Dual-task related neural connectivity changes in patients with Parkinson's Disease

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Abbreviations

AIT: Alternating Intakes Test

ANT: Alternating Names Test

CV: Coefficient of variability

D: Dominant

DT: Dual task

DTI: Diffusion Tensor Imaging

EEG: Electro-encephalography

FAB: Frontal Assessment Battery

FC: Functional Connectivity

FD: Framewise Displacement

FDR: False Discovery Rate

fNIRS: functional Near Infrared Spectroscopy

FOG: Freezing of Gait

GP: Globus Pallidus

MDS-UPDRS: Movement Disorder Society – Unified Parkinson's disease rating scale

MFG: Middle Frontal Gyrus

MMSE: Mini-Mental State Examination

MNI: Montreal Neurological Institute

MoCA: Montreal Cognitive Assessment

ND: Non-dominant

NFOG-Q: New Freezing of Gait Questionnaire

PD: Parkinson's disease

PPN: pedunculopontine nucleus

ROI: Region of Interest

rs-fMRI: Resting-state functional Magnetic Resonance Imaging

SMA: Supplementary motor area

ST: Single task

TMT: Trail Making Test

TR: Repetition Time

Abstract

Background and objectives: Dual-task (DT) gait impairment in people with Parkinson's disease (PD) and specifically in those with freezing of gait (FOG), reflects attentional dependency of movement. This study aimed to elucidate resting-state brain connectivity alterations related to DT gait abnormalities in PD with and without FOG.

Methods: PD patients (n=73) and healthy age-matched controls (n=20) underwent DT gait analysis and resting-state functional MRI while 'off' medication. Patients were classified as freezer (n=13) or non-freezer (n=60). Functional connectivity (FC) alterations between PD and controls and between patient subgroups were assessed in regions of interest (ROIs) within the fronto-parietal and motor network.

Results: PD had longer stance times, shorter swing times and more step length asymmetry during DT gait and needed more time and steps during DT turning compared to controls. Additionally, freezers showed similar impairments and longer double support times compared to non-freezers during DT gait. PD demonstrated hyper-connectivity between the inferior parietal lobule and premotor cortex (PMC) and between the cerebellum and the PMC and M1. FOG-specific hypo-connectivity within the striatum and between the caudate and superior temporal lobe and hyper-connectivity between the dorsal putamen and precuneus was correlated with worse DT performance.

Conclusion: PD showed FC alterations in DT-related networks, which were not correlated to DT performance. However, FOG-specific FC alterations in DT-related regions involving the precuneus and striatum were correlated to worse DT performance, suggesting that the balance between cognitive and motor networks is altered.

Key words: Parkinson's Disease, Freezing of gait, resting-state fMRI, dual-tasking

1. Introduction

Dopaminergic denervation in the basal ganglia is responsible for reduced movement automaticity in patients with Parkinson's disease (PD) and increased reliance on attention to execute movements [1]. Movement automaticity is often assessed using dual-task paradigms. Dual-tasking can be defined as the simultaneous execution of two tasks which have distinct goals and often involve motor and/or cognitive task sets [2]. Although several studies have shown dual-task (DT) interference on gait in PD [3-7], the effect sizes are variable and strongly rely on the nature of the task, cohort selection and medication state. Several studies have established that dual-tasking aggravates freezing of gait (FOG) [8-10]. FOG is defined as a brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk [11]. It is associated with reduced functional mobility, increased fall risk and reduced quality of life [12-14]. Nutt et al. (2011), proposed that an exaggerated loss of automaticity and/or frontal executive dysfunction may partially explain FOG [11], which also provides a possible rationale for its strong link with dual-tasking.

Several theoretical frameworks have been put forward to explain DT deterioration [15-18], but studies investigating its neural mechanisms in PD are scarce. Wu et al. (2008) showed greater activation in the prefrontal cortex, middle frontal gyrus (MFG), parietal cortex, temporal lobe and cerebellum in PD compared to controls while performing a tapping task together with letter counting [19]. In addition, increased brain activity registered in the cerebellum, the dorsolateral prefrontal cortex and precuneus was interpreted to have a supervisory role in DT integration both in PD and healthy subjects [20, 21]. As for FOG, a recent fMRI study showed decreased activation in prefrontal areas, the pre-supplementary motor area (pre-SMA) and posterior parietal cortex in freezers compared to non-freezers during a DT foot pedaling task performed in supine position in the scanner [22]. In addition, a Diffusion Tensor Imaging (DTI) study demonstrated that reduced structural connectivity of the right pedunculo-pontine nucleus (PPN) in freezers was strongly related to DT gait interference outcomes [23].

