

Frontoparietal-striatal network and nucleus basalis modulation in patients with Parkinson's disease and gait disturbance

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ABSTRACT

Background and Objectives: Neural computations underlying gait disorders in Parkinson's disease (PD) are multifactorial and involve impaired expression of stereotactic locomotor patterns and compensatory recruitment of cognitive functions. This study aimed to clarify the network mechanisms of cognitive contribution to gait control and its breakdown in patients with PD.

Methods: Patients with PD were instructed to walk at a comfortable pace on a mat with pressure sensors. The characterization of cognitive-motor interplay was enhanced by employing a gait with a secondary cognitive task (dual-task condition) and a gait without additional tasks (single-task condition). Participants were scanned using 3-T magnetic resonance imaging (MRI) and ^{123}I -ioflupane single photon emission computed tomography.

Results: According to gait characteristics, cluster analysis assisted by a nonlinear dimensionality reduction technique, t-distributed stochastic neighbor embedding, categorized 56 patients with PD into three subpopulations. The preserved gait (PG) subgroup ($n=23$) showed preserved speed and variability during gait, both with and without additional cognitive load. Compared with the PG subgroup, the mildly impaired gait (MIG) subgroup ($n=16$) demonstrated deteriorated gait variability with additional cognitive load and impaired speed and gait variability without additional cognitive load. The severely impaired gait (SIG) subgroup ($n=17$) revealed the slowest speed and highest gait variability. In addition, group differences were found in attention/working memory and executive function domains, with the lowest performance in the SIG subgroup than in the PG and MIG subgroups. Using resting-state functional MRI, the SIG subgroup demonstrated lower functional connectivity of the left and right frontoparietal network with the caudate than the PG subgroup did (FPN.Lt, $d=1.21$, $P<0.001$; FPN.Rt, $d=1.05$, $P=0.004$). Cortical thickness in the frontoparietal network and ^{123}I -ioflupane uptake in the striatum did not differ among the three subgroups. Contrastingly, the severity of Ch4 density loss was significantly correlated with the level of functional connectivity degradation of the frontoparietal network and caudate (FPN.Lt–caudate, $r=0.27$, $P=0.04$).

Discussion: These findings suggest that the functional connectivity of the frontoparietal network with the caudate, as mediated by the cholinergic Ch4 projection system, underlies the compensatory recruitment of attention and executive function for damaged automaticity in gait in patients with PD.

INTRODUCTION

Neural mechanisms underlying gait disorders in Parkinson's disease (PD) are multifactorial¹ and involve impaired expression of stereotactic locomotor patterns and compensatory recruitment of what are traditionally regarded as cognitive functions, including attention and executive function.^{1,2} Disturbed expression of automatic movements, including stereotactic locomotor patterns, is proposed to be attributable to dopamine depletion in the posterior putamen in PD.³ In fact, in classic decerebrate cat experiments, the stereotactic locomotor pattern itself have been elicited by the descending pathways from the brainstem to the spinal cord.⁴ It is suggested that the damage of automatic expression of locomotor pattern in PD is bypassed by the compensatory recruitment of cognitive function because rehabilitation strategies can potentially train patients to attain a more normal stride length by focusing their attention on their walking step size.⁵ Contrastingly, gait dysfunction in PD can be induced by the secondary task's consumption of limited cognitive resources, thus withdrawing the resource from supervising gait.

The cognitive contribution to gait control in PD should be represented by neural activity in distributed, yet interrelated cortical and subcortical networks. Actual as well as imagined gait in patients with PD have demonstrated activity in brain areas related to cognitive functions involving the frontoparietal cortices.^{6,7} Moreover, slowing of gait along with cognitive deterioration in patients with PD implicating an insufficient capacity for cognitive compensation of gait was observed in comorbid cortical cholinergic denervation rather than striatal dopamine depletion alone using positron emission tomography.⁸ Gait impairments in PD, therefore, are suspected to be, at least partly, related to cortical cholinergic and striatal dopaminergic denervation, causing aberrant network activity in surviving neurons.⁹ However, network mechanisms underlying cognitive control of gait are challenging to investigate because of the concurrent neural processing of cognition and gait.

