KU Leuven
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MOTOR AND NEURAL CORRELATES OF FREEZING OF GAIT IN PARKINSON'S DISEASE

Sarah VERCRUYSSE

Doctoral thesis in Biomedical Sciences

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Langzaam

Langzaam schuifelt de man door de straat
Langs de huizen schuifelt de man door de drukke straat
Tussen de mensen in de drukke straat schuifelt langzaam de man
Langs uitstalramen
Langs reclameborden
Schuifelt hij verder
Zo zijn ze samen onderweg
De haastige menigte en de trage man
Soms blijft hij even staan
Zijn bevende hand zoekt naar steun
Hij gaat moeizaam zitten op een bank
En staart wat moedeloos voor zich uit
Niemand kent de man
Niemand groet de man
Niemand ziet de man
Hij staat op en schuifelt verder door de straat
Langs uitstalramen en reclameborden schuifelt weer de man
Niemand weet waarheen
Niemand kent zijn stil verdriet

M. Colla
een bewerking van Melopee van Paul van Ostaijen

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LIST OF ABBREVIATIONS

AG: Angular gyrus
ANOVA: Analysis of variance
Ant.: Anterior
BA: Brodmann Area
BBS: Berg Balance Scale
BG: Basal ganglia
CED: Cambridge Electronic Design
CI: Confidence interval
CMRO2: Cerebral metabolic rate of oxygen
CONT: Continuous movement
COV: Coefficient of variability
CPG: Central pattern generator
CTRL: Control subject
D: Dopamine
DBS: Deep brain stimulation
DKI: Diffusion Kurtosis Imaging
DLPFC: Dorsolateral prefrontal cortex
DT: Dual task
DTI: Diffusion tensor imaging
EF: Executive functions
EMG: Electromyography
FC: Functional connectivity
FDR: False discovery rate
FFT: Fast Fourier transform
FI: Freezing index
fMRI: functional Magnetic Resonance Imaging
FOG: Freezing of gait;
FOGQ: Freezing of Gait Questionnaire;
FO-LL: Freezing of lower limb;
FO-UL (FOUL): Freezing of upper limb;
FWHM: Full width at half maximum
GABA: Gamma-aminobutyric acid
GEE: Generalized Estimation Equation
GM: Grey matter
GPe: Globus pallidus pars externa
GPi: Globus pallidus pars interna
H&Y: Hoehn and Yahr
Hb: Haemoglobin
HRF: Haemodynamical response function
ICC: Intra-class coefficient
LD (L-Dopa): Levodopa
LED: Levodopa-equivalent dose
M1: Primary motor cortex
MFG: Middle frontal gyrus
MLR: Mesencephalic locomotor region
MMSE: Mini Mental State Examination
MNI : Montreal Neurological Institute
NFOG-Q: New Freezing of Gait Questionnaire
OFF: Off medication
ON: On medication
OR: Odds ratio
PCI: Phase coordination index
PD: Parkinson's disease.
PD+FOG: PD patient with freezing of gait
PD-FOG: PD patient without freezing of gait
PET: Positron emission tomography
PFC: Prefrontal cortex
PIGD: Postural instability/gait disturbance
PM: Premotor cortex
PMd: Dorsal premotor cortex
PPN: Pedunculopontine nucleus
RCBF: Regional cerebral blood flow
RF: Radio frequency
ROI: Region of interest
RP: Relative phase
Rs (rs): Spearman's Rho
S1: Primary somatosensory cortex
SAS: Statistical Analysis Software
SCOPA-COG: Scales for Outcomes in Parkinson's disease - cognitive part
SEM: Standard error of measurements
SMA: Supplementary motor area
SNc: Substantia nigra pars compacta
SNr: Substantia nigra pars reticulate
SPECT: Single photon emission tomography
SPM: Statistical parametric mapping
STN: Subthalamic nucleus
TUG: Timed Up and Go test
UL: Upper limb
UPDRS-III: Unified PD Rating Scale part III
VBM: Voxel based morphometry
VTA: Ventral tegmental area
WM: White matter
WMH: White matter hypointesity

Chapter 1

General introduction

1. BACKGROUND OF THE RESEARCH PROJECT

Freezing of Gait (FOG) is a disabling gait disorder in patients with Parkinson's disease (PD). Patients who 'freeze' experience a sudden inability to start or continue walking. Because of the high prevalence, impact on patients' wellbeing and difficulty to manage therapeutically, FOG is a symptom of major clinical importance. This doctoral project aimed to increase our understanding of FOG and its causal mechanisms, mainly by comparing PD patients who present FOG with those who are free of FOG at the behavioral and neurological level.

Understanding FOG begins with recognizing its complexity. Therefore, a multi-disciplinary approach to the study of FOG is needed to identify key contributors of FOG in the cognitive, motor and neurological domain. The current dissertation mainly focuses on motor and neurological mechanisms of freezing in Parkinson's disease. The four studies described in this thesis investigated the freezing problem from the innovative viewpoint that core motor deficits related to FOG may extend beyond the control of gait. This was based on the observation of freezing-like motor blocks during various upper limb movements such as writing, brushing of the teeth and more experimental tasks involving bimanual coordination.[1-4] These types of upper limb motion share the repetitive character of the motor program, a feature that is also of crucial importance to attain fluent locomotion. The premise of shared mechanisms of gait and non-gait freezing enabled the use of functional magnetic resonance imaging (fMRI) to study how altered brain activation relates to altered motor behavior in patients with FOG. As such, the current doctoral project is situated at the intersection of the field of clinical neurology and the fundamental study of motor control.

In the next paragraph, a brief state of the art of epidemiology, symptoms, pathogenesis, pathophysiology and treatment in Parkinson's disease is provided. Next, the most characteristic features of freezing of gait are discussed. This paragraph (Paragraph 3), also reports on freezing episodes during other motor tasks ('non-gait freezing'), a phenomenon that opened a new avenue for FOG research. Key findings or uncertainties in literature that directly contributed to the research questions are emphasized. Paragraph 4 presents the overall aims of the doctoral project. Paragraph 5 provides an overview of the main methodological aspects applied in the studies. Last, the organization of Chapters 2 to 6 is outlined in Paragraph 6.

2. PARKINSON'S DISEASE

Epidemiology

Parkinson's disease is the second most common neurodegenerative disease after Alzheimer's disease and affects approximately 1-2% of the population aged over 60 years.[5] As a result of an aging population, the worldwide number of people suffering from PD by 2030 is estimated at 9 million.[6] In most cases, the cause of PD is unknown (idiopathic or sporadic PD).[7] Five to ten percent of patients present a hereditary form of PD. PD is a progressive disease, typically diagnosed at the age of 55 years[8] and lasts 20 years on average.[9] The risk for PD is 1.5 times greater in men compared to woman.[10]

Clinical picture

PD clinically manifests itself as a movement disorder accompanied by non-motor (cognitive, affective, psychiatric and autonomic) disturbances of varying severity (see **Box 1** for an overview of symptoms adapted from Jankovic, 2008[8]). The cardinal motor features on which a diagnosis of PD is primarily based, are bradykinesia, tremor and rigidity (muscle stiffness with increased resistance to passive movement) and gait and balance impairments.[8]

Bradykinesia or slowness of movement is the most characteristic feature of PD and is observable when patients have to perform rapid, sequential movements (such as finger tapping). Small, cramped handwriting (micrographia), reduced facial expression (hypomimia), impaired speech articulation (dysarthria) and other examples of lessened spontaneous movement have also been described as bradykinetic manifestations.[8] Similarly, Parkinsonian gait presents as slowed walking, with a marked reduction in stride length and arm swing. Tremor refers to unilateral, unintended movements at high frequencies (4-6Hz) that mostly appear in the distal extremities. Rest tremor commonly affects the hands and typically disappears during voluntary activity. Lips, chin, jaw and legs may also show rest tremor. In contrast, the amplitude of postural tremor assessed during outstretched position of the body part increases during action. Rigid muscles show an increased resistance to passive flexion, extension or rotation of the limb. Mild rigidity may be worsened by voluntary motion of the contralateral limb (Froment's manoeuvre). Rigidity occurs in distal and proximal body parts, can be painful and may result in postural deformities such as the typical stooped posture while walking in advanced PD.[8,11] Other examples of abnormal (axial) postures associated with PD are listed in Box 1. Postural instability or the loss of steady balance, is a major disabling problem in PD as it is a common cause of falls and injuries. [12,13] Balance and fall problems usually present with prolonged disease duration[13] though a recent follow-up study showed that also in early PD, 11% of subjects were habitual fallers and reported reduced quality of life as a consequence.[14] Postural instability arises due to the loss of postural reflexes[8], reduced central and peripheral sensation and knee extension strength.[13] Similar to gait disorders, balance problems and falls are less responsive to pharmacological treatment than the other cardinal features.[15] The cardinal features of PD can be evaluated by use of the Unified Parkinson's disease Rating Scale (UPDRS)[16] and the more recent version the MDS-UPDRS.[17] Based on the relative prominence of the scores of these symptoms, a patient can be categorized as belonging to the tremor-dominant or postural-instability-gait difficulty (PIGD) phenotype.[18] Freezing of gait, one of the most incapacitating gait problems, typically occurs in the PIGD type patients. It has been postulated that the rate of disease progression is more favorable in tremor-dominant compared to PIGD groups, though Vu et al. [15] recently found no such predictive value of subtypes over an 8-year follow up period.

Usually classified as a movement disorder, PD clearly involves a broad spectrum of cognitive, affective, sensory disturbances as well as impaired regulation of the autonomic nervous system. Motor and non-motor symptoms co-define patients' wellbeing and the burden to their lives and those of carers.[19] Cognitive deficits occur at a high frequency (23.5-28.2% have mild cognitive deficits[20]) and vary in severity between mild impairment in a single cognitive domain, global decline and dementia.[21] Typical problems involve attention, memory, language, visuospatial abilities and executive control.[21,22]

Box 1: Motor and non-motor symptoms in Parkinson's disease

Motor symptoms	Non-motor symptoms
Cardinal features: tremor, bradykinesia, rigidity, postural instability	**Cognitive impairment:** bradyphrenia, tip-of-the-tongue (word finding) phenomenon, executive dysfunction
Bradykinetic manifestations (1): hypomimia, dysarthria, dysphagia, sialorrhoea	**Psychiatric symptoms:** depression, apathy, anhedonia, fatigue, other behavioural problems
Bradykinetic manifestations (2): Decreased arm swing, shuffling gait, festination, difficulty arising from chair, turning in bed	**Sensory symptoms:** anosmia, ageusia, pain (shoulder, back), paresthesias
Bradykinetic manifestations (3): Micrographia, cutting food, feeding, hygiene, slow activities of daily living	**Dysautonomia**: orthostatic hypotension, constipation, urinary and sexual dysfunction, abnormal sweating, seborrhea, weight loss
Impaired inhibition of impaired reflexes: glabellar reflex, blepharospasm, dystonia, striatal deformity, scoliosis, camptocormia	**Sleep disorders:** REM behavior disorder, vivid dreams, daytime drowsiness, sleep fragmentation, restless leg syndrome

Explanation of motor symptoms: Hypomimia: reduced facial expression. Dysarthria: slurred, slow speech that is difficult to understand. Dysphagia: difficulty in swallowing. Sialorrhoea: excessive saliva. Festination: gait abnormality characterized by increasingly rapid but ever smaller steps.[23] Micrographia: abnormally small and cramped handwriting. Glabellar reflex: a primitive reflex of eye blinking in response to the first several taps on the forehead. Sustained blinking is abnormal. Bepharospasm: sustained, forced, involuntary closing of the eyelids. Dystonia: disorder that causes the muscles to contract and spasm involuntarily. Striatal deformity: abnormal postures mostly of the hand and feet.[24] Scoliosis: abnormal curving of the spine. Camptocormia: abnormal truncal flexion.

Explanation of non-motor symptoms: Bradyphrenia: slowness of thought. Apathy: state of emotional indifference. Anhedonia: the inability to experience pleasure from activities usually found enjoyable. Anosmia: olfactory disorder, namely the inability to perceive odors. Ageusia: loss of taste functions. Paresthesias: abnormal skin sensation described as burning, tingling or itching with no apparent physical cause. Dysautonomia: malfunction of the autonomic nervous system. Seborrhea: a form of skin inflammation mostly affecting face, scalp and torso. Rapid eye movement (REM) behavior disorder: sleep disorder with vivid, violent dreams and overt dramatic verbal and motor responses. Restless leg syndrome: a disorder in which there is an urge or need to move the legs to stop unpleasant sensations.

Source: Jankovic, 2008.[8]

Executive dysfunction is the most prominent cognitive feature, even in early stages of the disease.[22] Impairments in this domain are well documented though a uniform definition of executive functions (EF) is lacking.[21] On the one hand, EF is used as an umbrella term for attention and inhibition, task management, planning, monitoring and coding.[21,25] On the other hand, EF is associated with a supervisory system that flexibly distributes attentional resources to multiple facets of an ongoing task.[26,27] A recent review concluded medium to large effect sizes when comparing non-demented PD patients with controls on the 5 most commonly used tasks for EF: verbal fluency tasks, digit span, card sorting tests, Stroop tests,

Tower tests and Trail Making Tests.[21] As regards dementia, 80% of patients ultimately met the diagnostic criteria as disease progresses.[28-30]
There is a higher prevalence of psychiatric disturbances in people with PD compared to age-matched controls. Depression, apathy, anxiety, psychosis and problems with impulse-controlled behavior occur frequently. In line with symptoms of sensory (e.g. olfactory dysfunction) and autonomic failure (e.g. orthostatic hypotension), they often precede the classical motor complaints by years or decades.[31] Up to 40% of patients experience major or minor depressive disorders.[32] The prevalence of apathy is estimated at 60%, of anxiety disorders (mostly generalized anxiety disorder, panic disorder, social phobia and agoraphobia) between 20-49% and of visual hallucinations at 22-38% (see Gallagher et al., 2012[33] for review). Patients affected by neuropsychiatric problems have a higher risk for cognitive decline and nursing home placement.[22,34]

Pathogenesis and disease progression

The pathological hallmark of PD is the accumulation of the protein α-synuclein and formation of α-synuclein inclusion bodies, called Lewy bodies.[35] The exact cellular mechanisms of PD are not completely understood but the neurotoxic properties of misfolded α-synuclein are thought to play a pivotal role in inhibited synaptic vesicle recycling, altered mitochondrial respiratory regulation, oxidative stress and cell death.[36] Clinico-pathological correlations gave rise to the staging model of Braak and colleagues (2003).[37] In this framework, the cell-to-cell spread of Lewy pathology underlies disease progression (see **Figure 1** adopted from Halliday et al. (2001)[38] and Hawkes et al. (2010)[39]). According to Braak et al.[37] Lewy pathology in the periphery (entric nervous system), the olfactory bulb and the medulla oblongata accounts for olfactory and autonomous deficits (e.g. constipation) that characterize the preclinical stages (stages 1 and 2, before diagnosis).[36,38] In a prion-like manner[36], Lewy pathology further propagates into the brainstem (stages 3 and 4).[38] The appearance of cardinal motor symptoms is related to Lewy bodies in the substantia nigra pars compacta, the brain's main source of the neurotransmitter dopamine in the basal ganglia.[35] From micro to macro level, the consequences are depletion of dopaminergic neurons, altered neuronal oscillations, altered neuronal synchrony and impaired balance of basal ganglia-cortical circuits that drive motor and non-motor behavior.[7,40-42] These neuro-functional changes and particularly their effect on motor control are elaborated below. In stages 5 and 6, the limbic system and neocortical regions become affected causing neurobehavioral and cognitive impairment.[38] In clinical practice, the diagnosis of PD requires a combination of cardinal features, exclusion of other forms of Parkinsonism and response to the dopamine precursor levodopa.[8] By that moment, at least 50% of substantia nigra neurons is lost.[43] Hughes and colleagues[44] found that postmortem ascertained PD based on Lewy pathology concurred with neurologists' clinical diagnosis using the UK Parkinson's Disease Society Brain Bank Criteria in 91-92% of cases. After patients are diagnosed, functional decline can be classified into 5 stages of disease severity developed by Hoehn and Yahr[45] ranging from mild symptoms to increased immobility, balance impairment and chair or bed bound conditions (see Figure 1, lower panel).

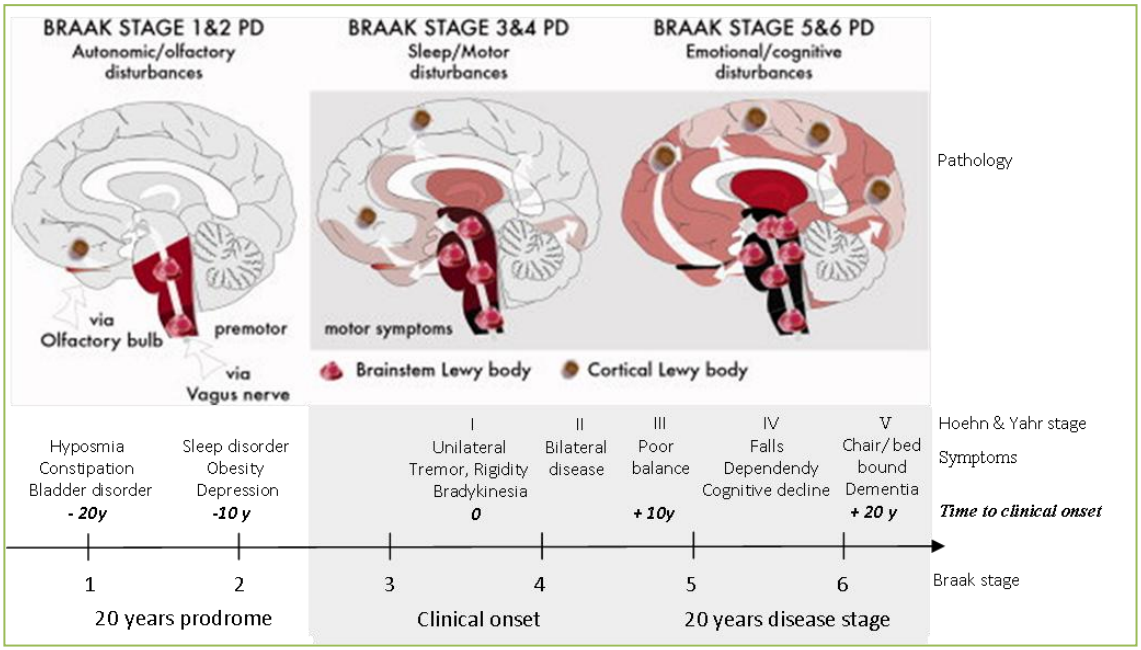


Figure 1: Progression of PD pathology and symptoms. The upper panel shows the spread of Lewy pathology from the medulla oblongata and olfactory bulb through the substantia nigra (clinical onset) to the later infiltration of Lewy bodies in cortical regions according to the Braak staging of PD (Braak et al., 2003). The lower part presents the Braak stages below a typical timeline of 20 years preclinical (before clinical onset, prodromal phase) and 20 years disease stage (after clinical onset). Above the timeline are the proposed symptoms. During the disease stage (grey zones), symptoms can be classified in 5 stages developed by Hoehn and Yahr in 1967.[45] Source: Halliday et al., 2001[38]; Hawkes et al., 2010.[39]

Impact of basal ganglia dysfunction on motor control

As emphasized by the staging model of Braak and colleagues[37], Lewy bodies affect the brain in a widespread manner. Still, the appearance of the classical motor features is mostly explained by neurodegeneration in the substantia nigra pars compacta (SNc) (**Figure 2**). Located in the midbrain immediately dorsal to the cerebral peduncles, the SNc regulates dopaminergic input to the basal ganglia.

The basal ganglia (BG) are deep grey matter structures located at the base of the cerebral hemispheres. The BG comprise a group of interconnected nuclei that are crucially involved in the regulation of movement through dense BG-thalamo-cortical connections and downstream projections to the brainstem and spinal cord (see **Box 2** for an overview of BG involvement in motor control adapted from Scott[46] and Redgrave et al.[47])The contribution of the BG in motor control is much debated but roughly entails the regulation of movement selection and initiation, also conceptualized as a 'gating function', suppression of unwanted motor behavior, generating automatic, well-learned skills, maintaining movement sequences, facilitation of motor learning and integrating motivational aspects of movement.[41,47,48] Cortical input is received in the striatal components of the BG: the caudate nucleus, the putamen and nucleus accumbens. These transmit excitatory glutamate either directly to BG output structures, i.e. the internal segment of the globus pallidus (GPi) and SN pars reticulate (SNr)) ('direct pathway') or first modulate signaling of the external part of the GP and subthalamus nucleus (STN) ('indirect pathway'). The connection between STN and output nuclei is inhibitory through the transmission of GABA. Stimulation of the direct versus indirect tracts within the BG has an opposite functional effect on BG targets. Inhibitory output of the direct pathway blocks tonic inhibition of the brainstem and thalamus resulting in a net

facilitation of movement. Conversely, the indirect output signals suppress movement. In this traditional view, the dual effect of dopamine transmitted from the SNc to striatal receptors serves as a core balancing mechanism between motor promotion through exciting D1 neurons of the direct pathway and motor suppression by inhibiting D2 indirect neurons.[41,47] In the Parkinsonsian state, reduced dopaminergic input to the striatum disrupts this internal BG balance which leads to hyperactive output structures (GPi and SNr) and to reduced motor behavior.[41,47]

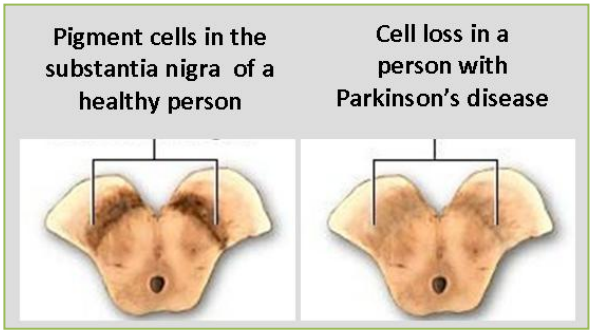


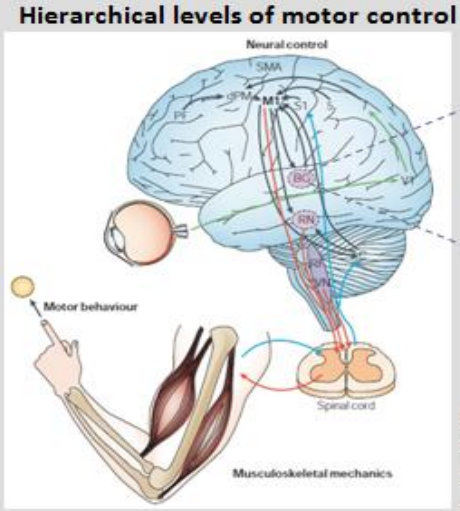
Figure 2: Illustration of cell loss in the substantia nigra in PD.

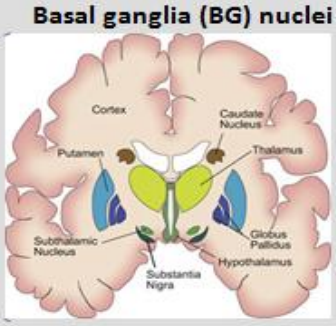
However, inspired by novel anatomical, behavioral and clinical findings, researchers have revisited the architectural and functional model of the BG described above. The lower panel of Box 2 shows the evolution of the original model of predictive or 'feedforward' signaling through direct and indirect pathways to a more complex organization of the BG.[47] For the purpose of this general introduction, two fundamental modifications of the traditional model will be discussed that refine our appreciation of BG dysfunction in Parkinson's disease:

1) Intrinsic signaling within the BG:[41,47] First, the 'direct' pathway may not be as direct as originally thought given the fact that branching collateral fibers on the input-output tract also terminate in the external part of the globus pallidus (GPe). Second, neural processing through the 'indirect' pathway may not be exclusively feedforward in nature as reciprocal connections exist between striatal nuclei, GPe and STN. The light grey arrows in the lower part b of Box 2 further indicate that dopamine projections from the SNc affect BG nuclei outside the striatum as well. Last, dopaminergic deprivation does not only affect the rate at which BG neurons fire, but deranges the degree of neuronal synchrony and thus the signal-to-noise ratio as well. This internal microcircuitry model impedes a straightforward prediction of how exactly local dopamine depletion in PD, changes BG output to brainstem and cortical motor networks and how this results in motor problems.

2) Topographical organization of parallel motor and non-motor loops:[41,47,49] It is now well established that besides a motor drive through the BG, associative (cognitive) and limbic commands are processed in parallel. **Figure 3** (adopted from Obeso et al.[41]) shows the spatially segregated loops from cortical motor, cognitive and limbic regions to their related subcortical target zones. The motor loop originates from the motor cortex (i.e. the primary motor (M1) and somatosensory cortex (S1), the premotor (PM) and supplementary motor area (SMA)) and projects through the sensorimotor portion of the striatum (posterior putamen) to dorsolateral parts of other BG nuclei. Cognitive information is mainly processed by the prefrontal cortex with dorsomedial BG targets including the 'associative striatum' (caudate nucleus and rostral putamen). Last, ventromedial domains of the BG including the nucleus accumbens receive input from frontal regions involved in limbic (affective, emotional) guidance of behavior.

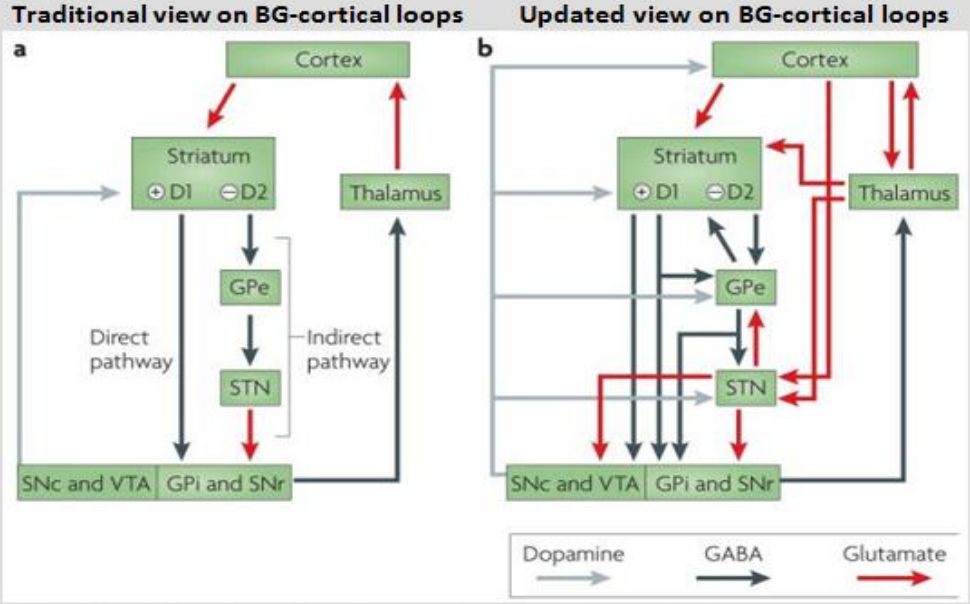
Box 2: Basal ganglia involvement in motor control





The basal ganglia comprise a group of interconnected nuclei.

Scott (2004) described three hierarchical levels of the central nervous system that drive the musculoskeletal system towards motor output: 1) Lowest level is the spinal cord including motor neurons and interneurons with a motor repertoire of stereotypical reflex patterns and basic locomotion patterns. 2) Brain stem motor regions (reticular formation, mesencephalic locomotor region, vestibular nuclei) select and enhance the spinal repertoire by improving postural control and oscillatory patterns of locomotion. 3) Cerebral cortex: supports a large and adaptable motor repertoire. **Source:** Scott, 2004.



In the traditional view, dopaminergic enervation from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) regulate the balance between the direct pathway that facilitates motor output and the indirect pathway that suppresses movement. In this model, increased BG output leads to reduced motor behavior in PD. Recent anatomical findings imply a more complex organization in which it is more difficult to predict how motor impairment arises. GPe, globus pallidus pars externa; GPi, globus pallidus pars interna; SNr, substantia nigra pars reticulata. **Source:** Redgrave et al., 2010

Two implications follow from this scheme with regards to the clinical image of PD: first, the anatomical location (and its functional specificity) of BG neurodegeneration determines the relative prominence of motor, cognitive and psychiatric symptoms and may imply a shift in the control mode of action. For example, dopaminergic depletion first affects the posterior part of the putamen and explains why early PD is best characterized by motor symptoms. These problems often involve movements that are normally performed automatically (habitual control) such as arm swing during walking. The associative striatum is comparatively spared and forces patients into an increased reliance on an attention-controlled (goal-directed) mode of behavior.[47] Second, dopaminergic dysfunction in PD appears to reduce the

degree of spatial segregation of BG loops[50,51] leading to an increased infiltration of non-motor information in the generation of motor output which may improve or disturb performance depending on the context.[51,52]

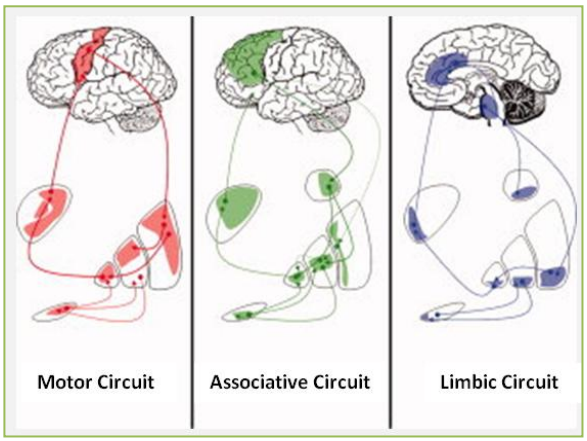


Figure 3: Topographical organization of motor, associative (cognitive) and limbic circuits through the basal ganglia. Source: Obeso et al., Mov Disord 2008.

In summary, the highly specified circuits of the BG in the normal state allow an integrative function of cortical cognitive, motor and emotional commands in behavioral control. PD pathology distorts the neurochemical and neurophysiological balance between multiple anatomical subregions of the BG and extends far beyond the dopaminergic nigrostriatal system. As a consequence, therapeutic approaches can have several target zones which are briefly elaborated in the next paragraph.

Therapeutic management of PD

Treatment of PD is not a main focus of this doctoral project and will only briefly be discussed. However, this paragraph serves as a theoretical background for the later description of different responses to medical and non-medical interventions in subgroups of patients with and without FOG that were included in the experiments of Chapters 2 to 5. Current medical therapies, most commonly pharmaceutical or surgical, are symptomatic, tackling motor and/or non-motor symptoms without modifying the disease progression. Typical pharmaceutical agents are designed to normalize the dopaminergic deficit either by replacing dopamine through dopamine precursors (e.g. Levodopa) or by activating dopamine receptors through dopamine agonists (e.g. pramipexole). Both are often combined with inhibitors of dopamine-metabolism enzymes (e.g. selegiline) that increase the duration of the drug effects.[53] Forty years after its introduction, Levodopa is still the gold-standard therapy for motor symptoms. LD significantly improves motor signs in a dose-dependent way and increases quality of life and survival. The effect of LD on postural instability, falls and cognitive deficits is however less beneficial.[54] In addition, severe motor complications such as involuntary repetitive movements (dyskinesias) and ON/OFF fluctuations are known sequelae of prolonged LD intake and disease duration.[55] Initially, periods in which the treatment effect is optimal (i.e. the 'ON' state of the medication cycle) are long and wear off (i.e. symptoms reappear, 'OFF' state of the medication cycle) only just before intake of the next dose. After 5 years, OFF periods become comparatively longer and occur unexpectedly in 39% of LD-treated patients and up to 60% of patients with a good response to LD.[55] In experimental settings, OFF periods are often induced by at least 12h withdrawal of medication (mostly

overnight) to better capture the symptoms under investigation. This practically defined OFF period is applied in the studies described in Chapters 2 to 4. Another related concept that is relevant to Chapters 2-5 is the levodopa equivalent dose (LED). This parameter describes the total daily antiparkinsonian medication a patient is receiving and is obtained by conversion of the active ingredient dose in non-levodopa drugs. As such, the LED of a drug is defined as '*that which produces the same level of symptomatic control as 100mg immediate release of levodopa*'.[56]

Non-dopaminergic treatments target the glutamatergic and cholinergic systems with varying benefits and side-effects (see Smith et al.[53] for an extensive review). It is noteworthy that cholinergic pathways involving the pedunculopontine nucleus (PPN) in the brainstem and the nucleus basalis of Meynert are currently under tight investigation as the basis of cognitive dysfunction, gait disorders and falls as well as their interplay (see Yarnall et al.[57] for review). In fact, alleviating cognitive impairment in synergy with motor improvement remains a challenge for pharmaceutical treatments, especially in the advanced stages of PD.[53]

Deep brain stimulation (DBS) is the most common neurosurgical intervention for PD patients and particularly for those with drug-refractory tremor and drug-induced motor complications.[58] Electrodes implanted into STN deliver continuous high-frequency stimulation and improve motor symptoms, ON/OFF fluctuations and dyskinesias. However, it has been put forward that the effects of STN stimulation wean off more quickly with time in axial compared to appendicular symptoms and particularly with respect to gait and falls.[59] Apart from STN and GPi, the PPN has been added to the list of potential target sites.[60]

Besides medical treatment, meta-analyses support the beneficial effect of motor training, physical and cognitive exercise on mobility, balance, quality of life and cognition as well as potential neuroprotective effects at least in animal models.[61-64] Physical therapy can reinforce remaining brain resources or bypass the dysfunctional BG circuits engaged in automatic control of movement by incorporating attentional strategies and external sensory information such as visual or auditory cueing in the generation and continuation of movement,[65-67] consistent with switching from habitual to goal-directed motor control.[47]

3. FREEZING OF GAIT

Definition and clinical importance of FOG

Freezing of Gait (FOG) is defined as '*a brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk*'.[68] FOG is a peculiar gait disorder whereby patients suddenly stop involuntarily as if their feet are glued to the floor. These short-lasting cessations of locomotion occur most frequently in the later stages of PD (70%)[69,70] but can also be seen in 26% of early stage patients who were not yet exposed to levodopa therapy.[71] FOG develops independently of the cardinal features of PD suggesting, in part diverging pathological mechanisms.[71,72] In addition to causing reduced mobility[73] and increased risk of falls and injuries,[13] FOG clearly downgrades socio-emotional dimensions of patients' quality of life.[73] Patients feel embarrassed and frustrated when they suddenly freeze in public.[73] The effect of medical and rehabilitation treatment on FOG is beneficial but not to the same extent as reported for other PD symptoms.[74,75] FOG is a transitory phenomenon, lasting only a few seconds, but occurs against a background of other, more continuous abnormalities that predispose patients to freeze in certain circumstances. As such, the study of FOG implies a challenging integration of episodic features such as the

circumstances that evoke FOG ('triggers') or the changes in motor output during an episode on the one hand, with background differences in the clinical profile of patients with and without FOG on the other hand.

Episodic features of FOG

Patients rarely freeze in open spaces without an apparent trigger.[68,76] FOG occurs most frequently when subjects turn, start, pass through narrow doorways, negotiate obstacles or reach their destination.[68,76-79] These circumstances require a flexible adaptation of the gait pattern with an elevated level of attentional control. If attentional resources are compromised by asking patients to perform a secondary task while walking (dual tasking), the risk for FOG further increases.[80-83] In fact, Spildooren et al.[82] showed that 360° turning in combination with a dual-task is the most important trigger for freezing. Emotional factors such as stress or anxiety also play a role.[80,84] This may explain why in daily living, FOG is often experienced in crowded areas or when trying to reach a ringing telephone. A FOG episode usually lasts less than 10 seconds after which regular gait is regained.[76,85] In most cases, subjects still make stepping movements but these are inefficient, small and rapid ('festinating behavior') with no or incomplete clearance from the ground surface ('shuffling movements'). Another common characteristic of FOG shown in **Figure 4**, is the presence of high frequency (3-8Hz), trembling-like movements in the legs.[76,86] In contrast to tremor, the alternating leg oscillations during FOG cover multiple frequencies in the absence of a dominant one, as determined by spectral analysis. This broadband frequency distribution has been associated with multiple anticipatory adjustments during a FOG episode[87] and can be applied in online detection devices for FOG.[85,88,89] FOG can also manifest itself without any stepping or leg movements but this complete akinetic type of freezing is rare.[76]

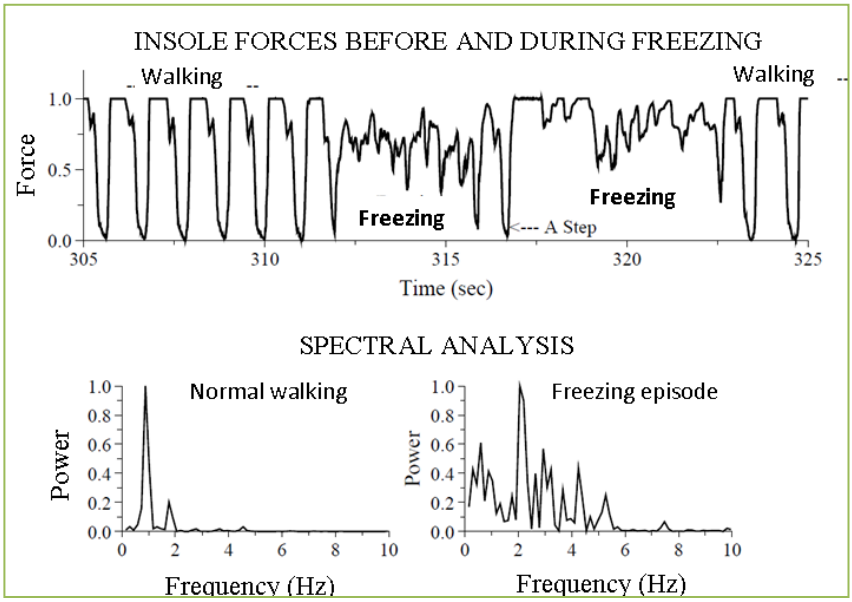


Figure 4: Example of freezing episodes in between periods of normal walking. The top panel shows the insole force during normal walking and freezing episodes. The bottom shows the corresponding frequency distribution indicating the presence of multiple high-frequency signals during FOG. Additional information on spectral analysis methods is given in **Box 3** (see Paragraph 5). Source: Hausdorff et al., 2003.[86]

Some spatiotemporal gait abnormalities precede the motor block. These include unusual timing of lower leg muscles[90] and a combination of progressively decreased stride length and increased cadence during the prefreezing steps.[91-93] When patients with FOG are forced to take smaller steps, the step-to-step reduction in stride length (sequence-effect) is more pronounced, resulting in a higher frequency of FOG episodes.[93]
With the identification of FOG-eliciting conditions, the question arises as to why these triggers cause a dramatic breakdown of locomotion in patients with FOG and not in their counterparts? This different response to triggers led to the hypothesis that patients with FOG have a certain clinical profile of more pronounced motor, attentional or emotional impairments that predisposes them to freeze. Before discussing the so-called background abnormalities, the next paragraph will summarize the literature on what is known about freezing in other movements.