While these studies provided essential information to unravel the neural correlates underlying DT performance, translation to gait is not straightforward due to movement restrictions inherent to a

scanner environment[24]. While proxy-measures such as mental imagery of gait have proven useful to investigate single task (ST) walking [25-27], they are less suitable for DT conditions. To overcome the problem of performance dependence [28], resting state fMRI (rs-fMRI) measures brain networks which remain temporally correlated during rest, providing a global window into functional connectivity (FC) changes. Therefore, this study used rs-fMRI to investigate FC changes underlying DT gait in PD and more specifically in an exploratory fashion in those with FOG. So far, only two rs-fMRI studies highlighted decreased connectivity in the visual and right fronto-parietal network and between the mesencephalic locomotor region and SMA in freezers compared to non-freezers, without modeling direct correlations with gait outcomes [29, 30]. For this purpose, we analyzed connectivity between regions of interest (ROIs) within the motor and fronto-parietal network, known to be implicated in DT and FOG [19, 22, 31] and performed a correlation analysis with DT gait outcomes obtained from 3D gait analysis in PD and age-matched controls. Based on the work of Wu and colleagues [19, 32, 33], we expected that reduced DT gait performance in PD patients compared to controls would correlate with hyper-connectivity in cognitive control areas. In addition, we hypothesized that DT gait impairment would be more pronounced in freezers compared to non-freezers and that this impairment would correlate to more widespread FC alterations in cognitive and motor control areas [29], including the prefrontal cortex, precuneus and cerebellum.

2. Experimental Procedures

2.1 Participants

Seventy-three PD patients and 20 healthy age-matched controls were recruited. Patients were included if they were diagnosed with PD according to the UK Brain Bank criteria and were able to walk independently for at least 10 minutes. Exclusion criteria were: a Mini-Mental State Examination (MMSE) score <24; presence of a Deep Brain Stimulator (DBS); neurological or motor comorbidities. The new Freezing of Gait questionnaire (NFOG-Q) was used to classify patients as freezers (NFOG-Q >1) (FOG: n=13) or non-freezers (NFOG: n=60) and was administered at the beginning of the behavioral test session. In addition, presence of FOG was determined by the physician in all but one

patient, who reported to have FOG after watching the video of the NFOG-Q. Disease severity was assessed by means of the MDS-UPDRS part III ‘off’ medication and disease duration was calculated from the moment of first motor symptom onset. All tests were performed ‘off’ medication, i.e. at least 12 hours after the last intake of medication (short and long working action) intake. All subjects gave written informed consent and the study was approved by the ethical committee of the University Hospitals Leuven according to the declaration of Helsinki.

2.2 Gait analysis

Gait was assessed using the VICON 3D motion analysis system (©Vicon Motion Systems Ltd. UK). Subjects were instructed to continuously walk back and forth over a 6 meter trajectory in the gait laboratory. They had to walk in a straight line in the part of the laboratory where the camera view was optimal and turn at the outer parts of the field of view. This was repeated until at least 30 steps were recorded (i.e. approximately 10 trials). Only straight line walking in the middle of the walkway was included in the analyses. In addition, patients were asked to turn in the center of the walkway for 6 trials. Sixteen reflective markers were placed on anatomical landmarks of the lower limb and pelvis according to the Plugin Gait model and were recorded at a sample frequency of 100Hz. Marker trajectories were processed using Nexus software (version 1.8.5) to calculate the position and timing of the heel strikes and toe-offs of each step. This information was used to calculate spatiotemporal gait kinematics[34]. Outcome measures for gait at self-preferred speed were: step time (s), % stance time, % swing time, % double support time, step length (mm), step width (mm) and gait speed (m/s). The average and variability were calculated for the disease dominant (D) and non-dominant (ND) leg for all parameters. Variability was expressed as the coefficient of variability (CV) and asymmetry was calculated between the D and ND leg for patients and between right and left leg for controls for all parameters except step width and gait speed. Stance time, swing time and double support time were expressed as a percentage of the total gait cycle. To maximize the DT impact, gait tests were performed while ‘off’ medication at self-preferred gait speed and during 360° turning [8, 35]. All

subjects performed 360° turns to the left and right side [8]. Outcome measures were step number, turn duration (s) and cadence (steps/min).

ST and DT conditions were applied in a semi-randomized order. An auditory version of the Stroop task was used as dual-task. During this task, subjects are presented with the Dutch words for ‘high’ and ‘low’ through a wireless headset. During the congruent task, the word ‘high’ is pronounced at a high pitch and the word low is pronounced at a low pitch. During the incongruent task, the word ‘high’ is pronounced at a low pitch and the word ‘low’ is pronounced at a ‘high’ pitch. Subjects were instructed to respond with the pitch that was presented through the microphone of the headset which was synchronized with the VICON system. The task was practiced and recorded while sitting and was subsequently performed during gait. The average and variability (CV) of the reaction time were chosen as outcome measures for DT performance.

2.3 Cognitive tests

All subjects underwent a cognitive test battery to assess general cognition (MMSE, Montreal Cognitive Assessment (MoCA) and SCOPA-COG), visuospatial function (Complex Figure of Rey copying) general executive function (Frontal Assessment Battery (FAB)) and task switching (Trail Making Test (TMT), parts A and B), Alternating Names Test (ANT) and Alternating Intake Test (AIT)).

