblemen en kan daarbij ook leiden tot 'freezing of gait' (FOG), het 'bevriezen van het stappen'. Hier hebben mensen het gevoel dat hun voeten aan de grond zijn vastgelijmd, ondanks hun intentie om te bewegen, wat kan leiden tot instabiliteit en vallen. Ondanks dat er al enkele interventies voorhanden zijn gefocust om het verbeteren van gangaspecten zoals gangsnelheid en staplengte, zijn deze huidige revalidatie technieken niet specifiek gericht om het verbeteren van de adaptieve component van het stappen. Dit is belangrijk, aangezien we tijdens dagelijkse situaties constant ons stappen moeten aanpassen aan de huidige omgevingscontext. Dit is waarom wij in het eerste deel van dit proefschrift onderzocht hebben of een training met herhaalde perturbaties tijdens het stappen kan leiden tot een verbetering van het adaptieve stappen in MmPD.

In **Hoofdstuk 2** hebben wij de beschikbare literatuur over herhaaldelijke gangverstoringstraining ('repeated gait perturbation training, RGPT) en de mogelijke effecten hiervan op het stappen in zowel MmPD alsook gezonde oudere volwassenen samengevat. In tegenstelling tot wat wij verwachtten vonden wij na het analyseren van alle resultaten samen geen gunstige effecten van RGPT in vergelijking met normale loopbandtraining, ondanks dat sommige individuele studies wel positieve en retentie effecten rapporteerden. Deze studies bestonden met name uit versnellingen en vertragingen van de loopband. Daarnaast was er slechts één studie die effecten rapporteerde over de adaptieve componenten van het stappen, waarbij significante verbeteringen werden gevonden voor staplengte asymmetrie en variabiliteitsuitkomsten. De hoeveelheid beschikbare literatuur was gelimiteerd aangezien slechts acht studies geïncludeerd konden worden in deze review en dat deze zich allemaal nog in een proof-of-concept fase bevonden met beperkte groepsgroottes en beperkte methodologische kwaliteit. Dit benadrukte de behoefte aan meer groter geschaalde trainingsstudies om de echte potentie van RGPT om het adaptief stappen bij MmPD te verbeteren verder te onderzoeken.

Daarom hebben wij in **Hoofdstuk 3** een grootschaligere gerandomiseerde trainingsstudie gedaan met 52 MmPD die ieder vier weken, drie keer per week, óf reguliere loopbandtraining óf adaptieve gespleten loopbandtraining kregen. Een gespleten loopband, ook wel een 'split-

belt' (SB) genoemd, heeft twee banden, een onder elke voet, waardoor de snelheid van ieder been apart geregeld kan worden. Door de snelheid van een been te verlagen kun je een asymmetrische verstoring tijdens het stappen geven, waardoor de deelnemer gedwongen wordt zijn stappatroon hieraan aan te passen. Gebaseerd op dit concept verwachtten wij dat we het adaptief stappen konden trainen, aangezien eerder werk van ons lab verbeteringen liet zien van het adaptief stappen op de loopband en een transfer van deze verbetering naar het draaien op de plaats in een groep met MmPD en FOG na slechts een enkele sessie SB training. Inderdaad, na vier weken verbeterden de MmPD die SB training hadden gekregen hun adaptief stappen op de loopband significant in vergelijking met de groep die reguliere loopbandtraining kreeg. Bovendien werden deze verbeteringen niet beïnvloed door interferentie van een dubbeltaak en bleven deze vier weken behouden zonder training. Onverwacht was dat deze verbeteringen niet doorwerkten in het op de plaats draaien, hoewel de gangsnelheid en staplengte in beide groepen wel waren verbeterd. Deze resultaten geven aan dat SB training zeer effectief is in het verbeteren van het adaptief stappen op de loopband met robuuste retentie effecten, hoewel toekomstig werk zich moet richten op hoe we de transfer van deze verbeteringen naar overgrondse situaties kunnen verbeteren, bijvoorbeeld door SB training te combineren met een overgrondse adaptieve staptraining.