This study aimed to clarify the network mechanisms of cognitive contribution to gait control and its breakdown in patients with PD. First, we classified heterogeneous gait disorders with a combination of various degrees of mobility disability and compensatory cognitive control among patients with PD. The characterization of cognitive-motor interplay was enhanced by employing a gait with a secondary cognitive task (dual-task condition) and a gait without additional tasks (single-task condition). Based on the idea that multiple gait parameters discriminate distinct underlying pathophysiological profiles among healthy older

participants and patients with pathology, we obtained 16 core gait parameters describing spatiotemporal characteristics and gait dynamics, such as step-to-step variability and asymmetry in both conditions.¹ These core parameters were then classified into five domains: pace, variability, rhythm, postural control, and asymmetry using a factor analysis approach.^{1,10} We assumed that some patients had well-preserved mobility ability, whereas others were divisible into disturbed mobility with and without cognitive compensation. As it is unknown which gait outcomes are appropriate for dissecting pathophysiological profiles, data-driven approaches were applied. Network mechanisms were subsequently compared among subgroups to identify possible effects of functional disconnectivity by measuring the level of co-activation of resting-state functional magnetic resonance imaging (rs-fMRI) time series. Furthermore, based on previous clinical observations, the nucleus basalis of Meynert, or basal forebrain Ch4, a major source of neocortical cholinergic projections,^{11,12} and striatal dopaminergic terminals were examined.

METHODS

Standard protocol approvals, registrations, and patient consents

The Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine approved this study (R0494). All patients provided written informed consent.

Participants

We recruited patients with PD at the Kyoto University Hospital from September 2016 to March 2020. Patients were diagnosed according to the Movement Disorder Society (MDS) clinical diagnostic criteria.¹³ Participants were excluded if they had major orthopaedic, proprioceptive or visual disorders that may interfere with gait. None of the participants had a history of neuropsychiatric disorders other than PD or were taking antipsychotic or antidepressant medications. All assessments were conducted in a drug-on state in which optimal dopaminergic medication was administered. We calculated the levodopa equivalent daily dose (LEDD).¹⁴

Gait data acquisition

Participants were instructed to walk at a comfortable pace around a 25-m long oval track under two conditions. Patients walked without an additional task during the single-task condition. Additionally, patients repeated the forward three-digit span test in which one digit was presented every 1 s, and one digit was subsequently answered every 1 s during the dual-task condition.¹⁵ Patients scored at least 4 in the forward digit span test. The dual-task condition followed the single-task condition. In the track, a 4.2-m long and 0.6-m wide mat with pressure sensors (WalkWay MW-1000; Anima, Tokyo, Japan) was placed, and quantitative gait data were measured at a 50-Hz sampling frequency. A minimum of 40 steps were recorded for each condition.

Quantitative gait data analysis

We obtained 16 core parameters describing five domains: pace, variability, rhythm, postural control, and asymmetry, as in a previous study.¹⁰ Moreover, the effect of the dual-task was evaluated on the core parameters by dual-task interference (DTI) ($[\text{dual-task performance} - \text{single-task performance}] / [\text{single-task performance}]$),¹⁶ except for the asymmetry domain in which the value in the single-task condition was zero in several participants. Therefore, we obtained a total of 29 parameters (16 core parameters in the single-task condition, and 13 DTI parameters).

The dimensionality of the quantitative gait parameters was reduced to nine by applying principal component analysis (PCA) with a commonly adopted cumulative contribution rate of 80%. Using t-distributed stochastic neighbor embedding (t-SNE), the 9-dimensional data were visualized in three-dimensional space and subsequently categorized into subpopulations with distinct coordinates¹⁷ to define the optimum number of clusters. Finally, a cluster analysis was conducted on the gait data processed by PCA using the k-means method and cosine distance. Analyses were performed using MATLAB 2019a (MathWorks, Natick, MA, USA).