2.4 Image acquisition

On a separate day than the behavioral tests, all subjects underwent a rs-fMRI scan while ‘off’ medication (Philips 3T ACHIEVA MRI scanner (Best, The Netherlands)) using a T2*-weighted Gradient Echo Planar Imaging (FEEPI) sequence (duration: 435s, slice number: 31; slice thickness: 4mm; slice gap: 0mm; repetition time (TR): 1700ms; echo time (TE): 33ms; flip angle: 90°; matrix: 64x64; FOV: 230x124x230mm) combined with a high-resolution anatomical T1-weighted sequence

(T1 Turbo Field Echo (TFE) sequence, duration: 383ms; slice number: 182; slice thickness: 1.2mm; slice gap: 0mm; TR: 9.624ms; TE: 4.6ms; flip angle: 8°; matrix: 256x256; FOV: 218.4x250x250mm). Six patients (FOG: n=1; NFOG: n=5) and 1 control could not undergo the MRI scan due to claustrophobia or presence of MRI incompatible implants. The MRI images of 2 patients (NFOG) and 1 control subject were corrupted by large image artifacts.

2.5 Image processing

Rs-fMRI images were pre-processed using SPM8 (Wellcome Department of Imaging Neuroscience, University College London, UK) implemented in Matlab® R2011b. All functional images were realigned to the reference (mean) image and slice-time corrected. The realigned images were co-registered to each subject's T1 image, which were segmented to generate a white matter, gray matter and cerebrospinal fluid mask. All images were normalized to standard Montreal Neurological Institute (MNI) space and smoothed with a 5mm full width at half maximum Gaussian kernel. The first 4 volumes of the rs-fMRI scan were omitted to account for saturation effects. First and second level analyses were performed using the CONN toolbox (v.15) (<http://www.nitrc.org/projects/conn>). All rs-fMRI images were motion-corrected using six translational and rotational realignment parameters which were visually inspected for excessive movement (>3mm). Instantaneous head motion was accounted for by calculating frame-wise displacement (FD) for each volume and performing all analyses on 'scrubbed' data [36], i.e. censoring frames displaying $FD > 0.5\text{mm}$ or frame-wise changes in brain image intensity $> 0.5\% \Delta \text{BOLD}$. If the proportion of corrupted volumes exceeded 50%, subjects were excluded from the analysis. FD correction led to exclusion of 9 patients (FOG: n=4; NFOG: n=5).

2.6 ROI-to-ROI analysis

Core regions from the motor control and fronto-parietal network [37], that were likely to be involved in DT gait, were selected as regions of interest (ROIs) in the rs-fMRI analysis (see Figure 1). At the

subcortical level the dorsal putamen, ventral putamen, caudate, substantia nigra pars compacta (SNc), subthalamic nucleus (STN), globus pallidus (GP), thalamus (parts projecting to premotor cortex (PMC) and M1) and pedunculopontine nucleus (PPN) were included. On the cortical level the M1, PMC, supplementary motor area (SMA), pre-SMA, dorsolateral prefrontal cortex (DLPFC), middle frontal gyrus (MFG), inferior parietal lobule (IPL), superior temporal lobe, precuneus, posterior cingulate cortex (PCC), anterior cingulate cortex (ACC) and insula were included. In addition, we also included the cerebellum (lobe VI) and cerebellar vermis. Subcortical ROI coordinates were based on previous work from our lab [38] and cortical ROI coordinates on Postuma and Dagher (2006) and Wu et al. (2012) [37, 39]. Coordinates originally reported in Talairach space, were transformed into MNI space using an application of the Yale BioImage Suite Package (<http://noodle.med.yale.edu/~papad/mni2tal/>). ROIs were constructed as spheres with 8mm radius (except for the SNc, which had a 3mm radius[37]) in standard MNI space using the MarsBaR toolbox[40] and were subsequently co-registered to each subject's structural and functional images. Each subject's time series for the defined ROIs were Pearson correlated to all other ROIs.

2.7 Statistics

2.7.1 Behavioral outcomes

Between-group comparisons for continuous subject characteristics were performed using unpaired T-tests and for categorical subject characteristics chi-squared tests were used. None of the outcome measures showed skewed distributions. Therefore, spatiotemporal kinematics during gait and turning and cognitive scores were compared between PD patients and controls using a MANOVA to account for covariance between gait parameters. All variables were entered in the initial model and this was reduced in step-wise manner until the largest amount of variability was explained by the least amount of variables. A similar approach was used to compare freezers to non-freezers with the addition of age, disease duration and MDS-UPDRS III scores as covariates. The level of significance was set at $\alpha=0.05$. Cohen's d effect sizes were calculated for all behavioral outcomes. Unlike in previous work

[23], we did not calculate % interference, because of the high within-subject variability and low test-retest reliability of this parameter [41, 42].