In deel II van dit proefschrift hebben we de onderliggende neurale en neuromusculaire mechanismen van het adaptief stappen bij MmPD bestudeerd en vergeleken met gezonde volwassen ouderen om beter te begrijpen waarom MmPD problemen ervaren met het adaptief stappen. Daarom hebben we in **Hoofdstuk 4** de corticale hersenactiviteit gemeten met functionele nabij-infrarood spectroscopie (fNIRS) tijdens TB en SB-stappen. We vonden dat SB stappen resulteerde in verhoogde activiteit in de posterieure pariëtale cortex (PPC) in zowel MmPD als controles, een gebied dat belangrijk is voor de sensomotorische integratie. Opvallend was dat MmPD de activiteit in het supplementaire motorgebied ('supplementary motor area', SMA) verhoogden van TB naar SB stappen, terwijl de controles dit niet deden. De SMA is belangrijk voor motorische planning en coördinatie. Tot slot vertoonden MmPD consistent verhoogde activiteit in de PPC en premotorische cortex (PMC) al tijdens het TB stappen, alsook tijdens het SB stappen in vergelijking met de controles. De PMC is een gebied belangrijk voor de selectie van bewegingen voor het motorprogramma en eventueel daaropvolgende aanpassing. De correlatieanalyse toonde aan dat deze toename in activatie zeer waarschijnlijk niet functionele compensatie is, aangezien hogere PPC-activatie tijdens SB stappen gerelateerd was aan een slechtere adaptieve prestatie in MmPD, wat hun adaptieve stapproblemen gedeeltelijk zou kunnen verklaren.

In **Hoofdstuk 5** bestudeerden we de neuromusculaire controle van adaptief stappen in MmPD en controles tijdens TB en SB stappen met behulp van elektromyografie (EMG). Hier

onderzochten we hoe spiergroepen met gelijkaardige functie (d.w.z. spiersynergiën) reageren op adaptief SB stappen. We vonden dat tijdens TB stappen beide groepen vier synergiën gebruikten en terugvielen naar drie synergiën tijdens SB-stappen, wat duidt op een meer vereenvoudigde controle tijdens de adaptieve taak. Bij het vergelijken van de activatiecurves van de vier TB synergiën tijdens alle TB en SB fases vonden we consistente fase effecten in beide benen, zij het meer prominent in het been dat werd vertraagd tijdens het SB-stappen. Er waren geen groep-fase interacties, wat duidt op een gelijkaardige response over de fases in beide groepen. De synergie bestaande uit de tibialis anterior vertoonde echter een groepseffect in het vertraagde been waar MmPD meer activatie hadden tijdens de zwaaifase en minder activatie rond de landing van de voet tijdens de adaptieve SB-fases in vergelijking met controles. Tot slot vonden we een associatie waarbij meer vereenvoudigde spiercontrole tijdens het SB stappen gerelateerd was aan een slechtere adaptieve stapprestatie op de loopband in zowel MmPD als controles. Dit suggereert dat hoewel de algehele synergie organisatie tijdens TB- en SB-stappen vergelijkbaar leek tussen de groepen, meer verfijnde veranderingen binnen synergiën beïnvloed kunnen worden door PD, en mogelijk ten grondslag ligt aan hun adaptieve stapproblemen.

De resultaten van dit proefschrift geven aan dat RGPT een veelbelovend revalidatievorm is in vergelijking met de meer traditionele staprevalidatie interventies om adaptief stappen in MmPD te verbeteren. Vier weken SB training liet robuuste verbeteringen zien in de adaptieve stapprestatie op de loopband met uitstekende retentie, hoewel het verbeteren van de transfer van deze effecten nog in meer detail onderzocht moet worden. We vonden ook dat de adaptieve stapproblemen die MmPD ervaren mogelijk veroorzaakt worden door veranderde corticale en neuromusculaire controle, en deze moeten daarom gemonitord en aangepakt worden in toekomstige revalidatie interventies voor een optimale impact van training.