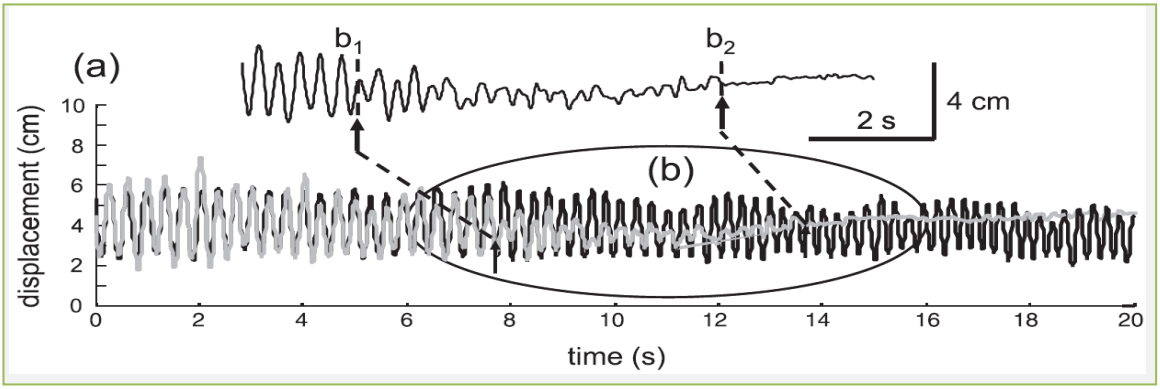


Figure 5: Example of upper limb freezing during an alternating writing task. Point b1 indicates the onset of amplitude attenuation leading up to freezing in the right hand (b2). Source: Nieuwboer et al. (2009).[4]

Freezing in other motor tasks ('non-gait freezing')

The research aims of this doctoral project were largely driven by reports in the literature of freezing-like motor blocks in various movements other than gait. Similar to locomotion, these motor tasks were rhythmic and repetitive in nature but they involved different effectors. Moreau et al.[94] found that oral festination during speech in PD patients was correlated with gait festination, a key feature of FOG. Motor blocks were also described during writing or tooth brushing.[1] Experimental settings confirmed the occurrence of upper limb freezing in a manual tapping task[2] and alternating hand movements.[3,4] Although, visually the freezing episodes seemed remarkably similar to FOG, the actual nature of the episodic signals had never been compared using quantitative methods. The kinematic signals just before an upper limb freezing episode seemed to resemble the stride length reduction preceding gait freezing (**Figure 5**).[98] Moreover, upper limb freezing (FOUL) severity was correlated to the severity of FOG, suggesting some shared underlying motor impairment.[98] However, general spatiotemporal abnormalities in functional hand movements (in the absence of freezing episodes) were less clear in freezers than expected based on their scaling and timing problems during ongoing gait. In addition, it was not clear if sensory cueing strategies such as auditory pacing would enhance upper limb performance in freezers to the same extent as in non-freezers. This question stems from a recent review on cueing effects on the walking pattern of PD patients which indicated reduced effectiveness of this rehabilitation technique in freezers as compared to non-freezers.[95] It was suggested that patients with FOG may have

a smaller capacity for compensation, especially in attention demanding situations.[95] Willems et al.[96] showed that freezers can increase stride length when auditory cues are offered at baseline frequency but, unlike non-freezers, their steps became smaller at higher frequencies. In addition, freezers showed evidence of greater dependence on the actual presence of the cue.[96] However, it was not clear if cueing and cueing withdrawal affected repetitive upper limb movement differently in freezers and non-freezers.

Altogether, **these findings raise the fundamental question as to how FOG is related to FOUL. To address these questions, Study 1 and 2 of this doctoral project were set-up to ascertain the behavioral correlates of the two phenomena.** In contrast to earlier studies[1,3,4] Study 1 systematically compared kinematic changes before and during upper limb freezing with FOG-related motor abnormalities using quantitative measurements sensitive to scaling and timing dyscontrol. Study 2 addressed the current uncertainty whether problems in amplitude-rhythm control that were found to persist during gait in between FOG episodes (ongoing gait), also affect upper limb control in freezers and whether their performance is worsened by the effect of cue-withdrawal. If a clear link between episodic and continuous motor abnormalities associated with FOG and FOUL could be established, the conceptualization of the notorious freezing phenomenon in PD would shift from a primary gait disorder to a generalized motor deficit. Building further on this new conceptualization, Study 3 examined the shared neural mechanisms of FOG and FOUL (see below: Neural determinants of FOG).

Background motor abnormalities associated with FOG

Comparing ongoing locomotion (i.e. gait in the absence of FOG) between freezers and non-freezers, numerous studies have shown abnormalities in spatiotemporal properties in freezers, particularly when OFF medication.[97-99] This suggests that freezers' locomotor control reaches a functional level without being entirely normal.[100] Patients with FOG produced smaller steps[92,101] and showed impaired regulation of rhythm, as expressed by greater stride-to-stride variability while walking.[97] In addition, inter-leg coordination in patients with FOG was less stable and more asymmetrical.[98-99] Freezers also displayed different turning behavior characterized by augmented cadence as the complexity of the turn increased[82] and an abnormal head-pelvis coupling.[102] The latter is also referred to as turning 'en-bloc' and might be compensatory for postural problems.[103] Indeed, postural components associated with FOG include impaired balance control[23] and a faulty coupling between postural preparation and the stepping command, most evident at gait initiation.[87] Whether more severe proprioceptive deficits in patients with FOG compared to those without[104] contribute to their balance problems, is currently unclear.

Cognitive and affective background abnormalities associated with FOG

Gait in PD is more attention-controlled than in healthy elderly.[105] It is further known that FOG is likely to occur in situations which pose additional attentional demands such as dual task walking conditions.[82,83] This is in line with the fact that attempts to (re-) focus attention on the locomotor task, for example by use of auditory cueing, sometimes alleviate symptoms.[95,96,106] The ability to keep different (motor or non-motor) ongoing tasks online and flexibly shift between them is called 'set-shifting'. There is converging evidence of a deficit in this component of executive function in freezers.[52,107] Findings of impaired

inhibition of unwanted responses and response selection (jointly called conflict resolution) support a frontal executive dysfunction hypothesis underlying FOG.[108,109] In addition, visuospatial deficits were reported in freezers and may influence the way gait is to be adapted to narrow passages.[110,111] Anxiety and panic attacks in freezers[80,112] may further increase the propensity for FOG. However, **it remains unclear if and how these motor and non-motor aspects interact and which is the most determining factor in developing FOG as most studies were based on univariate analyses or single-domain hypotheses. Therefore, study 4 of this doctoral thesis was aimed to clarify the relative contribution of the clinical features of patients in determining the FOG-symptom using for the first time a multivariate analysis.**

Neural determinants of FOG

In line with reduced automaticity due to a deficit in the striatofrontal drive of movement in PD, ample brain imaging studies have indicated increased engagement of the cerebello-premotor-parietal motor network in PD compared to healthy controls during various motor tasks including gait.[113-115] This relative hyper-activation is envisaged as compensatory in order to equate motor performance to the required output. Neuroimaging of gait in freezers and non-freezers is hampered by a number of technical difficulties related to the scanner environment. Therefore, current neural hypotheses of FOG rely on integrating the behavioral abnormalities in freezers described above with results of medical interventions and recent findings obtained by brain imaging methods that allow subjects to lie still in the scanner. **Table 1** gives an overview of such studies comparing structural or functional elements of brain organization in freezers with non-freezers.

The impact of levodopa and deep brain stimulation of the STN on FOG,[74,76,116] suggests an important involvement of nigrostriatal dopamine deficiency in the etiology of FOG. This is supported by altered dopamine and glucose metabolism in the putamen and caudate of freezers, possibly reflecting a more advanced disease stage.[117] Deficient BG output signaling may further pervert appropriate feedforward signaling to cortical motor preparation areas (Supplementary Motor Area (SMA) and premotor cortex (PMC)) and has been related to the difficulty of freezers to generate and maintain movement amplitude.[92,118,119] Interestingly, Snijders et al.[120] found reduced brain activation in the SMA of freezers during gait planning in combination with increased recruitment but reduced grey matter of the mesencephalic locomotor region (MLR). The MLR is a complex area located dorsomedially to the PPN and has dense connections with the BG, cerebellar and cortical regions.[121-123] Cholinergic neurodegeneration in the PPN region has received growing interest in the etiology of gait and posture difficulties in PD.[60,124-127] The possibility that FOG in part originates from PPN dysfunction is supported by Schweder and colleagues[128] who recently showed altered structural cortico-pontine-cerebellar connections in a small group of freezers compared to non-freezers. In addition, PPN DBS showed modest to large beneficial effects on the occurrence of FOG episodes.[129-132] This also would fit the behavioral observations of high-frequency trembling-like leg movements during freezing episodes, which have been related to misfiring spinal neurons that are driven by faulty PPN input[23,86,91] but this remains to be investigated.

Two recent studies found more severe grey matter atrophy in freezers than non-freezers in fronto-parietal areas.[133,134] These findings are corroborated by reduced cerebral blood flow and resting state functional connectivity in similar regions[135-137] and relate well with

converging evidence of executive dysfunction in freezers.[52,108,109] A parietal-premotor deficit in freezers has been associated with problems in sensorimotor integration.[133,134] Similarly, Lyoo et al., [138] found that FOG improvement by STN DBS was mediated by metabolic changes in parietal-occipito-temporal loop for sensorimotor processing. Difficulties in incorporating proprioceptive and external sensory information into the motor command may lead to the reduced efficiency and sustained dependence of auditory cues that was observed in freezers.[75,106]

To conclude, the studies described above point to a widespread origin of FOG including fronto-parietal cortical regions, basal ganglia and midbrain motor areas. The specific regions yielding differences between subgroups of freezers and non-freezers do not converge entirely and seem to depend on the brain tissue (e.g. white or grey matter) or neural mechanism (e.g. resting state blood flow or task-related BOLD) under investigation. Still, studies that employed a comparable neuroimaging technique revealed conflicting results as well. For example, it is currently unclear why Matsui et al.[136] found regional cerebral blood flow (RCBF) in the orbitofrontal cortex to be reduced in freezers vs non-freezers while anothor group[137] reported increased RCBF in freezers in this region. A heterogeneous clinical profile including patients of Hoehn and Yahr stage 5 and a lower methodological quality of the study of Imamura et al.[137] may contribute to the diverging results. Medication state might also have influenced the CBF findings but is not described by Matsui and colleagues.[136] Similarly, a more advanced patient group and different statistical approach (e.g. ROI[120] vs whole brain analysis with[133] or without[134] conjunction analysis) may explain why regions with grey matter atrophy in freezers revealed by VBM do not concur from one study to another. Some of the obtained results may also be confounded by small number of patients[128] and differences in disease severity between the subgroups of freezers and non-freezers.[133] Most importantly, **it is difficult to directly link these brain changes to the emergence of episodes of abnormal motor output. Study 3 was set up to address this lacuna in current knowledge on the neural correlates of freezing of gait. We investigated motor performance during the freezing-provoking upper limb task that was validated in Study 1 and 2, in freezers and non-freezers with comparable disease profiles in an fMRI environment. This approach allowed to measure brain activation during actual freezing episodes for the first time.**

Table 1: Overview of neuroimaging studies on FOG

Article	Participants	Imaging technique	Brain measure	FOG-related brain areas PD with FOG versus PD no FOG
Structural brain imaging studies				
Schweder et al. *Neuroreport 2010*[128]	2 PD+FOG (?) 8 PD-FOG (?) 17 CTRL	DTI *(around PPN)*	WM connectivity	Reduced WM connectivity in PD+FOG in: - **Pontine-cerebellar projections** increased WM connectivity in PD+FOG in: - **Cortico-pontine projections**
Snijders et al. *Brain 2011*[120]	12 PD+FOG (OFF) 12 PD-FOG (OFF) 21 CTRL	VBM (ROI)	GM volume	Reduced GM volume in PD+FOG in: - **MLR**
Kostic et al. *Neurology 2012*[133]	17 PD+FOG (OFF) 20 PD-FOG (OFF) 34 CTRL	VBM	GM volume	Reduced GM volume in PD+FOG in: - Left **Inferior frontal gyrus** - Left **Precentral gyrus** - Left **Inferior parietal gyrus**
Tessitore et al. *Am J Neuro-radiology 2012*[134]	12 PD+FOG (ON) 12 PD-FOG (ON) 12 CTRL	VBM	GM volume	Reduced GM volume in PD+FOG in: - Left **precuneus** - Left **cuneus and lingual gyrus** - Left **posterior cingulated gyrus**
Functional brain imaging studies				
Matsui et al. *Mov Disord 2005*[136]	24 PD+FOG (?) 31 PD-FOG (?)	(^{123}I-IMP) SPECT	RCBF	Reduced RCBF in PD+FOG in: - **Orbitofrontal cortex** : BA 11
Imamura et al. *Acta Neurologica Scand 2012*[137]	21 PD+FOG (OFF) 34 PD-FOG (OFF)	(^{123}I-IMP) SPECT	RCBF	Increased RCBF in PD+FOG in: - **Orbitofrontal cortex** (BA11) - Dorsal **Anterior Cingulate**
Bartels et al. *Mov Disord 2006*[117]	9 PD+FOG (ON) 7 PD-FOG (ON) 8 PD+FOG 6 PD-FOG	FDOPA-PET (striatum) FDG-PET (whole brain)	Dopamine uptake Glucose uptake	Reduced dopamine uptake in PD+FOG in: - **Putamen & Caudate Nucleus** Altered glucose uptake in PD+FOG in: - posterior **Putamen** (increased) - **Caudate Nucleus** (reduced) - **Inferior parietal regions** (reduced)
Snijders et al. *Brain 2011*[120]	12 PD+FOG (OFF) 12 PD-FOG (OFF) 21 CTRL	fMRI	Task related BOLD signal	Increased task related BOLD in PD+FOG in: - **MLR** Reduced task related BOLD in PD+FOG in: - **SMA**
Tessitore et al. *Parkinsonism Relat Disord 2012*[135]	16 PD+FOG (ON) 13 PD-FOG (ON) 15 CTRL	fMRI	Resting state FC	Reduced FC in PD+FOG in: - **Frontoparietal network**: MFG and AG - **Occipito-temporal network**

The results shown in this table are restricted to the comparison of Parkinson patients with freezing of gait (PD+FOG) to PD patients without FOG (PD-FOG). ON or OFF labels in brackets indicate whether patients were tested with medication intake or after medication withdrawal. Abbreviations: CTRL: control subjects; DTI: Diffusion Tensor Imaging; VBM; Voxel Based Morphometry; SPECT: Single Photon Emission Computed Tomography; PET: Positron Emission Tomography; fMRI: functional Magnetic Resonance Imaging; WM: White Matter; GM; Grey Matter; RCBF: Regional Cerebral Blood Flow; BOLD: Blood Oxygen Level Dependent; FC: Functional connectivity; MLR: Mesencephalic Locomotor Region; BA: Brodmann Area; SMA: Supplementary Motor Area; ROI: Region of interest.

4. AIMS OF THE DOCTORAL PROJECT

The above review of the literature has shown that though freezing of gait in Parkinson's disease has received considerable attention in recent years, it remains unclear what the nature of motor abnormalities related to FOG is, what the underlying neural correlates are and how motor and non-motor determinants interact in the development of the symptom. Therefore, the overall aim of this project is to understand the underlying mechanisms of FOG at the behavioral and neural systems level in four related studies. Study 1 and 2 pick up on the current lack of insight whether motor impairments related to FOG are gait-specific or represent a generic dysfunction in amplitude or timing-regulation of limb dynamics. A novel paradigm of upper limb freezing was developed to carefully compare spatiotemporal aspects of FOUL to FOG (Study 1) and to determine whether ongoing upper limb movement displays similar timing and scaling difficulties as described during functional gait in freezers (Study 2). This experimental paradigm further allowed to investigate which brain mechanisms mediate the motor components of freezing in PD in Study 3. Last, Study 4 focused on the co-occurrence of motor and cognitive features in patients with FOG and examined the independent contribution of these factors in a determinant model of FOG.

5. MAIN METHODOLOGICAL ASPECTS OF THE STUDIES

Patient recruitment and clinical examination

Patients with a Hoehn and Yahr stage II-III during ON phase were recruited from the Movement Disorders Clinic of the University Hospital Leuven. Patients who had DBS surgery or showed signs of clinical dementia (Mini Mental State Examination score (MMSE) <24) were not included in the studies. For Study 3 involving brain imaging, patients with claustrophobia and ferromagnetic implants were excluded. Before participation to any of the studies, all patients were visited at home for an extended clinical examination in the ON phase. This included the following assessments:

General disease characteristics were measured by the Unified Parkinson's Disease Rating Scale,[16] Hoehn and Yahr staging[42] and L-dopa equivalent dose (LED) intake (mg/day).[139]

Gait and balance tests included the Timed Up and Go Test (TUG),[140] a short version of the Berg Balance Scale[141] (BBS, items 8, 11, 13 and 14), 2 minutes walk test, the revised version of the Freezing of Gait Questionnaire NFOG-Q[142] and a questionnaire assessing falls and near falls during the last 3 months. The NFOG-Q was assessed in combination with a video showing examples of different types of FOG to ascertain valid responses on the 3 categories of questions: 1) item 1 discriminates between patients with or without FOG asking if freezing occurred during the last 3 months; 2) items 2-6 on objective severity of FOG (frequency and duration of FOG episodes) and 3) subjectively experienced severity of FOG (items 7-9). A positive score on item 1 of the NFOG-Q classified a patient as 'patient with FOG', a score of 0 as 'patient without FOG'.[142]

Though the unpredictive character of FOG poses challenges for measurement, recent work in collaboration with our research group showed that the NFOG-Q provides a reliabile and sensitive tool to detect FOG and assess its severity.[142] A non-gait freezing questionnaire was used to assess freezing in one of 8 known freezing-sensitive movements from daily life[1] (i.e. writing, tooth brushing, stirring while cooking, manipulating screw driver, feet wiping, typing, cutting food, talking) or another self-reported movement.

Cognitive outcomes were the MMSE and the cognitive section of the Scales for Outcomes in PD (SCOPA-COG).[143] The SCOPA-COG is a global cognitive measure, and reported to be more sensitive to cognitive decline in PD than the MMSE. It provides subscores on 4 domains: Memory, Executive function, Attention and Visuospatial abilities.

Studies 1-3 compared task performance of three groups: patients with FOG, patients without FOG and healthy age-matched control subjects. Patient groups were tested in the practical defined OFF phase and were matched for disease severity (Hoehn and Yahr stage, UPDRS score) and disease duration in OFF (Study 1 and 2) or ON (Study 3). Study 4 was based on clinical scores obtained in ON of 2 groups: patients with FOG and patients without FOG, matched for disease severity.

Experimental paradigm of upper limb freezing used in Study 1, 2 and 3

A freezing-provoking upper limb task served as central experimental paradigm for Study 1, 2 and 3 in which task performance was compared on the behavioral (Study 1 and 2) and neural level (Study 3) between patients with FOG, patients without FOG and healthy controls. Subjects performed a bimanual task of rhythmic flexion and extension movement of the index fingers in a 2x2x2 factorial design with manipulations in coordination pattern, amplitude and frequency. Subjects performed either in-phase movements requiring simultaneous activation of homologous muscles or anti-phase movements where left and right fingers alternate with a phase shift of 180°. The spatiotemporal coupling between fingers in these preferred coordination modes is considered part of the natural coordination repertoire and can be performed relatively automatically.[144] As is the case for gait, bimanual control may however be more effortful in elderly and PD patients, especially in the anti-phase condition. Therefore all subjects were given sufficient time to familiarize themselves with task requirements and achieve automaticity of movement. Before testing, a single anti-phase trial served to compute subject-specific comfortable amplitude and frequency. During testing, large-amplitude (comfortable=100%) or small-amplitude movements (66%) were required. Movement frequency was normal (comfortable=100%) or fast (133%). These settings were determined during pilot study. Based on the FOG-eliciting effect of small and rapid steps during gait, it was expected that small amplitude and fast movements during anti-phase coordination would provoke FOUL, whereas other conditions would be relatively freezing resistant. Auditory pacing guided the first 6 movement cycles to enable correct frequency manipulations. Hereafter, the cue was withdrawn and rhythm was to be maintained by means of internal movement generation for 25 seconds.
Using this experimental paradigm, we exploited the potential overlap in spatiotemporal bilateral movement control, crucially involved in fluent locomotion and bimanual coordination. Studies 1, 2 and 3 therefore looked at shared mechanisms of upper and lower limb (dys)control without disregarding the fact that gait-specific features (e.g. postural control) are also important in FOG.
Figure 6 shows the measurement equipment used in the behavioral studies (panel A and B) and fMRI study (panel C). In both settings, angular finger displacements were registered by means of encoders placed on the rotation axis of the index finger. For studies 1 and 2, analogue encoder signals were digitized via a Micro 1401 Acquisition Unit (CED, UK) and processed in Spike 2 and Matlab 7.7 (Mathworks, Sherborn, MA). In study 3, patients were lying supine in the scanner with their forearms in an orthosis that contained a non-ferromagnetic shaft encoder. Movement data were processed in Labview and Matlab.

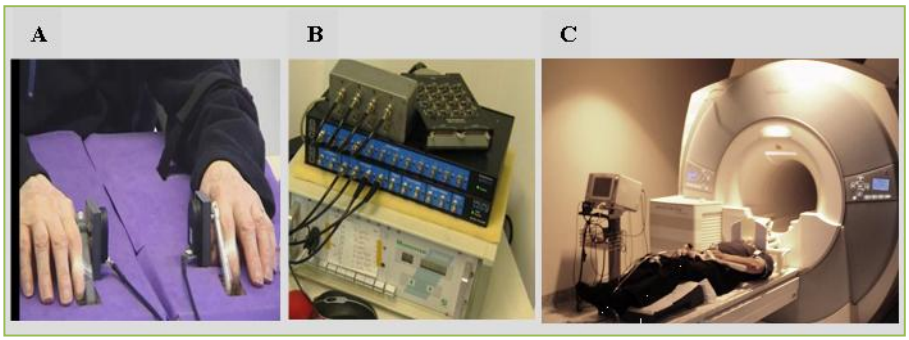


Figure 6: Measurement equipment for finger movement registration during behavioral (Study 1 and 2; panel A and B) and Neuroimaging studies (Study 3; panel C)

Behavioral analysis

Analysis of kinematic time series adopted in Study 1-3 generally included two steps: detecting FOUL episodes and determining spatiotemporal parameters of non-freezing upper limb movement (continuous data) (see **Figure 7**).

Detection of upper limb freezing episodes: In analogy to the definition of FOG, we defined FOUL episodes as '*a period of involuntary stop or clear absence of effective cyclic movements*'.[145] Thus, both periods with a complete halt as well as severely disrupted motion with a nearly complete loss of movement were classified as freezing episodes. At the start of the doctoral project, there was no consensus on how to detect freezing of gait episodes from obtained gait signals. Most gait analysis studies used post-hoc video observation to clinically detect FOG episodes.[72,76,88-91,93,97,146-148] Start- and end-criteria to demarcate the episodes were rarely validated.[76,93,146,147] In addition, quantitative assessment methods to measure the phenomenon of non-gait freezing were not available. A purely automated method to detect the episodes proved not feasible. Therefore, spatial and temporal criteria were developed in Study 1 which enabled subdividing kinematic time series into FOUL and continuous movement. More specifically, a previous study from our group had shown that a FOG-episode was preceded by highly abnormal stepping with an approximately 50% amplitude reduction and a non-linear cadence increase.[91] Therefore, we defined the beginning of an upper limb freezing episode as the onset of abnormally small motion cycles (<50% of initial amplitude) accompanied by an irregular cycle frequency, as illustrated in Figure 1 of chapter 2. Termination of the freezing episode was defined by the moment when a regular amplitude and rhythm was adopted for at least two movement cycles. These normal movement cycles were not included in the freezing episode. If a FOUL episode was not followed by a period of regular movement, the end of the episode was determined as the finish of the movement trial. The minimal duration of a freezing episode was set at 75% of the reference cycle duration to avoid misclassification of small disruptions due to pattern switches. Onset and termination of FOUL episodes were demarcated using visual markers by two independent observers blinded for freezing status of the subjects.
This method ensured an objective and reproducible detection method, which was found to be reliable between four investigators (ICC(2,4)=0.99). In Study 1, the frequency components of FOUL time series were further addressed by spectral analysis through which the 'freezing index'[85] of a signal could be defined (see **Box 3**). In Study 3, this quantitative indicator of FOUL was embedded in the detection method in Matlab which fine-tuned the demarcation steps (**Figure 8**). To validate this procedure, time series were exposed to two clinical experts

in FOG, who corroborated whether the episodes reflected freezing, using a random sample of signals. This proved to be a reliable detection method (ICC (2,2)=0.94).

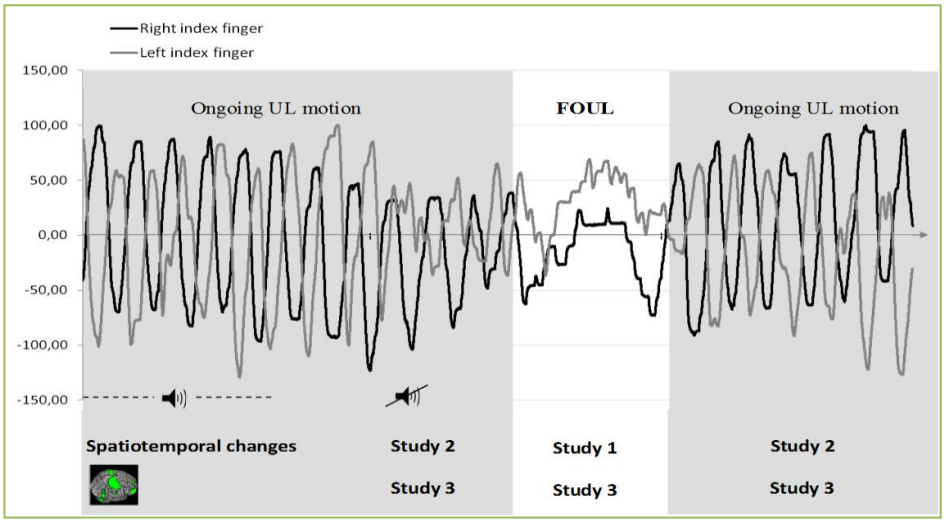
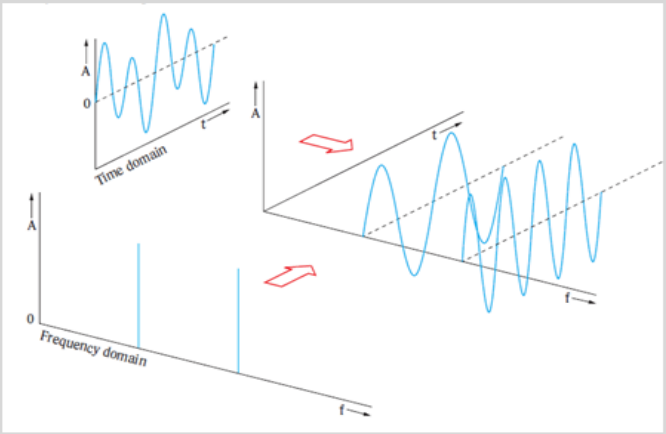


Figure 7: Subdivision of kinematic time series in ongoing movement with or without cue and freezing episodes (FOUL).

Box 3: Spectral analysis



Spectral analysis, also known as frequency analysis, is a common technique in signal processing and can be used to examine frequency components in (kinematic) time series. In the case of upper limb movement, these time series are oscillatory with amplitude that varies over time ('Time domain'). One could summarize the global temporal behavior of a signal by computing the average angular frequency of the wave form, accompanied by its variability. However, in some cases it is more meaningful to look at the relative proportion of multiple frequency components in the signal ('Frequency domain'). Think of a PD patient who moves his finger at a stable frequency of 1Hz, but at the same time shows tremor in the form of rapid oscillatory movement around a frequency of 4Hz. An average frequency of 2.5Hz would not be clinically representative in this case. Decomposing the main signal into a sum of sine and cosine signals of different frequency and amplitude results in a Fourier series and is applied in spectrum analysis based on the Fast Fourier Transform (FFT). The relative strength (i.e. the power) of each frequency-specific subsignal can be visualized in a power spectrum graph as was applied for gait signals in Figure 4. In the context of locomotion and FOG, the temporal abnormalities were quantified by the Freezing Index (FI). FI refers to the ratio of power in high (dysfunctional) frequency bands (3-8 Hz) to the power of low (functional) frequency bands (0.5-3Hz). Moore et al., (2008) showed that FOG can be detected with a sensitivity of 89% using individually defined cutoff values for FI. We used spectral analysis and the FI to examine if freezing during upper limb motion represents similar frequency abnormalities.

Continuous movement analysis: Of the remaining continuous (non-freezing) motor signals, amplitude, frequency and relative phase parameters were computed based on peak-to-peak measures of the end-effectors motions. This way, mean and variability of outcome measurements were determined for movement periods guided by the auditory cue (first 6 movement cycles) and movement after cue-withdrawal. Study 2 describes the spatiotemporal difficulties during ongoing movement and how these were influenced by cue-withdrawal.

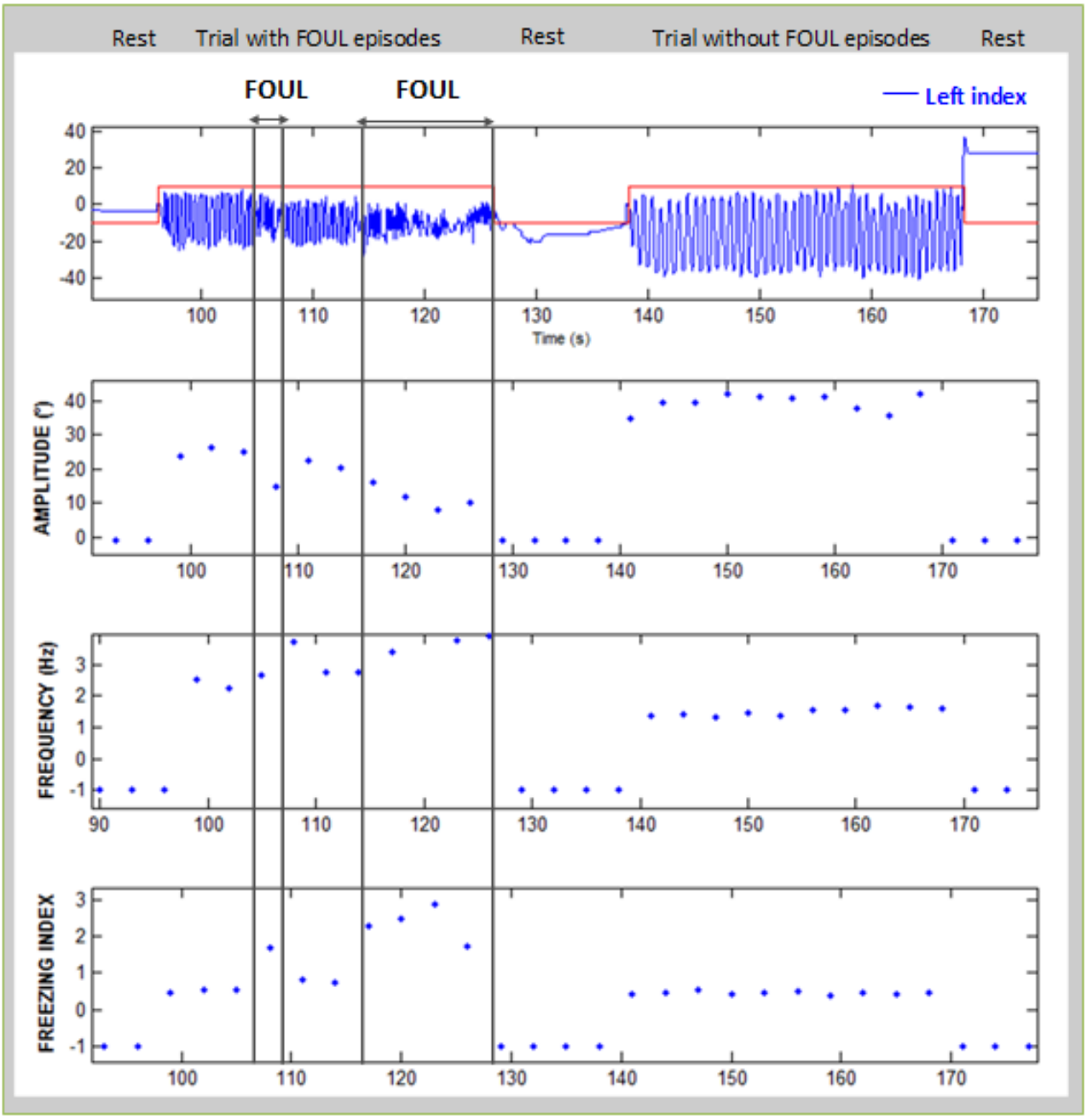


Figure 8: Freezing detection on upper limb kinematic time series obtained during scanning (Study 3). At the top panel, motion of the left index finger is depicted during 3 rest conditions (flat signal) and 2 movement trials (oscillating signals) in congruence with the red horizontal line (-1= rest; 1= movement trial). Below the evolution of movement amplitude, frequency and the freezing index is shown. During rest, these parameters are arbitrarily set at -1. Two freezing episodes (FOUL) occur during the first movement trial, none during the second. We used objective criteria to detect freezing episodes based on changes in amplitude (reduction > 50%), frequency (hastened, irregular), freezing index (>1). At least 2 of the criteria were to be met in order to identify a movement episode as FOUL. The vertical lines represent visual markers that were used by the 2 clinical experts in FOG to demarcate the freezing episodes.

Analysis of fMRI time series (Study 3):

We used fMRI to examine the neural basis of motor abnormalities associated with FOG and FOUL. Functional brain MRI is a non-invasive neuroimaging technique with a relatively high temporal and spatial resolution. Patients lie in the magnetic bore that elicits a strong but harmless magnetic field (for example with a strength of 3 Tesla). This static magnetic field makes hydrogen atoms in the human body spin with a certain (high) precession frequency in a low energy state. When a radio frequency (RF) pulse that oscillates at the resonance frequency of the
spins is applied, the dipoles of hydrogen atoms re-orientate back to their original orientation. Switching the RF pulse off induces a shift from a high-energy to a low-energy state of the atoms. The MRI image displays a tissue contrast that depends on the transversal relaxation time (T2): the time needed to return to the low-energy state. These T2-weighted images are sensitive to magnetic properties of blood haemoglobin oxygen which serves as a biomarker of neural activity. More specifically, fMRI provides an indirect measurement of neural activation associated with task performance through the principle of neurovascular coupling (see **Figure 9**).

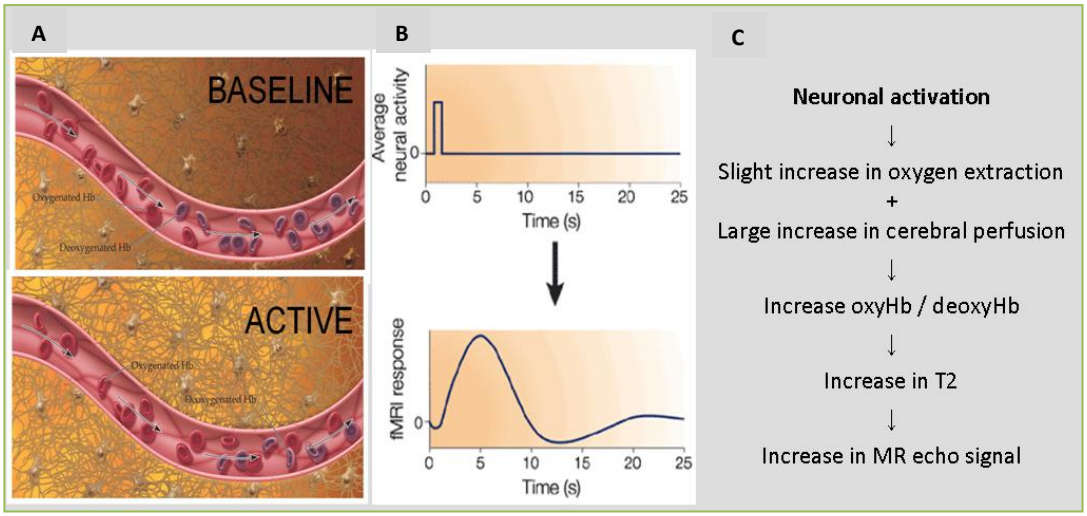


Figure 9: Principle of neurovascular coupling. Active neurons trigger an increased supply of oxygen transported by haemoglobin in the blood (A). This results in an increased fMRI response (B) according to the sequence of cerebrovascular changes in panel C. As shown in panel B, the blood oxygen level dependent signal shows an initial drop and reaches a maximum peak around 3 to 9 seconds after onset of neuronal activity. Source: Sunaert and Thomas [150]; Heeger and Ress.[151]

The principle of neurovascular coupling was introduced by Roy and Sherrington in 1890[149] and refers to the close relationship between local neural activity and subsequent changes in cerebral blood flow (rCBF) and blood oxygenation. When neurons become active, their metabolism increases. The increase in rCBF and regional blood volume (rCBV) serves to supply the active region with oxygen and glucose transported by haemoglobin (Hb), though this relation is not linear. As shown in part b of **Figure 9**, there is an initial drop in the oxygen level of the blood, reflecting increased cerebral metabolic rate of oxygen (CMRO2). This early response (1-3s after onset of neural activation) is followed by a slow, late response characterized by a huge increase in the oxygen concentration that exceeds the actual oxygen demand. The resulting increase in oxygenated blood reaches its maximum at 3-9s after neurons in the region became active. FMRI is sensitive to the magnetic state of the Hb iron which depends on the amount of oxygenation the Hb molecule is carrying. When Hb is

depleted from oxygen (deoxyhaemoglobin), the iron is paramagnetic. Conversely, it is diamagnetic when HB is saturated with oxygen. This Blood Oxygenation Level Dependent (BOLD) change in magnetic properties of Hb forms the basis of fMRI and is captured by T2 weighted images. As such, the BOLD signal reflects neural activation and can be compared between task conditions and between groups.

In Study 3, participants performed the freezing-provoking upper limb task while lying in a 3T MRI scanner. The paradigm was slightly modified towards a block-design in which 4 different randomly presented movement conditions alternated with baseline (rest) periods (**Figure 10**). Task-related brain activation was determined by subtracting neural activation during rest from neural activation during movement. For the current study, this contrast was expected to yield activation mainly in regions of the motor network. The results of this contrast allowed further comparison between different movement conditions (e.g. small-amplitude vs large-amplitude movements or FOUL vs continuous UL movement) and groups (PD with FOG, PD without FOG and Controls) to see how brain activation was modulated by spatiotemporal constraints and by pathology. More specifically, we were interested in two comparisons:
1) Between-group comparison of neural activation during ongoing upper limb movement, i.e. functional movement in the absence of freezing episodes.
2) Within-group comparison of neural activation during FOUL with activation during ongoing upper limb motion in patients that presented FOUL during testing.

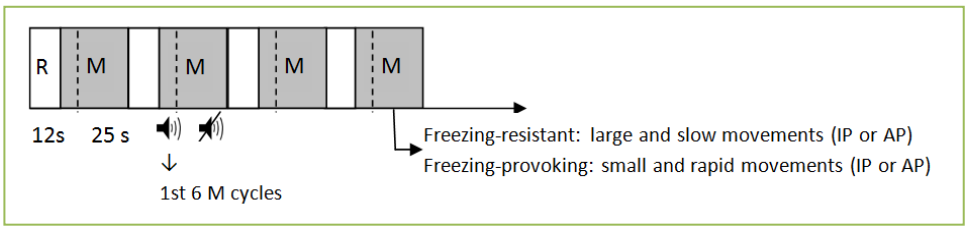


Figure 10: Block design used in the fMRI study. Rest (R) and movement (M) periods alternated. Movement was guided by an auditory cue during the first 6 movement cycles. Four movement conditions were presented in random order, namely two conditions that were thought to provoke freezing during upper limb movement ('freezing provoking') and two freezing-resistant conditions based on spatiotemporal constraints.

6. OVERVIEW OF THE CHAPTERS

Table 2 provides an overview of the four studies that are included in this doctoral project and described in detail in Chapter 2 to 5 (See **Table 2**). Studies 1 and 2 involve one experiment in which the obtained kinematic data were analyzed with a different focus: on spatiotemporal characteristics of freezing episodes in the upper limbs in Study 1 and on scaling and timing difficulties during ongoing upper limb movement and the effect of cue-withdrawal in Study 2. The same upper limb paradigm was used in the fMRI experiment of Study 3 to investigate the neural mechanisms of FOUL and FOG. Study 4 examined the relative independence of motor and non-motor determinants of FOG by use of a logistic regression model. The aims of the individual studies will be outlined in the following paragraphs. Some subjects participated in several studies. This overlap in participants across the four studies is shown in **Table 3**.

Table 2: Schematic overview of the four doctoral studies as described in Chapter 2 to 5

Chapter	Study type	General aim	Participants	Assessment	Analysis
Chapter 2	Behavioral	Spatiotemporal similarities of FOG and FOUL?	11 CTRL 11 PD+FOG (OFF) 12PD-FOG (OFF)	Motor performance during freezing-provoking UL task	Kinematic analysis Spectral analysis Correlation analysis
Chapter 3	Behavioral	Sustained UL motor abnormalities and effect of cue-withdrawal in PD+FOG?	11 CTRL 11 PD+FOG (OFF) 12 PD-FOG (OFF)	Motor performance during freezing-provoking UL task	Kinematic analysis
Chapter 4	Neuro-imaging (fMRI)	Brain activation during ongoing UL motion and FOUL?	16 CTRL 16 PD+FOG (OFF) 16 PD-FOG (OFF)	Motor performance and brain activation during freezing-provoking UL task	Kinematic analysis fMRI analysis
Chapter 5	Behavioral	Independent contribution of motor and non-motor factors on FOG?	27 PD+FOG (ON) 24 PD-FOG (ON)	Clinical test battery	Logistic regression analysis

Abbreviations of Table 2: FOG: Freezing of gait; FOUL: Freezing of upper limb movement; UL: upper limb; PD+FOG: Parkinson patients with FOG; PD-FOG: Parkinson patients without FOG; CTRL: Control subjects; fMRI: functional Magnetic Resonance Imaging.