2.7.2 Neuroimaging

Differences between patients and controls were tested using ANCOVA's with age as covariate and false discovery rate (FDR) corrected p-values with $\alpha=0.05$. Differences between freezers and non-freezers were tested using ANCOVA's with age, disease duration and MDS-UDPRS III as covariates and FDR-corrected values with $\alpha=0.05$ or uncorrected p-values with $\alpha=0.001$. In contrast to the MANOVA analysis, in which all subjects were included, rs-fMRI analyses were performed on 8 freezers and 47 non-freezers, as 4 FOG and 7 NFOG were excluded due to image artifacts and 1 FOG and 4 NFOG due to MRI contra-indications.

2.7.3 Correlations

Discriminatory PD-CTRL and FOG-NFOG FC measures were Pearson correlated to significant gait and cognitive outcomes of the final MANOVA DT model in the entire patient group. To check if correlations were specific to DT performance, the same gait parameters that were included in the DT correlation analysis were also correlated to cognitive outcomes and FC measures in the ST condition. Correlations were considered if $R \geq 0.35$ or $R \leq -0.35$ and were corrected for multiple comparison by accounting for the number of correlations performed using a Bonferroni correction ($\alpha=0.005$).

3. Results

3.1 Subject characteristics

Subject characteristics are shown in Table 1. PD patients and controls were matched for age and sex, but PD patients had lower MMSE-scores compared to controls. When comparing freezers and non-

freezers, there were no differences in disease duration, H&Y stage and Levedopa Equivalent Dose (LED). However, freezers were older and had higher MDS-UPDRS III scores. Hence, we controlled for these factors in the analysis.

Insert Table 1 about here

3.2 Behavioral analysis

The final models demonstrated that a much greater number of outcomes discriminated between PD and controls and between FOG and NFOG during DT versus ST conditions. The final DT MANOVA model ($F=2.81$; $p=0.004$) indicated that lower MMSE, MoCA and FAB-scores and higher TMTa and TMTb completion times discriminated PD from controls. In addition, PD needed more steps (D/ND) during turning compared to controls (Table 2). Although not significant, the final model also showed longer stance times ($p=0.06$), shorter swing times ($p=0.08$), increased step length asymmetry ($p=0.08$) and longer turn durations ($p=0.13$) in PD compared to controls. Reaction times on the auditory Stroop were not retained in the model. The final ST MANOVA model ($F=2.10$; $p=0.04$) comparing PD patients and controls showed lower MMSE, MoCA and FAB-scores and higher TMTa and TMTb completion times. In addition, PD patients had longer turn durations (D/ND) and an increased step number during turning (D/ND) compared to controls.

Insert Table 2 about here

When comparing freezers to non-freezers, the final DT MANOVA model ($F=2.41$; $p=0.02$), longer stance times (D/ND), shorter swing times (D) and longer double support times (D/ND). Freezers additionally had longer turn durations (D/ND) and needed more steps (D/ND) during turning compared to non-freezers (Table 2). These effects were generally stronger in the FOG-NFOG comparison (Cohen's d : 0.45–1.19) compared to the PD-CTRL comparison (Cohen's d : 0.27-0.93).

When comparing freezers to non-freezers, the final ST MANOVA model ($F=3.64$; $p=0.02$) indicated larger swing time variability (D), shorter swing times (ND) and longer turn durations (ND) (Table 3). When we explored the direct comparison between FOG and CTRL (data not shown) it appeared that the PD-CTRL contrast was largely driven by the inclusion of freezers.

Insert Table 3 about here

3.3 Rs-fMRI analysis

Figure 1a shows the FC matrix between DT-related ROIs for PD compared to controls. PD showed significant hypo-connectivity between the left M1 and the right M1 and right PMC compared to controls. In addition, the left IPL was hypo-connected to the bilateral caudate, as well as to the left SNc and the right PPN. Hyper-connections were found between the left cerebellum and the bilateral PMC and M1 in PD compared to controls. In addition, the right IPL was hyper-connected to the bilateral PMC and the left M1. The latter was also hyper-connected to the left SNc (Table 4).

Insert Table 4 and Figure 1 about here

Figure 1b shows the FC matrix between DT-related ROIs for freezers compared to non-freezers. Freezers had a striking pattern of decreased FC between the left caudate and the right superior temporal lobe and left cerebellum compared to non-freezers. The right caudate showed decreased FC with the bilateral dorsal putamen, the left Globus Pallidus (GP) and the bilateral superior temporal lobe. Freezers showed increased FC compared to non-freezers between the right precuneus and the left dorsal putamen (Table 4).

3.4 Correlation analysis

Figures 2a-f show the FC alterations that proved significantly correlated to DT outcomes after Bonferroni correction. This shows that worse step and swing times during DT gait were correlated with FOG-specific decreased FC between the left caudate and superior temporal lobe. In addition, FOG-specific hypo-connectivity between the right caudate and left GP correlated to a higher step number during DT turning. In contrast, FOG-specific increased FC between the left dorsal putamen and right precuneus was correlated to worse DT step (D and ND), swing (D and ND) and double support times (D and ND). Figures 3 a-c show that the increased FC between the left dorsal putamen and right precuneus was correlated to worse ST stance (ND) and swing (ND) times while hypo-connectivity between the right caudate and left dorsal putamen correlated to lower FAB-scores.