Clinical behavioral measures

Parkinsonian motor symptoms were assessed using the Hoehn and Yahr (HY) stage and MDS Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III score¹⁸ during dopaminergic medication on state. To evaluate PD motor signs, postural instability gait disturbance (PIGD) scores were calculated using items from the MDS-UPDRS, similar to a previous study¹⁹. A questionnaire was used to assess whether the patient had fallen in the year before recruitment to determine if a patient had an increased risk of falling. Patients who had experienced a fall at least once were defined as fallers, and those who had not were defined as non-fallers.²⁰

A neuropsychological battery was administered following the PD mild cognitive impairment level II criteria of MDS, which consist of five domains^{21,22}: trail-making test-A (TMT-A) and symbol digit modalities test (SDMT) for evaluating attention/working memory; trail-making test-B (TMT-B) and clock drawing test (CDT) for executive function; category fluency test (CFT) and similarities from the Wechsler adult intelligence scale 3rd edition for language; delayed recall of Hopkins verbal learning test–revised (HVLT-R) and delayed recall of the Rey–Osterrieth complex figure test (RCFT) for memory; judgment of line orientation (JLO) and copying pentagons test (CPT) for visuospatial function. Global functioning was examined using the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

MRI data acquisition

MRI scans were performed using a 3-T Magnetom Skyra system with a 32-channel head coil (Siemens Healthcare, Erlangen, Germany) (See Supplemental Methods).²³

¹²³I-ioflupane single photon emission computed tomography (SPECT) data acquisition

Scans were performed using the Infinia system (GE Healthcare, Waukesha, Wisconsin, USA), and 65 slice images were acquired as previously described.²³ The voxel size of the reconstructed image was 3.3×3.3×3.3 mm.

Functional connectivity analysis

Functional images were corrected for susceptibility-related distortion using topup implemented in the FMRIB Software Library.^{24,25} Surface-based rs-fMRI analysis was performed using the CONN toolbox version19.c²⁶ (See Supplemental Methods).

To specify regions of interest (ROIs) in key network nodes, group-level spatial independent component analysis (ICA) was performed. The ICA maps were assigned to one of Yeo's seven networks: visual network (VIS), sensorimotor network (SMN), dorsal attention network (DAN), ventral attention network (VAN), limbic network (LIM), frontoparietal network (FPN), and default mode network (DMN).²⁷ Thereafter, 16 ICA maps were identified as ROIs for a functional connectivity analysis (Fig. 1).

ROIs in the caudate nucleus and anterior and posterior putamen were defined with structural T1-weighted images using FreeSurfer (See Supplemental Methods).

For ROIs in the functional and structural images defined above, the characteristic activity during rs-fMRI was extracted by calculating the average time series. Individual ROI-to-ROI functional connectivity matrices were generated by computing Fisher-transformed bivariate Pearson's correlation coefficients between the pair of ROI time series, with age as the covariate of no interest.

Cortical thickness analysis

For ROIs that showed significant group differences in ROI-to-ROI functional connectivity, we investigated whether the observed differences were attributable to cortical neurodegeneration. We computed the average cortical thickness in each ROI with structural T1-weighted images using FreeSurfer version 6.0.²⁸

¹²³I-ioflupane SPECT analysis

Using DaTQUANT software for Xeleris3.1 (GE Healthcare, Waukesha, Wisconsin, USA), the uptake ratio of the volume of interest in the caudate, anterior and posterior putamen was calculated as previously described.²³

Basal forebrain Ch4 density analysis

The region-specific gray matter density of the nucleus basalis of Meynert or Ch4 was quantitatively evaluated using structural T1-weighted images by applying voxel-based morphometry methodology. We

used the computational anatomy toolbox (CAT12.8)²⁹ implemented in the statistical parametric mapping (See Supplemental Methods).³⁰