Table 3: Overview of overlap in participants of the four doctoral studies described in Chapter 2 to 5

	Controls	PD-FOG	PD+FOG
Chapter 2	**11**	**12**	**11**
Chapter 3	**11**	**12**	**11**
Chapter 4	**4** + 12 new = 16	**9** + 7 new = 16	**8** + 7 new + 1* = 16
Chapter 5	**0**	**12** + 6 + 6 new = 24	**10** + 7 + 10 new = 27

Abbreviations of Table 3: PD+FOG: Parkinson patients with FOG; PD-FOG: Parkinson patients without FOG. * One PD patient developed FOG after participating in the studies of Chapter 2 and 3.

Chapter 2: Freezing in Parkinson's disease: a spatiotemporal motor disorder beyond gait

In this first behavioral study, we sought to determine whether freezing of gait and freezing during other motor tasks are correlated and can be characterized by similar spatiotemporal changes in the kinematic signal. This study was the first to describe the motor changes during non-gait freezing episodes with detailed kinematic analysis.
Eleven freezers, twelve non-freezers and eleven age-matched healthy controls performed the rhythmic bilateral finger movement task described above. Patients were matched for disease duration and tested in the OFF phase. Freezing episodes were detected using the spatiotemporal criteria described above. The triggering effect of movement speed, amplitude and coordination pattern on FOUL was evaluated. Regression slopes and spectral analysis addressed the spatial and temporal kinematic changes inherent to freezing episodes.
We hypothesized that small-amplitude and fast alternating movements would provoke FOUL, similar to the effect of stride length and cadence manipulations on FOG. Secondly, we expected that a progressive decrease in movement amplitude and increase in frequency would precede FOUL in accordance to the sequence effect leading to FOG. Last, we assumed that the high-frequency components found during FOG would be present during FOUL as well and would translate in a broadband frequency spectrum and a higher freezing index compared to functional UL movement.

Chapter 3: Abnormalities and cue-dependence of rhythmical upper limb movements in Parkinson's disease patients with freezing of gait

The second behavioral study examined if problems in timing and scaling of movement observed in ongoing gait of freezers also affect ongoing upper limb coordination and if these motor abnormalities are emphasized by cue-withdrawal.
Data obtained from the same experiment as Chapter 2 were used to compare motor performance in the absence of freezing episodes (i.e. functional UL motion) between 11 freezers, 12 disease-matched non-freezers and eleven age-matched healthy controls. The stability and accuracy of movement amplitude, frequency and bilateral coordination were evaluated after exclusion of freezing trials. Outcome parameters were determined for movement periods guided by auditory cueing and after cue-withdrawal separately.
We hypothesized that patients with FOG would show sustained motor abnormalities demonstrated by smaller and more variable amplitude, increased and more variable frequency and less stable coordination patterns especially in the anti-phase mode. These problems would be consistent with the background locomotor difficulties in regulating stride length, stride timing and inter-leg coordination. Secondly and similar to the increased cue-dependency of freezers while walking, we expected cue-withdrawal to have a more detrimental effect on motor continuation of freezers compared to non-freezers and controls.

Chapter 4: The neural basis of disturbed motor control and upper limb freezing in Parkinson patients with freezing of gait

This neuroimaging study was designed to explore which altered patterns of brain activity underlie freezing episodes and the disturbed motor control in patients with FOG. This is the first fMRI study that compares brain activation during motor performance in freezers and non-freezers which has the potential of revealing how the brain behaves during actual freezing episodes in Parkinson patients.

A shortened version of the freezing provoking upper limb task was carried out by 16 PD patients with FOG, 16 disease-matched patients without FOG and 16 age-matched controls while lying in a MRI scanner. PD patients were OFF medication. Movement performance was registered and divided into periods of functional upper limb movement and freezing episodes based on the detection methods for FOUL described above. Brain activation during ongoing UL movement was compared between groups after correction for differences in motor behavior. Within patients who presented FOUL we established the difference in neural activation during FOUL versus ongoing motion.
We hypothesized that increased recruitment of cerebello-parietal networks described as compensatory for the deficient BG-frontal drive in PD would be absent or inefficient in freezers. Based on the results of current neuroimaging studies comparing structural and functional brain organization in freezers and non-freezers, it was also expected that widespread activation changes covering fronto-parietal cortical regions, basal ganglia and midbrain motor areas, may occur during actual freezing episodes.

Chapter 5: Explaining freezing of gait in Parkinson's disease: motor and cognitive determinants

In this cross-sectional study we aimed to determine which factors are associated with FOG by evaluating the relative independent contribution of motor and cognitive aspects in the prediction of FOG. We aimed to address the current diverging hypotheses on FOG, which postulate that motor abnormalities, postural components or executive dysfunction are single-domain origins for FOG. However, these impairments were never before addressed in a single, integrative model.
A group of 24 PD patients without FOG and 27 with FOG, matched for age, gender and disease severity underwent an extensive clinical test battery evaluating general disease characteristics, gait and balance, non-gait freezing and cognitive functions. Patients were ON medication during testing. The relative contribution of these outcomes to FOG was determined using logistic regression analysis.
We hypothesized that FOG would be best explained by a combination of motor and non-motor factors resulting in a multi-determinant model of FOG.

Chapter 6: General discussion

This last chapter will first summarize the main findings of each study and will discuss how the obtained results change our understanding of FOG. This chapter will also include sections on the study limitations of this doctoral project and highlight directions for future research.

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Chapter 2

Freezing in Parkinson's disease: a spatiotemporal motor disorder beyond gait

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ABSTRACT

Background: Freezing of gait (FOG) is an incapacitating problem in Parkinson's disease which is difficult to manage therapeutically. We tested the hypothesis that impaired rhythm and amplitude control is a common mechanism of freezing also present during other rhythmic tasks. Therefore, we compared the occurrence and spatiotemporal profiles of freezing episodes during upper limb motion, lower limb motion and freezing of gait.
Methods: Eleven freezers, twelve non-freezers and eleven controls performed a rhythmic bilateral finger movement task. The triggering effect of movement speed, amplitude and coordination pattern was evaluated. Regression slopes and spectral analysis addressed the spatial and temporal kinematic changes inherent to freezing episodes.
Results: The FOG-questionnaire score significantly predicted severity of upper limb freezing, present in 9 freezers and of foot freezing, present in 8 freezers. Similar to gait, small-amplitude movements tended to trigger upper limb freezing which was preceded by hastened movement and a strong amplitude breakdown. Upper limb freezing power spectra were broadband, including increased energy in the 'freeze band' (3-8Hz). Contrary to FOG, unilateral upper limb freezing was common and occurred mainly at the disease-dominant side.
Conclusions: The findings emphasize that a core motor problem underlies freezing which can affect various movement effectors. This deficit may originate at the disease-dominant body side and interfere with amplitude and timing regulation during repetitive limb movements. These results may shift current thinking on the origins of freezing as being not exclusively a gait failure.

Keywords: freezing of gait, upper limb motion, upper limb freezing, Parkinson's disease, spectral analysis.

1. INTRODUCTION

Freezing of gait (FOG) is experienced by approximately 50% of patients with advanced Parkinson's disease (PD)[1] and is defined as a transient inability to generate effective stepping.[2] As a significant predictor of falling, FOG is a major debilitating problem in PD.[3] FOG is partly responsive to dopaminergic medication[4] and STN-stimulation[5,6] but remains a challenging treatment target. The current understanding of its underlying mechanisms is dominated by diverging motor and non-motor hypotheses, based on specific factors known to elicit freezing, e.g. turns[4,7], postural perturbations[8], dual tasking[7,9], narrow spaces[10,11] set-shifting deficits[12,13] and stress.[14]

The present paper focuses on core aspects of motor control related to FOG. Irrespective of the trigger, FOG is mostly characterized by 1) a decrease in stride length; 2) an increase in stepping frequency preceding the episode and 3) the presence of a highly abnormal frequency of leg movements during the episodes.[15-20] This faulty scaling-timing interaction is crucial in the development of periodic freezing events, but is also implied in a more continuously disturbed gait pattern in patients with FOG (freezers) [20-23] compared to those without (non-freezers).

There is mounting evidence of freezing-like motor blocks in various rhythmic tasks such as speech[24], hand movements including writing and manual tapping and other anti-phase coordination tasks[24-28] such as those used as part of the new MDS-UPDRS.[29] Although previous study reported a correlation between freezing in different effectors[28], it is presently unclear whether movement breakdown during gait and other rhythmic movement reflect a generic deficit in automatic motor control. Therefore, the aim of this study was to investigate timing and scaling abnormalities of freezing episodes beyond the gait network. Following the hypothesis of an effector-independent spatiotemporal deficit leading up to motor blocks, we expected to observe specific similarities between FOG and upper limb freezing, namely that: 1) small-amplitude conditions would provoke freezing episodes during upper limb motion, like they do in FOG[19]; 2) the severity of FOG and freezing during finger movements would be highly correlated; 3) a gradual decrease in movement amplitude and increase in frequency would be precursors of freezing during upper limb motion (FO-UL); and 4) similar timing abnormalities would exist during FO-UL and FOG.

2. MATERIALS AND METHODS

Participants

Twenty-three PD patients were recruited from the University Hospital Leuven. A score ≥ 1 on the revised FOG-Questionnaire (FOGQ[30]) classified a patient as a freezer (n=11). Freezers and non-freezers (n=12) were matched for age, sex and disease severity (Hoehn and Yahr[31] stage II or III). Eleven age-matched controls also participated but these results are not reported in the current manuscript. Exclusion criteria were: (1) neurological disease other than PD, (2) having a deep brain stimulator, (3) suffering from significant upper limb tremor interfering with movement and 4) Mini Mental State Examination (MMSE) <24/30). Ethics approval was received by the Commissie Medische Ethiek K.U.Leuven.

Design and procedure

Clinical screening took place whilst patients were 'ON' medication and included the Unified Parkinson's Disease Rating Scale (UPDRS[32]), FOGQ, MMSE, Hoehn and Yahr staging and the cognitive section of the Scales for Outcomes in PD (SCOPA-Cog[33]). Testing occurred while OFF medication, i.e. after withholding anti-parkinsonian medications for at least 12 hours. On this day, UPDRS motor examination was repeated (UPDRS-OFF). Subjects performed two tasks entailing repetitive movement of the upper limbs (Task 1) and lower limbs (Task 2). Our main focus was on Task 1: a bimanual task of rhythmic flexion and extension movement of the index fingers in a 2x2x2 factorial design with manipulations in coordination pattern, amplitude and frequency. Subjects performed simultaneous or alternating movements, requiring in- or anti-phase coordination. Before testing, a single anti-phase trial served to compute subject-specific comfortable amplitude and frequency. During testing, large-amplitude (comfortable=100%) or small-amplitude movements (66%) were required. Movement frequency was normal (comfortable=100%) or fast (133%). Task order was randomized. Auditory pacing guided the first 6 movement cycles to enable correct frequency manipulations after which the rhythm was to be maintained for 25 seconds. A square box covering both hands prevented visual feedback. Angular finger displacements were registered by a Micro 1401 acquisition unit (CED, UK) through analogue encoders placed on the rotation axis of the fingers. Temporal and spatial resolutions were 2000 Hz and 0.0001 degrees. Subjects were given sufficient time to familiarize themselves with task requirements and achieve automaticity of movement. Task 2 was an exploratory study of alternating foot movements. Participants performed three trials of foot movements at a comfortable frequency and amplitude while lying supine. Each trial lasted 30 seconds. Foot movements were not registered but clinically screened for the occurrence of freezing episodes (see below).

Data processing

Regarding Task 1, we defined FO-UL episodes as '*a period of involuntary stop or clear absence of effective cyclic movements*'.[2] Thus, both periods with a complete halt as well as severely disrupted motion with a nearly complete loss of movement were classified as freezing episodes. We visually determined the beginning of an FO-UL-episode as the onset of abnormally small motion cycles (<50% of initial amplitude) accompanied by an irregular cycle frequency[15], as illustrated in **Figure 1**. The end of the freezing episode was defined by the moment when at least two movement cycles with regular amplitude and rhythm were resumed or by the finish of the trial when regular movement was not regained. The minimal duration of a freezing episode was set at 75% of normal cycle duration to avoid misclassification of disruptions due to pattern switches. Two independent observers demarcated the FO-UL-episodes on the basis of visual analysis of the movement signal using Spike 2 software in which the exact cycle amplitude and cycle duration could be obtained using a cursor. Each trial was also classified as a 'freezing trial' or a 'no-freezing trial'. Reproducibility of this detection method was established by a reliability study (ICC (2,4)=.99).

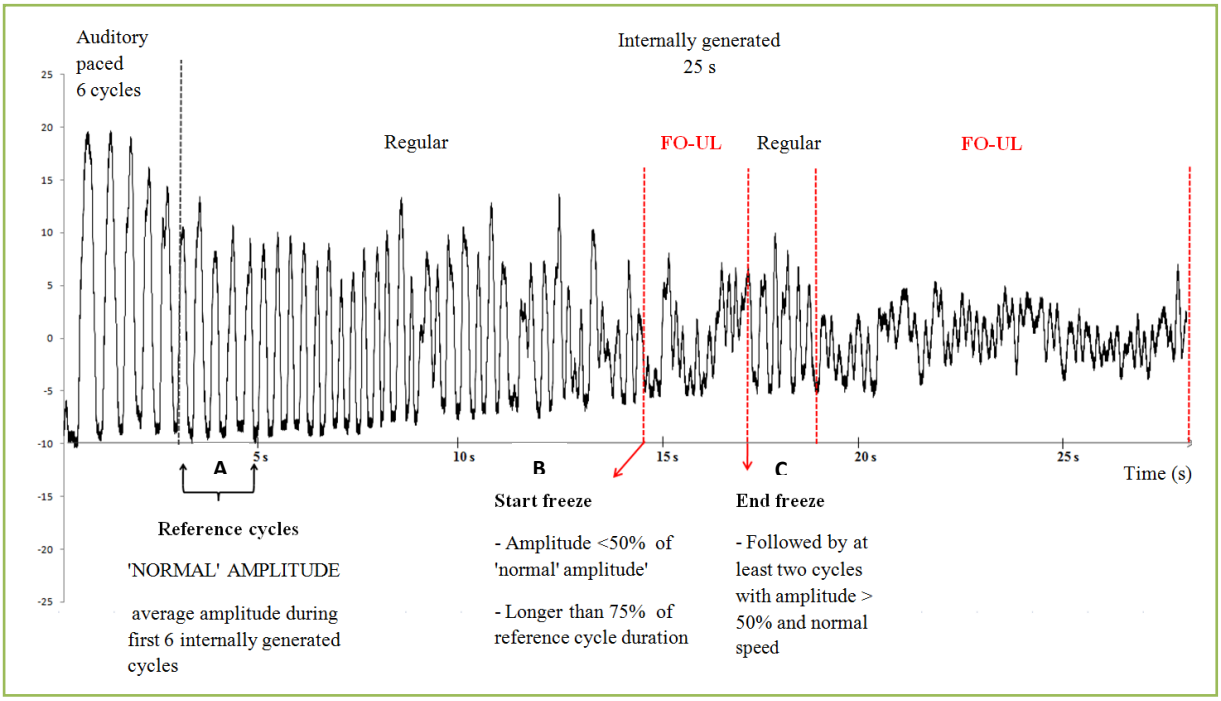

FIG. 1. Definition of upper limb freezing. Example of an upper-limb freezing episode FO-UL with a nearly complete loss of movement. Data of the right finger is shown and retrieved from a trial in which alternating movements with a comfortable amplitude and fast frequency were requested. Data of the left finger is not shown to increase visibility of the freezing episode and detection method. Based on spatial and temporal criteria, the time series is divided into regular motion and freezing episodes. A: The first 6 cycles after auditory pacing was removed, served as reference cycles for the computation of the normal (ie, average) amplitude and normal (ie, average) cycle duration for the given trial. B: The onset of the freezing episode was set when a reduction of amplitude above 50% of the normal amplitude lasted longer than 75% of the normal cycle duration. C: The freezing episode was considered as ended when at least 2 normal cycles were performed. These 2 normal cycles were included in the regular motion following the freezing episode.

Movement amplitude and cycling frequency were determined for each movement cycle using the difference in angular values of local maxima and minima (amplitude) and by taking the inverse of the time that elapses between successive peak positions (frequency). Linear regression coefficients (slopes, β) were computed to describe their change with time. In freezing trials, β calculation included at least 6 movement cycles preceding the FO-UL-episode or the tremor.[19] For each trial, scatter plots were used to check the distribution of amplitude and frequency data points of left and right finger separately. Outlying data points that would distort β calculation were removed from the data set (e.g. an unusually large amplitude at the beginning of the trial). Spectral analyses were performed on movement data lasting ≥ 1s (2000 data points). A freezing index (FI) was defined as the power in the freeze band (3-8 Hz) divided by the power in the normal motion band (0.5-3 Hz) [18] (see Supplement 1A for details).

We also performed spectral analyses of knee displacements during normal gait and FOG episodes in one freezer. This patient was tested while OFF medication 3 months later as part of another study[7] (see Supplement 1B).

In Task 2, freezing during lower limb movements (FO-LL) included periods with a clean arrest and a nearly complete loss of movement, similar to FO-UL. Foot movements were not registered but two independent raters scored each trial as with or without FO-LL based on online observation.

Statistical analysis

For all statistical testing we used STATISTICA (8.0) with significance levels of .05. Group comparisons on the normal trials are not reported in this manuscript. In case of abnormality or a discrete nature of the outcome variable, non-parametric statistics were used.

1) Clinical variables were compared between groups using one-way ANOVA (Disease duration, UPDRS-III scores, Levodopa-equivalent dose, comfortable speed), non-parametric Man-Whitney U test (Age, MMSE, Scopa-COG, Hoehn and Yahr stage and FOGQ) and logistic regression test (Gender).
2) The occurrence of freezing episodes was compared between freezers and non-freezers by means of a Mann-Whitney U test and between the two levels of PATTERN (in-phase, anti-phase), AMPLITUDE (normal, small) and SPEED (normal, fast) using a generalized estimating equation (GEE) logistic regression that accounts for clustered observations and binary outcomes (trial without FO-UL=0, with FO-UL=1).[34]
3) Non-parametric Spearman correlation tests (rs) were applied to relate severity of FOG (measured by the FOGQ), FO-UL, FO-LL and clinical outcomes within patients with FOG and/or FO-UL.
4) Amplitude and frequency regression coefficients (β values) of regular movement, not followed by FO-UL, were compared with β values of regular motion preceding an FO-UL episode, using non-parametric repeated-measures Friedman test within patients who presented FO-UL. Differences in FI between normal motion and freezing episodes were analyzed within patients with FO-UL by Friedman tests.

Significant effects were further analyzed by post-hoc tests yielding p-values that were corrected for multiple comparisons. Results are represented as mean and (standard error of measurement).

3. RESULTS

Subjects

Age, gender distribution and comfortable movement speed were similar between groups (**Table 1**). Freezers and non-freezers had comparable disease profiles. SCOPA-cog scores did not differ between freezers and non-freezers. Freezers' scores on the MMSE were lower than non-freezers but fell well within normal reference values.[35]

Occurrence of freezing episodes

Nine freezers (82%) and 2 non-freezers (17%) (p=0.001) demonstrated FO-UL. FO-LL was also more frequent in freezers (N=8, 73%) than in non-freezers (N=0) (p=0.002). Similar to FOG[4], duration of FO-UL episodes (total number=114) was quite variable, ranging from 0.34s to 23.3s and 5.98s on average.

Within a trial, FO-UL could be present bilaterally (31%) or unilaterally (69%). Unilateral FO-UL occurred more often at the disease-dominant (45 FO-UL trials, 75%) than the non-dominant body side (N=15, 25%) (p=.0002). The number of freezing trials was highest in the most complex condition (N=15, 17%), entailing alternating, fast and small-amplitude movements and lowest in the condition requiring simultaneous, slow and large-amplitude movements

(N=6, 7%). Using the GEE model, we found no difference in FO-UL frequency between in-phase and anti-phase (p=0.23) and between normal and high-frequency conditions (p=0.47). A trend for significance was found for the triggering effect of small versus large-amplitude conditions (50 vs. 37 freezing trials; p=.081) (**Figure 2**).

Table 1: Demographic and disease characteristics of participants.

Parameter	Non-Freezers	Freezers	*P*-value
Age (years)[1]	70 (64-72)	69 (65-72)	0.92
Disease duration (Years)[2]	7 (6-9)	9 (6-11)	0.43
Sex (M/F)[3]	9/2	10/2	0.82
UPDRS-III ON (0-108) [2]	36 (32-44)	31 (27-49)	0.97
UPDRS-III OFF (0-108)[2]	35 (28-37)	38 (28-42)	0.54
H & Y OFF (0-5) [4]	II (II-III)	III (II-III)	0.32
FOGQ (0-28)[4]	0 (0-0)	9 (8-16)	<0.01*
MMSE (24-30)[1]	30 (28-30)	28 (27-28)	<0.01*
SCOPA-COG (0-43)[1]	31 (28-33)	29 (25-31)	0.29
Levodopa-dose (mg)[2]	510 (413-626)	600 (468-708)	0.54
Comfortable speed (Hz)[2]	1.13 (0.80-1.37)	1.31 (0.90-1.70)	0.28

Demographic and clinical characteristics of 12 non-freezers, 11 freezers, and 11 control subjects (median and interquartile ranges).* Groups significantly different at *P*<.05; UPDRS-III, Unified Parkinson's Disease Rating Scale part III; H & Y, Hoehn & Yahr stage; FOGQ, Freezing of Gait Questionnaire; MMSE, Mini Mental State Examination; SCOPA-COG, Scales for Outcomes in Parkinson's Disease- Cognitive part. OFF= while off medication. 1Non-parametric Kruskal-Wallis test was used. 2One-way ANOVA was used. 3Logistic regression was used. 4Non-parametric Mann-Whitney U test was used.

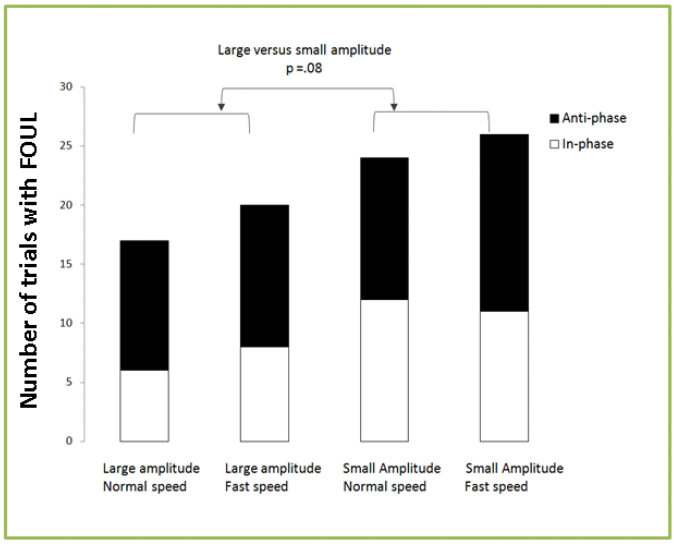

FIG. 2. The effect of manipulations in movement frequency, amplitude and coordination pattern on the occurrence of upper-limb freezing episodes. Frequency of FO-UL in each movement condition is shown. Most freezing episodes were elicited in the most complex movement condition; i.e. small and fast movements in an

anti-phase pattern. Small-amplitude conditions tended to provoke more freezing episodes compared to large amplitude conditions (p=0.08).

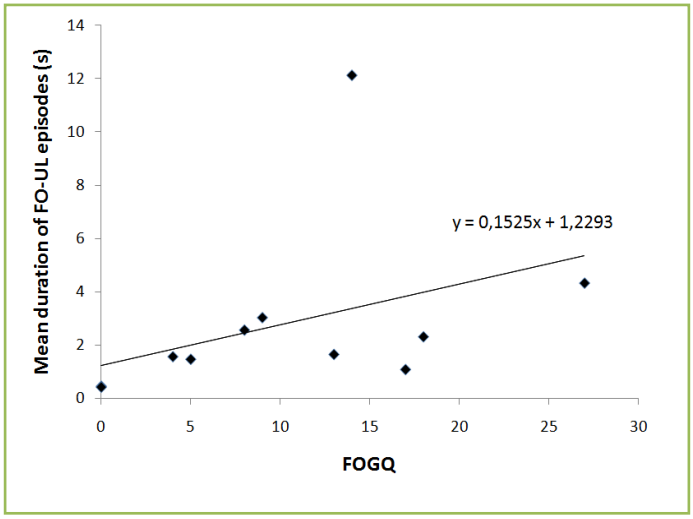

FIG. 3. Relation between severity of upper limb freezing and FOG. Relation between severity of upper limb freezing (FO-UL) and FOG within 11 patients who demonstrated FO-UL during testing; ie, 9freezers and 2 non-freezers. Spearman rank correlation (rs) = 0.64, P < .05. Solid line represents a linear trend. (As data points of the 2 non-freezers are very similar (x = 0, y = 0.48; and x = 0, y = 0.43) they are collated on the figure).

Correlation between FO-UL, FOG, FO-LL and clinical outcomes

Within patients with FOG and/or FO-UL, the FOGQ-score significantly predicted the number of FO-UL trials (r_S=.56, p<.05) and the number of FO-LL trials (r_S=.59, p<.05). The FOGQ was also significantly correlated with the duration of FO-UL episodes within patients who presented FO-UL during testing (r_S=.64,, p<.05) (**Figure 3**). In contrast, FO-UL and FO-LL were not explained by PD severity (**Table 2**) and cognitive scores. Lastly, the number of FO-UL and FO-LL trials were highly intercorrelated (r_S=0.80, p<.05).

Table 2: Correlations between severity of freezing in different effectors and disease severity.

	FO-UL # freezing trials	FO-LL # freezing trials
FOGQ (0-28)	0.56*	.59*
FO-LL # freezing trials (0-3)	/	0.80*
UPDRS-III OFF (0-108)	0.18	0.0047
H & Y OFF (0-5)	0.25	0.37
Disease duration (years)	-0.013	0.038

Spearman correlations between severity of freezing in different effectors (FOG, FO-UL, and FO-LL) and disease severity in 10 patients with FO-UL (8 freezers and 2 non-freezers). *P <.05. FO-UL, freezing of upper limb movement; FO-LL, freezing of lower limb movement; FOGQ, Freezing of Gait Questionnaire; UPDRS-III, Unified Parkinson's Disease Rating Scale part III; H&Y, Hoehn & Yahr stage; off, while off medication; FOG, freezing of gait.

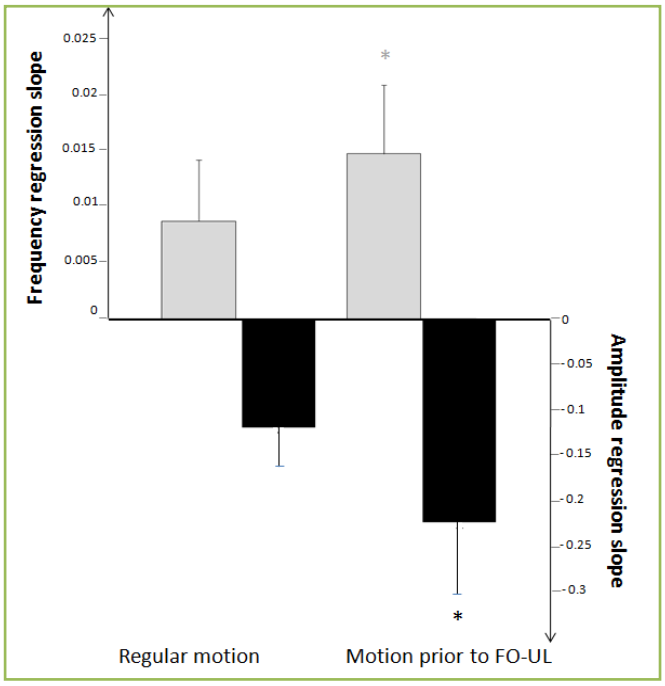

FIG. 4. Frequency and amplitude regression slopes in regular motion not followed by FO-UL and in motion preceding an FO-UL episode. Regression slopes of cycle-by-cycle frequency (upper part) and amplitude (lower part) in patients with FO-UL. Movement was scaled down more dramatically prior to an upper-limb freezing episode (more negative amplitude slope) than in normal motion not followed by FO-UL (''regular motion''). In contrast, movement was more hastened before FO-UL (larger frequency slope) compared to normal motion. (Data are represented by average slopes and standard error of measurements; *P < .05).

Spatiotemporal characteristics of upper limb freezing episodes

To evaluate the change in amplitude and frequency prior to a freezing episode, we compared the mean amplitude and frequency regression slopes of normal motion not followed by a freezing episode with the mean slopes of movement before FO-UL took place in 11 patients who presented FO-UL (**Figure 4**). In case of similar behavior of the left and right finger (both normal or both freezing), slopes were averaged for both body sides. In case of unilateral freezing, the slope of the freezing hand was entered in the category of 'motion preceding freezing' whereas the slope of the non-freezing hand was added to the 'motion not followed by freezing' category. For each subject, the average amplitude and frequency slope was computed for both categories. Amplitude β values were more negative during motion preceding a freezing episode (mean β= -0.23*(.07)*) than in motion not followed by freezing (mean β= -0.12 *(.04)*, p=.035). Conversely, frequency β values were larger when preceding a freezing episode (mean β=0.016 *(.007)*) than when motion was not followed by FO-UL (mean β= 0.0093 *(.006)*, p=.034).

These findings indicate a strong amplitude decline and frequency increase prior to the freezing episode (**Figure 4 and 5**). In fact, 71% of FO-UL episodes were preceded by frequencies above 2 Hz.

Ninety-three FO-UL episodes lasting longer than 1 second (82%) and 12 FOG episodes (retrieved from gait data of 1 freezer) were included for spectral analyses. Unlike regular finger movement and normal gait (**Figure 6A**), the power distribution of freezing episodes during both upper limb movement and gait was blurred, including local maxima within the 'freeze band' (3-8 Hz) (**Figure 6 B**). Freezing indices were significantly higher for FOG episodes than normal gait (FI = 1.26 (*0.2)* and 0.24(*.04*), p<.001). Similarly, FI values were higher during

FO-UL episodes indicating a relative increase in high frequency components compared to regular finger motion ((FI= 2.23(*0.16*) vs. 0.8 (*0.008*)) (p<.01)).

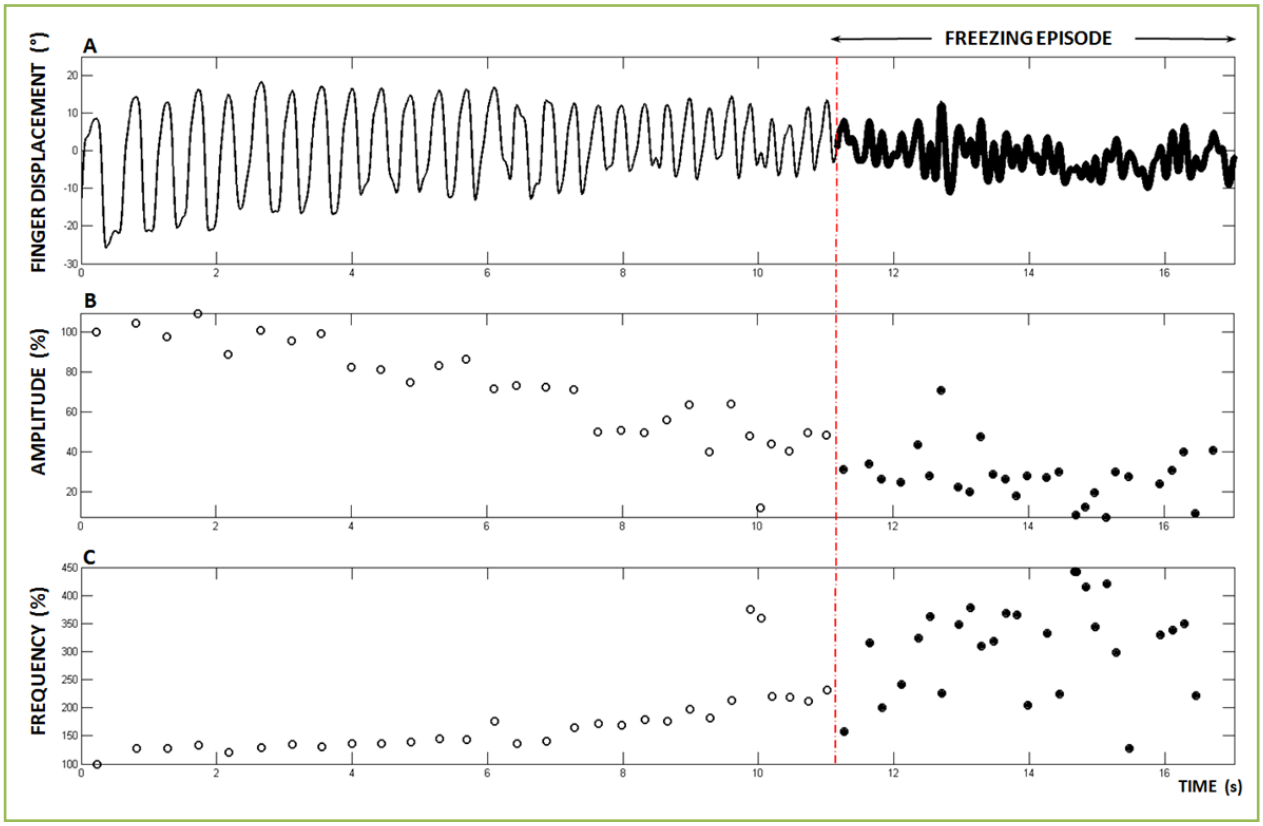

FIG. 5. Illustration of amplitude and frequency alterations preceding and during an upper limb freezing episode (FO-UL). A: This panel shows the angular displacement of the left index finger of a single subject while performing anti-phase movements with comfortable amplitude and frequency. The red dotted line indicates the beginning of the freezing episode. B: Movement amplitude is gradually reduced preceding the freezing episode. C: Movement frequency shows a progressive increase prior to FO-UL and is markedly variable (chaotic) during the episode. Amplitude and frequency are expressed as a percentage of the values obtained during the first movement cycle. Data from the first 17 seconds of the trial is shown. Normal movement was regained after the freezing episode but is not shown to aid clarity.

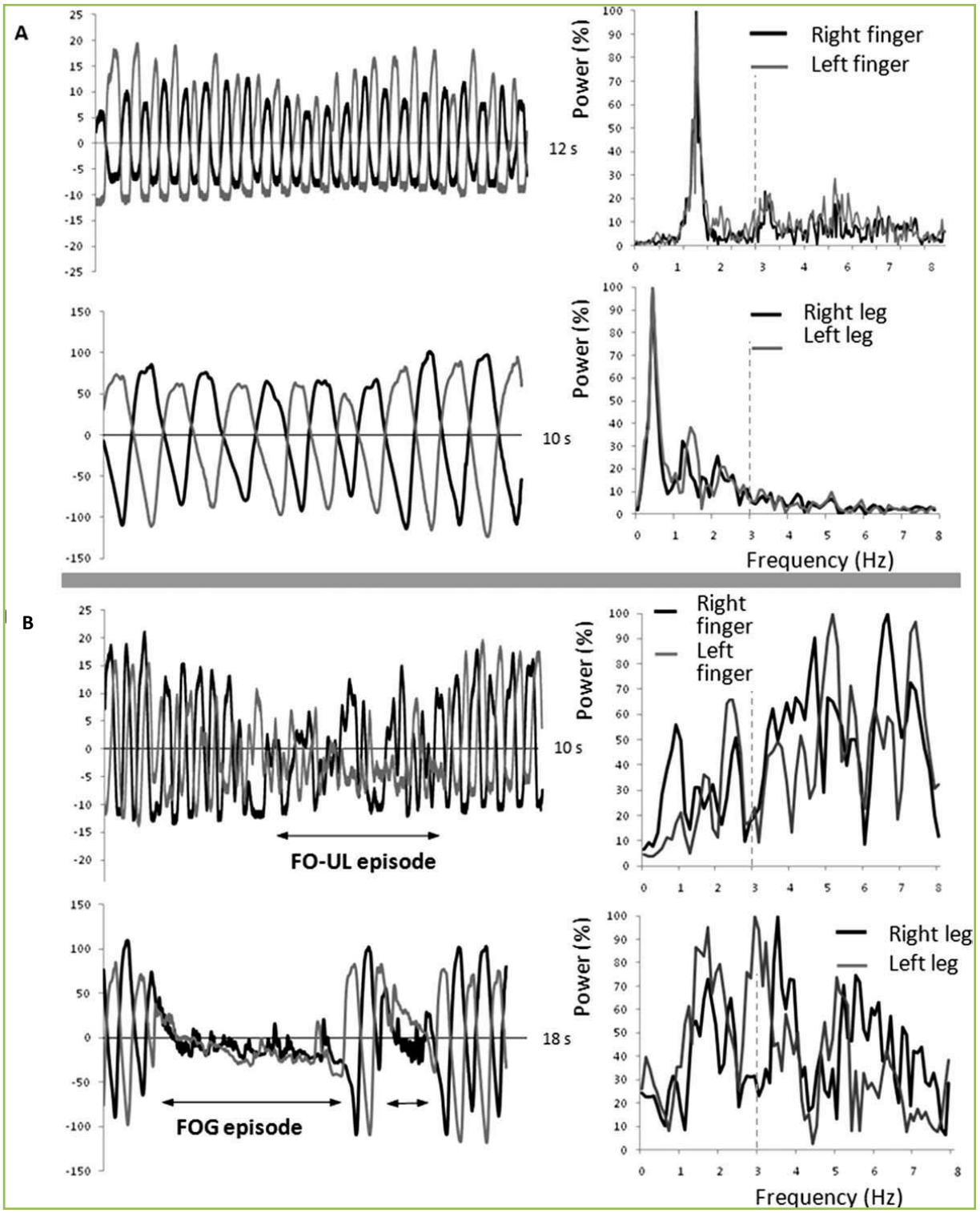

FIG. 6. Spectral analysis of gait (normal and freezing) and upper-limb motion (normal and freezing). A: Trials without freezing episodes during upper-limb motion (upper row) and gait (lower row). A clear peak in the power spectrum (right side) represents the main movement frequency within the normal motion band (0.5–3 Hz, left of vertical dashed line). B: Trials with freezing episodes during upper limb motion (upper row) and gait (lower row). Both types of freezing are characterized by a blurred energy distribution with increased energy in the freeze band (3–8Hz, right of vertical dashed line).

4. DISCUSSION

Our results support the hypothesis that a generic motor control problem underlies FOG. This effector-independent deficit interferes with amplitude and timing regulation during repetitive movement leading up to freezing events during upper as well as lower limb motion. First, imposing small amplitudes during bimanual finger movements increased the tendency of freezing. This is congruent with the results of Chee et al[19], who showed a strong association between reduced stride length and the occurrence of FOG. Second, the number and duration of upper and lower limb freezing episodes were related to FOG severity, not to disease severity or cognitive outcomes. Third, FO-UL episodes were preceded by a strong amplitude decrease and hastened movements. Fourth, FO-UL was characterized by high frequency components just like FOG.

FO-UL occurred more frequently in the present internally generated finger movement task without vision than in a hand drawing task, which was guided by vision.[28] Also, FO-UL was only observed in the absence of the initial auditory pacing. Interestingly, one of the two non-freezers who experienced FO-UL developed FOG a few months later. Possibly, this patient already had mild FOG symptoms at the time of testing pointing to the problems of distinguishing freezers from non-freezers using a questionnaire methodology.[28] Alternatively, FO-UL could be a precursor for the future development of FOG. Freezing as a generic deficit may be topographically distributed reaching either upper or lower limbs first, dependent on disease progression. This could also explain why two patients did not show FO-UL but did have FOG. It appears that the true nature of freezing is not as 'clear cut' as currently considered in clinical practice and research, but is better reflected by a continuous spectrum of abnormality with possibly a more gradual onset and affecting different body parts.[36]

Unilateral upper limb freezing

Another novel finding was that, contrary to FOG which is typically seen as a bilateral event, FO-UL sometimes emerged unilaterally while the contralateral limb kept moving regularly. FO-UL was not related to global disease severity but occurred more frequently in the disease-dominant
hand, consistent with the fact that during gait the first leg to enter a freezing state is usually the one at the disease-dominant side.[22]A unilateral onset and/or manifestation of FOG and FO-UL suggest that freezing originates from difficulties with within-limb spatiotemporal processing rather than a between-limb motor deficit. A pilot study by our group (unpublished data) also demonstrated that UL freezing could be elicited in single-limb finger tapping. The within-limb spatiotemporal deficit may be aggravated by bilateral coordination complexity, although freezing did not occur more often during alternating than simultaneous movements. Future studies on single (upper) limb movements might provide further insights on the relative contribution of bimanual coordination complexity to spatiotemporal impairments in the freezing problem.