Insert Figure 2 and Figure 3 about here

There were additional correlations that were significant but did not survive Bonferroni correction. Of note, hyper-connectivity between right PMC and right IPL (discriminatory for PD-CTRL) correlated with a decreased step number during DT and ST turning ($R=-0.35$; $p=0.01$). Decreased FC (discriminatory for FOG-NFOG) between the right caudate and dorsal putamen was correlated to worse performance during DT turning ($R=-0.36$; $p<0.01$). In addition, correlations were found between worse DT stance ($R=-0.35$; $p<0.01$), swing ($R=0.36$; $p<0.01$) and double support times ($R=-0.35$; $p<0.01$) and hypo-connectivity between the right caudate and left GP.

4. Discussion

The present study showed that gait at self-preferred speed was more impaired in DT conditions in PD patients compared to controls, whereas turning was affected in both ST and DT conditions. As shown

by Cohen's d values, a similar but more pronounced pattern was found when comparing freezers to non-freezers. Temporal gait kinematics were mostly affected by dual-tasking in freezers.

Longer DT stance and shorter swing times in PD compared to controls have been reported in earlier studies in addition to reductions in DT gait speed and stride length [10], increased gait asymmetry and variability [43, 44]. Similarly, other work demonstrated that dual-tasking induced more gait abnormalities in freezers compared to non-freezers [9, 45, 46] and that DT turning performance was significantly worse in freezers [8]. However, DT interference was also dependent on the nature of the dual-task and cohort selection. This was apparent from recent papers in early cohorts [5, 47], comparable to our own population of early to mid-stage PD patients, which showed different interference effects depending on the level of the cognitive dual-task. The fact that our PF group included a group of freezers may also partly account for the current results. The finding that, unlike in straight line gait, group differences were present during both ST and DT turning could be explained by the increased attentional demand during turning due to the higher burden on the postural control system, even in ST[48].

The rs-fMRI analysis showed a combined pattern of hypo –and hyper-connectivity in PD compared to controls in DT-related neural networks. In line with previous ‘off’ medication studies [49, 50], PD patients showed hyper-connectivity between the cerebellum and cortical motor areas compared to controls which was interpreted as compensatory for the reduced motor output stemming from the striato-thalamo-cortical system. However, our correlation analysis did not confirm a direct cerebellar involvement in DT performance in PD [21]. In contrast, better performance during both ST and DT turning was correlated to hyper-connectivity between the right IPL and PMC, although these correlations were not corrected for multiple comparisons. The IPL is known for its role in conscious movement control as it integrates spatial and sensory information to achieve optimal motor execution [51]. In addition, conscious control of movement in contrast to automatic performance has been associated with greater activation in the PMC and parietal cortex in PD compared to controls [1, 52, 53]. Although this hyper-activation was suggested to compensate for basal ganglia dysfunction, it may also indicate less efficiently organized neural interactions during movement execution [33].

The FOG-specific results of this study were the most striking, especially given the fact that only a small group of freezers could be included due to head motion artifacts. While the cerebellum was hyper-connected to cortical motor areas in PD compared to controls, it showed decreased FC with the left caudate in freezers compared to non-freezers. This is in line with a study that found weakened striatal-cerebellar connections in PD patients with akinesia/rigidity [54] and fits within the predominant pattern of decreased FC that was present in freezers compared to non-freezers. While this altered FC involving the left caudate might explain more impaired motor control in freezers compared to non-freezers, the right caudate was specifically hypo-connected to the striatum and pallidum. In line with earlier work on right-lateralization of neural impairment in FOG [31, 55] and dual-tasking [23], these FC alterations might represent impaired cognitive function in freezers compared to non-freezers. This finding is supported by the correlation between the decreased FC between the right caudate and left dorsal putamen and the lower FAB-scores. Moreover, this hypo-connectivity pattern is also in accordance with previous functional imaging studies that showed hypo-metabolism in the basal ganglia in freezers compared to non-freezers [56, 57] and fits with the gradual striatal depletion model [58]. Our results suggest that more severe dopamine depletion is underlying DT impairment, but also that this mechanism plays a greater role in those with FOG. Furthermore, the correlation between intra-striatal hypo-connectivity and FAB-scores corroborates previous functional imaging studies which found a correlation between reduced striatal dopamine transporter availability and executive impairment [59, 60]. In agreement, Tessitore et al. (2012) demonstrated lower FAB-scores in freezers compared to non-freezers as well as decreased resting-state activity in the right fronto-parietal network [29]. This all confirms cognitive involvement in FOG, in which fronto-striatal pathological changes in freezers might be mediated by striatal dopaminergic deficiency [61] indicating increased dopaminergic denervation in freezers compared to non-freezers [56].