Appendix I

TiDieR Checklist Split-belt RCT (Chapter 3)

The TIDieR (Template for Intervention Description and Replication) Checklist*:

TIDieR Template for Intervention Description and Replication

Information to include when describing an intervention and the location of the information

Item nr	Item	Description
	BRIEF NAME	
1.	Provide the name or a phrase that describes the intervention.	Split-Belt Treadmill training to improve gait adaptability overground (e.g. turning) and on the treadmill in patients with Parkinson's Disease (PD), when compared to regular treadmill training (tied-belt training, TBT).
	WHY	
2.	Describe any rationale, theory, or goal of the elements essential to the intervention.	Regular treadmill training is found to be very effective in improving gait, including gait speed and step length in patients with PD (Tomlinson et al. 2012, Mehrholz et al. 2016). However, these forms of training do not target the challenging demands of daily life ambulation, where gait needs to be adapted constantly in order to make turns or sudden changes in direction. One training form that specifically targets gait flexibility is split-belt treadmill training (SBT). A split-belt treadmill allows the control of the speed of the legs individually through separate belts, and can thereby impose asymmetrical perturbations to the gait pattern.
	WHAT	
3.	Materials: Describe any physical or informational materials used in the intervention, including those provided to participants or used in intervention delivery or in training of intervention providers. Provide information on where the materials can be accessed (e.g. online appendix, URL).	Both groups SBT and TBT received treadmill training on a split-belt treadmill. This is a treadmill with two belts which allows the regulation of speed for each leg separately. During SBT the two belts had a different speed compared to each other, during TBT the two belts ran at the same speed
4.	Procedures: Describe each of the procedures, activities, and/or processes used in the intervention, including any enabling or support activities.	Both training groups received training for 4 weeks, 3 times a week with a rest day scheduled in between sessions. Both groups received a short warm-up and cool-down period on the treadmill. The warm-up was followed by the main training of 30-45 minutes of walking divided in several blocks. Each week the total training duration was increased by 5 minutes. A 16-level training schedule was developed for both groups to ensure equal motivation in both groups.

		The training of the SBT group consisted of 6 blocks of 5 minutes of split-belt walking, and every week one 5-minute block was added regardless of the current level patients were in. The participants walked at their preferred gait speed and along the levels more switches between the belt speeds were imposed making the adaptation demands within a block increase over training period. Details on the amount of switches per block, the different belt contrasts used and levels can be found below this checklist. The TBT group training initially consisted of 6 blocks of 5 minutes walking at 80% of their comfortable walking speed. Every week, similarly to the SBT group the total training length was extended by 5 minutes. The training overall progressed by increasing the walking speed from 80% to 110% in the four weeks and increasing the length of the walking blocks from 5 minutes up to 20 minutes at one stretch at week 4. Details on the lengths of the blocks and walking speed per level can be found below this checklist.
	WHO PROVIDED	
5.	For each category of intervention provider (e.g. psychologist, nursing assistant), describe their expertise, background and any specific training given.	Trainers who were specialized in guiding PD patients in both the split-belt and tied-belt intervention protocol.
	HOW	
6.	Describe the modes of delivery (e.g. face-to-face or by some other mechanism, such as internet or telephone) of the intervention and whether it was provided individually or in a group.	Training sessions were individually given by the trainers at the respective training facilities. Both the SBT and TBT training were given on the split-belt treadmill. The treadmill had two belts, one for each leg. During the SBT the belts had different speeds under each leg initially causing an asymmetric stepping pattern. During TBT the two belts ran at the same speed, like a 'regular' treadmill.
	WHERE	
7.	Describe the type(s) of location(s) where the intervention occurred, including any necessary infrastructure or relevant features.	The training was given in two movement laboratories (KUL and CAUK) which was equipped with a split-belt treadmill.
	WHEN and HOW MUCH	
8.	Describe the number of times the intervention was delivered and over what period of time including the number of sessions, their schedule, and their duration, intensity or dose.	The training was given 3 times a week for 4 consecutive weeks. Each training session was approximately 1 hour, including 30-45 minutes of either TBT or SBT. Training days were alternated with a rest day in between.