Motor triggers of freezing

Contrary to the notorious difficulty to provoke FOG in laboratory settings, internally generated upper limb motion seems quite successful in triggering FO-UL. We found the highest number of FO-UL episodes in the most complex condition. However, there was only a

trend toward significance when manipulations in frequency, amplitude and coordination pattern were tested separately, probably due to limited statistical power. Almeida et al.[27] reported significantly more 'freezing' during anti-phase compared to in-phase movements. Unlike in the present study, these authors did not control for pattern corrections, which are more likely to occur when coordination complexity increases.[37]

Small-amplitude finger movements tended to increase the number of freezing trials and. amplitude decreased dramatically during movement preceding a freezing episode. This is consistent with earlier findings that a reduced stride length mediates the occurrence of FOG episodes[19], which was interpreted as a basal ganglia deficit compromising appropriate feedforward signaling to cortical motor preparation areas.[17,38,39]

FO-UL episodes were preceded by a gradual increase in cycle frequency (higher frequency slopes), although this was not confirmed by more FO-UL episodes during fast than normal speed conditions. Stegemöller and colleagues[40] showed that a nearly complete loss of movement during metronome paced finger movement was triggered by frequencies above 2 Hz. These rate-dependent movement disruptions occurred in PD patients without a documented history of FOG. Our data do not support the idea that exceeding a critical movement rate elicits motor impairments independent of freezing. In our study, comfortable and imposed movement frequencies were similar in freezers and non-freezers, and still movement disruptions were rare in non-freezers. We found that 71% of FO-UL episodes were preceded by frequencies above 2 Hz. This means that internally generated repetitive movement becomes hastened in freezers, but not in non-freezers and resembles the increased stepping frequency that often precedes FOG.[15,41-43]

FOG is known to occur in situations which pose environmental negotiation and demand elevated attention supporting a possible frontal executive hypothesis for FOG.[12,13] However, in this study FO-UL was triggered without additional cognitive, limbic or postural load suggesting a primary deficit in sequential movement generation. We consciously employed a bimanual task paradigm because it better resembled the inter-leg coordination during gait. Finger movements may be less automated than gait but the spatiotemporal coupling between the fingers is considered part of a natural coordination repertoire.[37] Similarly to walking, the fingers become integrated into a common 'motor gestalt' and can be performed effortlessly. [37,44] Attentional resources may have been invested by non-comfortable amplitude or frequency constraints but these should not interfere with the actual motor program. Anyhow, cognitive parameters were decreased but not abnormal in freezers in this study. Freezers scored less in the memory domain (MMSE) than non-freezers, but the SCOPA-COG, which is more sensitive to attention and executive functions, showed no significant differences between the subgroups. More importantly, cognitive functioning was not correlated to any of the freezing outcomes emphasizing that freezing is most likely a motor deficit even though it might be aggravated by non-motor triggers.

High-frequency components during freezing

High frequency components are common characteristics of FOG episodes.[4,15,16,21] A novel finding of this study was that trembling-like movements during upper limb freezing were very similar to gait freezing.[18,21,45] As in FOG, the broadband spectral distribution during FO-UL included multiple local maxima in frequency bands above 3Hz.[18,21] In gait, these temporal changes have been linked to multiple anticipatory adjustments[8] or to attempts to overcome

the motor block.[4] However, the complexity of the energy spectrum during FOG and FO-UL proposes multiple oscillatory inputs to the legs and fingers[16,21] rather than a compensatory phenomenon. In relation to FOG, the disturbed signaling is thought to be driven by misfiring central pattern generators (CPG's).[16,21] These spinal motor neurons remain silent during rest due to tonic inhibition from the basal ganglia to brainstem motor regions.[46,47] The high depolarization threshold in synapses of basal ganglia nuclei is crucial for the selective facilitation of movement.[47] Faulty facilitation may hamper the cortico-subcortical top down movement pathways or affect pathways from the mesencephalic locomotor region.[48,49] This in turn can hinder fine-tuned orchestration of limb-specific CPG's resulting in misfiring oscillations and uncontrolled trembling during FOG and FO-UL. Understanding the significance of these abnormal phenomena may be crucial for the development of novel treatment targets for FOG.

Assessment of freezing in PD.

A consensus on how to identify freezing episodes is presently lacking (See Supplement 2). We included as freezing episodes periods with a severely disrupted motion and a nearly but not complete loss of movement. The spatial and temporal criteria used in this study ensured an objective and reproducible detection method, which was investigator independent. A fully objective identification can only be reached using automated software. Spectral analyses seem promising to identify freezing episodes using a specific threshold of the freezing index, possibly defined separately for each subject.[18,21] A similar technique can be considered to detect FO-UL episodes, although delineation of 'normal' and 'freezing' frequency bands might need to be adjusted when detecting non-gait freezing. For the purpose of this study, foot movements were studied in an exploratory way. Although inter-rater agreement of FO-LL detection was sufficient, we acknowledge that future registration of these signals might provide valuable information with regards to scaling and timing difficulties in these movements as well. Complete akinesia and initiation difficulties were not observed during finger movements. These types of freezing might be more under intentional control and more dramatically observed in gait due to postural constraints. It is also possible that the hypothesized underlying mechanism of impaired amplitude-rhythm control is restricted to movement breakdown preceding and during freezing of ongoing motion.

5. CONCLUSION

This study demonstrated that hastened movement and a dramatic breakdown in movement amplitude constituted a prelude to freezing episodes during rhythmic upper limb motion which were highly correlated with FOG and exhibited highly similar motor changes. The results suggest that freezing can be conceptualized as primarily originating from impaired timing-amplitude control which is not restricted to the gait network but possibly represents a generic motor control problem.

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ABBREVIATIONS

FOG, freezing of gait; FOGQ, Freezing of Gait Questionnaire; FO-LL: freezing of lower limb; FO-UL, freezing of upper limb; PD, Parkinson's disease.

VIDEO LEGEND:

Video 1 is a short fragment of a Parkinson patient (freezer) performing the bilateral upper limb task in a simplified experimental setting (measuring equipment not shown). The freezing episode starts at 6s and is characterized by small-amplitude and high frequency movements that resemble the trembling like leg movements described for FOG. Between 6s and 10s nearly-normal movement cycles or half movement cycles still occur. After 10s both fingers are clearly 'stuck' in uncontrolled dysrhythmic behavior. In this example, the patient is not able to regain regular movement before the end of the video.

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SUPPLEMENTARY MATERIALS

Supplement 1 A. Spectral analyses

The Fast Fourier Transform (FFT) was used for spectral analyses. For the purpose of this study, only kinematic time series in the absence of auditory cueing were analyzed for both regular motion and freezing episodes separately. Signals were low pass filtered (cutoff-frequency of 30 Hz) using Matlab (7.4).
Preprocessing steps (e.g. signal filtering, smoothing and differentiation) and specific spectral methods (e.g. width of the time window, normalization procedures) were applied to calculate a freezing index (FI).[1-4] Pilot analysis using various methods described in previous studies[1-4] showed that these methodological aspects influenced absolute FI values, but not the relative difference between freezing episodes and regular motion. The power in each frequency band was calculated as the surface area under the spectral curve. The power in frequencies between 0.5 Hz and 8 Hz was expressed as a percentage of maximal power for each given trial. Signal preprocessing included differentiation and smoothing with a hamming window which corrected for edge artifacts in subdivided signals.

Supplement 1 B. Gait data

Angular knee displacement was determined every 0.01 second by a VICON data capturing system (Vicon Motion Systems, Workstation 612 with full body plug-in-gait marker placement) positioned at a ten meter walkway. Preprocessing and spectral methods were the same as those used for UL time series.

Supplement 2: Definition of freezing episodes

A consensus on how to identify freezing episodes is presently lacking. Previous studies described FOG as a sudden and/or transient difficulty or inability to initiate or continue walking[2, 5-7] or as a sudden and involuntary cessation of gait.[8-13] Post-hoc video observation was most commonly used to detect freezing episodes[2,4-6,8,9,11,12,14-17] but clear start- and end-criteria to demarcate the episodes were rarely validated.[9,14,15,16] We used 50% amplitude reduction as a cut-off point, as previous study on FOG indicated that reductions of this magnitude heralded freezing episodes.[8]

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Chapter 3

Abnormalities and cue-dependence of rhythmical upper limb movements in Parkinson's disease patients with freezing of gait

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ABSTRACT

Background. Freezing of gait (FOG) is a significant clinical problem in Parkinson disease (PD). Similar freezing-like episodes occur during finger movements, but little is known about ongoing motor problems during repetitive hand movements.
Objective. To investigate if the regulation of bimanual movements is impaired in those with FOG and if withdrawal of an auditory cue amplifies this problem.
Methods. A total of 23 PD patients (11 with and 12 without FOG) and 11 controls (CTRLs) performed repetitive finger movements, either externally paced or following cue withdrawal. Movement frequency, amplitude, and coordination pattern were manipulated. The stability and accuracy of movement were evaluated after exclusion of freezing trials.
Results. With auditory pacing present, movement performance was comparable between groups. Following cue withdrawal, motor control deteriorated in those with FOG, resulting in smaller and less stable amplitudes, hastened and more variable frequency, and decreased coordination stability. Conversely, the performance of those without FOG remained mostly similar to that of CTRLs.
Conclusions. Compared with those without FOG, those with FOG show greater continuous dyscontrol of bimanual movements, similar to the continuous timing and scaling difficulties during locomotion. Those with FOG also benefit from auditory cueing during upper-limb movements, but these improvements are highly cue dependent. This implies that internal timekeeping functions are more disturbed in those with FOG, who may require rehabilitation strategies for repetitive upper-extremity tasks that include cueing and imagery.

Keywords: freezing of gait, Parkinson disease, motor control, bimanual coordination, cueing

1. INTRODUCTION

One of the central hallmarks of Parkinson's disease (PD) is the disturbance of the basal ganglia motor circuitry, resulting in slow and hypokinetic movement.[1,2] Freezing of gait (FOG) represents a more dramatic movement breakdown which occurs very frequently in the later stages of PD.[3] As a significant predictor of falling, FOG is a highly debilitating problem in PD.[4] FOG is defined as a brief interruption of walking during which patients find it impossible to generate effective forward stepping movements despite the intention to walk.[5] The responsiveness of FOG to medical and surgical treatments is often limited.[6] Therefore, the development of effective rehabilitation strategies is of great clinical value. Training patients to use external sensory cues that coincide with the appropriate stepping rhythm or stride length generally improves the gait pattern[7-10] in PD. However, the beneficial effects of cues were not as obvious in freezers[11-14] as in non-freezers and were shown to be more dependent on the actual presence of the cue.[14] This may be because freezers (PD+FOG) and non-freezers (PD-FOG) have a distinct cognitive and motor profile, even in the face of similar disease severity.[15-21] FOG is associated with greater cognitive dysfunction, especially in the executive functioning domain.[15-17] PD+FOG patients also have a more severely disrupted gait pattern than non-freezers outside the momentary FOG episodes (hereafter referred to as ongoing or continuous abnormalities), including increased variability of step timing[18], disordered bilateral coordination[19,21] and a reduction of stride amplitude.[21] However, it is still unclear which of these abnormalities is most affected in patients with FOG.

Freezing-like motor blocks are not restricted to gait, but also occur in various other rhythmic tasks such as speech[22], writing, manual tapping, tooth brushing, feet wiping.[23-26] We recently showed that upper limb freezing episodes (FO-UL) were correlated to FOG and were also preceded by similar spatiotemporal changes namely a gradual decrease in movement amplitude and increase in frequency (hastening).[25,26] Abnormal high-frequency components were also found to be common characteristics of both FO-UL and FOG.[26] Hence, we argued that freezing is related to a general deficit in the organization of repetitive motion that is effector-independent.[26] In contrast to the continuous gait difficulties described for freezers,[18-21] little is known about motor problems during hand movements outside actual freezing episodes.[24, 25] It is also currently unclear whether these motor problems can be overcome by cueing as greater cue-dependency may also decrease the efficiency of cueing for upper limb movements in freezers just like in gait.[14]

Therefore, the first aim of the present study was to compare the general spatiotemporal characteristics of rhythmical finger movements between freezers, non-freezers and controls while movement amplitude[21] frequency[22] and coordination complexity[20] were systematically manipulated. In particular, we tested which of these determinants had the largest impact on movement quality in freezers. Secondly, we examined whether performance of rhythmical finger movements are more sensitive to auditory cue-withdrawal in freezers compared to non-freezers and control subjects.[14] This research question has high relevance for rehabilitation as cueing for other tasks than gait has been an unexplored area. Furthermore, insight in the underlying motor control deficits of freezing will aid in refining current rehabilitation strategies for PD subgroups.

2. MATERIALS AND METHODS

Participants

Twenty-three PD patients were recruited in the Movement Disorders Clinic of the University Hospital Leuven. Patients who had experienced FOG within one month before testing and thus scored positively on item 1 of the revised FOG-Questionnaire (FOGQ[27]) were classified as freezers (PD+FOG, n=11). Freezers and non-freezers (PD-FOG, n=12) were matched for age, sex and disease severity. Patients were in Hoehn and Yahr stage II or III[28] during the ON state. Exclusion criteria were: (1) diagnosis of a neurological disease (other than PD), (2) presence of a deep brain stimulator, and (3) dementia (Mini Mental State Examination (MMSE) score <24/30). Eleven age-matched, healthy control subjects (CTRL, male=10) also participated. Participants gave informed consent consistent with the Declaration of Helsinki. Ethics approval was received by the Commissie Medische Ethiek K.U.Leuven.

Design and procedure

Prior to testing, the following clinical data were collected: the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS[29]), FOGQ, MMSE, Hoehn and Yahr staging and the cognitive section of the Scales for Outcomes in PD (SCOPA-Cog[30]). This clinical screening occurred at participants' homes when ON-medication.

The experimental session in the laboratory took place in the early morning during the 'practically defined OFF-phase', i.e. after omitting the morning dose of medication (12–15h after last intake). On this day, UPDRS motor examination was repeated. Seated on a height-adjustable chair, subjects performed a bimanual task consisting of rhythmic flexion and extension movement of the index fingers. We chose a bimanual rather than a unimanual paradigm because it allows well controlled manipulations of coordination complexity within an experimental setting. Movement complexity, amplitude and frequency were manipulated according to a 2 x 2 x 2 factorial design. Subjects of three subgroups (PD+FOG, PD-FOG, CTRL) performed either an easy coordination pattern requiring simultaneous finger movements (in-phase coordination) or a complex pattern requiring alternating movements (anti-phase coordination). Before testing, a single trial of alternating movements lasting 30 seconds enabled the computation of participants' comfortable amplitude and frequency. During testing, they were instructed to make movements at their comfortable amplitude, or at small amplitude (66% of the comfortable amplitude). Similarly, cycle frequency was either comfortable or fast (i.e. 100% and 133% of comfortable frequency). The size of the amplitude decrement and frequency increment was determined in a pilot study and chosen to challenge movement control so that freezing-like movement breakdown could be triggered. Movement was initially guided by auditory pacing (first 6 movement cycles, 'cue present'), after which it became internally generated ('cue withdrawn'). Subjects performed three trials per condition. A square box covering both hands prevented visual feedback during movement. A Micro 1401 acquisition unit (Cambridge Electronic Device, UK) with Spike 2 software recorded angular displacements with temporal and spatial resolutions of 2000 Hz and 0.0001 degrees through analogue encoders placed on the rotation axis of a device connected to each index finger. Subjects were given sufficient time to practice and familiarize themselves with task requirements.

Data processing

This study focused on ongoing motor regulation in freezers outside the freezing episodes. Therefore, we excluded trials in which FO-UL occurred for this analysis. FO-UL was defined as 'a period of an involuntary absence of or markedly reduced cyclic movements'.[26] A detailed description of the freezing detection method is described elsewhere (see Chapter 2).[26]
Kinematic time series were low pass filtered (digital Butterworth filter with cutoff-frequency of 30 Hz) using Matlab (version 7.4). The following movement parameters were obtained:
Within-limb movement amplitude: Movement amplitude was calculated based on point-by-point measures of the end-effectors motions using the Hilbert transform.[31,32] This way, mean movement amplitude and its coefficient of variability (COV), i.e. the ratio of standard deviation (SD) over the mean expressed as a percentage (%) were obtained.
Within-limb frequency measures: Mean frequency, the deviation from the requested frequency (frequency error (Hz)) and frequency COV (expressed as %) were computed using peak-to-peak measures of the end-effectors motions.
Consistent with previous research[25, 27], results of the disease-dominant hand in patients and the non-writing hand in control subjects will be reported for within-limb amplitude and frequency parameters.
Interlimb coordination: Coordination parameters were based on the relative phase (RP) which corresponds to the difference in position of the left and right finger in their movement cycle at a given moment.[33] RP is expressed in degrees and was calculated on a point-by-point estimation using the instantaneous phase of each signal, produced by the Hilbert transform.[31,32] The following equation was used:

$$\phi = \theta_{\mathrm{R}} - \theta_{\mathrm{L}} = \tan^{-1}\left(\frac{\mathrm{d}X_{\mathrm{R}}/\mathrm{d}t}{X_{\mathrm{R}}}\right) - \tan^{-1}\left(\frac{\mathrm{d}X_{\mathrm{L}}/\mathrm{d}t}{X_{\mathrm{L}}}\right)$$

where XR and XL are the instantaneous displacements of the right and left end-effectors, respectively, and dXR ⁄ dt and dXL ⁄ dt are the instantaneous velocities. Circular variability of RP (RPvar) was computed. Absolute error scores (RPerror) indicated the degree of deviation from the target RP (i.e. 0° and 180° for in-phase and anti-phase pattern respectively).
Movement outcomes of trials within a same condition were averaged (3 trials per condition or less in case of omitted freezing-trials).

Statistical analysis

Statistical analyses were performed using STATISTICA (8.0). Clinical parameters were compared between groups by use of a one-way analysis of variance (ANOVA) or non-parametric Mann-Whitney U-tests (2 groups) or Kruskal-Wallis tests (3 groups) in case of a skewed distribution of the data. A repeated measures ANOVA was used to test influence of within-subject factors CUE (present, withdrawn), PATTERN (in-phase, anti-phase), AMPLITUDE (comfortable, small) and FREQUENCY (comfortable, fast) in addition to the between-subject factor GROUP (PD+FOG, PD-FOG, CTRL). The relative contribution of within-subject manipulations during movement after cue withdrawal was further explored in freezers using a one-way ANOVA comparing the two levels of factors FREQUENCY, AMPLITUDE and PATTERN. Significance levels were set at .05. Significant effects were addressed by Newman-

Keuls post-hoc tests. Results are reported as means and standard errors of measurements (SEM).

3. RESULTS

Subjects

Data of one patient from the PD+FOG group were excluded from the analysis because freezing episodes occurred in all but one trial. Apart from severe FOG, this patient showed no other signs of greater disease severity or cognitive impairment than the other patients. Eight other freezers and 2 non-freezers demonstrated FO-UL resulting in a total of 64 trials with FO-UL (on average 6.4 (1.4) per person). These trials were excluded from analysis. Details on the occurrence and characteristics of freezing episodes can be found elsewhere.[26] All but one freezing episode occurred after cue-withdrawal. **Table 1** represents the clinical characteristics of the remaining 10 freezers, 12 non-freezers and 11 controls. Age, gender distribution and comfortable movement frequency and amplitude measured before actual testing were similar in the 3 groups. Mean disease duration, UPDRS motor scores in on and off, Hoehn and Yahr staging, daily levodopa-equivalent dose and MMSE scores were not significantly different between PD+FOG and PD-FOG, indicating comparable disease profiles. SCOPA-COG scores were lower in freezers compared to non-freezers and controls but fell within the range of normal cognition.[34]

Ongoing movement performance (independent of freezing episodes)

Omitting trials in which freezing occurred led to missing values in 6 patients of the PD+FOG group (none in the PD-FOG group). Data were therefore pooled for the levels of the factor FREQUENCY. Factor FREQUENCY was preferred over factors PATTERN and AMPLITUDE because 1) it eliminated all missing values in contrast to pooling for the other factors and 2) it was considered a less determining factor than AMPLITUDE[21] and PATTERN[20]. The results reported below are based on repeated measures ANOVA with CUE, PATTERN and AMPLITUDE and GROUP as factors. Similar results were obtained when data were pooled over other factors.

<u>Movement amplitude</u>

Amplitude requirements were based on subject-specific comfortable amplitudes which were similar between groups (table 1). All participants made smaller movements in small-amplitude compared to comfortable-amplitude conditions ($p < .0005$). The significant group x cue x amplitude interaction ($p = .044$) showed that only in PD+FOG amplitude decreased after withdrawal of auditory pacing in comfortable-amplitude conditions (from 47.14 (5.64) to 42.36 (5.33), $p = .039$)). In contrast, controls increased their amplitude after cue withdrawal in the comfortable- (64.70 (3.08) vs. 69.15 (3.94), $p = .00023$) and small-amplitude conditions (40.58 (3.12) vs. 43.52 (3.46), $p = .016$). This resulted in a significantly smaller amplitude in freezers (42.36 (5.33)) compared to controls (69.15 (3.91)) during uncued, comfortable-amplitude conditions ($p = .039$). No group differences were found when the cue was present.

We found a significant group x cue interaction effect ($p = .00043$) on amplitude variability. For externally guided movement, no group differences were found (**Figure 1**). However, during internally guided movement, amplitude COV (which corrects for differences in mean

amplitude) was higher in freezers compared to controls (p=.044). A cue effect was only found in freezers where amplitude variability was significantly higher in the absence of the cue compared to while the cue was present, (p=.000521).

Table 1: Demographic and Clinical Characteristics of 12 Participants Without FOG, 10 With FOG, and 11 Controls (Median and Interquartile Ranges)

Parameter	Non-Freezers	Freezers	Controls	P-value
Age (years)	70 (64-72)	69 (65-71)	67 (60-71)	0.80
Disease duration (Years)	7 (6-9)	9 (6-12)	----	0.47
Sex (M/F)$^{\Delta}$	10/2	8/2	10/1	0.78
UPDRS-III ON (0-108)	36.5 (32-44.3)	29.5 (27-46.3)	----	0.55
UPDRS-III OFF (0-108)	35 (28-37)	36 (28-43)	----	0.61
H & Y OFF (0-5)°[1]	II (II-III)	III (II-III)	----	0.46
FOGQ (0-23)°[1]	0 (0-0)	9 (7-17)	----	0.00*
MMSE (24-30)°[2]	30 (28-30)	28 (27-28)	30 (29-30)	0.09*
SCOPA-COG (0-43)	31 (28-33)	30 (27-31)	34 (30-37)	0.05*
Levodopa-dose (mg)	510 (413-626)	555 (466-688)	----	0.70
Comfortable frequency (Hz)	1.13 (0.80-1.37)	1.14 (0.89-1.69)	1.38 (0.97- 1.69)	0.32
Comfortable amplitude (°)	36.6 (32.0-48.7)	35.3 (22.7-44.5)	45.33 (35.4-50.9)	0.18

* Groups significantly different at P<.05; °Non-parametric tests were used: °[1] Man-Whitney-U test; °[2] Kruskal-Wallis test ΔLogistic regression was used. UPDRS-III, Unified Parkinson's Disease Rating Scale part III; H & Y, Hoehn & Yahr stage; FOGQ, Freezing of Gait Questionnaire; MMSE, Mini Mental State Examination; SCOPA-COG, Scales for Outcomes in Parkinson's Disease- Cognitive part.

Movement frequency

Frequency requirements were based on subject-specific comfortable frequency which was comparable between groups (table 1). Mean frequency increased when auditory pacing was removed (*p*=.0052). When we explored the almost significant group x cue interaction (*p*=.088), the post-hoc test suggested that this hastening effect after cue withdrawal occurred mainly in freezers (*p*=.0053). Unlike controls, both patient groups moved faster during small-amplitude conditions than comfortable-amplitude conditions (1.24 (*0.13*) Hz during comfortable- and 1.43 (*0.16*) Hz during small-amplitude conditions for PD-FOG (*p*=.00027) and 1.55 (*0.17*) Hz and 1.74 (*0.20*) Hz in PD+FOG (*p*=.00036)).

Main effects of the factors amplitude and cue showed that frequency error increased during small-amplitude compared to comfortable-amplitude conditions (*p*=.044) and after cue withdrawal (*p*=.012). The group x cue x amplitude interaction (*p*=.023) revealed that an increase in frequency error following cue withdrawal occurred only in PD+FOG during small amplitude-conditions (from 0.22 Hs (*0.070*) to 0.44 Hz (*0.11*), *p*=.00016). The frequency error increase in small versus comfortable-amplitude conditions was only significant in freezers when the cue was withdrawn (from (0.28 Hz (*0.079*) in comfortable- to 0.44 Hz (*0.11*) in small-amplitude conditions *p*=.00045).

Variability of movement frequency increased in PD patients when small amplitude compared to comfortable amplitude movements were required (in PD-FOG: *p*=.00015; in PD+FOG: *p*=.017, group x amplitude interaction, *p*=.039). **Figure 2** illustrates the significant group x cue interaction (*p*=.0023). Frequency variability was similar between groups when external cueing was present. However, after cue withdrawal, variability increased significantly in freezers only (*p*=.0028) resulting in a higher frequency COV (frequency COV= 13.47% (*1.93*)) compared to PD-FOG (10.19% (*1.61*), *p*=.012) and controls (6.80% (*0.63*), *p*=.00017).

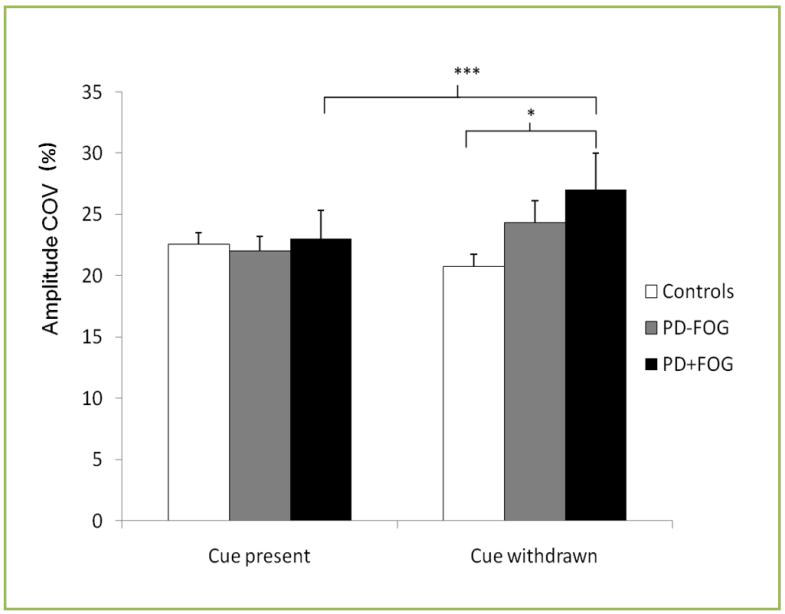

Figure 1. Amplitude coefficient of variability (COV) as a percentage in controls (CTRLs), those without FOG, and those
with FOG during movement guided by auditory pacing (cue present) and after cue withdrawal (cue withdrawn). After removal of the cue, the amplitude COV of those with FOG significantly increased and became higher than that of CTRLs. Vertical bars represent standard error of measurements. Abbreviations: PD, Parkinson disease; FOG, freezing of gait. *$P < .05$; ***$P < .005$.

Interlimb coordination.

Although the main effect of group failed to reach significance (*p*=.088), freezers showed a clear trend to have a higher relative phase error compared to controls (*p*=.069). In all participants, RP error was lower while the cue was present than after cue withdrawal (*p*=.041).

Coordination variability was higher during anti-phase than in-phase coordination trials (*p*<.000005). The significant group x cue x pattern interaction is depicted in **Figure 3** (*p*=.018). In freezers, RPvar increased following cue withdrawal (*p*=.055 and *p*=.00012 for in-phase and anti-phase conditions respectively). In non-freezers, this cue effect was only present during anti-phase conditions (*p*=.012). In the most complex condition, namely anti-phase coordination after cue withdrawal, RPvar was significantly higher in freezers than non-freezers (*p*=.0024) and controls (*p*=.00027).

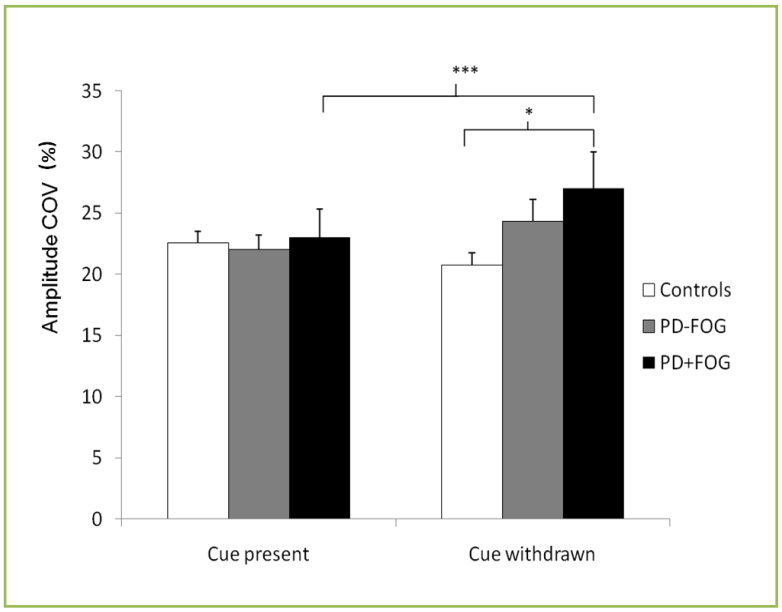

Figure 2. Variability of movement frequency (COV) in CTRLs, those without FOG, and those with FOG during movement guided by auditory pacing (cue present) and after cue withdrawal (cue withdrawn). Withdrawal of external cueing resulted in significantly increased frequency COV in those with FOG than in those without FOG and CTRLs. Vertical bars represent standard error of measurements. Abbreviations: PD, Parkinson disease; FOG, freezing of gait. **P* < .05; ***P* < .005; ****P* < .0005.

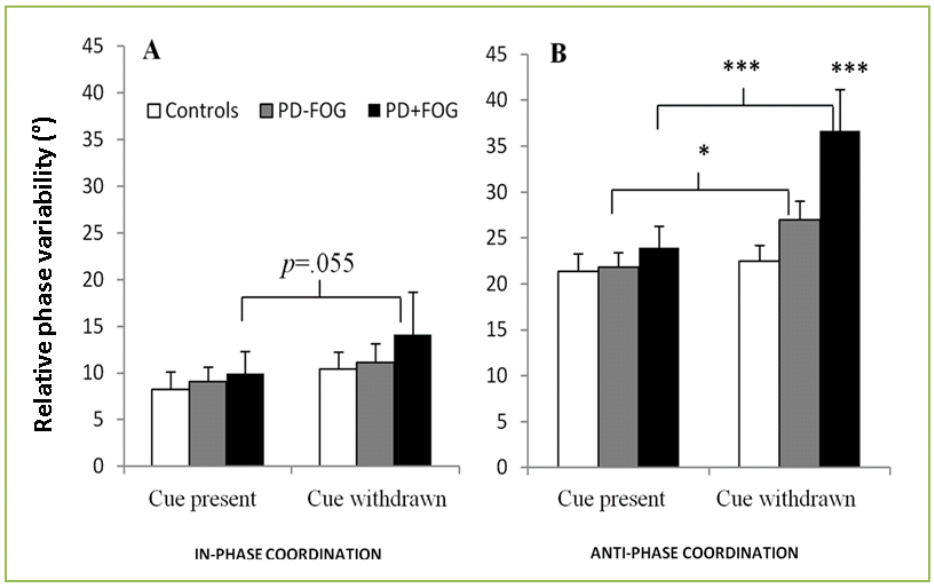

Figure 3. Variability of relative phase (RP) in CTRLs, those without FOG, and those with FOG during movement guided by auditory pacing (cue present) and after cue withdrawal (cue withdrawn) during (A) in-phase coordination and (B) antiphase coordination. RP variability during in-phase coordination was higher in those with FOG after cue withdrawal compared with when the cue was present. During antiphase coordination, variability increased after cue withdrawal in those without FOG and even more strongly in those with FOG, resulting in significantly higher RP variability than in those without FOG and CTRLs. Vertical bars represent standard error of measurements. Abbreviations: PD, Parkinson disease; FOG, freezing of gait. **P* < .05; *** *P* < .0005.

Exploratory subanalysis in freezers

We further explored which movement constraint (amplitude, frequency and pattern) had the most deteriorating influence on uncued motor performance in freezers (**Table 2**). We were especially interested in the influence of a given factor (e.g. amplitude) on outcome parameters that did not directly depend on it (e.g. on frequency outcomes instead of mean and variability of amplitude). Unlike frequency and pattern constraints, the manipulation of amplitude affected outcomes within the scaling domain as well as frequency and coordination measures. In small-amplitude conditions, freezers' frequency became hastened (11.78 % increase in mean frequency from 1.65 (*0.18*) Hz to 1.86 (*0.21*) Hz, *p*=.0086) and deviated more from the requested frequency (83.03 % increase in frequency error from 0.28 (*0.067*) Hz to 0.44 (*0.11*) Hz, *p*=.013) compared to comfortable-amplitude conditions. Similarly, inter-manual coordination in freezers became worse in small-amplitude compared to comfortable-amplitude conditions resulting in a 52.26 % increase in RP error (from 9.99 (*1.45*) ° to 15.06 (*2.67*) °, *p*= .018) and a 22.58% increased RP variability (from 22.57 (*2.49*) to 27.41 (*2.91*), *p*=.0026).

Table 2: Exploratory analyses of the relative Influence of amplitude, frequency, and pattern manipulations on motor performance in those with FOG after cue withdrawal

		Outcome parameters						
Manipulations		**Amplitude Mean**	**Amplitude COV**	**Frequency Mean**	**Frequency Error**	**Frequency COV**	**Relative phase Error**	**Relative phase Var**
AMPLITUDE	***p*- value**	<0.01*	0.09	**<0.01***	**0.01***	0.14	**0.02***	**<0.01***
	% change *(SEM)*	-38.12 *(3.96)*	11.45 *(4.67)*	**11.78** ***(2.74)***	**83.03** ***(30.44)***	21.90 *(9.78)*	**52.26** ***(14.72)***	**22.58** ***(5.28)***
FREQUENCY	***p*- value**	0.38	0.81	0.03*	0.09	0.04*	0.69	0.67
	% change *(SEM)*	6.06 *(4.04)*	1.40 *(2.41)*	13.98 *(4.66)*	-16.34 *(11.22)*	12.48 *(4.90)*	11.40 *(14.71)*	0.47 *(9.29)*
PATTERN	***p*- value**	0.22	0.26	0.24	0.88	0.24	0.19	<0.01*
	% change *(SEM)*	-5.71 *(6.66)*	17.78 *(10.92)*	-7.54 *(4.82)*	36.33 *(23.59)*	22.28 *(14.80)*	-30.00 *(18.55)*	168.23 *(18.04)*

Abbreviations: COV, coefficient of variability; SEM, standard error of measurements; FOG, freezing of gait. Percentage change indicates the change in outcome parameters when comparing the most difficult level of a given factor to the least difficult level (ie, small vs comfortable amplitude; fast vs comfortable frequency; antiphase vs in-phase pattern). Cells in grey indicate the influence of a given factor (eg, amplitude manipulation) on outcome parameters within the same movement domain (eg, mean and variability of amplitude). Unlike speed and pattern constraints, the manipulation in amplitude affected outcomes within the scaling domain as well as frequency and coordination measures in those with FOG. *Significant *P* values <.05.

4. DISCUSSION

The aim of the study was to investigate the motor abnormalities in freezers and non-freezers during ongoing repetitive upper limb movements and how this is influenced by cue withdrawal. We found that within-limb spatiotemporal and coordinative control of internally generated finger movements is much more affected in freezers than in non-freezers. These findings coincide remarkably with the pronounced FOG-related motor abnormalities present during gait.[20-23] Secondly, the dramatic effect of cue withdrawal indicates that freezers

benefit from external guidance but show increased cue-dependency. This suggests that internal motor control is more affected in freezers than non-freezers[35], which is important for rehabilitation.

Motor abnormalities in freezers

Internally generated finger movements in freezers were characterized by a small and unstable amplitude, a variable, hastened frequency and decreased coordinative stability. In contrast, movement in non-freezers did not differ significantly from control subjects in most parameters. The fact that visual feedback was absent and interlimb coordination was required, may explain why our paradigm revealed a faulty scaling-timing mechanism in freezers which was not clearly shown previously.[24, 25]

Although correlations were found between FOG and upper limb freezing[25, 26], the generic nature of the freezing phenomenon is still a matter of debate. We previously showed that changes in the kinematic signals prior to a freezing episode are quite similar in gait and upper limb movement.[26] FOG has been related to a continuously disrupted gait pattern even outside freezing episodes.[18-21] The present results therefore expand the similarity between FOG and upper limb freezing to a faulty organization of ongoing movement and add strength to the conceptualization of freezing as a generic spatiotemporal motor control problem.[26] It is plausible that this background of abnormal motor physiology culminates in episodic breakdown during gait and finger movement.

FOG is known to occur in the face of heightened attentional or perceptuomotor demands, for example when turning[36], passing through a narrow door[37,38] or performing a secondary task while walking.[36] Here, freezing related motor abnormalities such as hastening were triggered without additional cognitive, limbic or postural load but were amplified in conditions where small-amplitude finger movements were requested. Though based on exploratory analyses, this finding is important as it is congruent with the dramatic effect of ongoing amplitude adjustments on cadence regulation during straight line walking[21] and turning[36], and with the triggering effect of small-amplitude movements on freezing episodes during finger movement.[26] These results underscore the core role of impaired amplitude regulation in the freezing phenomenon.[21,26] Hence, re-learning patients to maintain a regular amplitude may improve writing skills and other repetitive upper limb movement and prevent hastening or freezing episodes, but this awaits further study.

To focus on ongoing motor abnormalities, we excluded trials in which FO-UL occurred, inherently leading to missing values in the PD+FOG group. Pooling data for both levels of factor FREQUENCY eliminated all missing values but may have influenced the present results. This methodological concern may be avoided by incorporating more repeated trials per condition in future research.

Neuroanatomical correlates of freezing

Bilateral coordination of automated movement sequences requires a tight connection of brain areas within a distributed network.[39,40] The basal ganglia are crucial in regulating movement amplitude.[39-44] As a result of striatal dysfunction, people with PD tend to shift from automatic to controlled movement, associated with decreased brain activity in the striato-supplementary motor areas (SMA) and increased activation of the cerebellum and

premotor-parietal areas.[40,45, 46] The distinct motor profile in freezers shown here suggests a more profound striatofrontal disruption that does not allow further tapping into compensatory neural reserves and may as such cause motor breakdown. A recent study[47] comparing brain activity during motor imagery in freezers and non-freezers supports this idea. The authors found similar striatal activation levels in both groups but relative underactivation in the SMA in freezers, which they related to stride length dyscontrol. Freezers did not show increased cerebellar and premotor-parietal activations but demonstrated increased activation in the mesencephalic locomotor region (MLR), a densely connected region that is thought to drive gait via central pattern generations in the spinal cord.[48,49] Grey matter and connectivity changes in the MLR were also found in freezers.[47,50] Further study is needed to understand whether the MLR is a similar key player in upper limb freezing or whether it is gait specific.