Statistical analysis

Demographic measures were compared among the subgroups identified in quantitative gait data analysis using one-way analysis of variance (ANOVA) with post-hoc Tukey–Kramer test for continuous variables and Fisher’s exact test with post-hoc Bonferroni correction, which multiplies the raw *P*-values by the number of tests ($n=3$), for the rate of fallers/non-fallers. Cognitive measures were compared using an analysis of covariance (ANCOVA) adjusted for age with a post-hoc Tukey–Kramer test, because the performance is influenced by age. Statistical analyses were conducted using MATLAB 2019a and JMP pro15, with a threshold set at $P < 0.05$.

Group-level statistical analysis of functional connectivity measures in subgroups was performed using a multivariate parametric general linear model. Family-wise error rate was controlled by adopting cluster-level inference, as implemented in the CONN toolbox. Cluster-level inferences deliberate groups of connections that share similar characteristics, instead of considering individual connections between all possible pairs of ROIs.³¹ This methodology effectively evaluates the entire set of connections between ROIs with regard to between- and within-network connectivity. A significance value was set to a cluster-level *P*-value < 0.05 , applying a false discovery rate (FDR) controlling procedure,³² with an uncorrected *P*-value < 0.05 at a ROI-to-ROI connection level.³³

Cortical thickness values in ROIs that revealed significant group differences in functional connectivity were compared among the subgroups using ANCOVA adjusted for age. The ¹²³I-ioflupane uptake ratio in the caudate and anterior and posterior putamen was also compared among the subgroups using ANCOVA, controlling for age. Furthermore, region-specific gray matter density of Ch4 was compared among the subgroups with age and total intracranial volume (TIV) as nuisance covariates, following previous studies.^{34,35} Finally, in ROIs that showed group differences in functional connectivity, the linear relationship between the degree of decline in functional connectivity and the severity of reduction in gray matter density of Ch4 was investigated with age and TIV as nuisance covariates.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Data-driven identification of subgroups based on gait characteristics

Gait characteristics in 56 patients with PD (mean±standard deviation [SD]: age, 64.0±10.1 [range, 35–85] years; education, 14.2±2.2 [range, 9–18] years; disease duration from cardinal Parkinsonism symptom onset, 9.6±4.8 [1.0–26.6] years; 18 male) were categorized into three subpopulations with distinct t-SNE coordinates (Fig. 2: subgroup A, blue; B, red; and C, yellow). Therefore, we regarded the optimum number of clusters as three, and using a cluster analysis, we obtained the following subgroups (Supplemental Table S1): A) the preserved gait (PG) subgroup ($n=23$), which had preserved speed and gait variability both during the single- and dual-task conditions; B) the mildly impaired gait (MIG) subgroup ($n=16$), which had slower speed and preserved gait variability during the single-task condition and deteriorated gait variability during the dual-task condition; and C) the severely impaired gait (SIG) subgroup ($n=17$), which had the slowest speed and highest gait variability both during the single- and dual-task conditions.

A forward three-digit span test was used in the dual-task condition for gait analysis. We assessed the performance of the forward digit span test among the subgroups and confirmed no significant difference (the PG subgroup, 6.7 ± 1.3 ; MIG subgroup, 6.7 ± 1.1 ; and SIG subgroup, 6.2 ± 1.1 ; $F(2,53)=0.85$, $P=0.43$).

Clinical behavioral characteristics of the three subgroups

Comparisons among the subgroups revealed significant age differences, although post-hoc tests of group means did not reach a significant level (Table 1). Contrastingly, no significant group differences existed in disease duration. The PG subgroup had a milder HY stage, MDS-UPDRS Part III severity score, and PIGD score during dopaminergic medication on state and a lower rate of fallers than the SIG subgroup did. The PG subgroup also had a milder HY stage than the MIG subgroup did. Significant group differences were found in the LEDD,¹⁴ with a higher dose in the PG subgroup than in the MIG subgroup. Group differences were also observed in attention/working memory and executive function, as indicated by TMT-A, SDMT, and TMT-B scores, with favorable performance in the PG and MIG subgroups than in the SIG subgroup (Table 2).