	TAILORING	
9.	If the intervention was planned to be personalised, titrated or adapted, then describe what, why, when, and how.	To ensure comparable training load and cater for the different levels of walking ability, the training progression was standardized. If exertion was too high, trainers could choose to repeat the same level in the next session or the opposite, if participants were insufficiently challenged a level could be skipped (max 1 level skipped per week). In case a participant was not able to follow the training protocol due to fatigue, the following training adjustment measures were applied (in this order): lengthening the in-between block breaks, shortening the duration of a block, and leaving out blocks if necessary. Heart rate was monitored to ensure patients did not exceed their individual maximum heart rate. This was calculated in advance using a formula based on resting heart rate (Nes et al., 2013).
	MODIFICATIONS	
10.‡	If the intervention was modified during the course of the study, describe the changes (what, why, when, and how).	N/A
	HOW WELL	
11.	Planned: If intervention adherence or fidelity was assessed, describe how and by whom, and if any strategies were used to maintain or improve fidelity, describe them.	Adherence was registered by the trainers. If patients did not show up the trainers contacted them to make sure nothing bad had happened along the way. Some patients regularly forgot their appointments and this was mitigated by sending reminders of the training the day beforehand. Furthermore, training sessions were always scheduled together with the participants to avoid agenda conflicts and possible drop-outs because of this. Trainers also closely monitored exhaustion and other complaints and tailored the subsequent sessions based on this feedback.
12.‡	Actual: If intervention adherence or fidelity was assessed, describe the extent to which the intervention was delivered as planned.	Five people dropped out during the intervention period due to restrictions caused by the COVID pandemic. Two participants dropped out during training due to personal reasons and transportation issues. All other participants completed the training period.

** **Authors** - use N/A if an item is not applicable for the intervention being described. **Reviewers** – use '?' if information about the element is not reported/not sufficiently reported.

† If the information is not provided in the primary paper, give details of where this information is available. This may include locations such as a published protocol or other published papers (provide citation details) or a website (provide the URL).

‡ If completing the TIDieR checklist for a protocol, these items are not relevant to the protocol and cannot be described until the study is complete.

Appendix II

Between-Stride Variability Influence on Synergy Extraction (Chapter 5)

Is the reduction of synergies reported possibly explainable by the increased between stride variance of the EMG signal making the mean signal over the 5 strides not representative and therefore invalid?

1. T-test between BL- and EA- between stride variability

First, is there increased EMG variability during SB vs TB strides?

Looked at the main contributors for each synergy (TA, GL, VL, BF) in slow and fast leg

Table 1. T-tests between BL vs EA for the four main contributing muscles.

Slow leg	**BL vs EA**	**p-value**	**Fast leg**	
TA	EA>BL	**<0.001**	EA>BL	0.448
GL	EA>BL	0.144	EA>BL	**<0.001**
VL	EA>BL	**<0.001**	EA>BL	0.408
BF	EA>BL	1.000	EA>BL	**<0.001**

Bonferroni corrected for multiple testing. Abbreviations: TA, tibialis anterior; GL, gastrocnemius lateralis; VL, vastus lateralis; BF, biceps femoris.

Generally, there is more variability in the EMG during the EA vs the BL phase. However, there are different patterns for the slow and fast leg with regards to differences in variability between phases. Despite this, the overall synergy structure came out similarly between the two legs, illustrating that variability and synergy structure are relatively independent.

- The results suggest that there is variability but with limited impact on the synergy extraction

2. Repeated measures ANOVA all phases * group

Second, since synergies were extracted during all phases, I also checked the influence of variability across all phases and the possible influence of the groups on this.

- For all models there was a main effect of phase but no group effects or interactions meaning that the variability between the groups behaved similarly across phases.
- Post-hoc tests showed a similar pattern as the t-tests**.**

Table 2. Post-hoc tests for phase.

Slow leg	**Main phase differences**	**p-value**	**Fast leg**	
TA	EA>BL	**<0.001**	EA>BL but main effect driven by LA>BL & LA>EA	0.595
GL	EA>BL but main effect driven by EA>LA & LA>EP	0.206	EA>BL	**<0.001**
VL	EA>BL	**<0.001**	EA>BL but main effect driven by LA>EA & EP>LA	0.436
BF	EA>BL but main effect driven by EP>LA	0.596	EA>BL	**<0.001**

Bonferroni corrected for multiple testing. Abbreviations: TA, tibialis anterior; GL, gastrocnemius lateralis; VL, vastus lateralis; BF, biceps femoris.