Cue-dependency in freezers

The use of sensory cues is an efficient rehabilitation tool in PD as it bypasses the deficient striato-frontal system in favor of cerebello-parietal-premotor pathways.[10,51] As such, scaling and timing of steps can be improved in PD and translates in increased functional mobility after a training period.[7-10] In a systematic review in 2008, Nieuwboer concluded that the benefits of sensory cueing were less obvious in freezers than in non-freezers, indicating reduced capacity for compensation.[11] The present findings propose that auditory cues can partially normalize motor abnormalities during upper limb movement in freezers while they are in the off-phase of the medication cycle. This is in line with recent evidence of improved gait parameters and turning behavior in freezers using rhythmic cueing.[52, 53, 54] Few studies focused on cueing of movements in the upper extremities in PD, yielding inconsistent results. In contrast to earlier work[24,55] Ringenbach et al[56] found that timing, scaling and coordination during a drawing task improved by auditory pacing. Benefits due to visual cueing were less evident. However, augmented visual feedback enhanced upper limb motor learning in PD.[57, 58] The effect of cueing on bimanual movements in subgroups of freezers and non-freezers was recently addressed for the first time.[25] Visual target lines aided both groups in achieving a more stable and accurate drawing performance, but increased the relative phase variability in freezers. In our study, movement parameters, including coordination stability, improved in freezers when auditory cueing was present, but dropped dramatically after cue-withdrawal.
These results strongly suggest reduced retention of external cueing following cue-withdrawal in freezers. A previous study demonstrated short-term carry-over effects of sensory cueing during walking in freezers when optimally medicated.[52] However, congruent with Willems et al.,[14] our results showed the opposite. This implies that freezers may suffer from a specific deficit in automating cued responses. Cued conditions were short and non-randomized, which is a drawback of the study. As an order effect cannot be ruled out, the findings need replication by future studies.
The neural processes underlying internal continuation of bimanual movements after removal of an auditory cue have recently been investigated by Cerasa et al.[59] This fMRI study showed no neurofunctional differences between the two timing phases in PD and matched controls. However, in both the synchronization and continuation phase compensatory activity increased in the cerebello-thalamic pathway in PD patients associated with similar motor performance compared to the control group. It is therefore possible that this compensatory

neural reserve is insufficient or not sustained in freezers resulting in a continuous need for external information to drive the motor network. Motor learning strategies aimed at internalizing external information, for example using mental practice, may be especially relevant in freezers. Motor imagery was recently shown to be feasible in patients with PD in general[60] as well as in freezers in particular.[47, 61] Moreover, the mutual influence of mental practice and external cues recently shown by Heremans and colleagues,[62] may form a promising new direction in the development of rehabilitation strategies for repetitive upper limb tasks which may also be applied in freezers. The feasibility of such a motor learning approach should be examined in future clinical studies.

Conclusions and implications for future research and rehabilitation

Our findings of exaggerated dyscontrol of repetitive upper limb movements in freezers corroborate previous studies on FOG-related abnormalities during ongoing gait. In view of these observations, future motor control research would benefit from either selecting the study population based on the absence or presence of freezing or describing the freezing profiles to enhance the interpretation of the findings. We showed for the first time that freezers may benefit from external sensory cueing to normalize scaling, timing and coordination difficulties in an upper limb repetitive task but show increased dependency on the presence of the cue. These findings may be of use when developing guidelines for rehabilitation interventions for the training of functional upper limb tasks such as writing, stirring, screw driving, wiping and tapping in patients with freezing. Further research on the retention of cueing effects in freezers is warranted, particularly when patients are tested in the ON-phase. These insights are especially important in the light of translating laboratory training studies to patients' home settings.

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ABBREVIATIONS

FOG, freezing of gait; FOGQ, Freezing of Gait Questionnaire; FO-UL, freezing of upper limb; PD, Parkinson's disease.

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Chapter 4

The neural basis of disturbed motor control and upper limb freezing in Parkinson patients with freezing of gait

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In preparation

ABSTRACT

Background: Freezing of Gait (FOG) is a highly disabling clinical problem in Parkinson's disease (PD). A growing number of brain imaging studies have addressed the neural mechanisms of FOG indirectly by measuring neural activation during rest and motor planning. Patients with FOG present freezing episodes and continuous motor abnormalities during rhythmic upper limb movement that resemble their gait problems. Upper limb freezing offers a novel paradigm to examine changes in cerebral motor control related to freezing, using functional Magnetic Resonance Imaging (fMRI).
Objective: We used fMRI to identify the neural correlates of freezing and related timing-amplitude dyscontrol in PD during movement generation.
Methods: Brain activation was measured during the performance of bilateral repetitive finger movements in 16 PD patients with FOG, 16 disease-matched patients without FOG and 16 age-matched controls. Kinematic time series obtained during scanning were divided into ongoing (no freezing) movement and upper limb freezing episodes (FOUL). We contrasted brain activation during 1) ongoing upper limb movement between groups and 2) during ongoing movement versus upper limb freezing within 8 patients who presented FOUL during testing.
Results: There were two main findings: 1) Brain activity during ongoing movement was decreased in the right dorsolateral prefrontal, left dorsal premotor (PMd) and primary motor cortex (M1) in patients with FOG compared to PD without FOG and controls. In contrast, right dorsal putamen, bilateral pallidum and bilateral subthalamic nucleus showed greater activation in PD with FOG compared to controls and PD without FOG. 2) Brain activation during freezing episodes showed an inverse pattern with increased cortical activity in the right supplementary motor area, the right M1, right PMd and left prefrontal cortex compared to ongoing movement, whereas the pallidum and putamen showed decreased brain activation during FOUL as compared to CONT.
Conclusion: The shared motor mechanisms of FOG and FOUL (amplitude and rhythm dyscontrol) were related to altered patterns of brain activity within the striatofrontal circuitry. Subcortical hyperactivity may dampen cortical activation in motor and cognitive areas, resulting in spatiotemporal abnormalities during ongoing upper limb movement in freezers. In contrast, a shift to an increased cortical drive which has been described as a compensatory mechanism in PD, only occurred in freezers when FOUL had emerged and may reflect an attempt to overcome the motor block.

Keywords: Freezing of gait, Upper limb freezing, Neuroimaging, fMRI, Parkinson's disease

1. INTRODUCTION

Freezing of gait (FOG) is one of the most debilitating gait disorders in Parkinson's disease (PD) as it causes falls[1] and reduces mobility and quality of life.[2,3] During a FOG episode, patients experience a '*marked reduction of forward progression of the feet despite the intention to walk'*, as if their feet were glued to the floor.[4] FOG is common in advanced PD but does not affect all patients.[5] Patients with FOG have a distinct neuropathological profile in which dopaminergic, motor, cognitive and postural impairments play a synergistic role.[4,6] In this paper, we focus on the core motor aspect of freezing, namely a generalized disturbance in amplitude and timing regulation.[7-9] Timing-amplitude dyscontrol is evidenced by abnormal motor output with high-frequency trembling during a freezing event.[10-13] However, this deficit is also present during ongoing (functional) gait causing impairments in step timing, interlimb coordination, step amplitude and cadence.[14-19] We refer to these motor pattern generation problems underlying freezing as 'generalized' because they are not restricted to gait. Interestingly, repetitive upper limb movements showed similar episodic motor blocks that were correlated and strikingly similar to FOG.[7,9] In the latter task, freezers presented with marked impairment in maintaining a stable movement amplitude and frequency even when no freezing episodes were experienced, in line with their continuous gait abnormalities.[8] In view of these converging patterns, upper limb freezing offers a novel and unique paradigm to examine changes in cerebral motor control related to freezing, with distinct advantages for application within a functional Magnetic Resonance Imaging (fMRI) environment.

Ample brain imaging studies indicate increased engagement of the cerebello-premotor-parietal network in PD compared to healthy controls in order to equate motor performance to the required output during various motor tasks including gait.[20-22] Comparing freezers to non-freezers, recent studies found grey matter atrophy and divergent metabolic changes in fronto-parietal regions of freezers.[23-28] In addition, functional[29] and structural[29,30] imaging studies pointed to structures downstream of the deficient basal ganglia as playing a key role in FOG. The non-dopaminergic mesencephalic locomotor region (MLR) in the brainstem, projecting to spinal central pattern generators (CPG), showed greater grey matter atrophy,[29] altered white matter connectivity with cortical and cerebellar regions[30] and hyperactivation during gait planning[29] in patients with FOG compared to those without. These findings contribute to our understanding of the neuropathology of FOG but remain difficult to directly link to the emergence of the episodes of abnormal motor output since none of these studies measured brain activity during movement production (rather the patients imagined to walk).

The purpose of the present study was to identify the neural correlates of freezing and related motor abnormalities in PD during movement generation. We exploited the convergent pattern of the spatiotemporal dyscontrol involved in freezing of gait and freezing during upper limb motion in a fMRI environment, allowing us to study brain activation during actual freezing episodes. Admittedly, measuring cerebral activity during upper limb motion does not address postural and balance components of FOG[1,4,6] but has the benefit of directly relating changes in neural activation to altered motor control associated with freezing in contrast to structural imaging, resting state, and motor imagery fMRI paradigms.[23-30]

2. MATERIALS AND METHODS

Subjects

We studied 32 patients with Parkinson's disease recruited in the Movement Disorders Clinic of the University Hospital Leuven. Patients with FOG (N=16) and without FOG (N=16) were matched for disease severity and disease duration (**Table 1**). A score ≥ 1 on the new FOG-Questionnaire (NFOG-Q) classified a patient as 'patient with FOG', a score of 0 as 'patient without FOG'.[31] Matching occurred through a clinical assessment at the patients' home while on medication to reduce the testing burden for patients during the scanning session. Disease severity was measured by the Unified Parkinson's Disease Rating Scale (UPDRS)[32] and Hoehn and Yahr staging.[33] Patients with a deep brain stimulator or excessive rest tremor were excluded. A group of 16 healthy age-matched subjects served as controls. All participants had no diagnosis of a neurological disease other than PD and had no signs of clinical dementia (Mental State Examination (MMSE) score > 24). Executive functioning assessed by the cognitive section of the Scales for Outcomes in PD (SCOPA-COG)[34] and other clinical variables were similar across groups, except for the levodopa equivalent dose which was higher in freezers (Table 1). Participants gave informed consent consistent with the sixth version of the Declaration of Helsinki. Ethics approval was received by the local Medical Ethics Committee UZLeuven.

Behavioral task

One or two days before scanning, patients were invited to the laboratory to receive testing instructions and they practiced the required motor task in a dummy scanner to achieve stable performance.

Testing in the actual scanner took place in the early morning after patients had withdrawn medication for at least 12h (off medication). Subjects performed a bimanual task consisting of rhythmic flexion and extension movement of the index fingers, validated to elicit FOG-related upper limb freezing (FOUL).[7,8] In the interest of comparable task difficulty across participants, amplitude and frequency constraints were expressed as a percentage of subject-specific preferred values that were defined in the dummy scanner session (see below). To reduce scanning time and avoid fatigue, a fractional factorial design was used with 2 freezing-resistant and 2 freezing-provoking movement conditions based on previous work.[7] Freezing-resistant conditions allowed comfortable-amplitude movements at a comfortable frequency while the two index fingers were moved in-phase (Condition 1) or anti-phase (Condition 2). Freezing-eliciting conditions required small-amplitude movements (i.e. 50% of comfortable amplitude) at high frequency (i.e. 133% of comfortable frequency) according to an in-phase (Condition 3) or anti-phase coordination pattern (Condition 4). Movement conditions were presented in a random order and alternated with a rest condition using a block design. Each movement condition was prompted by a short instruction projected on the screen in the scanner at the end of the rest period. Subjects were given sufficient time (3 s) to prepare and were instructed to start moving when an auditory pacing signal started. Auditory pacing enabled frequency manipulations and was present during the first six movement cycles after which movement was continued using internal movement generation until the instruction to rest reappeared on the screen. Each movement trial lasted 30 seconds.

Subjects were lying supine in the scanner with the upper arms positioned along the body and elbows slightly flexed. Care was taken to avoid head movements using foam padding to fix the head and a bite-bar in some cases. The forearms were positioned in an orthosis, enabling only flexion and extension movements of the index fingers in the sagittal plane. The angular displacements of the index fingers were registered by means of non-ferromagnetic shaft encoders fixed to the rotation axis of the orthosis which was aligned with the metacarpophalangeal joint axis of the index finger. The shaft encoders registered movement with a spatial resolution of 1° and a sampling frequency of 200 Hz.

Functional MRI procedure

Imaging was carried out in a 3 Tesla Magnetom Trio Magnetic Resonance scanner (Siemens, Erlangen, Germany). For each subject, we acquired high-resolution T1-weighed anatomical scans and T2-weighted functional images using the following gradient echo planar imaging pulse sequence: 50 transversal slices, slice thickness: 2.8mm, slice gap: 0.28mm, TE=30 ms, TR=3000ms, flip angle= 90°, matrix: 80x80, in-plane resolution= 2.5mm x 2.5mm. The protocol consisted of 5 runs in which each of the 4 movement conditions was repeated twice. Accordingly, each run lasted 5.6 min and had 8 active conditions of 30 seconds and 8 rest conditions of 12 seconds.

Table 1: Clinical details of participants

Parameter		Controls (n=16)	PD without FOG (n=16)	PD with FOG (n=16)	*P*
Gender (M/F) [1]	Frequencies	11/5	12/4	13/3	0.72
Age (years) [2]	Mean (+/- SD)	67.3 (61.1 – 73.4)	67.4 (62.3 – 72.6)	66.1 (59.2- 73.1)	0.81
SCOPA-COG (0-43) [2]	Mean (+/-SD)	30.7 (26.0 – 35.4)	30.3 (26.2 – 34.4)	27.6 (23.2- 32.0)	0.12
Hoehn & Yahr staging (0-5) [3] (on medication)	Median (IQR)		2.5 (2.0 - 2.5)	2.5 (2. 0 - 3.0)	0.90
Disease duration (years) [2]	Mean (+/- SD)		7.4 (2.6 – 12.2)	9.5 (6.2 – 12.7)	0.17
UPDRS motor score (0-108) [3] (on medication)	Median (IQR)		34.0 (26.0 - 44.3)	32.0 (22.0-42.8)	0.72
L-dopa dose (mg/day) [2]	Mean (+/- SD)		443.8 (258.8 – 628.7)	659.7 (435.0 – 884.4)	0.01 *

* Groups significantly different at p <0.05. [1]Chi-Square test was used. [2]Two-sample t-test was used. [3]Non-parametric Wilcoxon two-sample t-test was used. Abbreviations: SD: Standard Deviation. IQR: Inter-Quartile Range (Q1-Q3). SCOPA-COG: Scales for Outcomes in Parkinson's Disease- Cognitive part. UPDRS motor score: Unified Parkinson's Disease Rating Scale part III (motor examination); L-dopa dose: Levodopa Equivalent Dose.

Data analysis

Behavioral data analysis

We processed kinematic time series in Matlab 7.7 (Mathworks, Sherborn, MA) in 2 steps. First, upper limb freezing episodes (FOUL) were detected using objective criteria. FOUL was defined as 'a period of involuntary stop or clear absence of effective cyclic movements'.[35] As validated previously[7] ineffective movement included at least 2 out 3 phenomena: 1) abnormally reduced amplitude < 50% of reference cycle; 2) irregular frequency and 3) a freezing index >1 and FOUL-episodes were demarcated by means of visual markers (**Figure 1**). Reproducibility of the FOUL detection method was established by a reliability study between two independent clinical experts blinded for freezing status of the subjects (ICC (2,2)= 94%). Number and duration (s) of FOUL episodes per movement trial were the primary outcomes.

Secondly, amplitude and frequency measures were computed for the remaining continuous (non-freezing) motor signals based on peak-to-peak measures of the end-effectors motions. Mean amplitude and frequency per movement cycle were the main outcomes. Group comparisons of amplitude and frequency were restricted to continuous movement during the freezing-resistant conditions. For this purpose, kinematic outcome parameters were pooled across the freezing-resistant Conditions 1 and 2, hereafter called 'CONT'. Mean amplitude and frequency during CONT were compared between PD with FOG, PD without FOG and controls using one-way ANOVA. Significant group effects were further addressed with Tukey's HSD post-hoc test.

Brain activity analysis

Functional imaging data were preprocessed and analyzed with SPM5 (Wellcome Department of Imaging Neuroscience, University College, London) implemented in Matlab. They were spatially realigned to the mean image in the time series for motion correction, unwarped, then corrected for differences in slice acquisition time by temporal interpolation to the middle slice (reference slice = 25) and spatially co-registered to the individual's anatomical T1 image. Anatomical images were normalized to the MNI template using the SPM5 segmentation procedure and this transformation was also applied to all realigned EPI images. Finally, the normalized functional images were smoothed with an isotropic 8 mm FWHM Gaussian kernel.

At the first level, the preprocessed fMRI data of each subject were analyzed on a voxel-by-voxel basis using an epoch-related approach in the context of the General Linear Model. REST, CONT and FOUL epochs were modeled as box-car functions convolved with the canonical hemodynamic response function (HRF). Additionally, we included regressors with mean signal intensity values for three compartment signals (white matter, cerebral spinal fluid and out-of-brain voxels) as covariates of no interest.

At the second level, two types of analyses were performed. The first analysis included the contrast image representing the effect of CONT versus REST that was defined in all subjects. CONTvsREST contrast images (1 per subject) were entered in a second level random effects analysis in the context of the General Linear Model with pre-planned comparisons using t-tests within and between groups ($p<0.05$, FDR corrected). Cerebral activation was compared between 1) PD with FOG versus PD without FOG and 2) PD with FOG vs controls. We restricted the search volume for between group analyses to grey matter voxels that showed task-related activation as defined by the CONTvsREST contrast. The second analysis involved a fixed effects model including all runs (across all subjects) in which FOUL had occurred.

Contrast images representing the effect of FOUL versus CONT were specified per FOUL-run and were used for within group analysis (pre-planned comparison of FOULvsCONT within those with FOUL, p<0.05, FDR corrected).

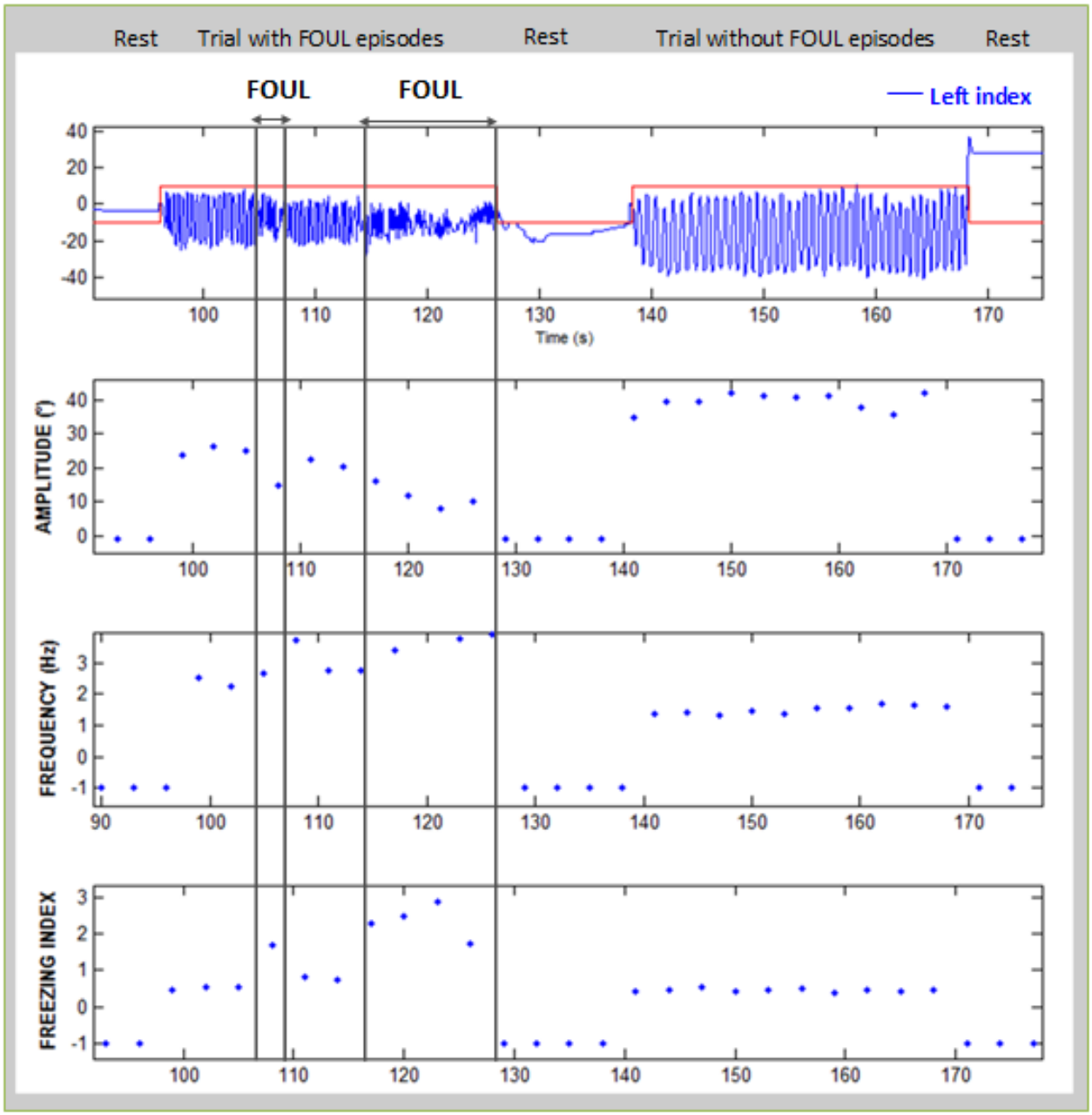

Figure 1: Freezing detection in upper limb kinematic time series obtained during scanning. At the top panel, motion of the left index finger is depicted during 3 rest conditions (flat signal) and 2 movement trials (oscillating signals) in congruence with the red horizontal line (-1= rest; 1= movement trial). Below the evolution of movement amplitude, frequency and the freezing index is shown. During rest, these parameters are arbitrarily set at -1 for clarity's sake. Two freezing episodes (FOUL) occur during the first movement trial, none during the second. We used objective criteria to detect freezing episodes based on changes in amplitude (reduction > 50%), frequency (hastened, irregular), freezing index (>1). At least 2 of the criteria were to be met in order to identify a movement episode as FOUL. The vertical lines represent visual markers that were used by the 2 clinical experts in FOG to demarcate the freezing episodes.

Region of interest analysis

Recent studies pointed to deficits in the neural circuitry connecting the basal ganglia, subthalamic nucleus (STN), brainstem structures and cortical regions as probable origins of FOG (see Nutt et al.[4]). We therefore included the following subcortical areas as regions of interest (ROI) defined in Marsbar[36]: the putamen, caudate nucleus, STN, pallidum, the motor parts of the thalamus, the mesencephalic locomotor region (MLR) and the pedunculopontine nucleus (PPN). All ROIs were bilateral. The caudate nucleus, pallidum and dorsal and ventral parts of the putamen were delineated according to Postuma and Dagher (2006).[37] Motor subregions of the thalamus included two ROIs according to their predominant anatomical connections with the PMC and M1 in accordance with Behrens et al. [38] The MLR ROI was defined as a 8 x 8 x 8 cube centered around coordinate x, y, z = 0, -28, -20.[29] The STN ROI was centered around coordinate x, y, z = +/-10, -15, -5.[39] Stereotactic coordinates reported by Zrinzo et al.[40] were used for the PPN ROI with x, y, z coordinates ranging from 5 to 7, -25 to -30, -7 to -16. For all ROIs, contrast values of CONTvsREST were extracted for each run in all subjects and FOULvsCONT contrast values for each run with FOUL.

3. RESULTS

In the following, data are represented in 2 sections: 1) the behavioral and brain imaging results of the continuous motion in the 3 groups; 2) the behavioral and brain imaging data for the upper limb freezing episodes within the group with FOUL.

Continuous movement

Group comparison of kinematics during continuous movement (CONT)

During CONT, mean amplitude was larger in controls (55.25° (18.2)) compared to patients without FOG (37.55° (14.90), p=0.009) and patients with FOG (36.42° (15.17), p=0.006) (see **Figure 2A**). Mean frequency was higher in patients with FOG (1.49Hz (0.50)) than controls (1.13Hz (0.22), p=0.009) and patients without FOG (1.11Hz (0.33), p=0.009) (See **Figure 2B**).

Whole brain analysis within groups during CONT

Task-related brain activation within each group is depicted in **Figure 3**. While performing continuous movements at comfortable amplitude and frequency (CONT), control subjects activated the bilateral supplementary motor area (SMA), bilateral pre- and postcentral motor areas, bilateral middle cingulum, bilateral angular gyrus, bilateral superior frontal lobe, bilateral orbitofrontal region, left anterior cingulum, left middle temporal lobe, bilateral precuneus, the cerebellum (vermis, bilateral areas 4, 5, 6 and 8, crus 2) and right caudate nucleus (one sample t-test, p<0.05 FDR corrected, see Figure 3A).

During CONT, PD patients without FOG recruited the bilateral supplementary motor area (SMA), bilateral primary motor area (M1), dorsal premotor cortex (PMd) and post-central gyrus, bilateral superior frontal lobe, the cerebellum (vermis, bilateral areas 4 and 5 and right cerebellar hemisphere areas 6 and 8) and the left caudate nucleus (one sample t-test, p<0.05 FDR corrected, see Figure 3B).

PD patients with FOG engaged the bilateral supplementary motor area (SMA), bilateral M1, PMD and postcentral gyrus left superior frontal lobe, left orbitofrontal gyrus, right middle

temporal lobe, the cerebellum (vermis, bilateral areas 4, 5, 6), left caudate nucleus and bilateral putamen (one sample t-test, p<0.05 FDR corrected, see Figure 3C).

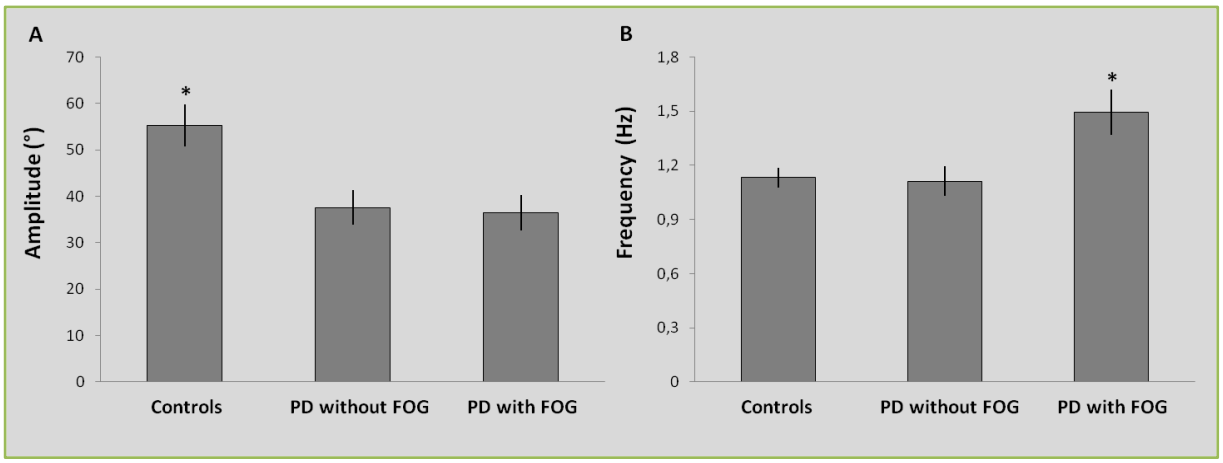

Figure 2: Kinematic group comparison during CONT. Mean movement amplitude (A) and frequency (B) during continuous movement (CONT) of Controls (n=16), PD without FOG (n=16) and PD with FOG (n=16). Vertical bars represent standard error of measurement (SEM). * Groups significantly different at *p* <0.05.

Whole brain analysis between groups during CONT

To ascertain that differences in brain activation between PD with FOG, PD without FOG and controls were not confounded by differences in behavioral performance, we included a regressor containing the mean frequency values during CONT for each subject as a covariate of no interest in the 2nd level ANOVA model. Comparing brain activation during CONT between PD with FOG and PD without FOG, corrected for differences in movement frequency, showed relatively decreased activation in PD with FOG in the right middle frontal gyrus (anterior dorsolateral prefrontal cortex (PFC)) and the left PMd and M1 (two sample t-test, p<0.05 FDR corrected, see **Figure 4** and **Table 2**). Similarly, a strong tendency towards decreased activation in right anterior dorsolateral PFC and the left PMd was found in PD with FOG compared to control subjects (two sample t-test, p=0.057 FDR corrected, see **Figure 5** and Table 2). No areas showed increased activation in PD with FOG compared to PD without FOG or controls. With a more liberal threshold, activation in the right putamen was increased in PD with FOG compared to control subjects (two sample t-test, p<0.001 uncorrected, see Figure 5 and Table 2).

ROI analysis between groups during CONT

PD subjects with FOG showed increased activation in the bilateral STN compared to PD without FOG (ROI analysis, two-sample t-test corrected for differences in movement frequency, p=0.017) and increased activation in the bilateral dorsal putamen compared to controls (ROI analysis, two-sample t-test corrected for movement frequency, p=0.011). As shown in **Figure 6**, the relative increase in STN activation is due to decreased de-activation in PD with FOG. Brain activation in the MLR, PPN and ventral putamen did not differ between groups (p>0.2).

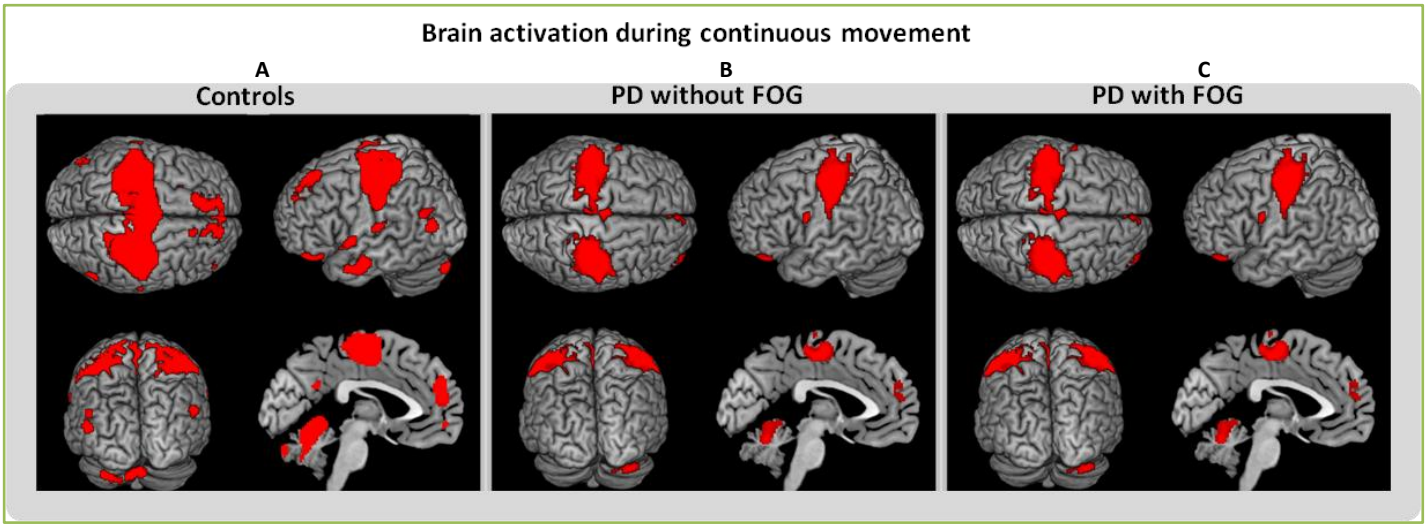

Figure 3: Motor network activated during continuous movement in controls, PD without FOG and PD with FOG. Results are based on one sample t-test thresholded at p<0.05 with FDR correction to identify brain areas that were activated during continuous movement (contrast CONT > REST) within the three groups.

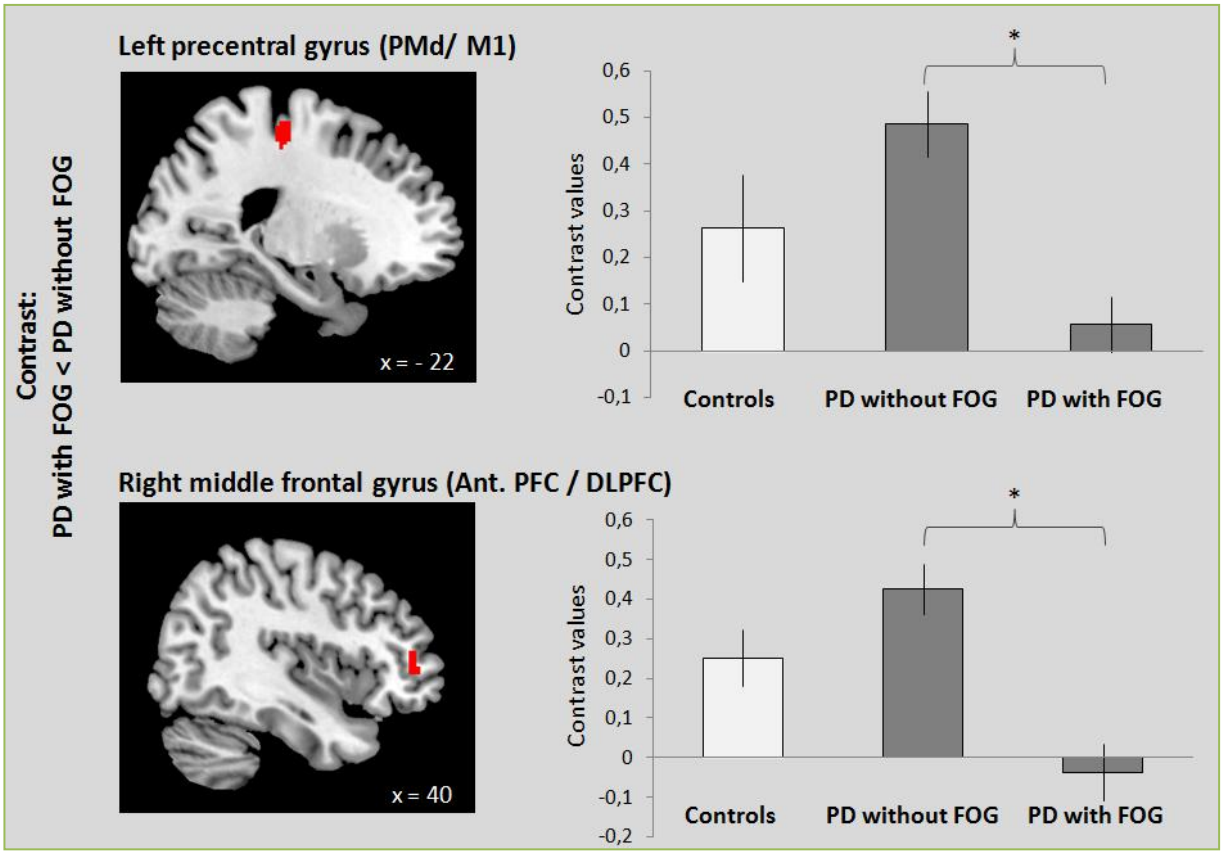

Figure 4: Differences in brain activation during continuous movement between PD without FOG and PD with FOG. Anatomical location (left side) and contrast values (right side) of brain regions that were less activated in PD with FOG compared to PD without FOG. Results are based on two-sample T- tests with movement frequency as covariate of no interest and are significant at p<0.05 with FDR correction. The subject group with a white bar was not included in the given contrast but is shown to provide the reader with a complete view on contrast values in all 3 groups. Abbreviations: PMd: dorsal Premotor cortex; M1: Primary motor area; Ant. PFC: Anterior prefrontal cortex: DLPFC: Dorsolateral prefrontal cortex.

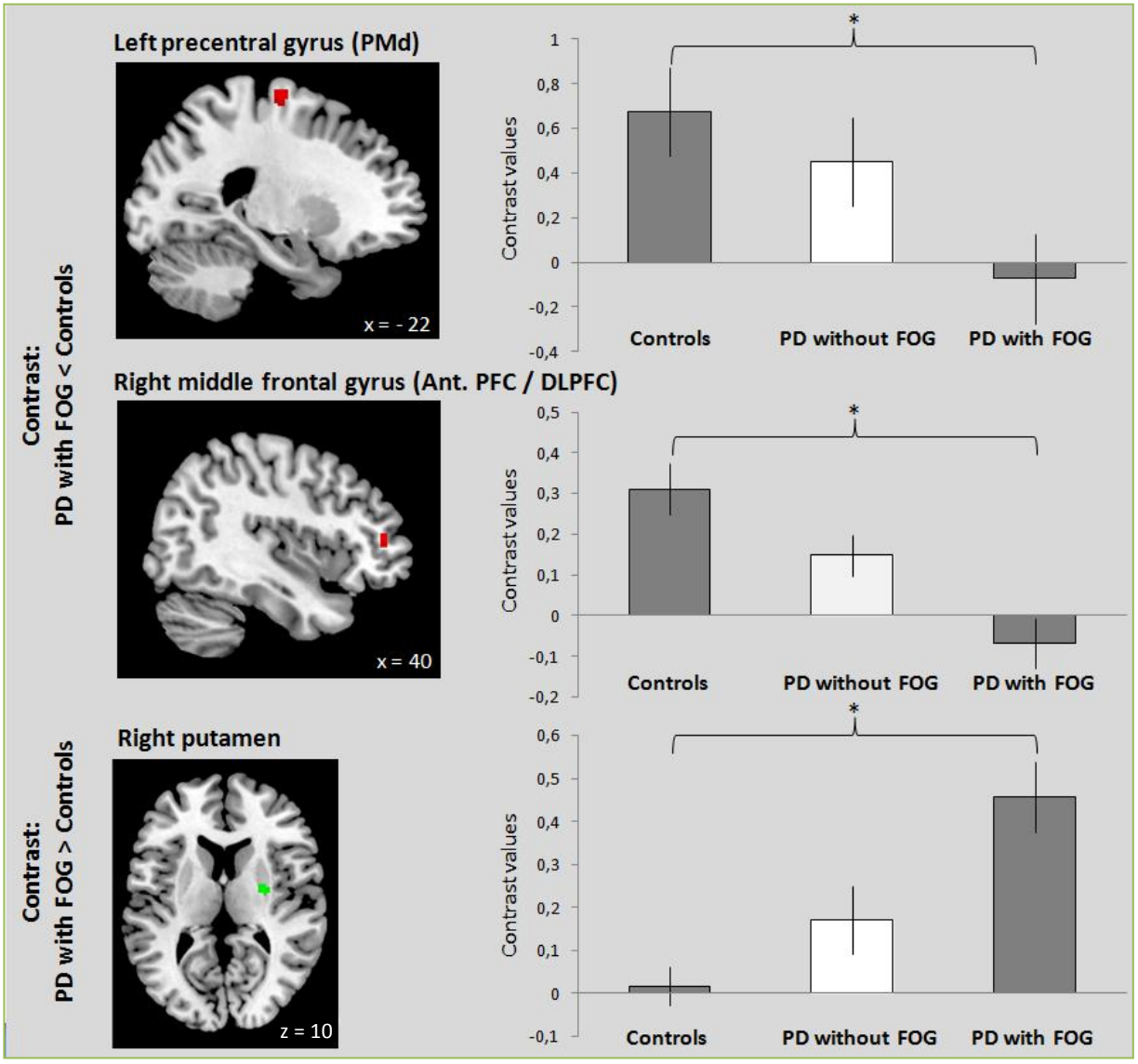

Figure 5: Differences in brain activation during continuous movement between Controls and PD with FOG. Anatomical location (left side) and contrast values (right side) of brain regions that were differently activated in PD with FOG compared to Controls. Results are based on two-sample T- tests with movement frequency as covariate of no interest and are significant at p<0.001 (uncorrected). Exact p-values after FDR correction can be found in Table 2. The subject group with a white bar was not included in the given contrast but is shown to provide the reader with a complete view on contrast values in all 3 groups. Abbreviations: PMd: dorsal premotor cortex; Ant. PFC: Anterior prefrontal cortex: DLPFC: Dorsolateral prefrontal cortex.

Correlation analysis

We tested whether group differences in motor-related brain activation (right PMd, left primary motor and premotor areas, right putamen, bilateral STN and bilateral pallidum) were related to clinical characteristics using Pearson correlations. Within patients with FOG, a longer disease duration was associated with a stronger reduction in activity of the right prefrontal cortex (R= - 0.56, p<0,05) and with a stronger increase in activity of the dorsal and ventral putamen (dorsal putamen: R=0.57, p=0.02; ventral putamen: R=0.60, p=0.02). Higher UPDRS motor scores were also positively correlated to contrast values of the CONTvsRest contrast, indicating that higher UPDRS scores related to a stronger increase in pallidal activity within freezers. No areas showed significant correlations with cognitive variables (MMSE and

SCOPA-cog), disease severity (UPDRS, Hoehn and Yahr stage), the FOG-Q and movement amplitude or frequency.