Dysconnections between the caudate and prefrontal areas have been related to FOG pathophysiology as dopamine release in both areas is necessary for gait execution [62, 63]. However, our results did not show altered FC between the caudate and prefrontal regions, but rather indicated hyper-connectivity between the dorsal putamen and precuneus. The precuneus has been suggested to play a central

executive role in DT performance and was more activated in PD patients during a DT fMRI study [19]. Although we did not find hyper-connectivity involving the precuneus in the PD-CTRL comparison, the increased FC in freezers compared to non-freezers might suggest an ineffective compensatory strategy as it proved inversely correlated to DT gait performance. It may indicate recruitment of additional central executive capacity and attention to perform dual-tasks [64]. The lack of altered FC involving prefrontal areas might be explained by the relatively small ROI-sizes that were used for the relatively large prefrontal areas. Future studies might benefit from using ROI-masks that cover larger regions of the prefrontal cortex, although this might come at the cost of reduced spatial specificity.

Furthermore, we found decreased FC between the bilateral caudate and superior temporal lobe that correlated to worse DT gait quality. Since projections from superior temporal gyrus to the caudate are part of a complex associative network through which cognitive control areas may influence motor output [65], it is not surprising that this proved to be a factor in DT impairment. This is in sharp contrast with the increased activity within the temporal lobe, as well as the MFG, cerebellum, prefrontal and parietal cortex that was associated with combining finger tapping and letter counting in PD in earlier work using a task-based analysis [19].

In contrast to previous studies [66], we found no FC alterations involving the PPN, except for hypo-connectivity between the PPN and IPL in PD compared to controls. This brainstem area was also previously shown to be hyper-connected to the SMA in freezers compared to non-freezers in rest [30] and showed asymmetric structural connectivity that correlated with DT interference in freezers [23]. Our lack of PPN-related results might be attributed to the less severely affected freezers in the present study or to different coordinates used to define the PPN ROI compared to previous work[55]

Finally, the main limitation of our study was the small number of freezers. Next to the power issues in the FC analyses, this may also have also led to a compromised detection of behavioral differences between freezers and non-freezers. Future studies should combine larger sample sizes with imaging modalities that enable direct investigation of neural alterations during DT gait, using

electroencephalography (EEG) [67] or functional Near Infrared Spectroscopy (fNIRS) [68, 69] together with modalities that offer good anatomical resolutions (e.g. MRI).

In conclusion, our results support FC alterations in PD compared to controls in areas that have previously been associated with DT performance, such as the IPL, PMC and cerebellum. In addition, we found pronounced FC alterations of the right caudate nucleus, cerebellum and superior temporal lobe when comparing patients with and without FOG, supporting their role in executive impairment and loss of movement automaticity in FOG. However, the current study did not provide evidence for direct involvement of frontal executive function in DT performance in PD. We did demonstrate that the precuneus, which is suggested to have an integrative role in DT performance, showed hyper-connectivity implying that the balance between the motor and cognitive control network is altered and may underlie DT impairment in PD, and especially in those who have FOG.

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Tables

Table 1. Subject characteristics

	PD (n=73)	CTRL (n=20)	FOG (n=13)	NFOG (n=60)	Between- group p	Between- subgroup p
Age (years)	59.5 ($\pm$ 10.2)	57.7 ($\pm$ 8.8)	65.8 ($\pm$ 9.9)	58.2 ($\pm$ 9.9)	0.42	0.02
Sex (M/F)	43/30	14/6	1/12	31/29	0.81	0.01
Disease duration (years)	6.2 ($\pm$ 3.8)	NA	7.9 ($\pm$ 5.4)	5.8 ($\pm$ 3.3)	NA	0.21
Disease dominant side (L/R)	31/42	NA	7/6	24/36	NA	0.54
MDS-UPDRS III (0-132)	28.1 ($\pm$ 13.2)	NA	38.3 ($\pm$ 13.9)	25.9 ($\pm$ 12.0)	NA	<0.01
H&Y (1-5)	2.0 ($\pm$ 0.7)	NA	2.2 ($\pm$ 0.8)	2.0 ($\pm$ 0.6)	NA	0.36
NFOG-Q (0-29)	NA	NA	15.8 ($\pm$ 7.5)	NA	NA	NA
LED (mg/day)	444.9 ($\pm$ 284.1)	NA	604.8 ($\pm$ 380.0)	409.7 ($\pm$ 248.9)	NA	0.1
MMSE (0-30)	28.4 ($\pm$ 1.6)	29.3 ($\pm$ 0.8)	28.0 ($\pm$ 1.6)	28.5 ($\pm$ 1.6)	< 0.01	0.32

Means and standard deviations (SD) are shown. Between group p-values represent comparison between PD patients and controls. Between subgroup p-values represent comparison between FOG and NFOG. CTRL: controls; FOG: Freezing of Gait; H&Y: Hoehn and Yahr; LED: Levodopa Equivalent Dose; MDS-UPDRS: Movement Disorder Society Unified Parkinson's Disease Rating Scale; MMSE: Mini-Mental State Examination; NA: not applicable; NFOG: No Freezing of Gait; NFOG-Q: New Freezing of Gait Questionnaire; PD: Parkinson's Disease.