3. Scatter plots between delta number of synergies (EA-BL) and EMG variability

Third, does heightened variability possibly explain the drop in synergies observed between BL and EA?

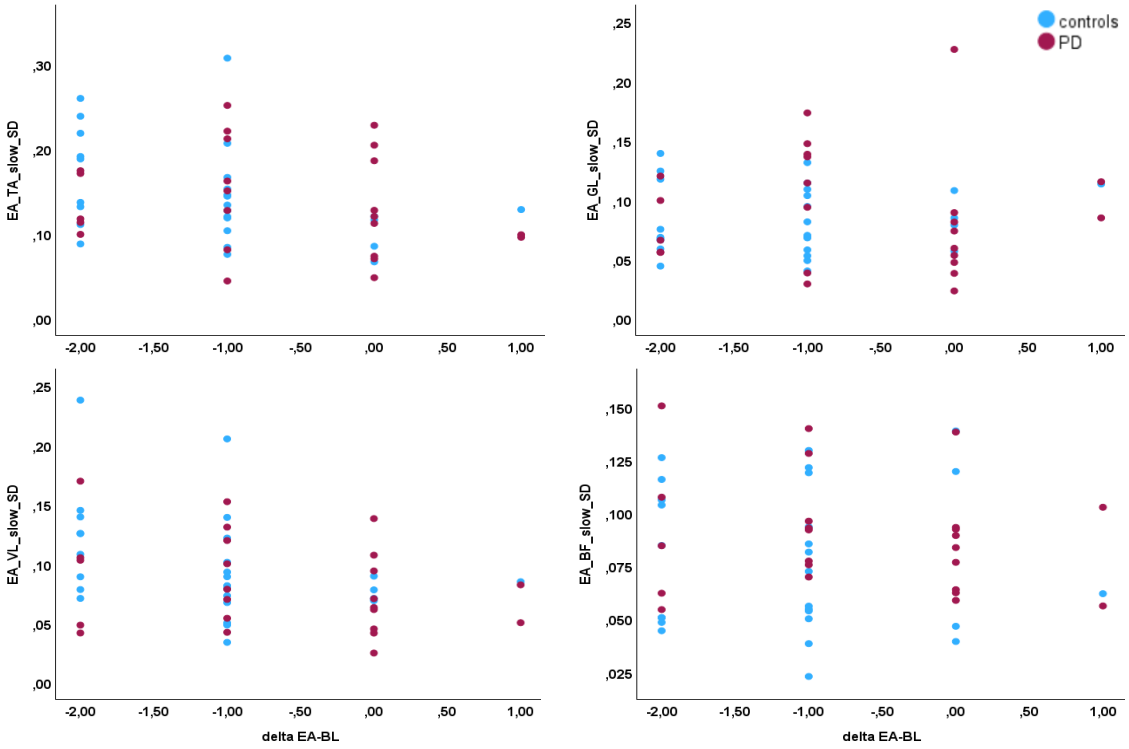

Figure 1. Scatter plots of the data of the slow leg in the four main contributing muscles (TA, GL, VL, BF), indicating that participants who did not change synergies between EA and BL displayed comparable variability to the people decreasing synergies between phases.