Due to the observed differences in the LEDD among the subgroups, we investigated potential confounders from the LEDD on gait performance and found a significant correlation between the LEDD and gait

parameters, including step velocity ($P=0.02$), without correction for multiple comparisons (Supplemental Results).

Functional connectivity in the three subgroups

Key network nodes were specified using group-level spatial ICA. We identified 16 ICA maps as ROIs for functional connectivity analysis, which were assigned to one of Yeo's seven networks, as follows: VIS, SMN, DAN, VAN, LIM, FPN, and DMN (Fig. 1).²⁷

Functional connectivity of the PG, MIG, and SIG subgroups exhibited generally similar patterns in descending order of density (Fig. 3). All subgroups demonstrated positive correlations of the SMN2 with the anterior and posterior putamen in addition to positive correlations of the left frontoparietal network (FPN.Lt) and the right frontoparietal network (FPN.Rt) with the DMN1, DMN2, and DMN3. The PG subgroup exhibited positive correlations of the FPN.Lt and FPN.Rt with the caudate (Fig. 3). In the MIG subgroup, significant correlations of the FPN.Lt and FPN.Rt with the caudate were not observed, whereas negative correlations of the VIS and LIM with other networks were found. The SIG subgroup exhibited significant negative correlations of the FPN.Lt and FPN.Rt with the caudate, whereas correlations of the caudate with the VIS1 and VIS2 were not observed. The VIS network degraded in the MIG and SIG subgroups.

Subgroup comparisons using a multivariate general linear model with age as a nuisance covariate showed higher functional connectivity of the FPN and striatum in the PG subgroup than in the SIG subgroup (Table 3). Moreover, the post-hoc ROI-to-ROI functional connectivity analysis showed significantly higher functional connectivity of the FPN.Lt, FPN.Rt, and caudate and the FPN.Lt and posterior putamen in the PG subgroup than in the SIG subgroup (Fig. 3). No significant difference was found between the PG and MIG subgroups or between the MIG and SIG subgroups.

Because of the differences in the LEDD among the subgroups, subgroup comparisons were conducted with age and LEDD as nuisance covariates, and demonstrated similar results (Supplemental Fig. S1 and Table S2).

Cortical thickness and ¹²³I-ioflupane uptake in the three subgroups

Given the significant group differences in functional connectivity of the FPN.Lt and FPN.Rt with the caudate and posterior putamen, we assessed group differences in cortical thickness of the frontoparietal regions and ¹²³I-ioflupane uptake ratio of the caudate and anterior and posterior putamen.

Cortical thickness was not different among the gait subgroups in the FPN.Lt (mean $\pm$ SD; PG subgroup, 2.52 $\pm$ 0.12 mm; MIG subgroup, 2.54 $\pm$ 0.13 mm; SIG subgroup, 2.47 $\pm$ 0.16 mm; $F(2,52)=0.46$, $P=0.63$) or FPN.Rt (PG subgroup, 2.60 $\pm$ 0.14 mm; MIG subgroup, 2.60 $\pm$ 0.12 mm; SIG subgroup, 2.54 $\pm$ 0.16 mm; $F(2,52)=0.04$, $P=0.96$) after controlling for age.

The ^{123}I -ioflupane uptake ratio was not different among the subgroups in the caudate (PG subgroup, 0.91 $\pm$ 0.33; MIG subgroup, 1.05 $\pm$ 0.37; SIG subgroup, 0.72 $\pm$ 0.30; $F(2,52)=2.25$, $P=0.12$), anterior putamen (PG subgroup, 0.61 $\pm$ 0.20; MIG subgroup, 0.64 $\pm$ 0.25; SIG subgroup, 0.51 $\pm$ 0.18; $F(2,52)=0.44$, $P=0.65$), or posterior putamen (PG subgroup, 0.36 $\pm$ 0.14; MIG subgroup, 0.34 $\pm$ 0.15; SIG subgroup, 0.33 $\pm$ 0.13; $F(2,52)=0.31$, $P=0.73$) after controlling for age.