Table 2. MANOVA models for DT gait with cognition included

DT gait									
Model parameters	PD (n=73)	CTRL (n=20)	p-value	Cohen's d	Model parameters	FOG (n=13)	NFOG (n=60)	p-value	Cohen's d
MMSE (0-30)	28.4 (± 1.5)	29.3 (± 0.8)	0.01	0.75	Stance time D (%)	61.3 (±1.7)	59.7 (± 1.2)	0.03	1.09
MoCA (0-30)	26.0 (± 2.9)	27.5 (± 1.5)	0.03	0.65	Stance time ND (%)	61.4 (± 2.7)	59.9 (± 1.2)	0.02	0.72
TMTa (s)	37.3 (± 12.0)	27.8 (± 8.0)	<0.01	0.93	Swing time D (%)	38.9 (± 2.6)	40.3 (± 1.0)	0.02	0.71
TMTb (s)	82.2 (± 42.1)	54.1 (± 12.8)	<0.01	0.90	Swing time ND (%)	39.3 (±2.6)	40.2 (±1.1)	0.04	0.45
FAB (0-18)	16.0 (± 1.8)	17.2 (± 1.0)	<0.01	0.82	Double support time D (%)	11.4 (± 2.9)	9.8 (± 1.2)	0.03	0.72
Stance time D (%)	60.0 (± 1.4)	59.4 (± 0.6)	0.06	0.56	Double support time ND (%)	11.2 (± 2.3)	9.9 (± 0.9)	0.03	0.74
Swing time D (%)	39.9 (± 1.4)	40.2 (± 0.7)	0.08	0.27	Turn duration D (s)	8.7 (± 3.4)	5.5 (± 2.2)	<0.01	1.12
Step length asymmetry (mm)	15.9 (± 22.9)	6.7 (± 5.6)	0.08	0.55	Turn duration ND (s)	9.0 (± 3.9)	5.4 (± 2.1)	0.04	1.15
Turn duration D (s)	5.9 (± 2.7)	5.0 (± 1.5)	0.13	0.41	Turn steps D	16.5 (± 5.6)	11.1 (± 3.2)	0.01	1.18
Turn steps D	11.8 (± 4.1)	9.6 (± 1.7)	0.05	0.70	Turn steps ND	16.9 (± 6.7)	10.7 (± 3.0)	<0.01	1.19
Turn steps ND	11.5 (± 4.3)	9.6 (± 1.8)	0.02	0.58					

Means and standard deviations (SD) are shown. P-values represent the individual parameters within the significant general MANOVA model. CTRL: controls; D: disease dominant side; FAB: Frontal Assessment Battery; FOG: Freezing of Gait; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; ND: disease non-dominant side; NFOG: No Freezing of Gait; PD: Parkinson's disease; TMT: Trail Making Test.

Table 3. MANOVA models for ST gait with cognition included

ST gait									
Model parameters	PD	CTRL	p-value	Cohen's d	Model parameters	FOG	NFOG	p-value	Cohen's d
MMSE (0-30)	28.4 ($\pm$ 1.5)	29.3 ($\pm$ 0.8)	0.04	0.75	Swing time ND (%)	(40.0 $\pm$ 2.2)	(41.2 $\pm$ 1.0)	0.05	0.70
MoCA (0-30)	26.0 ($\pm$ 2.9)	27.5 ($\pm$ 1.5)	0.05	0.65	Swing time CV D (%)	(10.2 $\pm$ 6.6)	(6.8 $\pm$ 2.9)	0.03	0.67
TMTa (s)	37.3 ($\pm$ 12.0)	27.8 ($\pm$ 8.0)	<0.01	0.93	Turn duration ND (s)	(9.9 $\pm$ 6.1)	(4.9 $\pm$ 1.7)	0.06	1.12
TMTb (s)	82.2 ($\pm$ 42.1)	54.1 ($\pm$ 12.8)	<0.01	0.90					
FAB (0-18)	16.0 ($\pm$ 1.8)	17.2 ($\pm$ 1.0)	<0.01	0.82					
Turn duration D (s)	(5.2 $\pm$ 1.7)	(4.1 $\pm$ 1.2)	0.01	0.75					
Turn duration ND (s)	(5.3 $\pm$ 1.9)	(4.2 $\pm$ 1.3)	0.03	0.68					
Turn steps D	(10.8 $\pm$ 2.8)	(8.5 $\pm$ 1.2)	<0.01	1.07					
Turn steps ND	(10.9 $\pm$ 1.9)	(8.6 $\pm$ 1.6)	<0.01	1.31					

Means and standard deviations (SD) are shown. P-values represent the individual parameters within the significant general MANOVA model. MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; TMT: Trail Making Test; FAB: Frontal Assessment Battery; D: disease dominant side; ND: disease non-dominant side; PD: Parkinson's disease; CTRL: controls; FOG: Freezing of Gait; NFOG: No Freezing of Gait.