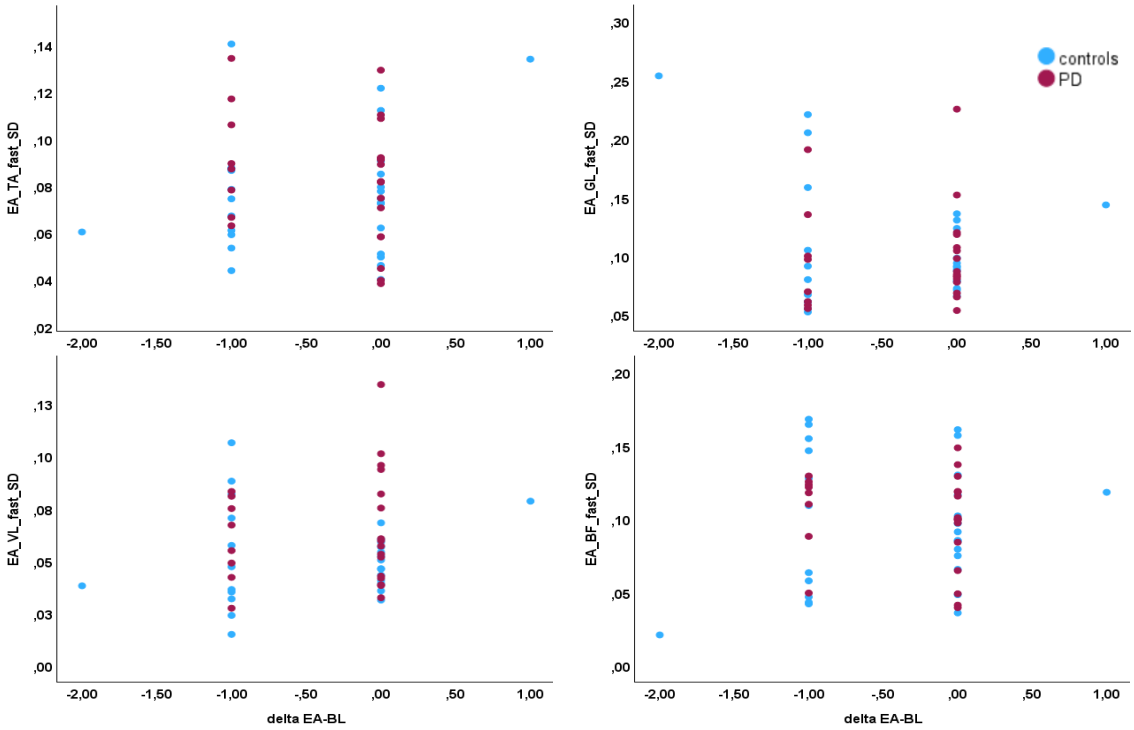

Figure 2. Scatter plots of the data of the fast leg in the four main contributing muscles (TA, GL, VL, BF). Showing similar variability in people changing and not changing synergies between BL and EA.

Concluding remarks

1. Generally, there seems to be increased variability between the 5 strides in the EMG signal during EA (recall T-test and ANOVA results), however not in all muscles and differentially present in the slow and fast leg.
   Suggesting a limited effect of variability in synergy extraction since for both the slow and fast leg similar synergies were extracted and similar patterns of reduction were observed
2. This variability is similar between groups across phases (recall ANOVA results)
   Suggesting a similar 'noise' possibly caused by the variability across phases therefore not majorly interfering with group comparisons.
3. Exploration of the data by scatterplots including the change in number of synergies and EMG variability indicated that for both the slow and fast leg there were people who reduced the number of synergies, irrespective of high and low variability. Similar results were displayed for people who had no or little change between BL and EA.
   Suggesting that although variability was larger in some muscles during EA as compared to BL the drop in number of synergies found in **Chapter 5** seems not to be solely explained by this.

Appositions

Bijstellingen

Academia is notoriously competitive and funding scarce. Investing in financial stability for researchers will lead to research based on due diligence, less mental health issues, less loss of valuable knowledge, and a decrease in research fraud and abuse of power.

De academische wereld is berucht om haar concurrentie en schaarse financiering. Investeren in financiële stabiliteit voor onderzoekers zal leiden tot onderzoek op basis van zorgvuldigheid, minder geestelijke gezondheidsproblemen, minder verlies van waardevolle kennis en een afname van onderzoeksfraude en machtsmisbruik.

Critical reflection in and on the scientific community is important for progression, but skepticism towards science is growing and thereby endangering evidence-based practices. Therefore, it is our scientific duty to communicate our work and its importance comprehensibly to the outside world.

Kritische reflectie in en op de wetenschappelijke gemeenschap is belangrijk voor vooruitgang, maar het scepticisme ten opzichte van wetenschap groeit en brengt evidence-based practice in gevaar. Daarom is het onze wetenschappelijke plicht om ons werk en het belang hiervan begrijpelijk te communiceren aan de buitenwereld.