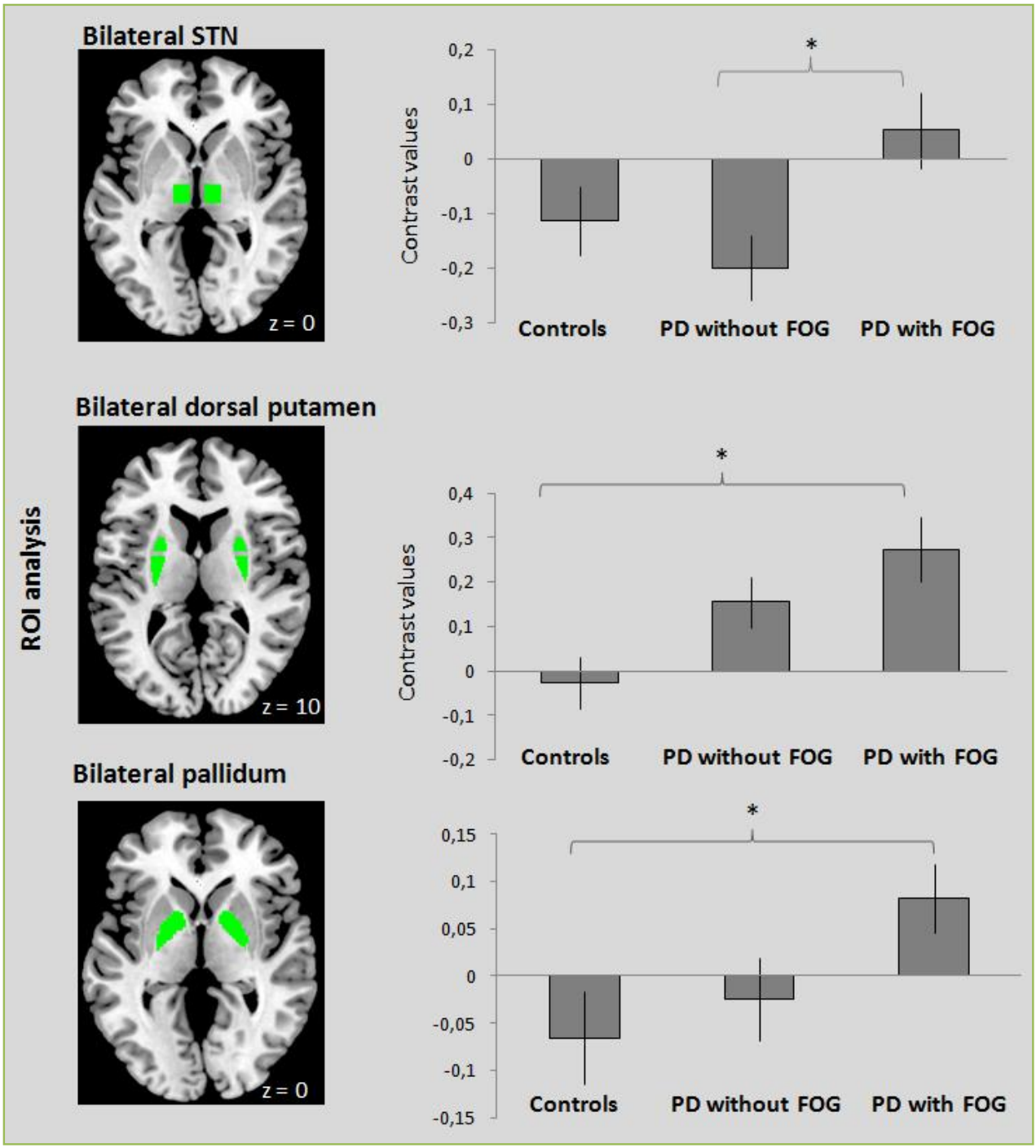

Figure 6: Differences in activation in subcortical regions of interest (ROIs) between Controls, PD without FOG and PD with FOG during continuous movement performance. Anatomical delineation (left side) and contrast values of bilateral subthalamic nucleus (STN) and dorsal putamen which showed increased activation in PD with FOG compared to PD without FOG (STN) and controls (putamen). In analogy to whole brain analysis, two-sample T tests were used with movement frequency as covariate of no interest. *p<0.05.

Table 2: Brain areas with different activation patterns between groups during continuous movement (A) and within patients with FOUL during FOUL compared to CONT (B)

Brain region	Functional label	BA	Coordinates x	y	z	T	p (FDR)	Cluster size
A. Whole brain analysis during CONT								
PD with FOG < PD without FOG								
Right middle frontal gyrus	Ant. DLPFC	10, 46	40	46	6	4.46	0.042	63
Left precentral gyrus	PMd & M1	6, 4	-22	-22	58	5.25	0.042	82
PD with FOG < Controls								
Right middle frontal gyrus	Ant. DLPFC	10, 46	40	46	10	4.53	0.057	84
Left precentral gyrus	PMd	6	-24	-12	70	4.87	0.057	47
PD with FOG > Controls								
Right putamen			26	-6	10	3.51	0.455	24
B. Whole brain analysis during FOUL								
FOUL > CONT								
Right superior frontal gyrus	SMA	6	8	8	48	5.83	0.015	54
Right precentral gyrus	PMd & M1	6, 4	42	-22	58	5.86	0.015	157
Left superior frontal gyrus	Ant. PFC	10	-18	66	18	5.08	0.018	18

A. Whole brain analysis of task-related activation during continuous movement (CONT) between PD with FOG (n=16), PD without FOG (n=16) and Controls (n=16) using two-sample T- tests. All areas and reported cluster sizes are significant at p<0.001 (uncorrected). P-values after False Discovery Rate (FDR) correction for multiple comparisons at the voxel level are shown. **B.** Whole brain analysis of activation during freezing of upper limb (FOUL) and continuous movement (CONT) based on a one-sample T-test within patients who demonstrated FOUL (n=8). All areas and reported cluster sizes are significant at p<0.05 after FDR correction at the voxel level. Coordinates of local maxima at *x y z* are in MNI space. Abbreviations: PD: Parkinson's disease; FOG: Freezing of gait; FOUL: Freezing of upper limb; Ant.: Anterior; PFC: Prefrontal cortex; M1: Primary motor cortex; SMA: Supplementary motor area.

Upper limb freezing episodes (FOUL)

Occurrence of FOUL episodes

FOUL was detected in 9 patients with FOG and 1 patient without FOG. Of the 289 episodes in total, 150 were bilateral FOUL (51.90%), 93 unilateral left FOUL (32.18%) and 46 unilateral right FOUL (15.92%). The duration varied between FOUL episodes with a median of 4.89s (IQR= 2.10 – 14.97s) (see **Figure 7A**). More frequent and longer freezing episodes were observed during freezing provoking conditions (small-amplitude, fast-frequency conditions) compared to freezing-resistant conditions (comfortable amplitude and speed) (see **Figure 7B**). Within patients with FOUL, the total FOUL time tended to be related to FOG severity but

this was not significant (R_S=0.41, p=0.27). The freezing index of FOUL was 2.36 (1.08 SD) compared to 0.63 (0.13 SD) during CONT (p=0.0013). Patients with FOG who demonstrated FOUL had a similar clinical profile as FOG patients without FOUL (non-parametric Wilcoxon t-test p>0.2 for age, UPDRS, disease duration, Hoehn and Yahr score, LED, FOG-Q, SCOPA-cog and preferred movement frequency) but movement amplitude in FOG patients with FOUL was smaller (FOG with FOUL: 26.89° (13.03) versus FOG without FOUL: 48.95° (7.97); non-parametric Wilcoxon t-test, p=0.0097).

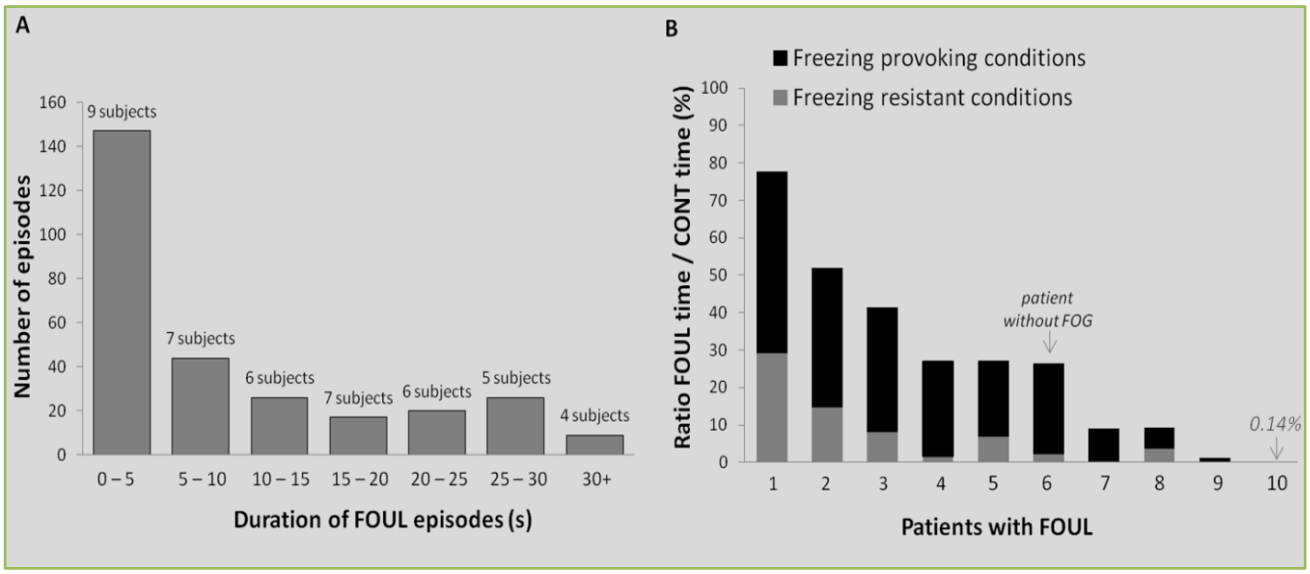

Figure 7: Characteristics of FOUL episodes. Freezing of upper limb movement (FOUL) was detected in 10 subjects (9 PD with FOG, 1 PD without FOG). Panel **A** displays the number of FOUL episodes for each duration bin. Above each column, the number of subjects who demonstrated FOUL within the given duration bin is shown. In panel **B**, the proportion of FOUL time (summed duration of FOUL episodes) to CONT time (summed duration of CONT) expressed as a percentage is shown for each patient who demonstrated FOUL during freezing provoking conditions (in black: small amplitude- fast frequency- conditions) and freezing resistant conditions (in gray: comfortable amplitude and frequency conditions). For example, the total movement time (100%) of patient 1 can be divided in 22% CONT, 29% FOUL in freezing resistant conditions and 49% FOUL during freezing-provoking conditions. Patients 9 and 10 showed only a short duration of FOUL and were not included in the analysis.

Whole brain analysis during FOUL

Brain activation during FOUL was compared to CONT within patients who demonstrated FOUL using a fixed effects model. Five runs with only short periods of FOUL (summed duration of FOUL episodes < 5% of total motion time were excluded from the analysis in view of the slowness of the BOLD signal (see Figure 7). The following results are based on 274 FOUL episodes (out of 289 in total) distributed over 35 runs of 8 subjects (7 PD with FOG, 1 PD without FOG; median duration 5.76s (IQR= 2.25 – 15.73s)). The right SMA, right PMd and M1, and left superior frontal gyrus (anterior PFC) showed increased activation during FOUL compared to CONT (p<0.05, FDR corrected, see **Figure 8** and Table 2). No areas showed decreased activation during FOUL versus CONT.

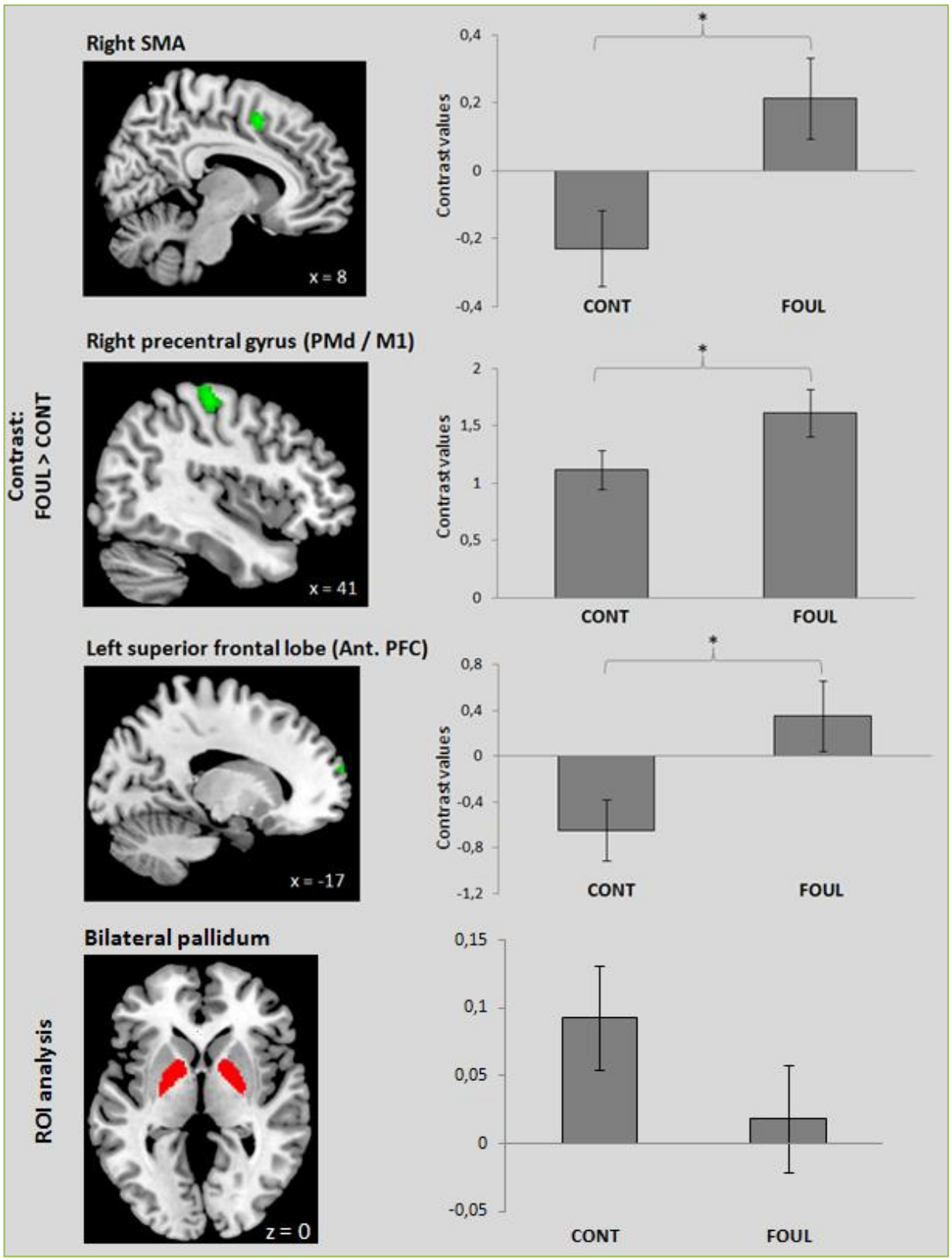

Figure 8: Differences in brain activation during FOUL compared to CONT within patients with FOUL. Anatomical location (left side) and contrast values (right side) of brain regions that showed increased (green) or decreased (red) activation during FOUL. Results of the whole brain analysis are based on one-sample T- tests and are thresholded at p<0.05 (FDR correction). Abbreviations: SMA: Supplementary Motor Area; PMC: Premotor cortex; M1: Primary motor area; Ant. PFC: Anterior prefrontal cortex; ROI: Region of interest.

ROI analysis during FOUL

Brain activation in the STN, MLR, PPN did not differ between FOUL and CONT (p>0.3) but the results showed decreased activity in the pallidum bilaterally during FOUL compared to CONT (ROI analysis, p=0.03, Figure 8) and there was a trend for decreased activation in the ventral and dorsal putamen during FOUL compared to CONT (ROI analysis, p=0.073 for ventral putamen, p=0.078 for dorsal putamen).

4. DISCUSSION

The purpose of the present study was to identify the neural correlates of freezing and related timing-amplitude dyscontrol in PD. This is the first study that uses functional magnetic resonance imaging to address differences in cerebral activation during motor pattern generation in Parkinson patients with and without freezing of gait. Patients with FOG have been shown to exhibit more pronounced movement disturbances than patients without FOG both during gait and other repetitive movements.[4,6-9,14-19] Plotnik et al.[19] hypothesized that the co-occurrence of multiple gait problems is a crucial aspect in the origin of FOG. In line with this, the current study showed that behavioral performance during a repetitive finger movement task in freezers was characterized by a combination of small and fast movements whereas non-freezers only presented with reduced amplitude compared to controls. Furthermore, at the neural level, there were two main findings: First, during performance of continuous repetitive movement, patients with FOG showed decreased activation in cortical frontal areas (left PMd and M1, right PFC) compared to patients without FOG and controls. In contrast, subcortical activity in the right dorsal putamen, bilateral pallidum and bilateral STN was increased in PD with FOG compared to controls and non-freezers. These findings were obtained after statistically controlling for differences in movement frequency. The between-group analyses were crucial in determining if and how the involvement of typical areas of the motor network is changed in patients with FOG when movement production is successful. Secondly, comparing the BOLD signal during continuous and 'frozen' upper limb movement in patients who demonstrated FOUL in the scanner, an inverse pattern of neural activation was found compared to the between-group differences described above: during freezing episodes, cortical (right SMA, PMd and M1, left PFC) brain activity was now increased while subcortical activity in the pallidum and putamen bilaterally was decreased. These novel findings indicate that the neural drive for rhythmic movement generation and more specifically the balance between subcortical and cortical activation, is altered in patients with FOG.

Reduced cortical and increased subcortical brain activity during continuous motion in PD+FOG

During task performance, healthy controls and both Parkinson groups showed widespread activation of brain areas within a representative network for rhythmic sensorimotor coordination.[41] This network included areas typically related to aspects of movement execution (e.g. M1) as well as areas indicative of higher-level motor control (SMA, PMC, parietal cortex, BG, prefrontal cortex and cerebellum). These regions play a crucial role in rhythmical movement generation and have also been found to be part of the gait network.[4,42,43] Previous functional MRI studies of upper limb motion in PD have consistently shown increased activation in premotor-parietal and cerebellar regions, presumably to

compensate for the dysfunctional striato-supplementary motor loop.[21,44-46] The reduced BG-SMA drive for volitional movement was found to be correlated to disease severity[21] and partly normalized after Levodopa intake[22] or was by-passed when external cueing was provided to guide movement.[47] In the current study we found that, unlike in non-freezers, there was no evidence of increased, compensatory cortical recruitment during continuous movement in freezers. Instead, activation of M1, PMd and DLPFC was reduced in freezers compared to non-freezers and controls. Conversely, comparing contrast values between non-freezers and controls as shown in Figure 4 and 5 suggests that non-freezers were still able to increase activity in part of these areas. Freezers did show increased activity in the pallidum, STN and putamen during ongoing movement. The combination of hyperactivity in these three subcortical regions and cortical hypoactivity during movement regulation in freezers is an important finding. It suggests increased involvement of the so-called indirect BG-pathway, known to suppress cortical motor regions through the thalamus.[48] This neural circuitry is crucial for inhibitory action control, a fundamental component of regulating goal-directed motor behavior.[49-51] Recruitment of this pathway and its connections to prefrontal areas underlies several response selection processes including successful task switching and multitasking[52,53], abilities that has been shown to be impaired in PD patients with FOG.[54-57] In line with this, the current study revealed decreased activation in freezers in the DLPFC, including the frontopolar region (BA 10) that has been linked with core attentional functions necessary to plan, monitor, adapt and switch behavior.[53] In PD patients in general, a recent fMRI study showed that selection processes and more specifically the behavioral cost of motor switching was found to rely on the striatofrontal circuitry with an increased involvement of the middle frontal cortex as disease progresses.[58] The disturbance in the frontostriatal circuitry along with the indirect pathway as suggested by the current results, also coincides well with the fact that clinically STN-stimulation has been found to reduce FOG.[59] Although the exact mechanism underlying this effect is poorly understood, STN-DBS was shown to modulate pallidal-prefrontal coupling during decision making processes.[60] Hyperactivity of areas involved in inhibition, may well explain why frontal cortical activation in freezers was reduced, though alternative explanations are also possible. For instance, cortical hypoactivity may point to reduced striatofrontal compensatory activity due to cognitive decline in patients with FOG.[54-57]

In addition, the pallidal ROI entailed both internal and external parts of the globus pallidus as well as the ventral pallidum, making a clear interpretation of hyperactivity in this region more difficult than in more segregated ROIs such as the putamen. As main motor structure of the BG, the posterior putamen has strong output channels to cortical motor areas (PMC, SMA, S1/M1)[37] through thalamocortical pathways.[61] Within the FOG group, activity in the posterior putamen was positively correlated with disease severity. This is in line with the finding that this part is more severely affected by dopamine depletion than the anterior or ventral striatum.[24] With regards to upper limb coordination, putamen activity has been found to be particularly increased during the movement initiation phase.[62] As such, patients with FOG may need additional subcortical input to preserve movement continuity. This finding also relates well with increased glucose metabolism in the putamen of freezers as revealed by FDG-PET compared to non-freezers.[24] Whole brain analysis revealed increased activity in the putamen of freezers compared to controls in the right hemisphere in combination with reduced activity of motor areas located in the left hemisphere and right DLPFC. This is consistent with predominantly contralateral (crossing) projections from the putamen to

cortical areas involved in motor control.[37] Helmich et al.[63] showed that functional segregation between BG-cortical motor, cognitive and limbic loops was reduced in PD patients. In addition, increased inter-hemispherical functional connectivity was found between several structures of the basal ganglia in PD.[46] In the pathophysiological model of FOG proposed by Lewis and Barker,[64] increased cross-talk between competing basal ganglia networks was envisaged as a candidate mechanism for excessive inhibition of the thalamus and PPN which may trigger FOG. Whether increased interplay of multi-domain processing through the BG, lies at the base of abnormal sensorimotor integration in PD[63] or facilitates compensatory recruitment in non-motor regions[65] merits further investigation.
Interestingly, comparing cerebral activation during gait planning in PD patients with and without FOG, decreased activation of mesial frontal and posterior parietal cortices in freezers was revealed.[29] This was interpreted as that compensatory brain activity is insufficient in patients with FOG which may result in difficulties in stride length regulation including the sequence effect, i.e. the successive reduction of stride length.[16,29] The sequence effect is thought to be mediated by dysfunctional feedforward output from the BG to SMA and premotor areas.[16,24,66] The current findings of reduced M1 and PMd activation in freezers are consistent with this idea and show that this reduced cortical drive was associated with increased subcortical activity. To conclude, the above mentioned findings suggest that increased subcortical activity during ongoing movement in freezers may suppress activation in frontal cortical regions involved in spatiotemporal as well as cognitive properties of action control which may relate to a reduced capacity to recruit compensatory brain activation and scaling-timing motor problems.

Increased cortical and decreased subcortical brain activity during upper limb freezing

In contrast to the pattern of neural activation during ongoing movement, upper limb freezing episodes were associated with increased engagement of SMA, PMd, M1 and the anterior prefrontal cortex and decreased subcortical activity as compared to continuous motion within PD with FOUL. Behaviorally, upper limb freezing episodes were characterized by highly abnormal motor output resembling the kinematic changes during FOG namely severely reduced amplitude, irregular frequency and the presence of high-frequency trembling-like components.[4,6,10-13] This may imply that a cortical drive only increases in freezers when motor output is severely at odds with the intended motor program. It is important to note that, considering the reduced amount of movement during FOUL, it is unlikely that increased brain activation is merely a result of altered motor output. Instead, we hypothesize that the increased cortical activation represents the attempt to intentionally correct motor behavior and overcome the motor block. The reason why increased cortical activation previously described as compensatory in PD occurs in freezers only when FOUL has emerged, is currently unclear. Recent findings of more pronounced grey matter atrophy and reduced functional connectivity in fronto-parietal regions of freezers,[26,27] were interpreted as mediating the executive dysfunction that has been described in freezers,[54-56] as well as impaired integration of sensorimotor and proprioceptive information.[67] Whether these aspects contribute to ineffective error-monitoring of movement in freezers awaits further investigation.

Involvement of brainstem motor structures in freezing: specific to gait freezing?

In addition to the view that dysfunctions in basal ganglia, frontal motor and cognitive areas underlie FOG, Nutt et al.[4] hypothesized that disturbances in the midbrain locomotor regions play a key role in FOG etiology. Brainstem motor regions (the PPN and MLR) showed altered white matter connectivity, grey matter atrophy and hyperactivity during gait planning in freezers.[29,30] In addition, it has been put forward that disturbed output signaling from the brainstem-central pattern generator pathways may result in the abnormal high-frequency components during FOG[4,10-13] and FOUL,[7] but this hypothesis has never been tested. The current study found no significant differences in activation during ongoing upper limb motion between PD with FOG, PD without FOG and controls in these areas as revealed by whole brain and region of interest analysis. Also, no such differences were apparent when comparing CONT versus FOUL within PD with FOUL in which high-frequency output was clearly present. Consequently, we suggest that the role of brainstem motor areas in the origin of freezing may be specific to gait and not necessarily related to the generalized impairment of scaling and timing control underlying freezing. The recent finding that PPN stimulation reduces the number of FOG episodes but does not improve background abnormalities in stride length and timing control,[68] appears to support this hypothesis. Brainstem motor areas may exert a gait-specific influence on FOG through loss of cholinergic cells that are involved in postural control and cognitive performance (see ref[69] for review).

Limitations of the study and suggestions for future research

Overall, between group effects in brain activation included a small number of voxels and sometimes only reached significance when relatively liberal statistical thresholds were used. The limited statistical power in some instances is possibly due to a high variability in the neural profiles of these patient populations and has to be taken into consideration when interpreting the findings.

Secondly, fewer subjects showed FOUL than expected based on a previous study using the same experimental paradigm.[7] There were no clinical differences between patients with FOG who demonstrated FOUL during testing (n=9/16) or those who did not (n=7/17), but PD with FOUL had a smaller baseline movement amplitude, adding further strength to the major role of scaling difficulties in freezing[7,8,9,16,70]

To reduce the testing burden for patients during the scanning session, clinical assessment was not repeated on the test day. As such matching occurred through a clinical assessment at the patients' home while on medication. Consistent with prior study,[6] levodopa dose was higher in freezers compared to non-freezers. In the OFF state, this may have resulted in worse disease severity parameters (Hoehn and Yahr stage and UPDRS scores) in freezers which is an important limitation of the current study. General motor output during the upper limb tests was however comparable between patient groups for movement amplitude during off.

Recent studies indicated that motor-related hypo- and hyperactivation patterns in PD occured in parallel with functional connectivity (FC) changes within the striato-thalamo-cortical networks (decreased FC) and cerebello-thalamo-cortical loop (increased FC).[21,46] In fact, Palmer et al.[46] found altered FC in PD between regions without apparent group differences in amplitude of the BOLD signal. FC may be a sensitive parameter for pathology-induced changes in brain organization which can be used in future studies comparing task-related neural activation in freezers and non-freezers.

Last, the current study addressed brain activation associated with shared motor problems in amplitude-timing control of repetitive upper and lower limb movement generation. It showed that the DLPFC and anterior PFC, which play an important role in cognitive controlled (motor) behavior,[51-53,71] were part of the network related to ongoing motor abnormalities and freezing in PD with FOG. Though this is consistent with findings of executive dysfunction in PD with FOG,[54-56] the role of cognitive impairment in the origins of FOG and FOUL requires further research.

5. CONCLUSION

The shared motor mechanisms of FOG and FOUL related to amplitude and timing dyscontrol were associated with altered patterns of brain activity within the striatofrontal circuitry. Subcortical hyperactivity may inhibit cortical activation in motor and cognitive areas, resulting in spatiotemporal abnormalities during ongoing upper limb movement of freezers. In contrast, an increase of cortical drive which has been described as a compensatory mechanism in PD, only occurred in freezers when UL freezing had emerged and was interpreted as an attempt to overcome the motor block.

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Chapter 5

Explaining freezing of gait in Parkinson's disease: motor and cognitive determinants

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ABSTRACT

Background: Freezing of gait is part of a complex clinical picture in Parkinson's disease and is largely refractory to standard care. Diverging hypotheses exist about its origins but a consolidated view on what determines freezing of gait is lacking.
Objectives: To develop an integrative model of freezing of gait in people with Parkinson's disease.
Methods: This cross-sectional study included 51 Parkinson subjects: 24 patients without freezing of gait and 27 with freezing of gait, matched for age, gender and disease severity. Subjects underwent an extensive clinical test battery evaluating general disease characteristics, gait and balance, non-gait freezing and cognitive functions. The relative contribution of these outcomes to freezing of gait was determined using logistic regression analysis.
Results: The combination of the following four independent contributors provided the best explanatory model of freezing of gait (R^2=0.49): non-gait freezing, Levodopa equivalent dose, cognitive impairment, and falls and balance problems. The model yields a high risk profile for freezing of gait (P>95%)) when Parkinson patients are affected by at least one type of non-gait freezing (e.g. freezing of other repetitive movements), falls or balance problems during the last three months, and a SCOPA-COG score below 28. A high Levodopa equivalent dose further increases the risk of freezing of gait to 99 per cent.
Conclusions: Non-gait freezing, increased dopaminergic drug dose, cognitive deficits, and falls and balance problems are independent determinants of freezing of gait in people with Parkinson's disease and may play a synergistic role in its manifestation.

Keywords: Parkinson's disease, Gait disorders, Cognitive disorders, Postural control, Freezing of gait.

1. INTRODUCTION

Freezing of gait (FOG) is a disabling gait disorder defined as a 'brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk'.[1] Longer disease duration and greater disease severity increase the likelihood of developing FOG although not all patients ultimately do so.[2] FOG is accompanied by motor and cognitive abnormalities, but it is currently unclear how these aspects interact and which factor is the most determining in the development of FOG. Important motor correlates of FOG are postural instability which causes falls[3], and impaired regulation of rhythmic stepping movements.[1,4,5] In addition, recent work has shown that spatiotemporal dyscontrol and freezing episodes were reported beyond the gait network during writing and repetitive finger movements.[6,7,8,9] Freezing during movements other than gait is henceforth called 'non-gait freezing'. In the cognitive domain, components of executive functioning[10] (e.g. conflict resolution[11], set-shifting[12]) and visuospatial abilities[13,14] were reported as impaired in patients with FOG. Unlike the cardinal symptoms of PD, FOG is less efficiently improved with dopaminergic medication.[15] This suggests that FOG has a unique neuropathology that exceeds typical dopaminergic regions and requires adequate alternative therapeutic approaches.[16] Although neuroprotective therapy is still under investigation, early risk identification of FOG may improve its treatment in the near future.[17]
Therefore, we performed a cross-sectional regression analysis to investigate potential risk factors in having FOG from four domains: demographic and disease characteristics, gait and balance variables, non-gait freezing, and cognition. The study goal was to develop an integrative model of the factors determining FOG and to obtain a clinically applicable prediction equation to estimate its probability. Secondly, we aimed to investigate which factors were most closely associated with FOG severity.

2. MATERIALS AND METHODS

Participants

Fifty-one PD patients were recruited from the Movement Disorders Clinic of the University Hospital Leuven. A score ≥ 1 on the new FOG-Questionnaire (NFOG-Q) classified 27 patients as freezers and a score < 1 categorized 24 as non-freezers.[18] All patients had previously undergone a gait test in the context of other studies.[6,7,19] Patients were identified as definite freezers when freezing episodes were observed during this gait analysis. Using the algorithm of Snijders et al.[20], self-reported freezers without observed FOG were classified as probable freezers. Freezers and non-freezers were matched for age, gender and disease severity (UPDRS scores[21], Hoehn and Yahr[22] stage II or III). Exclusion criteria were: (1) diagnosis of a neurological disease other than PD, (2) presence of a deep brain stimulator and 3) Levodopa-induced ON-freezing. Participants gave informed consent and Ethics approval was received by the local Medical Ethics Committee KU Leuven.

Clinical assessment

All patients underwent an extensive clinical test battery while 'ON' medication:

1. General disease characteristics: Unified Parkinson's Disease Rating Scale (UPDRS[21]), Hoehn and Yahr staging and L-dopa equivalent dose (LED) intake (mg/day).[23]

2. Gait and balance tests: Timed Up and Go Test (TUG)[24], a short version of the Berg Balance Scale (BBS, items 8, 11, 13 and 14),[25] the NFOG-Q[18] and a questionnaire assessing falls and near falls during the last three months, according to Ashburn et al.[26] This questionnaire contained four items: falls caused by FOG, falls independent of FOG, near falls caused by FOG, and near falls independent of FOG. To address intrinsic balance problems, only fall/near fall scores not induced by FOG episodes were analyzed. Patients scored 1 (or 0) if they had (or had not) experienced falls and/or near falls independent of FOG during the last three months.

3. A non-gait freezing score was used to assess freezing during eight known freezing-sensitive movements from daily life[27] (i.e. writing, tooth brushing, stirring while cooking, screw driving, feet wiping, typing, cutting food, talking) or during another self-reported movement. Patients scored 1 (or 0) when reporting at least one type of (or no) non-gait freezing.

4. Cognitive outcomes: MMSE and the cognitive section of the Scales for Outcomes in PD (SCOPA-COG).[28] A SCOPA-COG < 28 identified subjects with or without cognitive problems (1/0).[29] A binary SCOPA-COG score was preferred over its total score for purposes of model simplicity and clinical use.

Statistical analysis

All analyses were performed using SAS 9.3 and SAS Enterprise Guide.[30] A Kolmogorov-Smirnov distribution analysis was carried out to determine the appropriate test for group comparison of all clinical variables: Chi-Square tests for binary outcomes, non-parametric Wilcoxon two-sample tests for ordinal and not normally distributed interval/ratio data, and two-sample t-test for normally distributed ratio data. Correlations between variables that differed significantly between groups and their relation to the decision variable Group (freezers/non-freezers) were calculated using Point Biserial (interval/ratio predictors) and Rank Biserial (ordinal predictors) correlation coefficients.[31] Variables that correlated highly (|R|>0.30) with Group but weakly with other predictors (avoiding colinearity) were examined in a univariate logistic regression analysis (RA). Variables with high univariate predictive accuracy (R^2) were entered in the full-model, multivariate logistic RA.[32] A prediction model for FOG was obtained using the formulas below:

Log (Y) = $A + \beta 1\, X1 + \beta 2\, X2 + \beta 3\, X3 + ... + \beta n\, Xn$ *Logistic regression equation*

P(FOG) = $P(Y=1) = \dfrac{e^{(A + \beta 1\, X1 + \beta 2\, X2 + \beta 3\, X3 + ... + \beta n\, Xn)}}{1 + e^{(A + \beta 1\, X1 + \beta 2\, X2 + \beta 3\, X3 + ... + \beta n\, Xn)}}$ *Prediction model*

Y represents the decision variable Group (freezers/non-freezers), A the regression intercept and βiXi the weighted predictors. Significance testing of predictors was based on Wald χ^2 statistics.

The relationship between predictors and FOG severity was examined in the freezer population using the same approach but employing a linear regression model. The summed score of items 2-6 of the NFOG-Q[18] on frequency and duration of FOG episodes served as outcome of freezing severity. P values < 0.05 were considered significant.

3. RESULTS

From the 27 reported freezers, 23 had shown FOG episodes during the gait tests. Four probable freezers were thus not retained in the analysis. All definite freezers experienced FOG in OFF. In nine confirmed freezers, FOG was also observed during clinical assessment in ON. There were no clinical differences in patients in whom FOG had been observed during OFF and ON versus in OFF only ($p \geq 0.10$) (See **Appendix 1**)

The final dataset included 47 patients. **Table 1** shows the group comparisons for all clinical, gait and balance, non-gait freezing and cognitive variables. No differences were found for gender, age, Hoehn and Yahr stages, UPDRS (III) total and sub-scores. Freezers had longer disease duration and higher LED than non-freezers. Freezers had similar TUG scores with and without dual tasking but scored significantly worse on the BBS than non-freezers. Falls or near falls (irrespective of FOG) were reported by 52 per cent of freezers compared to 21 per cent of non-freezers. An important distinguishing variable was the presence of non-gait freezing reported by 83 per cent of freezers and 33 per cent of non-freezers. Of the eight items, freezing while feet wiping (present in 57 per cent of freezers and 8 per cent of non-freezers), talking (44 per cent of freezers, 13 per cent of non-freezers) and writing (30 per cent of freezers, 13 per cent of non-freezers) occurred most frequently. All other items were reported by at least two freezers. Cognitive (MMSE and SCOPA-COG) scores were lower in freezers than non-freezers. Based on the SCOPA-COG cut-off score, 60 per cent (N=14) of freezers were identified as having cognitive problems versus 21.7 per cent (N=5) of non-freezers.

A model for FOG occurrence

Variables with significant group differences were examined using Pearson correlation derivatives for binary outcomes. To avoid over-fitting, no more than four candidate predictors could enter the multivariate logistic model of FOG.[32] All variables correlated significantly ($|R|>0.30$, $p<0.05$) with decision variable Group (**Appendix 2**).

LED, disease duration, BBS, non-gait freezing and the binary SCOPA-COG had the strongest correlations with Group. Disease duration correlated with LED ($R=0.54$, $p<0.01$) and Group but not with motor or cognitive variables. LED had a stronger correlation with Group than disease duration ($R=0.47$, $p<0.01$ versus $R=0.31$, $p=0.03$). As the BBS showed evidence of collinearity, only the falls/near falls score (irrespective of FOG) was adopted in the model.

Table 1: Group comparison of clinical outcomes

		Non-freezers (N = 24)	Freezers (N = 23)	
DEMOGRAPHIC ANC DISEASE CHARACTERISTICS				***p***
Gender (M/F) [1]	Frequencies	17/7	19/4	0.34
Age (years) [2]	Mean (+/-SD)	66.3 (60.1-72.6)	68.3 (60.5-76.0)	0.35
Disease duration (years) [2]	Mean (+/-SD)	6.9 (2.7-11.2)	9.7 (5.4-14.0)	0.03 *
Hoehn and Yahr stage (on) (0-5) [3]	Median (IQR)	2.5 (2-2.5)	2.5 (2-3)	0.42
UPDRS III (0-108) [3]	Median (IQR)	29.5 (24.5-39.5)	35 (24-51)	0.32
LED (mg/day) [2]	Mean (+/-SD)	464.0 (281.3-646.8)	674.9 (447.1-902.7)	<0.01 *
GAIT AND BALANCE VARIABLES				
TUG (s) [2]	Mean (+/-SD)	11.2 (9.2-13.1)	13.5 (7.5-19.5)	0.08
TUG motor DT (s) [2]	Mean (+/-SD)	11.5 (9.3-13.8)	14.4 (7.7-21.1)	0.06
TUG cognitive DT (s) [2]	Mean (+/-SD)	13.2 (9.6-16.8)	18.5 (3.2-33.8)	0.11
BBS (items 8, 11, 13, 14; 0-24) [3]	Median (IQR)	20 (19.8-21.3)	19 (15-21)	0.03 *
Falls and balance problems (0/1) [1]	Frequencies	19/5	11/12	0.03 *
FOG-questionnaire (0-28) [3]	Median (IQR)	0 (0-0)	15 (9-20)	<0.01 *
UPDRS III SUBSCORES				
Rest tremor (item 20; 0-20) [3]	Median (IQR)	0 (0-2)	0 (0-1)	0.53
Action and Postural tremor (item 21; 0-8) [3]	Median (IQR)	1 (0-2)	1 (0-2)	0.48
Rigidity (item 22; 0-20) [3]	Median (IQR)	7 (6-8.5)	7 (5-12)	0.72
Repetitive movements (items 23-26; 0-32) [2]	Mean (+/-SD)	12.4 (7.8-17.0)	14.7 (8.5-20.9)	0.16
NON-GAIT FREEZING				
Non-gait freezing (0/1) [1]	Frequencies	16/8	4/19	<0.01 *
COGNITIVE VARIABLES				
MMSE (0-30) [3]	Median (IQR)	29.0 (27.0-30.0)	28.0 (27.0-28.0)	0.03 *
SCOPA-COG (0-43) [2]	Mean (+/-SD)	29.9 (26.2-33.6)	25.2 (18.9-31.4)	<0.01*
SCOPA-COG (0/1) [1]	Frequencies	18/5	9/14 [4]	0.01 *
Memory (items 1-3, 10; 0-22) [3]	Median (IQR)	12 (9-14)	9 (6-12)	0.01 *
Attention (items 4-5; 0-4) [3]	Median (IQR)	4 (3-4)	3 (3-4)	0.01 *
Executive functions (items 6-8; 0-12) [3]	Median (IQR)	11 (9-11)	10 (7-11)	0.05
Visuospatial functions (item 9; 0-5) [3]	Median (IQR)	4 (4-5)	4 (4-5)	0.81

* Groups significantly different at p <0.05. SD: Standard Deviation. IQR: Inter-Quartile Range (Q1-Q3). [1]Chi-Square test was used. [2]Two-sample t-test was used. [3]Non- parametric Wilcoxon two-sample t-test was used. [4]One score on the SCOPA-COG was missing due to practical reasons. UPDRS-III: Unified Parkinson's Disease Rating Scale part III (motor examination); LED: Levodopa Equivalent Dose; TUG: Timed get Up and Go test; DT: Dual Task; BBS: Berg Balance Scale; MMSE: Mini Mental State Examination; SCOPA-COG: Scales for Outcomes in Parkinson's Disease- Cognitive part.