Gray matter density of Ch4 in the three subgroups

Gray matter density of Ch4 was significantly different among the subgroups (PG subgroup, 0.46 $\pm$ 0.04; MIG subgroup, 0.44 $\pm$ 0.04; SIG subgroup, 0.41 $\pm$ 0.05; $F(2,51)=3.60$, $P < 0.05$). The TIV did not differ among the subgroups (PG subgroup, 1483 $\pm$ 164 mL; MIG subgroup, 1437 $\pm$ 179 mL; SIG subgroup, 1433 $\pm$ 118 mL; $F(2,52)=0.66$, $P=0.52$). The gray matter density of Ch4 was significantly lower in the SIG subgroup than in the PG subgroup ($P < 0.03$), whereas the density did not differ between the SIG and MIG subgroups ($P=0.13$) or between the MIG and PG subgroups ($P=0.85$) after controlling for age and TIV.

Given that the subgroups according to gait characteristics demonstrated differences in gray matter density of Ch4 and functional connectivity of the FPN.Lt and FPN.Rt with the caudate and posterior putamen, we examined the linear relationship between gray matter density reduction in Ch4 and decline in functional connectivity with age and TIV as nuisance covariates (Fig. 4). The degree of Ch4 density reduction was significantly positively correlated with the level of functional connectivity degradation of the FPN.Lt with the caudate ($r=0.27$, $P=0.04$) and posterior putamen ($r=0.30$, $P=0.03$) and tended to be positively correlated with a decline in functional connectivity of the FPN.Rt with the caudate ($r=0.25$, $P=0.07$).

DISCUSSION

According to gait characteristics with and without additional cognitive load, patients with PD were categorized into three subpopulations using cluster analysis assisted by a nonlinear dimensionality reduction technique t-SNE.¹⁷ The PG subgroup showed preserved speed and variability during gait, both with and without additional cognitive load. Compared with the PG subgroup, the MIG subgroup demonstrated deteriorated gait variability with additional cognitive load and impaired speed and gait variability without additional cognitive load. The SIG subgroup revealed the slowest speed and highest gait variability, both with and without additional cognitive load. The SIG subgroup showed a significantly impaired HY stage and a higher MDS-UPDRS Part III score and rate of fallers than the PG subgroup did. Moreover, group differences were found in the TMT-A, SDMT, and TMT-B scores, which belong to the attention/working memory and executive function domains, with the lowest performance in the SIG subgroup compared with the PG and MIG subgroups. The analysis of rs-fMRI demonstrated significantly lower functional connectivity of the bilateral FPN with the caudate in the SIG subgroup than in the PG subgroup. The decreased functional connectivity was unlikely to be attributable to cortical degeneration or striatal dopaminergic terminal degeneration alone because cortical thickness in the bilateral FPN and ¹²³I-ioflupane uptake in the striatum were not different among the SIG, PG, and MIG subgroups. In contrast, the Ch4 density was significantly lower in the SIG subgroup than in the PG subgroup. Furthermore, the severity of Ch4 density loss was significantly correlated with the level of functional connectivity degradation of the FPN.Lt with the caudate and posterior putamen, and tended to be correlated with the functional connectivity reduction of the FPN.Rt with the caudate. Our findings suggest that functional connectivity of the frontoparietal cortical network with the caudate, mediated by the cholinergic Ch4 projection system, underlies the compensatory recruitment of attention and executive function for damaged automaticity in gait in patients with PD.