Table 4. Resting state functional connectivity differences

	Seed region	Target region	T-value	FDR-corrected p	Uncorrected p
PD (n=57) < CTRL (n=18)					
	Left M1	Right PMC	-3.43	0.020	< 0.001
	Left M1	Right M1	-3.01	0.037	0.002
	Left IPL	Right caudate	-3.61	0.011	<0.001
	Left IPL	Left caudate	-2.71	0.043	0.004
	Left IPL	Left SNc	-3.02	0.036	0.002
	Left IPL	Right PPN	-2.67	0.043	0.004
PD > CTRL					
	Left M1	Right IPL	2.90	0.041	0.002
	Left M1	Left SNc	2.97	0.041	0.002
	Left M1	Left cerebellum, lobule VI	2.84	0.041	0.003
	Right M1	Left cerebellum, lobule VI	2.70	0.045	0.004
	Left PMC	Right IPL	3.84	<0.01	<0.001
	Left PMC	Left cerebellum, lobule VI	2.91	0.049	0.002
	Right PMC	Right IPL	2.79	0.046	0.003
	Right PMC	Left cerebellum, lobule VI	2.82	0.042	0.003
FOG (n=8) < NFOG (n=49)					
	Left caudate	Right superior temporal lobe	-3.48	0.017	<0.001
	Left caudate	Left cerebellum, lobule VI	-2.99	0.039	0.002
	Right caudate	Right dorsal putamen	-2.62	0.048	0.005
	Right caudate	Left dorsal putamen	-2.61	0.048	0.005
	Right caudate	Left GP	-2.63	0.048	0.005
	Right caudate	Right superior temporal lobe	-2.69	0.037	0.004
	Right caudate	Left superior temporal lobe	-2.78	0.048	0.003
FOG > NFOG					
	Left dorsal putamen	Right precuneus	3.13	0.051	0.001

Functional connectivity (FC) differences between seed and target regions within the motor control and fronto-parietal network are shown for PD patients compared to controls. Negative T-values represent hypo-connectivity in patients compared to controls while positive T-values represent hyper-connectivity in patients compared to controls. CTRL: controls; FDR: False discovery rate; FOG: Freezing of Gait; GP: Globus Pallidus; IPL: Inferior Parietal Lobule; M1: Primary motor cortex; MFG: Middle

Frontal Gyrus; NFOG: No Freezing of Gait; PCC: Posterior Cingulate Cortex; PD: Parkinson's disease; PMC: Premotor cortex; PPN: Pedunculopontine nucleus; SNc: Substantia Nigra pars compacta.

Figures

Figure 1. FC matrices for the motor control and fronto-parietal network

Matrices represent the Fisher transformed Pearson correlation coefficients of the comparison time series of ROIs within the motor control and fronto-parietal network. a) shows the FC matrix for PD patients compared to controls. Orange and red colors represent positive beta-values for ROIs that were hyper-connected in PD compared to controls, while green and blue colors represent negative beta-values for ROIs that were hypo-connected in PD compared to controls; b) shows the FC matrix for freezers compared to non-freezers. Orange and red colors represent positive beta-values for ROIs that had increased FC in freezers compared to non-freezers, while green and blue colors represent negative beta-values for ROIs that had decreased FC in freezers compared to non-freezers. ACC: anterior cingulate cortex; GP: globus pallidus; DLPFC: dorsolateral prefrontal cortex; IPL: inferior parietal lobule; M1: primary motor cortex; MFG: middle frontal gyrus; PMC: premotor cortex; PPC: posterior cingulate cortex; PPN: pedunculopontine nucleus; SMA: supplementary motor area; SNc: substantia nigra pars compacta; STN: subthalamic nucleus; thal-M1: thalamus projecting to M1; thal-PMC: thalamus projecting to PMC.

Figure 2. Bonferroni-corrected correlations between FC and DT behavioral measures.

Figures represent correlations of discriminatory FC measures between freezers and non-freezers with discriminatory behavioral measures. White dots and black squares represent non-freezers and freezers respectively. Lines indicate correlations within the entire patient group and represent Pearson correlations between FC outcomes and DT behavioral measures that discriminated between freezers and non-freezers. DT: dual-task; FC: Functional Connectivity; FOG: freezing of gait; GP: globus pallidus; L: left; NFOG: No freezing of gait; R: right.

Figure 3. Bonferroni-corrected correlations between FC and ST behavioral measures.

Figures represent correlations of discriminatory FC measures between freezers and non-freezers with discriminatory behavioral measures. White dots and black squares represent non-freezers and freezers respectively. Lines indicate correlations within the entire patient group. a. represents a Spearman correlation

between FAB-scores and FC between the right caudate and left dorsal putamen. b-c. represent Pearson correlations between FC outcomes and ST measures. FAB: Frontal Assessment Battery; FC: Functional Connectivity; FOG: Freezing of Gait; L: Left; ND: Non-dominant; NFOG: No freezing of gait; R: right; ST: single task.