Even though we are more connected than ever through numerous platforms, there is a lack of compassion for people outside our algorithm based social bubble, leading to polarization. Exposure to different points of view and human perspectives is enriching and can restore a healthy sense of community.

Hoewel we via talloze platforms meer dan ooit met elkaar verbonden zijn, is er een gebrek aan compassie voor mensen buiten onze op algoritmen gebaseerde sociale bubbel, wat leidt tot polarisatie. Blootstelling aan verschillende standpunten en menselijke zienswijzen is verrijkend en kan een gezond gemeenschapsgevoel herstellen.

Dankwoord

Word of Thanks

Donderdag 22 November 2018, Sydney, Australië. Daar begon het, de dag voordat ik op mijn solo backpackreis zou vertrekken, achter mijn kleine bureautje in mijn gedeelde slaapkamer, had ik een Skype-gesprek met Alice, Moran, Pieter en Evelien over een mogelijke PhD positie in hun team. Niet wetende dat dit de start zou zijn van dit academische avontuur in Leuven! Wat ben ik dankbaar dat ik toentertijd deze kans heb gekregen en blij met iedereen die ik de afgelopen vijf jaar om me heen heb gehad en heb mogen ontmoeten. Het uitvoeren van een doctoraatstraject is geen solo-avontuur, zonder jullie was dit werk dan ook nooit in deze vorm tot stand gekomen! Via deze weg wil ik dan ook iedereen bedanken die hier direct, indirect, bewust of onbewust aan heeft bijgedragen.

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About the Author



Femke Hulzinga was born in Zoetermeer, the Netherlands on the first of February 1994. Her interest in clinical populations and rehabilitation developed during her bachelor studies Human Movement Sciences at the VU University Amsterdam. Here she also obtained a minor degree in Psychomotor Therapy focused on movement centered therapy for depression, bipolar and post-traumatic stress disorders. During her master at the Radboud University Nijmegen, she specialized in Clinical Human Movement Sciences with a focus on neurorehabilitation and science communication. She performed an internship at the Dutch Neuromuscular Disease Association (Spierziekten Nederland), writing science-based brochures for patients and general practitioners. The year after, Femke got familiar with neurorehabilitation research within the group of Prof. Weerdesteyn, where she worked on a project about treadmill perturbation based balance training in stroke patients. Here, she validated a 2D camera system for measuring backward stepping leg angles, which is a strong predictor for balance recovery. In parallel, she was a research assistant and supported gait analysis assessments with neurological patients in the Radboudumc hospital. During her graduation project, Femke deepened her research experience abroad in the lab of Prof. Delbaere at Neuroscience Research Australia (NeuRA) in Sydney, Australia where she developed a fall risk classification model based on data of a large home-based study focused on balance and gait in older adults. In 2019, she graduated cum laude and started her PhD in Leuven under supervision of Prof. Alice Nieuwboer, Prof. Moran Gilat and Dr. Pieter Ginis in the Parkinson Rehabilitation Research Lab (PRO-labo). In 2020 she obtained a 4-year personal research grant from Research Foundation Flanders (FWO). In her PhD project, Femke focused on the potential of split-belt treadmill training to improve adaptive gait in people with Parkinson's disease, as well as the underlying neurophysiological mechanisms of adaptive gait.

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Personal Statements

Personal contributions

Chapter 2 – Review design, search strategy, study selection, data extraction, quality assessment, qualitative review, quantitative review, manuscript draft and revision.

Chapter 3 – Development of treadmill training programs including scripts for the treadmill, participant recruitment, data collection Leuven, data processing, statistical analysis regarding overground turning task and other overground outcome measures, manuscript draft and revision.

Chapter 4 – Development of treadmill task including scripts for the treadmill, participant recruitment, data collection, data processing, statistical analysis, manuscript draft and revision.

Chapter 5 – Development of treadmill task including scripts for the treadmill, participant recruitment, data collection, data processing, statistical analysis, manuscript draft and revision.

Conflict of interest statement

The author (FH) declares no conflict of interest for any of the contributing authors with regards to the work presented in this doctoral thesis.