Several lines of studies have argued that gait impairments in patients with PD are not attributable to locomotor deficits in isolation but to the inability to appropriately allocate attention and executive function to deal with distractors during gait control.³⁶⁻³⁸ This argument agrees with the present observation of lower locomotor performance along with lower attention and executive function in the SIG subgroup than that in the PG subgroup. Our observation is in line with epidemiologic evidence indicating the co-occurrence of

gait disturbance and attention and executive dysfunction resulting from aging and neurological diseases.³⁹ Moreover, our findings showed that additional cognitive load exacerbated gait variability in the MIG subgroup, suggesting compensatory recruitment of cognitive function for impaired gait. Our findings are also consistent with the thought that more cognitive demand is needed for the maintenance of gait as mobility ability declines⁴⁰; hence, similar levels of attention and executive function despite a higher stage of HY represent more severe mobility disturbances in the MIG subgroup than in the PG subgroup. In contrast, the maintained gait with additional cognitive load in the PG subgroup is probably because of the low cognitive demand for sufficient attentional and executive function capacity or low compensatory need for preserved gait ability. The limited deterioration of impaired gait with additional cognitive load in the SIG subgroup is likely to reflect a lack of cognitive compensation because of deficient levels of cognition and locomotion. Indeed, gait variability without additional cognitive load in the SIG subgroup was even worse than the variability with additional cognitive load in the MIG subgroup.

Our findings support the notion that network activities among the frontoparietal cortices and subcortical nuclei, including the caudate, underlie compensatory cognitive control linked to the impaired expression of automatic locomotor movement in patients with PD. Functional decoupling between the frontoparietal cortices and basal ganglia was observed during discrete motor arrests in the virtual reality gait task in PD patients with freezing of gait, and the degree of impairment of functional decoupling was associated with the number of motor arrests.⁷ Regional cerebral blood flow in the frontoparietal cortices increased when transverse lines on the walking surface improved gait performance in patients with PD, suggesting enhanced network activity as a compensatory mechanism by strengthening visual attention function.⁶ Brain activity measured with fMRI in the frontoparietal cortices was also greater in PD patients than in healthy controls while performing automatic movements after proper training with more difficulty.^{41,42} As the frontoparietal network was found to carry signals adjusting error and initiating task performance on a trial-trial basis across a wide range of tasks in mixed blocked/event-related fMRI data,⁴³ it seems reasonable to assume that the network was recruited for adaptive online control to deal with distractors during gait to cover the damaged automaticity in patients with PD. Although cortical thickness in the frontoparietal network was not significantly reduced in the SIG subgroup compared with the PG subgroup, widespread cortical atrophy involving frontoparietal regions was reported in PD patients with HY stage 3 and higher⁴⁴ and in PD patients with mild cognitive impairment. Therefore, it is estimated that frontoparietal cortical

degeneration is likely to occur as the disease progresses, further degrading cortical neural computation, leading to gait deterioration in patients with PD.

Based on the present findings, we propose that Ch4, a major source of cholinergic input into the neocortex, modulates network activity among the frontoparietal cortices and caudate, serving as a cognitive mechanism of gait control in patients with PD. Animal experiments demonstrated an increased fall rate by combined cortical cholinergic-striatal dopaminergic deafferentation compared to isolated striatal dopaminergic deafferentation, correlation of fall rate with the performance of a sustained attention task, and a decline in the performance of the attention task by cholinergic lesions but not by dopaminergic lesions alone.⁹ The experiments proposed that additional cortical cholinergic damage in PD makes compensatory recruitment of attentional resources unavailable for diminished striatal control of complex locomotor movement. Moreover, correct responses were reduced, and omissions were increased in a visual and olfactory target detection task in cortical cholinergic lesioned rats compared to sham-lesioned rats, and accurate detection of the sensory targets was related to increased local field potential coherence between the frontal and parietal cortices.⁴⁵ These findings suggest that the cortical cholinergic system underlies supramodal attention by modulating the frontoparietal cortical network activity. These lines of animal experiments are in line with our observation that deterioration in the attention/working memory and executive function domains and gait performance, as well as Ch4 atrophy, were accompanied by impaired network activities among the frontoparietal cortices and caudate in the SIG subgroup compared with the PG subgroup. Moreover, clinical *in vivo* imaging studies have shown that reduced Ch4 gray matter volume and decreased cortical cholinergic innervation are associated with slower gait speed in patients with PD.^{8,46} Additionally, short-latency afferent inhibition, a putative marker of cholinergic function measured by conditioning motor evoked potentials elicited by transcranial magnetic stimulation with electrical stimuli delivered to the contralateral median nerve, also demonstrated a relationship between cholinergic denervation and slower gait among patients with PD.⁴⁷ Finally, ameliorating cholinergic damage with the acetylcholinesterase inhibitor rivastigmine improved gait speed and step time variability while reducing the fall rate in patients with PD.⁴⁸