Table 2: Results of the univariate logistic regression analysis on factor Group (Non-freezers; Freezers)

Odds Ratio estimates					
Effect	**Point Estimate**	**95% Wald Confidence Limits**		**R^2**	***p***
Non-gait freezing (1 versus 0)	9.50	2.41	37.47	0.23	<0.01*
LED (dg)	1.69	1.17	2.43	0.21	<0.01*
Cognitive problems (1 versus 0)	5.60	1.53	20.49	0.15	0.01*
Falls and balance problems (1 versus 0)	4.15	1.15	14.92	0.10	0.03*
Disease duration (years)	1.18	1.00	1.38	0.10	0.05

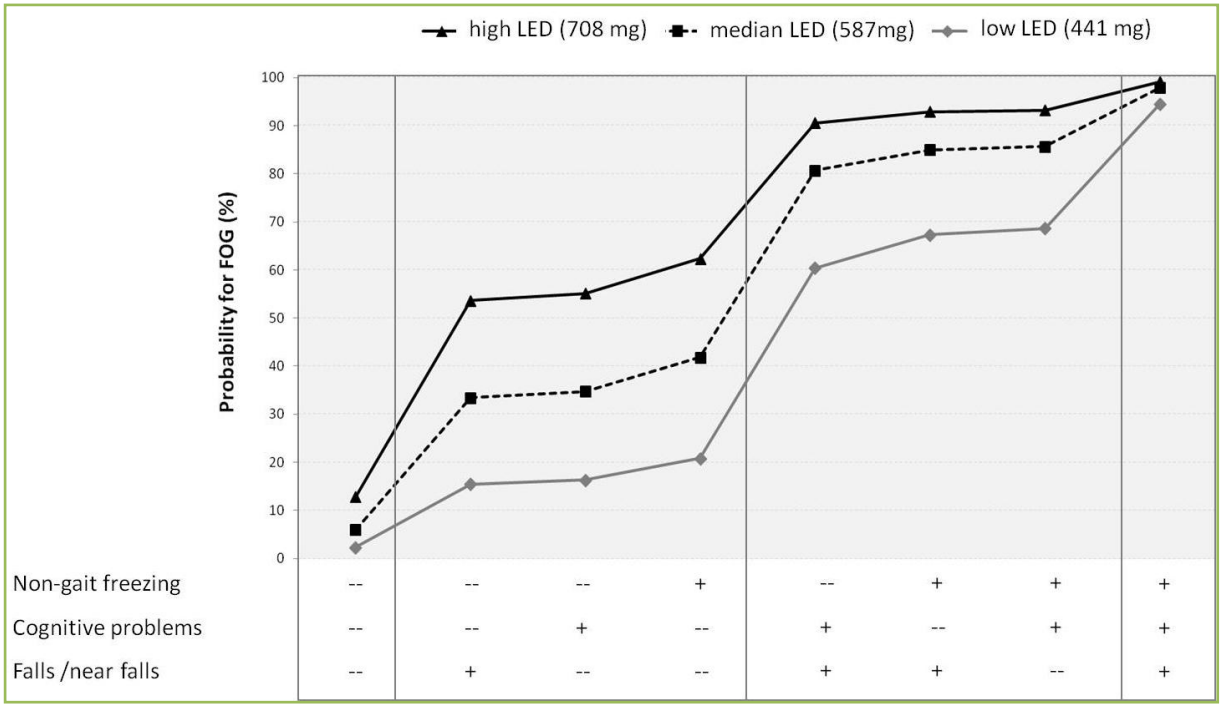
Odds ratios indicate the increase in odds for FOG due to a 1-unit change in the effect parameters: 1 dg (100mg) for levodopa equivalent dose (LED), or from 0 to 1 (problem absent to problem present) for falls and balance problems, non-gait freezing and cognitive problems. For example, for a one-unit increase in LED score (100mg), there is a 69% increase in the odds of developing freezing. All variables except disease duration had a significant univariate predictive accuracy (R^2; *p<0.05).

The univariate predictive accuracy (R^2) was examined for LED, disease duration, falls/near falls, non-gait freezing and SCOPA-COG (1/0) (**Table 2**). Non-gait freezing had the largest R^2 (R^2=0.23, p<0.01). The odds of having FOG in patients with non-gait freezing symptoms compared to patients without this feature was 9.5 (95% CI=2.51-37.37). LED values were converted from mg to dg (100mg) to aid interpretation of the odds ratios. LED explained 21 per cent of the variability (OR= 1.69 (CI=1.17-2.43); p<0.01), SCOPA-COG 15 per cent (OR= 5.6 (CI=1.53-20.49); p=0.01) and falls/near falls10 per cent (OR= 4.15 (CI=1.15-14.92); p=0.03). Given the stronger correlation of LED with Group (freezer/non-freezer) and higher R^2 than disease duration (R^2=21% versus R^2=9.8%), only LED was included in the multivariate model.
LED, non-gait freezing, falls/near falls and SCOPA-COG (0/1) were entered into the multivariate logistic regression model to determine FOG. These four contributors jointly explained 49 per cent of variability between freezers and non-freezers (**Table 3**). Having falls or near falls was the least important factor (p=0.06).
Given these β estimations, a prediction model for FOG could be determined:

$$\mathbf{P(FOG)} = \frac{e \wedge (-3.5 - 1.21\ \text{Non-gait freezing} + 0.69\ \text{LED} - 1.06\ \text{SCOPA-COG} - 1.03\ \text{Falls/near falls})}{1 + e \wedge (-3.5 - 1.21\ \text{Non-gait freezing} + 0.69\ \text{LED} - 1.06\ \text{SCOPA-COG} - 1.03\ \text{Falls/near falls})}$$

Figure 1 compares the contribution of the predictor variables to the presence of FOG at three levels of LED (1st quartile, median, and 3rd quartile of the test population). As depicted, the risk of FOG in patients receiving a median LED of 587mg, who were free from non-gait freezing, falls/near falls, and cognitive problems was estimated at 6 per cent. The risk of FOG increased dramatically to 98 per cent for patients with the LED suffering from non-gait freezing, falls/near-falls and cognitive problems. The estimated probability of FOG was similar between LED levels for patients with either a 0 score (2, 6 and 12%) or a 1 score on all (94, 98 and 99 %) explaining variables. LED levels had greater predictive value in patients suffering from at least one motor or cognitive problem.

Figure 1: Probability of FOG (%) determined by LED and the presence of non-gait freezing, falls and balance problems and cognitive problems.



LED levels were based on 1st, 2nd and 3rd quartiles of the study population. Vertical lines in the figure indicate whether no, at least one, two or all areas are affected. Note that the P(FOG) approaches 100 for patients that experience problems in all spheres, independent of LED (low dose (1st quartile), median, high dose (3d quartile)). Cognitive problems: no cognitive problems: SCOPA-COG ≥ 28; cognitive problems: SCOPA-COG < 28.[27] '--' indicates the problem is present; '+' indicates the problem is absent. LED = Levodopa Equivalent Dose.

Table 3: Results of the multivariate logistic regression on factor Group (Non-freezers; Freezers)

Log (Y) =	$a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$				
	Intercept	**Non-gait freezing** (0/1)	**LED** (dg)	**Cognitive problems** (0/1)	**Falls and balance problems** (0/1)
β estimate	-3.50	-1.21	0.69	-1.06	-1.03
Standard error	1.42	0.51	0.26	0.49	0.56
Wald χ^2	6.13	5.65	7.18	4.63	3.44
p	0.01	0.02	0.01	0.03	0.06
	Overall explained variance between Non-Freezers and Freezers: **R^2= 0.49**				

Variables with significant univariate predictive accuracy were entered in the multivariate logistic regression model. Levodopa-equivalent dose (LED), non-gait freezing, cognitive problems and falls and balance problems were found to be significant independent contributors and jointly explained 49% of variability between non-freezers and freezers. *β* estimations allow to determine the prediction equation for FOG. (Note that negative *β* estimations are due to the class level design of 1/-1 for 0/1 response variables in the SAS system.

A model for FOG severity

Linear regression analysis was applied to test the influence of descriptive, motor and cognitive features on FOG severity in freezers (N=23). FOG severity correlated highly with the falls/near-falls questionnaire (R = 0.51, p=0.01) and moderately with the total score of the non-gait freezing items (R=0.29, p=0.18). Only falls/near falls had a significant univariate explanation of FOG severity (R^2= 27%, p = 0.01). A multivariate regression analysis was therefore not required.

4. DISCUSSION

FOG is a complex gait disorder in which disease characteristics and motor and cognitive factors may play a convergent role.[1]This is the first study that has aimed to identify the unique contribution of some of these factors in a multi-determinant model of FOG. A combination of non-gait freezing, LED, falls/near falls, and cognitive impairment provided the best prediction of having FOG in PD patients of equal disease severity. Some of these factors have previously been associated with FOG but were often regarded as single contributors.[13] Combining these factors in an integrative model of FOG is novel. Only patients with observed FOG ('definite freezers') were included in the freezer group, which adds strength to the model. The findings substantiate the view that a breakdown of multiple neurological systems may be involved in the occurrence of FOG.[1, 33]

Earlier studies reported increased gait asymmetry, problems in gait rhythmicity and left-right coordination as motor correlates of FOG.[4,5,34] In addition, our group and others showed that motor features beyond gait, such as freezing in repetitive hand and feet movements, were associated with FOG.[6-9] The non-gait freezing questionnaire used in this study, had the highest univariate predictive value of FOG. Since a validated tool to assess non-gait freezing in daily activities has yet to be developed, a self-report bias cannot be fully excluded, pointing to a limitation in the present study. Non-gait freezing episodes were reported most often during wiping of the feet. Other items of the questionnaire, particularly hand writing, were also found to be freezing-provoking. Interestingly, patients commented that they tended to avoid these activities in daily life. The non-gait freezing questionnaire was predictive of FOG but not the finger tapping and other repetitive movement items embedded in the UPDRS. Although these items can also induce freezing, scoring is mainly based on the observed slowing and reduction of movement amplitude. In contrast, when we assessed patients using the non-gait freezing questionnaire, we explicitly asked whether actual motor blocks occurred that resembled FOG.

The role of reduced cognitive resources, either as a primary or a compensatory factor contributing to the motor abnormalities of freezers, is a matter of debate. Additionally, it is currently not known which cognitive function is principally involved. One of the cognitive hypotheses of FOG states that freezing is a consequence of frontal executive dysfunction based on evidence that freezers demonstrated reduced cognitive flexibility and verbal fluency compared to non-freezers.[1,10] Recently, Tessitore and colleagues underscored these behavioural findings by showing grey matter and resting state MRI changes in fronto-parietal regions in freezers.[35,36] Accordingly, our results confirm that the SCOPA-COG significantly contributes to the presence of FOG in synergy with motor symptoms. Cognitive decline in freezers, however, was unrelated to freezing severity. In particular, attention and memory functions were impaired in freezers, unlike visuospatial function which was comparable to

non-freezers. The latter finding contradicts recent work that suggests a visuospatial perception deficit in freezers; this may be explained by the fact that the figure assembly task included in the SCOPA-COG is insufficiently sensitive to discriminate between freezers and non-freezers.[13,14] This points to the general limitation that studying the cognitive correlates of FOG is highly dependent on the choice of cognitive measures, indicating a cautious interpretation.

The SCOPA-COG was used to investigate the role of global cognitive decline (score<28) in FOG. Using the recently described cut-off score for PD dementia (PDD) (SCOPA-COG <23),[37] one non-freezer and five freezers would have been classified as having PDD. The possible presence of early dementia in our subject sample (mostly freezers) could well be a study limitation, but it is nonetheless an inherent feature of the population under investigation, where cognitive decline evolves faster with time.[38] Factor et al.[39] recently explored the risk factors of FOG in PD patients of the Postural instability/gait disturbance (PIGD) subgroup. The MMSE was not discriminative between two PIGD sub-types: a group with postural instability and falls (but no FOG) and a group with FOG. However, patients with PIGD and FOG had more frequent psychotic symptoms that have been linked to cognitive deterioration. Genetic differences were most convincing in distinct profiles for PIGD sub-types with and without FOG. This is in contrast to our finding that falls and balance problems per se contributed to the presence of FOG, albeit the least important factor (p=0.06). In addition, falls/near falls was the only determinant of FOG severity, confirming an overlap in neuropathology.[40] Although a failure to couple balance and voluntary locomotor synergies was recently found to be related to FOG, the exact nature of the postural deficits underlying FOG is not yet understood.[40,41]

LED was significantly higher in freezers than non-freezers, which may reflect a higher dose needed to alleviate FOG than other symptoms, especially in later disease stages.[15,42] Patients were matched for UPDRS-scores and Hoehn and Yahr stage in ON but not for disease duration. Thus, a higher LED may have masked a greater underlying disease severity in freezers and may explain why LED discriminated between freezers and non-freezers. However, LED had a stronger correlation with the presence/absence of FOG compared to disease duration, and the explained variance was twice as high as that of disease duration. This suggests that LED captures differences in disease profiles between freezers and non-freezers that are additional to those merely reflecting increased duration/severity. The Levodopa-dependent element of FOG remains difficult to interpret since freezers more often receive Levodopa-therapy as an initial drug than non-freezers.[43] Chronic medication intake leading to reduced synaptic dopa-sensitivity could be a possible explanation for FOG in later stages,[44] and may also explain that, once FOG exists, its severity is not adequately alleviated by Levodopa. In the present study, some patients also demonstrated FOG during clinical assessment while they were otherwise optimally medicated. These patients showed no clinical differences with freezers in whom FOG was only observed during OFF and most likely presented ‘pseudo-ON-freezing’, i.e. FOG that persists in the ON state but may be alleviated by a higher dose of dopaminergic medication.[15] This is consistent with the idea that the therapeutic threshold for FOG may be higher than for other dopa-responsive symptoms.[15] Reduced dopa-sensitivity does not explain FOG in early PD patients. The latter is more suggestive of neural depletion outside the nigrostriatal motor pathway.[16] Ample studies support a non-dopaminergic origin for FOG with the brainstem locomotor regions as key structures (see[1] for a review). The risk profile for FOG yielded by our study underscores this

hypothesis, as cognitive problems and falls and balance problems are also typically seen as poorly responsive to dopaminergic treatment and indicative of cholinergic depletion in the pedunculopontine nucleus (PPN).[45] Studies that examine the influence of dopa-therapy on non-gait freezing are lacking but impaired bimanual coordination, which was associated with upper limb freezing[7], does not seem to improve with dopamine replacement.[46] Therefore, satisfactory treatment of FOG may require a tight regulation of dopaminergic and possibly cholinergic levels.

The determinant model derived from the current study explained only 48 per cent of the variance and did not take into account the contribution of emotional factors (e.g. depression and anxiety) to FOG.[47] However, it provides fresh evidence for the multi-faceted character of FOG, a notion that had not been tested in a single, integrative study before. The assessment of non-gait freezing, cognitive impairment, LED and falls/near falls is not time consuming, adding to the clinical utility of the model. Longitudinal evidence is needed to validate whether the identified determinants predict the occurrence of FOG over time.

5. CONCLUSION

Non-gait freezing, increased dopaminergic drug dose, falls/near falls and cognitive problems are independent determinants of freezing of gait in people with PD. In contrast to earlier studies that focused on a single mechanism to explain freezing in PD, our data indicate that dopaminergic, motor, postural and cognitive deficits play a synergistic role in the manifestation of FOG.

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ABBREVIATIONS

FOG, freezing of gait; FOGQ, Freezing of Gait Questionnaire; PD, Parkinson's disease; OR, Odds Ratio; CI, Confidence interval, LED, Levodopa- equivalent dose; BBS, Berg Balance Scale, MMSE, Mini Mental State Examination; SCOPA-COG, Scales for Outcomes in Parkinson's disease- cognitive part; TUG, Timed Up and Go test; UPDRS-III: Unified Parkinson's Disease Rating Scale part III (motor examination); DT, Dual Task.

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APPENDIX 1

Clinical comparison between freezers of which FOG had been observed only in OFF versus freezers of which FOG had been observed in ON and OFF.

Variable		Freezing observed in OFF (N=14)	Freezing observed in ON and OFF (N=9)	*p*
DEMOGRAPHIC AND DISEASE CHARACTERISTICS				
Gender (M/F)[1]	Frequencies	12/2	7/2	1.00
Age (years)	Median (IQR)	70.5 (59.8-74.8)	69.0 (65.0-72.0)	1.00
Disease duration (years)	Median (IQR)	9.5 (6.5-10.4)	10.0 (8.0-14.0)	0.55
Hoehn and Yahr stage (0-5)	Median (IQR)	2.5 (2-3)	2.5 (2-3)	0.76
LED (mg/day)	Median (IQR)	646.0 (562.9-751.3)	780.0 (600.0-940.0)	0.23
UPDRS III (0-108)	Median (IQR)	40.0 (20.5-50.5)	31.0 (28.0-41.0)	0.99
GAIT AND BALANCE VARIABLES				
TUG (s)	Median (IQR)	10.7 (9.5-13.5)	13.3 (10.6-15.9)	0.13
TUG motor DT (s)	Median (IQR)	11.0 (9.7-14.7)	13.5 (12.8-16.9)	0.10
TUG cognitive DT (s)	Median (IQR)	14.6 (11.6-16.3)	16.0 (12.9-18.6)	0.36
BBS (0-24)	Median (IQR)	19.0 (15.3-21.8)	15.0 (15.0-20.0)	0.43
Falls and balance problems (0/1) [1]	Frequencies	5/9	6/3	0.21
FOG-questionnaire (0-28)	Median (IQR)	14.0 (9.3-20.8)	16.0 (13.0-20.0)	0.78
NON-GAIT FREEZING				
Non-gait freezing (0/1) [1]	Frequencies	3/11	2/7	1.00
COGNITIVE VARIABLES				
MMSE (0-30)	Median (IQR)	28.0 (26.3-28.0)	28.0 (27.0-28.0)	0.77
SCOPA-COG (0-43)	Median (IQR)	25.5 (20.5-30.5)	27.0 (25.0-28.0)	0.90
SCOPA-COG (0/1) [1]	Frequencies	6/8	3/6	1.00

[1]Chi-square test was used. In all other cases, non-parametric Wilcoxon two-sample T-Test was used. IQR = Interquartile range. During gait tests in the context of other studies, all freezers demonstrated FOG in the OFF phase of the medication cycle. Some patients also showed FOG during clinical assessment in ON at their homes. These patients showed no clinical differences with freezers in whom FOG was only observed during OFF and most likely presented 'pseudo-ON-freezing', i.e. FOG that persists in the ON state but may be alleviated by a higher dose of dopaminergic medication.[15] Note that the current study was not designed to examine ON freezing explicitly.

APPENDIX 2

Pearson correlation between explanatory variables and with factor group (Non-freezers; Freezers)

	Disease duration	LED	BBS	Fall and balance problems (0/1)	Non-gait motor blocks (0/1)	MMSE	SCOPA-COG (0/1)	GROUP (0/1)
Disease duration		**0.54** *<.0001*	-0.10 *0.50*	0.16 *0.28*	0.11 *0.44*	0.14 *0.35*	0.21 *0.17*	**0.31** *0.03*
LED	**0.56** *<.0001*		**-0.31** *0.03*	0.008 *0.96*	0.24 *0.10*	-0.11 *0.44*	0.14 *0.37*	**0.47** *<0.01*
BBS	-0.10 *0.50*	**-0.34** *0.02*		-0.11 *0.45*	**-0.46** *0.001*	**0.36** *0.01*	**-0.65** *<.0001*	**-0.40** *0.01*
Falls and balance problems (0/1)	0.16 *0.28*	-0.1 *0.51*	-0.11 *0.45*		0.20 *0.18*	-0.05 *0.76*	0.18 *0.23*	**0.33** *0.03*
Non-gait freezing (0/1)	0.1143 *0.444*	0.222 *0.133*	**-0.46** *<0.01*	0.200 *0.178*		**-0.33** *0.02*	0.17 *0.27*	**0.50** *<0.001*
MMSE	0.14 *0.35*	-0.12 *0.42*	**0.36** *0.01*	-0.05 *0.76*	**-0.33** *0.02*		**-0.36** *0.01*	**-0.33** *0.02*
SCOPA-COG (0/1)	0.21 *0.17*	0.15 *0.32*	**-0.65** *<.0001*	0.18 *0.22*	0.17 *0.27*	**-0.36** *0.01*		**0.40** *<0.01*

Pearson correlations and p- values (in italic) are shown. LED: Levodopa Equivalent Dose; BBS: Berg Balance Scale; MMSE: Mini Mental State Examination; SCOPA-COG: Scales for Outcomes in Parkinson's Disease- Cognitive part. (Correlations with binary outcomes were obtained using appropriate Pearson correlation derivatives).

Chapter 6

General discussion

This doctoral project aimed to increase our understanding of the underlying mechanisms of freezing of gait in Parkinson's disease at the behavioral and neural systems level. Therefore, a series of studies were conducted in which motor performance during a freezing-provoking upper limb task, its underlying brain activation and clinical data were compared between patients with and without FOG who were matched for disease severity. The first paragraph of this general discussion summarizes the key questions and answers of each study and highlights how the obtained results add to the knowledge of the origins of FOG. By and large, these studies were based on the assumption that by investigating upper limb freezing, we tackled mechanisms that were shared with freezing of gait. Paragraph 2 critically reviews similarities and differences of FOUL and FOG and proposes a neural model of generic versus gait-specific features of freezing in PD. Suggestions for clinical practice that follow from the obtained results are addressed in Paragraph 3. Study limitations and ideas for future research, are addressed in Paragraph 4. Last, Paragraph 5 formulates the final conclusions following from this research project.

1. SUMMARY OF OBTAINED RESULTS

Study 1 (Chapter 2) investigated the behavioral correlates of FOG and non-gait freezing with a focus on episodic motor abnormalities inherent to these types of freezing. More specifically, we addressed the research question whether freezing of gait and freezing during other motor tasks were correlated and characterized by similar spatiotemporal changes in the kinematic signal. The novelty of this study was situated in the systematic comparison of the kinematic changes before and during upper limb freezing with FOG-related motor abnormalities using quantitative measurements sensitive to scaling and timing dyscontrol. Therefore, a group of 11 PD patients with FOG, 12 PD patients without FOG and 11 control subjects performed two freezing-provoking motor tasks: 1) bilateral repetitive movement of the index fingers with varying constraints in movement amplitude, frequency and pattern coordination and 2) rhythmic, alternating foot movements at comfortable pace and amplitude. This paradigm enabled to determine the correlation between the occurrence of freezing in different effectors and their similarities in terms of triggering conditions and spatiotemporal movement changes prior and during the freeze.
The results demonstrated that severity of freezing during repetitive upper limb and foot movements was correlated to severity of FOG. Similar to FOG, freezing in the upper limb was 1) best triggered by small-amplitude movements, 2) preceded by a progressive decrease in movement amplitude and increase in frequency and 3) characterized by high-frequency trembling-like movements.
Contribution to the understanding of FOG: The findings suggest a generic (effector-independent) motor problem underlying the freezing problem in PD that affects repetitive movement generation beyond the locomotor system.

Study 2 (Chapter 3) further analyzed behavioral similarities between FOG and FOUL but now with a focus on motor abnormalities that persist during non-freezing (continuous) movement and on how these are influenced by auditory cueing. Two research questions were formulated: 1) Do problems in timing and scaling of movement observed in ongoing gait of freezers also affect ongoing upper limb movement and 2) are these motor abnormalities emphasized by cue-withdrawal? In contrast to earlier work of our group that demonstrated relatively spared spatiotemporal control during functional hand writing movements in

freezers,[1] the current study involved a more freezing-sensitive upper limb task and included the effect of cue-withdrawal. Therefore, movement trials obtained from Study 1 that were free of FOUL were compared between freezers, non-freezers and control subjects during performance with auditory pacing and after cue withdrawal.
Similar to their continuous gait abnormalities, PD patients with FOG showed 1) more pronounced scaling, timing and bilateral coordination problems during ongoing bimanual movements and 2) greater cue-dependency than PD without FOG and control subjects.
Contribution to the understanding of FOG: The findings of Study 2 add that continuous problems in controlling amplitude, rhythm, bilateral coordinating and internal timekeeping of external sensory information are apparent in the generic aspects of freezing of PD.

Neuroimaging studies so far had revealed widespread structural and functional changes in the brain in patients with FOG including fronto-parietal cortical regions, basal ganglia and midbrain motor areas.[2-9] However, none of these findings were directly related to the emergence of abnormal motor output such as freezing episodes. **Study 3 (Chapter 4)** addressed this lacuna in current research on the neural basis of freezing by exploiting the overlap in motor disturbances between FOG and FOUL as demonstrated in Study 1 and 2 in an fMRI environment. With this study, we wanted to explore which altered patterns of brain activity underlie the shared motor mechanisms of FOG and FOUL that lead to continuous spatiotemporal difficulties and freezing episodes. A group of 16 PD patients with FOG, 16 PDs without FOG and 16 controls performed a shortened version of the freezing provoking upper limb task in an MRI scanner. Two planned comparisons were effectuated. We contrasted brain activation during 1) ongoing, functional, upper limb movement between groups and 2) during ongoing movement versus upper limb freezing within patients who presented FOUL during testing.
There were two main results: 1) During ongoing upper limb movement, brain activity in PD with FOG was decreased in the right dorsolateral prefrontal, left dorsal premotor (PMd) and primary motor cortex (M1) in patients with FOG whereas right dorsal putamen and subthalamic activation were more active compared to PD without FOG. 2) In contrast, brain activation during freezing episodes was increased in the right supplementary motor area, the right M1 and PMd and left prefrontal cortex compared to continuous movement, whereas activation in the putamen now tended to be decreased.
Contribution to the understanding of FOG: The shared motor mechanisms of FOG and FOUL were related to altered patterns of brain activity within the striatofrontal circuitry. Subcortical hyperactivity may inhibit cortical activation in motor and cognitive areas, resulting in subtle abnormalities during ongoing upper limb movement of freezers. In contrast, a shift to cortical drive which has been described as a compensatory mechanism in PD, only occurred in freezers when UL freezing had emerged and probably reflects an attempt to overcome the motor block.

Study 1 to 3 focused on motor components that are common to FOG and non-gait freezing. The aim of **Study 4 (Chapter 5)** was to put these motor determinants in perspective of a more complex clinical picture of FOG including postural, cognitive and affective disturbances that were found more pronounced in freezers than non-freezers. The strength of the current study was that, unlike earlier studies, a multivariate approach was used to examine the independent contribution of motor and non-motor determinants of FOG. In this cross-

sectional study, a group of 27 patients with FOG and 24 PD patients without FOG were assessed with an extensive clinical test battery evaluating general disease characteristics, gait and balance, non-gait freezing and cognitive functions. The relative contribution of these outcomes to FOG was determined using logistic regression analysis.
We showed that the occurrence of FOG was best predicted by a combination of four independent factors: the presence of non-gait freezing, increased dopaminergic drug dose, cognitive deficits ascertained by a SCOPA-COG score < 28 and history of falls or near falls within the last 3 months.
Contribution to the understanding of FOG: Adding to earlier univariate hypotheses, the findings suggest that dopaminergic, motor and non-motor deficits co-determine FOG and play an independent and probably synergistic role in its manifestation.

2. GENERIC VERSUS GAIT-SPECIFIC FEATURES OF FREEZING IN PARKINSON'S DISEASE

The crucial and overarching question that determines the true scientific contribution of this doctoral project is what the study of UL freezing revealed about the origins of FOG? To answer this question, we first review the similarities and differences between FOG and FOUL (**Table 1**). Converging findings based on gait analysis as well as on the presented UL studies of this doctoral thesis point to the fact that the generic or effector-independent aspects of freezing in PD are related to amplitude and rhythm control. The role of other features may be specific to gait or remain to be tested in the context of upper limb freezing.
A recent review by Plotnik et al.[41] offers an elegant theoretical framework of FOG through which the emergence of movement breakdown during gait and upper limb movement will be compared. In this framework, overall gait performance of freezers is expressed as a combination of several locomotor impairments that fluctuate over time. As listed in Table 1, these background abnormalities affect the regulation of stride length, stride timing, bilateral coordination, symmetry, and dynamic postural control. Ample studies (see Table 1) support the presence of these multiple gait abnormalities but the relation between FOG and impaired anticipatory postural control however, was recently contradicted by Paul et al., (2012).[42] Postural dyscontrol was an independent determinant of FOG in the multivariate model of Study 4 but other factors such as the presence of non-gait freezing and cognitive dysfunction seemed to play a bigger role.
Certain circumstances, previously identified as triggers of FOG lead to episodic changes in the gait pattern and increase the interdependence of individual gait features. For example, asymmetry between the left and right motor program is associated with the sequence effect during turning, but does not affect stride length during straight-line walking. This synergistic malfunction of background gait abnormalities is a crucial element in the model and also accounts for the effect of non-motor triggers such as dual tasking which acts through the worsening of all 5 gait impairments.[43-47]

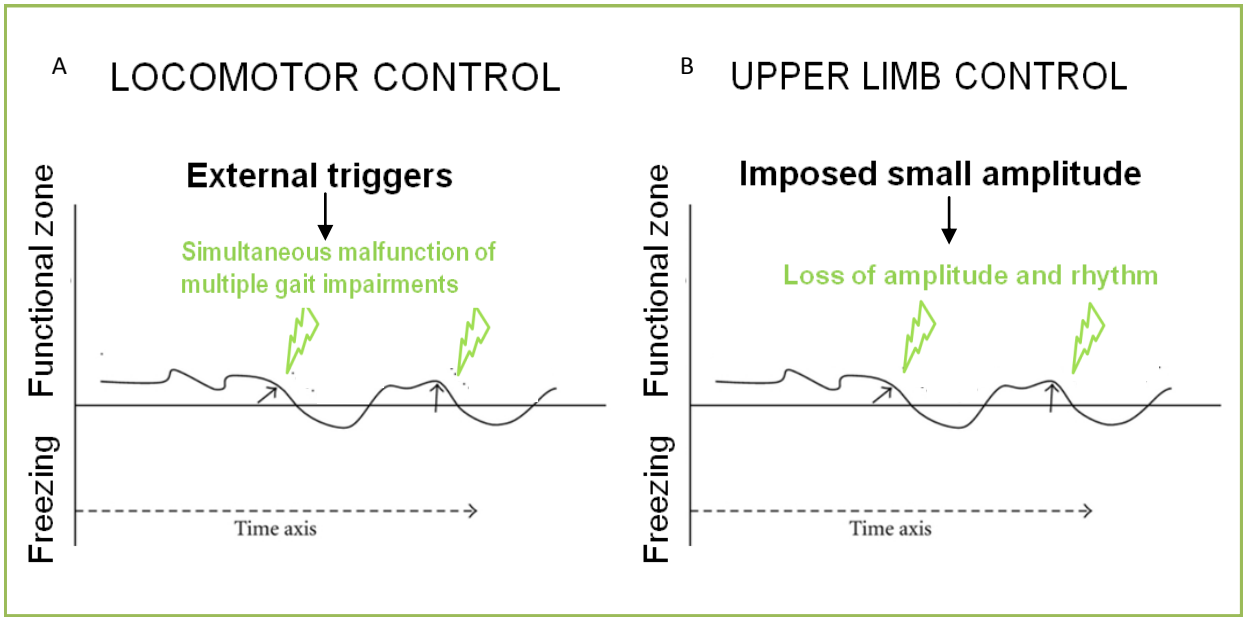

Figure 1: Comparison of the emergence of movement breakdown during locomotor (A) and upper limb (B) control. In gait, the compound walking signal reflects a combination of background abnormalities that affect the regulation of stride length, stride timing, bilateral coordination, symmetry, and dynamic postural control. When external triggers push the overall gait pattern under the imaginary threshold (horizontal line), FOG emerges. A similar conceptualization can be applied to the occurrence of FOUL based on background abnormalities in scaling, timing and coordination of UL movement in freezers. Especially, the joined occurrence of progressive amplitude reduction and frequency increase in response to an imposed small amplitude resulted in FOUL. Adapted from Plotnik et al., 2012.[41]

As shown in **Figure 1** (part A), the simultaneous deterioration of multiple gait properties pushes the compound walking pattern below an imaginary threshold resulting in a transition from functional to frozen gait. Subsequently, the effect of treatment on FOG can be evaluated in three ways: 1) does it improve background abnormalities, moving the overall performance further away from the threshold for FOG? 2) Does it reduce the response to triggers**,** visualized by a smaller drop of the compound gait signal towards the threshold? 3) Does it aid in overcoming the FOG episode by enhancing a return to the functional zone? According to Plotnik et al.,[41] the beneficial effect of Levodopa on FOG is situated in improving baseline gait aspects such that the overall gait pattern does not cross the critical threshold in the influence of triggers. More pronounced executive dysfunction may however make locomotion in freezers more prone to movement breakdown although it is not clear at present whether this effect is mediated by worsening the background abnormalities or by aggravating the response to triggers resulting in a so-called simultaneous mental and motor collapse.[48] The association between cognitive dysfunction and FOG is controversial at present. Amboni et al.[29] reported a significant correlation between FOG-Q and scores on executive functioning (frontal assessment battery, phonemic verbal fluency, Stroop test, and ten-point clock test) but these associations may have been overestimated as data of non-freezers were included in the correlation analysis. Other authors reported no significant relations between FOG-Q and cognitive scores when determined within PD with FOG.[14,30] There is converging evidence of a deficit in a specific component of executive function in freezers, namely set-shifting or the ability to keep different (motor or non- motor) ongoing tasks online and flexibly shift between them.[30,49,50] The gating operating processing in basal

Table 1: Evaluation of similarities and differences of freezing of gait and upper limb freezing

FOG-related feature	Results from gait studies	Results from UL studies
Episodic features of FOG		
Triggers	Gait initiation[10,11]	Not observed
	Gait adaptation[10-14]	Not tested
	Destination[10]	Not tested
	✓ Walking with small steps[15,16]	Small-amplitude UL motion[S1]
	Dual tasking[15,17,18]	Not tested
	Stress[17,19]	Not tested
Kinematic changes prior to freezing episode	Abnormal muscle timing[20]	Data to be analyzed
	✓ Progressive decrease in stride length[16,21]	Progressive decrease in UL amplitude [S1]
	✓ Progressive increase in cadence[21]	Progressive increase in UL frequency [S1]
Kinematic changes during freezing episode	✓ High frequency leg movements[10,22]	High frequency finger movements [S1]
	Complete akinesia[10]	Not observed
Background motor abnormalities related to FOG		
Impaired motor scaling	✓ Smaller steps[14,16,23]	Smaller UL amplitude [S2]
		More variable UL amplitude [S2]
Impaired motor timing	Hastened cadence[14]	Hastened UL frequency [S2]
	✓ Increased stride time variability[24]	More variable UL frequency [S2]
Impaired coordination	✓ More variable stepping phase[25]	More variable UL relative phase [S2]
	More asymmetric gait[26]	Data to be analyzed
Impaired posture and balance	Abnormal postural adjustments[27]	Gait-specific
	Poor balance and falls[28,S4]	Gait-specific
Background non-motor abnormalities related to FOG		
Cognitive disturbance	Executive deficits correlated to FOG[29] and independent predictor of FOG [S4]	FOUL not to correlated cognitive scores[S1]
	Visuospatial deficits associated with FOG[12,13]	Not tested
Affective disturbance	Anxiety and depression related to FOG[17,19,30]	Not tested
Treatment effect on FOG		
Dopaminergic drugs	Less FOG and background motor problems in ON versus OFF[10]	Not tested
DBS	STN DBS reduces FOG and background motor problems[31,32]	Not tested
	PPN DBS reduces FOG but has no effect on background motor problems[33-36]	Not tested
Cueing	✓ Cueing reduces FOG and background motor problems[27-40]	Cueing improves FOUL and UL background problems [S2]
	✓ Greater cue-dependency in freezers[37-39]	Greater cue-dependency in freezers [S2]

Table 1: Converging findings based on gait analysis as well as the presented UL studies are marked. These communalities represent generic or effector-independent aspects of freezing in PD. The role of other features may be specific to gait or remain to be tested in the context of upper limb freezing. S1-4= Study 1to 4 of the proposed doctoral research.

ganglia-frontal networks related to dynamic response selection may be critically involved in set-shifting.[51] Further research is warranted to unravel the involvement of executive processes possibly mediated by striato-prefrontal cognitive pathways in the etiology of FOG and more specifically how they lead to movement breakdown during walking.

Fitting the results of Study 1 and 2 within this theoretical model, overall performance of upper limb movement in freezers can be viewed as fluctuating in the functional or freezing zone depending on whether the baseline abnormalities associated with FOUL, i.e. poor control of movement scaling, rhythmicity and bilateral coordination jointly occur (Figure 1, part B). Especially, the combination of a sequence effect and increased frequency was found to result in FOUL. This is in contrast to hypokinetic deficits in PD such as micrographia which is characterized by a profound deterioration in the amplitude domain only.[52]
Similar to FOG,[15,16,21] imposing small-amplitude movements had a detrimental effect on upper limb movement parameters in freezers leading to the emergence of freezing episodes. Therefore, a generic aspect of freezing in PD is the difficulty to maintain largely scaled movements in synergy with a stable rhythm in response to triggers that operate through a reduction of movement amplitude. Study 3 further demonstrated that the neural basis of this problematic scaling-timing interaction during ongoing upper limb movement was related to increased basal ganglia activity and reduced frontal cortical drive in freezers. This suggests that dysfunction of the striato-frontal neural circuitry which is considerably affected in PD, also plays a prominent role in the generic aspects of freezing. In line with this, treatment interventions that target the dysfunctional BG-frontal pathway such as dopaminergic medication, STN stimulation or cueing strategies were shown to decrease the propensity of FOG by partially normalizing background abnormalities in stride length and timing control.[10,31-33,37-40] Following the idea that generic motor aspects of freezing are located within the basal ganglia-cortical network, FOUL may also be improved after dopaminergic medication intake, but this question was not addressed in the current studies. Based on observation and pilot work, patients seemed to show less FOUL during the training sessions for Study 3 in ON compared to OFF situations in Study 1, 2 and 3. A case-report of Snijders et al (personal communication) however revealed that STN-DBS had alleviated FOG completely in a patient, but had worsened speech freezing pointing to the possibility that STN-DBS, of which the exact mechanisms are not fully understood[31] may also exert diverging effects on gait and non-gait freezing.

Once freezing emerges, be it during gait[10,22] or upper limb control (Study 1), it is generally characterized by the presence of abnormally small and high frequent oscillatory motor output. As for gait, these trembling-like leg movements have been associated with an attempt to overcome the motor block[10] and multiple anticipatory postural adjustments.[27] On the neuronal level they are thought to result from disturbed output signaling from the brainstem-central pattern generator pathway.[21,22,53] FOUL also presented with high-frequency components outside the context of postural control. This favors the idea that they either reflect the ultimate endpoint on the continuum of spatiotemporal disruption that is present in freezers (and thus bringing the overall performance deeper in the freezing zone) or in contrast, reflect the attempt to regain functional movement. The results of Study 3 may aid in understanding the role of high-frequency components during freezing. Brain activity during FOUL was reduced in the putamen and increased in cortical motor and cognitive areas compared to ongoing movement. This inverse pattern of neural activation during FOUL compared to functional UL movement, strengthens the idea that the highly disrupted

temporal signals during freezing represent an attempt to overcome the motor block. This increased volitional error-correction during freezing would also explain why the observed changes in brain activations were found within a higher order striato-frontal network and not in previously suggested brainstem motor areas such as the PPN. Region of interest analysis also revealed no significant differences in activation during ongoing upper limb motion between PD with FOG, PD without FOG and controls in these areas. However, brainstem motor regions (the PPN and MLR) showed altered white matter connectivity[2], grey matter atrophy[3] and hyperactivity during gait planning in freezers[3] compared to non freezers in other studies. Consequently, we suggest that the role of brainstem motor areas in the origin of freezing is specific to gait and not necessarily related to the impaired scaling and timing control underlying FOG. The recent finding that PPN stimulation improves the number of FOG episodes but not background abnormalities in stride length and timing control supports this hypothesis.[4]

Brainstem motor areas may exert a gait-specific influence on FOG through loss of cholinergic cells that are involved in postural control.[54] Indeed, dopaminergic medication has limited effects on postural instability, falls and cognitive deficits.[55] As mentioned above, impairments in balance control and cognitive tasks that are sensitive to executive functioning are generally worse in freezers compared to non-freezers, were significant independent contributors to FOG in the multivariate model of Study 4 but their specific role in causing sudden cessation of gait is not fully understood. At present, there is no direct evidence for more severe cholinergic degeneration in freezers compared to non-freezers. The PET study of Bohnen et al.[56] indirectly supports this idea by showing that cholinergic hypofunction, and not dopaminergic activity, discriminated PD patients with a history of frequent falls from PD-non-fallers. Reduced cholinergic activity was found in cortical areas and in the thalamus which receives major cholinergic projections from the PPN. Though attention and executive functioning is largely mediated by striato-frontal (dopaminergic) pathways through the associative striatum (see Chapter 1), the decline in these cognitive processes has also been clearly associated with cholinergic hypoactivity[57] and increased burden of white matter hypointensity (WMH) within cholinergic pathways.[58] According to Shin et al.,[58] cholinergic WMH may even be worsened by chronic levodopa intake. This points to the possibility that the contribution of an increased LED dose in determining FOG as shown in Study 4, reflected worsened cholinergic function of freezers in addition to dopaminergic dysregulation, but this remains speculative. Though Levodopa is thought to influence the threshold for FOG, it may reach a certain saturation level after which increasing the dose does not results in further improvement.[59] In some cases, creating a supra-ON state by augmenting the LED above its optimal effect, was shown to worsen FOG and produce motor blocks in hand and feet movements.[59] These findings point to parallel effects of enhancing dopaminergic pathways on gait and non-gait freezing with either improvement or worsening depending on a whether a saturation level has been reached.

In summary, we propose a neural model on freezing in PD whereby generic aspects, such as amplitude and rhythm control in both gait and other repetitive movements rely on striato-frontal mechanisms whereas gait-specific elements of postural control are mostly located downstream of the basal ganglia network. Cognitive impairment, mainly in the executive and attentional domain, may aggravate central motor control disturbances in freezers although the exact behavioral and neural mechanisms underlying this effect merit further studies. Probably, both dopaminergic and cholinergic projections to cortical cognitive areas are involved in executive problems of freezers. This perspective offers a testable hypothesis that FOG may originate from more pronounced cholinergic dysfunction superimposed on the

more severely affected dopaminergic pathways. This final, though hypothetical, model is depicted in Figure 2. Last, dopaminergic treatment was found to have a more pronounced effect on stride length generation compared to rhythmicity of gait.[24] These findings suggested that the non-dopaminergic, possibly, cholinergic neurotransmitter system may also be involved in timing problems which challenges the proposed model and emphasizes the need for future research.

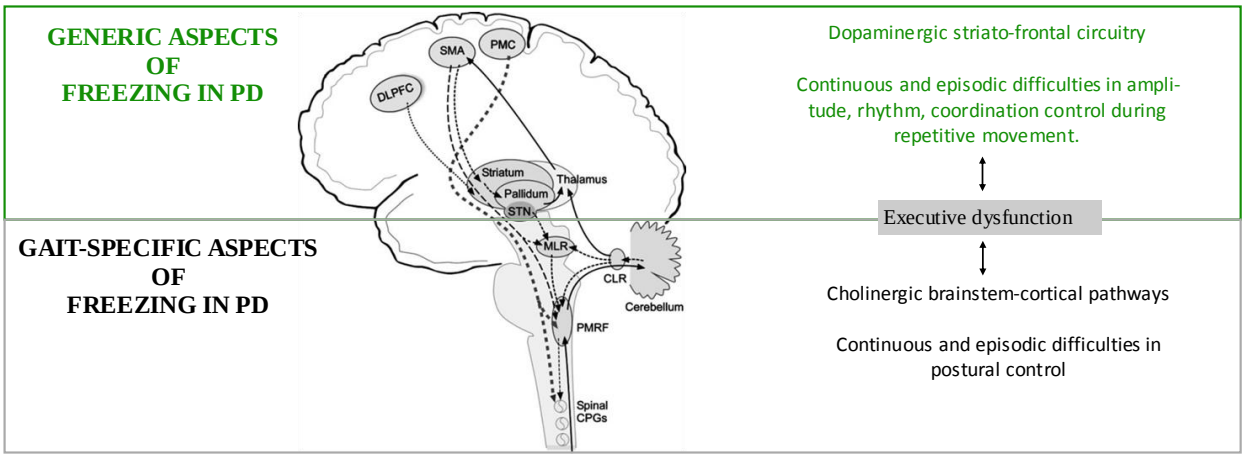

Figure 2: Proposed neural model on freezing in PD whereby generic aspects rely on striato-frontal mechanisms whereas gait-specific elements are largely located downstream of the basal ganglia network. Adapted from Nutt et al., 2011.[60]

3. CLINICAL IMPLICATIONS

FOG is a well-recognized clinical problem. However, clinicians may be less familiar with the existence of non-gait freezing. The current project may contribute to broadening the clinicians' perspective on FOG and facilitating the recognition of motor blocks in hand movements as part of the same problem. As such, patients' reports of upper limb freezing in daily practice (for example one patient complained about freezing during the repetitive hand movements he made to wash his hair) may be better understood or even be indicative of the presence of FOG. The functional implications of freezing in other movements have not been elucidated by this doctoral project and need additional investigation. Rehabilitation strategies for PD patients with FOG may improve when taking three key findings of this doctoral project on board:

A rehabilitation focus on amplitude and rhythm generation in PD patients with FOG

As central motor components of freezing in PD, difficulties in amplitude and rhythm generation should be a primary focus of training interventions for patients with FOG. Freezers will benefit from the instruction to initiate gait with a bigger step and try to maintain this amplitude. Although slowness of gait is typically regarded as symptomatic for PD, PD patients with FOG should be encouraged to decrease their cadence to achieve a more safe and functional walking pattern. This way, the combined occurrence of decreased stride length and festinating steps known to provoke FOG[15,16,21] may be prevented. In a similar vein, motor learning aimed at generating large-amplitude and slower hand movements, especially when these are to be performed in a repetitive way, may improve patients' upper limb skills. Handwriting for example, is of major importance in daily life and is often avoided by patients

due to severe difficulties including micrographia and freezing episodes. The development of a novel relearning program for handwriting skills would have great functional meaning and is currently undertaken in combination with neuroimaging tools to assess the accompanying plastic changes in the brain. The results of Study 2 when comparing the deteriorating effect of amplitude, frequency and interlimb pattern constraints on several UL movement parameters, suggest that large-amplitude movements should be emphasized in particular to prevent upper limb freezing. Taking things a step further, patients with severe balance problems may even benefit from spatiotemporal motor learning first applied to repetitive hand movements and later to locomotion, although such a transfer from upper to lower limb training is to be investigated.

Rehabilitation strategies that bypass cue-dependency in PD patients with FOG

The implementation of external sensory cues may help patients in achieving specific spatiotemporal motor goals but is challenged by the risk of cue-dependency in freezers. Hardly any UL freezing episodes occurred in the presence of an auditory cue but Study 2 revealed that cue-withdrawal induced more pronounced motor abnormalities in patients with FOG compared to those without FOG and healthy controls. Although external pacing was only briefly presented (1st 6 movement cycles) and was not studied in counterbalanced way, the results complement earlier findings of greater cue-dependency in freezers while walking[37-39] These results argue in favor of offering continuous cueing methods or of a rehabilitation program in which patients learn to better internalize the cue. Continuous cueing has the benefit of bypassing the effect of cue-withdrawal but is very attention-demanding and not practical in daily life. Pilot data presented by Plotnik et al.,[61] showed promising results of a motor learning paradigm in which auditory cues were presented during walking only when steps showed pre-freezing behavior. This way, cueing is feedback controlled and delivered to prevent FOG instead of normalizing ongoing gait. An important finding was that freezers experienced less FOG episodes even in the retention phase (in the absence of cues) suggesting they had learned to detect pre-freezing steps themselves and correct them based on internal motor commands.

Interventions that take cognitive impairment of patients with FOG into account

In line with previous research, Study 4 showed that executive dysfunction is an independent contributor to FOG in PD. Physiotherapists should be aware of the fact that attentional deficits may hinder successful retrieval and completion of the applied intervention. Instructions to the patient should be clear and simple and offered preferably in a non-noisy environment. In a later stage, when automaticity of the target movement is improved, the training can be continued at home or at least in a more clinically relevant setting that confronts patients with distracting features on their way (e.g. a ringing telephone). At that point, dealing with dual task situations and/or task prioritizing can become new treatment goals. In addition, informing patients with FOG and their carers of possible cognitive difficulties may also reduce frustration in some cases. Alternatively, recent studies showed that cognitive training improves executive functions.[62-64] and normalizes brain activation associated with cognitive task performance.[65] Mobility outcomes such as gait speed and 'walking while talking' abilities were also shown to improve in healthy seniors after a cognitive remediation program.[66] The effect of cognitive training on motor and non-motor

features associated with FOG is not studied yet but could be an important future research direction (see below).

4. STUDY LIMITATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

This section formulates objectives and directions for future research that mainly originate from the results or limitations of the studies that were presented in this doctoral thesis. These suggestions are structured in 4 domains which roughly concur with the research aims of study 1 to 4.

Future studies aimed at identifying generic versus gait-specific elements of freezing in PD

Studies 1 and 2 were designed to investigate shared motor mechanisms of freezing in different effectors. This way, impairment in regulating movement amplitude and frequency in a coordinative way, were found to occur both during gait and UL movement in response to an imposed smaller amplitude, which is a common trigger of FOG and FOUL. An important limitation is that the effects of external triggers that require divided attention and/or a flexible adaptation to the ongoing motor program were not directly studied. A future study that compares UL motor performance under dual task situations (e.g. a color decision or a counting task) with single task conditions may be helpful in understanding how executive or attentional problems promote the emergence of movement breakdown and the extent to which they are generic or specific to locomotor control. Following the idea that the FOG-eliciting effect of dual task conditions during gait is partly mediated by decreased stride length, we hypothesize that a secondary task during UL movement would be performed at the expense of amplitude control and thus would also provoke FOUL. However, this remains to be tested. In addition, if the generic features of freezing in PD are thought to rely on disturbed striato-frontal interaction, UL freezing should be improved by dopaminergic medication. This hypothesis could be tested by comparing UL task performance while patients are in ON and OFF states. Clinical studies that evaluate the presence of non-gait freezing events (e.g. freezing while writing) before and after medical or surgical interventions may further complement this behavioral experiment but await a validated questionnaire on non-gait freezing items. Studying the validity and reliability of a research instrument for non-gait freezing allows further research into the effect of interventions to reduce freezing of gait and other effectors. The validation of a similar questionnaire as used in Study 4 may also be essential to determine the temporal relation between the occurrence of upper limb and gait freezing through disease progression. According to our proposed model where generic aspects of freezing rely on severe dopaminergic dysfunction in contrast to gait-specific aspects that may predominantly involve cholinergic pathways, freezing in the upper limbs may occur earlier in the disease and serve as a biomarker of future development of FOG. A long-term follow-up study including behavioral and neurological assessments is currently undertaken in this perspective.

Last, the neurophysiological profiles of upper limb muscle activity need to be further analyzed to understand the nature of freezing parameters that were previously studied in association with FOG. For this aim muscle activation during performance of the upper limb paradigm was measured in Study 1 using electromyography (EMG). EMG profiles during pre-freezing UL movement cycles need to be analyzed to investigate if, similar to FOG,[20] abnormal muscle timing precedes FOUL. In addition, coordinative asymmetry that was described as a

characteristic of freezers' gait pattern requires further investigation for UL movements as well by means of the phase coordination index (PCI)[25] to unravel the overlapping and diverging mechanisms of both types of freezing

Future studies that investigate novel rehabilitation strategies for patients with FOG

Cueing is a common rehabilitation strategy in PD that can improve spatial and/or temporal aspects of walking quality. Study 2 investigated how motor performance in freezers, non-freezers and controls was influenced by auditory cueing and cue-withdrawal. An important limitation of this study was that cueing conditions were not offered in randomized or counterbalanced way. To better capture the full extent to which freezers benefit from additional sensory information during hand movements, we suggest a randomized controlled trial including motor assessment in the retention phase. Given the major impairment of amplitude generation leading to freezing episodes, freezers may also show greater improvement by using visual stimuli that emphasize scaling properties of the ongoing movement as compared to auditory, rhythm-focused cues. As mentioned above, 'smart cues' or dynamic cues that vary depending on actual motor performance and thus enable patients to correct for subtle deviations in the motor output before actual freezing occurs, showed promising results but await further investigation. Freezers may particularly benefit from such a feedback-controlled guidance of movement as it bypasses the dependence effect of fixed (feed-forward) cues.[67] By enhancing error-monitoring processes, this approach has the potential to induce actual learning in freezers which is important in view of functional independence in these patients. Still, future studies need to examine whether motor learning strategies aimed at internalizing external information decrease the cue-dependency in freezers. As demonstrated by Heremans et al.[68] the combination of mental practice and cueing could be a promising new direction in the development of rehabilitation strategies for repetitive upper limb tasks which may also be applied to freezers.
Given the tight interplay of executive functions and motor control in PD patients and especially in those with FOG, we also see a future for studies aimed at determining the effect of cognitive remediation on motor and non-motor outcomes in PD patients with and without FOG. Cognitive training that enhances attentional capacities in PD[62-64] may improve patients' ability to negotiate obstacles or to walk in dual task conditions, both factors known to elicit freezing. By normalizing the response to triggers, a smaller drop of the compound gait signal towards the threshold can be achieved. For example, promising results have been shown of an attention orientated training program on dual task performance in people with mild cognitive impairment and executive dysfunction.[69] A pilot study by Yogev-Seligmann et al.[70] pointed to feasibility and similar benefits of dual task training in PD patients. It was also shown that a training program to heighten attention and decrease distractibility increased resting state CBF in prefrontal regions of healthy elderly.[71] This offers a sensitive parameter to study the benefits of cognitive training in the context of brain plasticity.

Future studies that tackle the neurological mechanisms of FOG

Up to now, only one study explored differences in integrity of white matter tracts using DTI in a small group of freezers and non-freezers.[2] All subjects who participated in the fMRI experiment of Study 3 also underwent a Diffusion Kurtosis Imaging (DKI) scan that, like DTI, provides information on fiber structures based on water diffusion properties in the brain.

These data will be analyzed to increase our understanding on white matter degeneration in freezers and how this relates to altered behavior in cognitive and motor domains.
Furthermore, we would like to gain more insight into the brain activity during the pre-freezing events and functional connectivity measures during ongoing UL movement compared to freezing episodes. The latter comparison would be a first step in defining the relation between compensatory brain recruitment and functional connectivity. Functional connectivity may be particularly important in future studies as it been suggested as a sensitive parameter for pathology-induced changes in brain organization, even more so than changes in the amplitude of the BOLD signal.[72] An alternative fMRI compatible approach to study locomotion-related brain activity in PD with and without FOG may be based on an action observation paradigm. Similar to motor imagery, action observation engages several brain activation sites that concur with the neural activation during motor performance.[73] In this perspective, we would propose to measure brain activation while patients observe their own gait on video in standardized freezing-provoking and freezing-resistant conditions.
The hypothesis that dysfunction of the cholinergic neurotransmittor system is involved in cognitive, postural and higher order gait disturbances (such as FOG) has received growing interest in PD literature[54,56,,57] and fits well within the neural model of FOG that was proposed in this dissertation. Future studies could use specific molecular PET agents to determine if the evolution of dopaminergic, cholinergic dysfunction and even amyloid plaque distribution shows a different pattern over time in PD patients with or without FOG.
In general, there lies a great challenge in integrating results of neuroimaging studies that compare different modalities of brain organization in PD patients with and without FOG. This relates however to a more fundamental neuroscientific question as it remains difficult to predict how for example alterations in structural grey or white matter affect the BOLD signal during rest or task performance measured by fMRI. Future studies are indicated using combined methodological approaches to elucidate these relationships.

Future studies targeting the interplay between motor and non-motor determinants of FOG

In Study 4, we developed a multi-determinant model of FOG using a cross-sectional design with 24 patients without FOG and 23 definite freezers. A first limitation of this study is that no data were available on affective disturbances which were previously associated to FOG.[17,18] In addition, a larger study sample would allow more predictor variables to be included in the multivariate model of FOG or would promote the use of factor analysis. In contrast to regression models, factor analysis allows interdependency of explaining variables and uses it to group variables into contributing factors. This way the relative contribution of these factors (e.g. cognitive impairment) to FOG can be determined as well as the weight of different variables within the factor (e.g. executive dysfunction test). Such an analysis would provide a more holistic approach to the freezing problem in PD but has the drawback that a prediction equation for the risk for FOG does not follow easily from the obtained results making it less suitable in clinical practice. The prediction model for FOG we obtained in Study 4 awaits further longitudinal evidence to validate whether the identified determinants predict the occurrence of FOG with time. As previously mentioned, such a long-term clinical follow-up study is currently undertaken in combination with neuroimaging assessments.

5. GENERAL CONCLUSION

The principal aim of this dissertation was to increase our understanding of motor, non-motor and neural origins of freezing of gait in Parkinson's disease. Motor correlates of gait and non-gait freezing were predominantly found within the control of timing, scaling and coordination of repetitive movement. A particular central (generic) aspect of freezing in PD is the difficulty to maintain largely scaled movements in synergy with a stable rhythm in response to triggers that operate through a reduction of movement amplitude. The study of brain activation during performance of a freezing-provoking upper limb motor paradigm was pioneering and provided insights in the neural overlap of FOG and upper limb freezing. The obtained results fit well within a neural model on freezing in PD that accounts for the fact that dopaminergic, motor and non-motor factors in synergy determine freezing. In the proposed model, generic motor aspects mainly rely on striato-frontal mechanisms whereas gait-specific components related to postural control are probably to a large extent located downstream of the basal ganglia network. Cognitive impairment, mainly in the executive and attentional domain, may aggravate motor control disturbances in freezers although the exact neural mechanism underlying this effect awaits further research. This perspective offers a testable hypothesis that FOG may originate from more pronounced cholinergic dysfunction superimposed on more severely affected dopaminergic pathways but this awaits future confirmatory research.

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Appendices

ENGLISH SUMMARY

Freezing of Gait (FOG) is a disabling gait disorder in patients with Parkinson's disease (PD). Patients who 'freeze' experience a sudden inability to start or continue walking as if their feet are glued to the floor. Because of the high prevalence, impact on patients' wellbeing and difficulty to manage therapeutically, FOG is a symptom of major clinical importance. The origins of FOG are largely unknown at present. The aim of this doctoral project was to increase our understanding of FOG and its causal mechanisms at the behavioral and neural systems level.

The four studies described in this thesis investigated the freezing problem from the innovative viewpoint that core motor deficits related to FOG may extend beyond the control of gait. This was based on reports in the literature of freezing-like motor blocks in various repetitive movements other than gait (non-gait freezing). The first study investigated the behavioral correlates of FOG and non-gait freezing by systematically comparing spatiotemporal changes in the kinematic signal before and during upper limb freezing (FOUL) with FOG-related motor abnormalities. Therefore, three subject groups (PD patients with FOG, PD patients without FOG, control subjects) performed freezing-provoking upper and lower limb motor tasks. Severity of freezing during repetitive upper limb and foot movements was correlated to severity of FOG. Similar to FOG, freezing in the upper limb was best triggered by small-amplitude movements, preceded by a progressive decrease in movement amplitude and increase in frequency and characterized by high-frequency trembling-like movements. Previous research had shown that patients with FOG show persistent difficulties in regulating their step length and walking rhythm even when no actual freezing episodes occurred. Using the same experimental paradigm as the first study, the second study further demonstrated that these continuous difficulties in timing and scaling of movement were also present during ongoing upper limb movement and that these motor abnormalities worsened after withdrawal of an auditory cue. These findings suggest that episodic and continuous problems in controlling amplitude, rhythm, bilateral coordination and internal timekeeping of external sensory information are apparent in the generic aspects of freezing in PD.

The overlap in motor disturbances between FOG and FOUL was exploited in an fMRI environment in the third study to address the neural basis of freezing. Three subject groups (freezers, non-freezers and controls) performed a shortened version of the freezing provoking upper limb task while lying in an MRI scanner. During ongoing upper limb movement, brain activity in freezers was decreased in cortical motor (dorsal premotor, primary motor cortex) and cognitive areas (dorsolateral prefrontal cortex) as compared to non-freezers and controls whereas subcortical activation in the putamen and subthalamic nucleus was increased as compared to controls and non-freezers respectively. Brain activation during actual freezing episodes showed an inverse neural pattern with increased cortical activity in motor and cognitive areas compared to ongoing movement, whereas activation in the putamen tended to be decreased. These novel findings indicate that the neural drive for rhythmic movement generation and more specifically the balance between subcortical and cortical activation, is altered in patients with FOG.

In the fourth study, we further examined the role of motor problems in FOG within the perspective of a more complex clinical picture including postural, cognitive and affective disturbances that were found more pronounced in freezers compared non-freezers. An extensive clinical test battery was used to identify the parameters that best discriminated between patients with and without FOG. The combination of four independent factors gave the best prediction of the occurrence of FOG. For example, a patient who presents with non-

gait freezing in daily life (e.g. motor blocks during writing), increased dopaminergic drug dose, cognitive deficits and history of falls or near falls within the last 3 months had an estimated risk for FOG above 95%. The findings thus suggest that dopaminergic, motor and non-motor deficits co-determine FOG and play an independent and probably synergistic role in its pathogenesis.

In summary, this doctoral project provided novel and quantitative evidence that difficulties in controlling movement amplitude and rhythm form a central, generic mechanism of freezing in PD. We propose a neural model of FOG whereby dysfunction of the dopaminergic striato-frontal neural circuitry which is considerably affected in PD, also plays a prominent role in the generic aspects of freezing. Cognitive impairment, mainly in the executive and attentional domain, may aggravate motor control disturbances in freezers although the exact neural mechanism underlying this effect awaits further research. Cholinergic cell loss in brainstem motor areas may exert a gait-specific influence on FOG by inducing posture and balance problems that, in synergy with dopaminergic, amplitude-timing and cognitive deficits, make the walking pattern of patients with FOG prone to movement breakdown in certain challenging conditions.

DUTCH SUMMARY

Het optreden van gangblokkades of 'freezing of gait' (FOG) is één van de meest invaliderende bewegingsstoornissen bij de ziekte van Parkinson. Freezing wordt gekenmerkt door het kortstondig en onvrijwillig stilvallen van het stappen. Patiënten ervaren dit alsof hun voeten aan de grond blijven plakken. Freezing episodes kunnen een val veroorzaken en belemmeren de kwaliteit van leven en mobiliteit van de patiënt. De oorzaken van dit belangrijk klinisch probleem zijn grotendeels ongekend. Het doel van dit doctoraatsproject is om de motorische en onderliggende neurologische mechanismen van freezing beter te begrijpen.

Recente bevindingen toonden aan dat motorische blokkeringen (freezing) niet exclusief gerelateerd zijn aan het stappen maar ook in een gelijkaardige vorm optreden tijdens ritmische, cyclische bewegingen van de bovenste ledematen. Tijdens het schrijven bijvoorbeeld, kan de beweging van de hand plots ongewenst stilvallen. Dit doet vermoeden dat er een algemeen motorisch probleem aan de grondslag ligt van freezing dat verder reikt dan de controle van het stappen. De 4 studies die deel uitmaakten van dit doctoraatsproject onderzochten het freezing probleem vanuit deze vernieuwende invalshoek. In de eerste studie voerden drie groepen (freezers, niet-freezers en controles) ritmische bilaterale bewegingen uit in de vingers en voeten waarvan uit vóóronderzoek was gebleken dat deze twee taken freezing uitlokten bij sommige patiënten. We vonden een sterke relatie tussen de ernst van freezing tijdens deze ritmische vinger- en voetbewegingen enerzijds en de ernst van freezing bij het stappen anderzijds. De sterke afname in amplitudo en toename in snelheid van het bewegingspatroon net vóór en tijdens een vinger freezing episode vertoonden bovendien overtuigende gelijkenissen met spatiotemporele veranderingen voorafgaand en tijdens freezing van het stappen. Net als bij freezing tijdens het stappen, werd het blokkeren in de bovenste ledematen voornamelijk uitgelokt wanneer proefpersonen gevraagd werden kleine bewegingen te maken.

Hoewel het stilvallen van de gang maar kort duurt, meestal minder dan 3 seconden, toonde vorig onderzoek aan dat de controle van stapgrootte en stapritme continu verstoord is bij patiënten met freezing. Dit leidt tot een minder stabiel en nauwkeurig stappatroon in freezers, ook als ze niet geblokkeerd zijn. In de tweede studie bouwden we verder op de vingerbewegingsdata van Studie 1 en vonden we dat gelijkaardige continue problemen in het genereren van bewegingsgrootte en –tempo ook voorkwamen tijdens vingerbewegingen. Wanneer het bewegingsritme gestuurd werd door een auditieve cue, verbeterde de bewegingsprestatie van freezers maar dit therapeutisch voordeel viel weg nadat de auditieve toon werd stopgezet. Deze bevindingen geven aan dat kortstondige en continue problemen in de controle van amplitudo, ritme, coördinatie en het internaliseren van externe sensorische informatie deel uitmaken van de gegeneraliseerde motorisch aspecten van freezing bij de ziekte van Parkinson.

De derde studie benutte de overlap in motorische problemen in freezing in de vingers en freezing bij het stappen om de neuronale basis van freezing te onderzoeken. Hierbij werd gebruik gemaakt van medische beeldvorming aan de hand van een MRI scanner waarin hersenactiviteit kan gemeten worden terwijl proefpersonen vingerbewegingen uitvoeren (maar niet terwijl ze stappen). Tijdens continue beweging, bleek dat bepaalde corticale motorische (primaire motorische en premotorische cortex) en cognitieve prefrontale hersengebieden minder geactiveerd waren in freezers in vergelijking met niet-freezers en controle subjecten. In tegenstelling hiermee, was activiteit in diepergelegen (subcorticale) hersengebieden groter in freezers ten opzichte van niet-freezers (subthalamische nucleus) en controles (putamen). De hersenactiviteit tijdens freezing episodes vertoonde een omgekeerd

patroon namelijk een verhoogde corticale activiteit in motorische en cognitieve gebieden in combinatie met een verlaagde activiteit in het subcorticale putamen in vergelijking met continue beweging. Deze nieuwe bevindingen tonen aan dat de neuronale sturing van ritmische bewegingen en in het bijzonder het evenwicht tussen subcorticale en corticale hersenactiviteit, veranderd is in patiënten met freezing.

De vierde studie plaatste de motorische problemen gerelateerd aan freezing, in de context van een meer complex klinisch beeld van freezers waarin naast problemen in het sturen van bewegingsamplitudo en -ritme, ook moeilijkheden thuis horen op vlak van evenwicht, cognitie, aandacht en emoties. Een uitgebreide klinische testbatterij werd gebruikt om na te gaan welke motorische en niet-motorische parameters het best freezers van niet-freezers konden onderscheiden. De combinatie van 4 onafhankelijke factoren gaf de beste voorspelling van het voorkomen van gangblokkades. Bijvoorbeeld werd bij een patiënt die blokkeringen ondervindt in andere bewegingen dan het stappen (vb tijdens het schrijven) en cognitieve problemen vertoont in combinatie met een verhoogde dosis dopaminerge medicatie en evenwichtsproblemen, het risico op freezing bij het stappen geschat op minstens 95%. De resultaten benadrukken dus dat dopaminerge, motorische en niet-motorische gebreken samen, het al dan niet voorkomen van freezing bij het stappen bepalen en dat deze factoren waarschijnlijk een synergetische rol hebben in de pathogenese van freezing.

Samengevat leverde dit doctoraatsproject nieuwe en kwantitatieve evidentie dat problemen in de controle van bewegingsgrootte en ritme een centraal, gegeneraliseerd motorisch mechanisme van freezing vormen. De bevindingen passen binnen een neuronaal model van freezing waarin storingen in het dopaminerge striato-frontale hersencircuit dat sterk aangetast is door de ziekte van Parkinson in het algemeen, ook een cruciale rol speelt in de gegeneraliseerde aspecten van freezing. Cognitieve problemen, voornamelijk in aandachtsfuncties, kunnen de motorische problemen versterken, hoewel de onderliggende neuronale mechanismes van dit effect nog niet duidelijk zijn. Degeneratie van cholinerge hersencircuits die de hersenstam met bovengelegen gebieden verbinden, kunnen een eerder gang-specifieke rol spelen in het ontstaan van freezing door evenwichtsproblemen te veroorzaken. Evenwichtsproblemen in synergie met dopaminerge, cognitieve en amplitudo- en timingproblemen zouden kunnen leiden tot een gangpatroon in freezers dat vatbaar is voor plotse, ongewenste blokkeringen.

ENGLISH APPOSITIONS

Apposition 1

Informing volunteers after participating in scientific studies is an expression of respectful gratitude and forms the basis of successful future recruitment.

Apposition 2

Intensively revising a paper based on comments of external reviewers is an important factor in guaranteeing that research papers that are accepted for publication in scientific journals are of high-quality. Still, the slowness of this peer review process sometimes undermines the competitive profile of the researcher.

Apposition 3

The ability to change is man's most precious tool in life that enables us to react dynamically in the face of adversities affecting ourselves, friends or family. It allows seizing a second chance to move forward without 'freezing' in place.

DUTCH APPOSITIONS

Bijstelling 1

Het informeren van proefpersonen na afloop van wetenschappelijke studies waaraan ze vrijwillig deelnamen is een uiting van respectvolle dankbaarheid en vormt de basis van succesvolle vervolgstudies.

Bijstelling 2

Een intensieve revisie van artikels op basis van externe reviewers bepaalt mede de kwaliteit van het geschreven werk dat aanvaard wordt voor publicatie in wetenschappelijke tijdschriften. Het gebrek aan snelheid van dit peer-review-proces kan het concurrentieel profiel van de onderzoeker echter ondermijnen.

Bijstelling 3

Het aanpassingsvermogen van de mens is een kostbaar gegeven dat ons de mogelijkheid biedt dynamisch om te gaan met tegenslagen bij onszelf, vrienden of familie en een tweede kans te grijpen om terug vooruit te gaan en niet ter plaatse te ‘freezen’.

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ABOUT THE AUTHOR

Sarah Vercruysse was born on September 10th 1984 in Kortrijk, Belgium. She graduated High School at the Lyceum Onze Lieve Vrouw van Vlaanderen in Kortrijk in 2002. She studied Psychology at the Faculty of Psychology and Educational Sciences of the Katholieke Universiteit Leuven. The final two years of the program allowed her to specialize in aspects of Theoretical Psychology and to develop a profound interest in scientific research. Her research internship took place at the Université de la Méditerrannée, Marseille, France, where she studied bimanual motor control in the context of aging. In 2007, she graduated magna cum laude as Master in Psychology. After graduation, she started her PhD training at the Faculty of Kinesiology and Rehabilitation Sciences of the Katholieke Universiteit Leuven. Under supervision of Prof. Dr. Alice Nieuwboer and Prof. Dr. Nici Wenderoth, her PhD project focused on behavioral and neural mechanisms of freezing of gait, a disabling gait disorder in patients with Parkinson's disease. Her work was carried out in the Research Group of Neuromotor Rehabilitation, in close collaboration with the Research Centre for Movement Control and Neuroplasticity.

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1. Articles in internationally reviewed academic journals

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1. Vercruysse S, Temprado JJ, Salesse R & Berton E. Aging and bimanual coordination. (2007). 12th International ACAPS (Assocation des Chercheurs en Activités Physiques et Sportives) Conference, Leuven, oct 31-nov 2.

2. Vercruysse S, Spildooren J, Deroost N, Vandenberghe W, Nieuwboer A. (2009). Upper limb freezing in patients with Parkinson's disease. 13th International Congress of Parkinson's Disease and Movement Disorders. Paris, France June 7-11.

3. Vercruysse S, Spildooren J, Vandenbossche J, Heremans E, Levin O, Vandenberghe W, Nieuwboer A. (2010). Spatiotemporal similarities of upper limb and gait freezing in Parkinson's disease. 3d International Congress on Gait & Mental Function. Washington, USA, February 26-28.
   → *Selected for oral session*
   → *Awarded with Oral Presentation Prize Award*

4. Vercruysse S, Spildooren J, Heremans E, Kerckhofs E, Swinnen SP, Wenderoth N, Vandenberghe W, Nieuwboer A. (2010). Freezing in Parkinson's disease: a pilot fMRI-study. 3d International Congress on Gait & Mental Function. Washington, USA, February 26-28.
   → *Selected for guided poster tour*

5. Vercruysse S, Spildooren J, Heremans E, Deroost N, Swinnen SP, Wenderoth N, Vandenberghe W, Nieuwboer A. (2010). Freezing in Parkinson's disease: a pilot fMRI study. 14th International Congress of Parkinson's Disease and Movement Disorders. Buenos Aires, Argentinia, June 13-17.
   → *Travel Grant received for attending this conference*

6. Vercruysse S, Spildooren J, Heremans E, Vandenbossche J, Wenderoth N, Swinnen S, Vandenberghe W, Nieuwboer A. (2011). Spatiotemporal coordination deficits in PD patients with freezing of gait. 15th International Congress of Parkinson's Disease and Movement Disorders. Toronto, Canada, June 5-9.

7. Vercruysse S. (2012). Neuroimaging of freezing of gait in Parkinson's disease. Joint World Congress of ISPGR and Gait & Mental Function. Trondheim, Norway, June 24-28.
   → *Invited speaker*

8. Vercruysse S, Nieuwboer A. (2012). Treatment for freezing of gait in Parkinson's disease: new insights based on its pathology. 85th Deutsche Gesellschaft für Neurologie Kongress. Hamburg, Germany, September 26-29.
   → *Invited speaker*

9. Nieuwboer A, (Invited speaker) Vercruysse S, Spildooren J, Vandenbossche J, Soetens E, N Deroost, Kerckhofs E. (2010). Cognitive correlates of freezing of gait in Parkinson's disease. The 7th International Congress on Mental Dysfunctions & Other Non-Motor symptoms in Parkinson's disease and Related disorders. Barcelona, Spain, December 9-12.

10. Nieuwboer A, Vercruysse S, Spildooren J, DeVos H, Münks L, Heremans E, Vandenberghe W. (2012). Can we predict freezing of gait in Parkinson's disease using motor and cognitive variables? 1st Joint World Congress of ISPGR and Gait & Mental Function. Trondheim, Norway, June 24-28.

11. Spildooren J, Vercruysse S, Desloovere K, Vandenbossche J, Callewaert B, Vandenberghe W, Nieuwboer A. (2009). Turning behavior in patients with Parkinson's disease with and without freezing of gait. 13th International Congress of Parkinson's Disease and Movement Disorders. Paris, France, June 7-11.

12. Spildooren J, Vercruysse S, Desloovere K, Vandenberghe W, Nieuwboer A. (2010). The influence of disease-dominance on turning problems and freezing of gait in patients with Parkinson's disease. 14th International Congress of Parkinson's Disease and Movement Disorders. Buenos Aires, Argentinia, June 13-17.

13. Spildooren J, Vercruysse S, Galna B, Vandenbossche J, Vandenberghe W, Nieuwboer A. (2011). Differences in turning strategies between Parkinson's disease patients with and without freezing of gait. 15th International Congress of Parkinson's Disease and Movement Disorders. Toronto, Canada, June 5-9.

14. Spildooren J, Vercruysse S, Desloovere K, Vandenberghe W, Vandenbossche J, Nieuwboer A. (2010).Unilateral auditory cueing to improve freezing during turning in Parkinson's disease. 2nd World Parkinson Congress. Glasgow, Scotland, September 28 - October.

15. Spildooren J, Vercruysse S, Desloovere K, Vandenberghe W, Nieuwboer A. (2011). The influence of disease-dominance on turning problems and freezing of gait in patients with Parkinson's disease. World Confederation for Physical Therapy. Amsterdam, The Netherlands, June 20-23.

16. Spildooren J, Vercruysse S, Galna B, Vandenberghe W, Nieuwboer A. (2012).Turning strategies in Parkinson's Disease patients with freezing of gait. 1st Joint World Congress of ISPGR and Gait & Mental Function. Trondheim, Norway, June 24-28.

17. Nieuwboer A, Levin O, Vercruysse S, Spildooren J, Swinnen S. (2008). Cueing for a bimanual coordination task in Parkinson's disease: a comparison of patients with and

without freezing of gait. 2nd International Congress on Gait & Mental Function. Amsterdam, The Netherlands, February 1-3.

18. Nieuwboer A, Spildooren J, Vercruysse S. (2010).Training the untrainable: dual task walking in Parkinson's disease. 3rd International Congress on Gait & Mental Function. Washington, USA, February 26-28.

19. Nieuwboer A, Nuttin B, Vercruysse S, Spildooren J, Billiet L, Vandenberghe W. (2009). A prospective and controlled study into the effect of subthalamic nucleus stimulation on freezing of gait. 13th International Congress of Parkinson's Disease and Movement Disorders. Paris, France, June 7-11.

20. Spildooren J, Desloovere K, Vercruysse S, Vandenberghe W, Huenaerts C, Kerckhofs E, Nieuwboer A. (2009). Turning problems in Parkinson patients with freezing of gait: preliminary data. 19th International Conference on Posture and Gait. Bologna, Italy June 21-25.

21. Spildooren J, Strouwen C, Vercruysse S, Heremans E, Galna B, Vandenberghe W, Nieuwboer A. (2012). Cueing and attention strategies for axial movement impairment related to freezing of gait in Parkinson's disease. 1st Joint World Congress of ISPGR and Gait & Mental Function. Trondheim, Norway, June 24-28.

22. Spildooren J, Mohammadi F, Vercruysse S, Heremans E, Galna B, Vandenbossche J, Desloovere K, Nieuwboer A. (2012). Head-pelvis dissociation during turning in healthy subjects who mimic bradykinetic gait. 1st Joint World Congress of ISPGR and Gait & Mental Function. Trondheim, Norway, June 24-28.

23. Vandenbossche, J, Spildooren, J, Vercruysse S, Deroost, N, Soetens, E, Nieuwboer, A, & Kerckhofs, E. (2010). Attention networks in Parkinson's disease: Freezing of gait related to impaired conflict resolution? 3d International Congress on Gait & Mental Function. Washington, USA, 26-28 February.

24. Heremans E, Helsen W, Nieuwboer A, Vercruysse S, Vandenberghe W, Sharma N, Feys P. (2010) Clinical assessment of motor imagery ability in patients with Parkinson's disease. Presented at: 3rd International Congress on Gait & Mental Function, Washington, USA, February 26-28.

25. Heremans E, Helsen W, Nieuwboer A, Vercruysse S, Vandenberghe W, Sharma N, Feys P. (2010) External cueing improves temporal characteristics of motor imagery in patients with Parkinson's disease. Presented at: 14th International Congress of Parkinson's Disease and Movement Disorders. Buenos Aires, Argentina June 13-17.

26. Heremans E, Feys P, Nieuwboer A, Vercruysse S, Vandenberghe W, Helsen W. (2010). Motor imagery: an eye-catching newcomer in PD rehabilitation? Presented at: 1st Neuropsychology Workshop. Leuven, Belgium, November 18-19.

27. Vandenbossche J, Zeischka P, Coomans D, Vercruysse S, Spildooren J, Deroost N, Soetens E, Kerckhofs E. (2010) Interference control in Parkinson's Disease and Freezing Of Gait. Presented at: 8th Sepex conference. Granada, Spain, April 15-17.

28. Vandenbossche J, Deroost N, Soetens E, Spildooren J, Vercruysse S, & Kerckhofs E. (2010). Freezing of gait in Parkinson's disease: a cognitive profile. 2nd Meeting of the Federation of European Societies of Neuropsychology (ESN). Amsterdam, The Netherlands, September.

29. Vandenbossche J, Deroost N, Soetens E, Spildooren J, Vercruysse S, & Kerckhofs E. (2010). Executive dysfunction in Parkinson's disease: Freezing of gait associated with impaired conflict resolution. 7th International Congress on Mental Dysfunctions & Other Non-motor symptoms in Parkinson's disease and Related disorders. Barcelona, Spain, December 9-12.

3. MEETING ABSTRACTS PRESENTED AT OTHER PROFESSIONALLY ORIENTED CONFERENCES

30. Vercruysse S, Spildooren J, Heremans E, Deroost N, Swinnen SP, Wenderoth N, Vandenberghe W, Nieuwboer A. (2010). Freezing in patiënten met de ziekte van Parkinson: bevindingen uit gedrags- en fMRI-studies. Presented at: Vakgroep Bewegingsstoornissen, U.Z. Gasthuisberg Leuven, May 20.

31. Vercruysse S, Spildooren J, Heremans E, Vandenberghe W, Nieuwboer A. (2011). Neuronale aspecten van „freezing of gait' bij de ziekte van Parkinson. Presented at: Symposium NeuroInteresseGroep, U.Z. Gasthuisberg Leuven, October 22.