Gait performance was evaluated during dopaminergic medication on state to model real-life situations in patients with PD. This possibly influences the present findings. Indeed, a higher LEDD tended to correlate with faster step velocity. Contrastingly, lower functional connectivity in the frontoparietal network with

the caudate was still found in the SIG than the PG subgroup, even if LEDD was added to a nuisance covariate. Based on the findings in PD patients and animal models, we propose comorbid striatal dopaminergic and cortical cholinergic degeneration contributes to locomotor dysfunction.^{8,9}

This study has some limitations. First, we evaluated the main neocortical cholinergic source, Ch4, and striatal dopaminergic terminals, although other neurotransmitter systems and subcortical structures have also been implicated in the pathophysiology of gait and cognitive abnormalities in PD. Cholinergic neurons in the pedunculopontine nucleus supplying projections to the thalamus are diminished in PD, which is associated with falls.⁴⁹ Noradrenergic systems may also influence gait disorders in PD patients⁵⁰. Second, rs-fMRI is an indirect measure of neural activity that is vulnerable to inevitable noise, including cardiac and respiratory-related activity and head movements, and is evaluated during rest. Thus, our findings should be confirmed by the direct measurement of neural activity during gait. Third, the performance of gait and cognitive functioning was assessed using a cross-sectional design, although a decline in gait and cognitive testing should be confirmed based on a participant's premorbid abilities in future studies. Fourth, the present proposal that the functional connectivity of the frontoparietal network with the caudate, as mediated by the cholinergic Ch4 projection system in patients with PD, needs to be supported by healthy control data, which was not examined in the present study.

In conclusion, this study proposes that a functional network composed of the frontoparietal cortex and caudate, mediated by the Ch4 cholinergic projection system, regulates the compensatory recruitment of attention and executive function for impaired automaticity in gait in patients with PD. These findings may accelerate the development of medical therapy that stimulates the cholinergic and dopaminergic systems and rehabilitation strategies that enhance attention and executive function for gait deficits in PD.

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CONFLICTS OF INTERESTS AND DISCLOSURE

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TABLES

Table 1. Clinical characteristics of subpopulations of patients with Parkinson's disease classified according to quantitative gait data analysis

	Preserved gait (n=23)	Mildly impaired gait (n=16)	Severely impaired gait (n=17)		Post-hoc <i>P</i> -value (95% CI) and Cohen's <i>d</i>		
					Preserved vs. Mildly impaired	Preserved vs. Severely impaired	Mildly impaired vs. Severely impaired
Age (years)	62.0±8.0	61.8±8.7	69.0±12.4	F(2,53)=3.20 <i>P</i> =0.049	<i>P</i> =1.00 (-7.4, 7.8) <i>d</i> =0.03	<i>P</i> =0.07 (-14.5, 0.4) <i>d</i> =0.70	<i>P</i> =0.09 (-15.4, 0.9) <i>d</i> =0.67
Education (years)	14.7±2.4	14.6±2.2	13.2±1.7	F(2,53)=2.92 <i>P</i> =0.06	<i>P</i> =0.99 (-1.6, 1.7) <i>d</i> =-0.03	<i>P</i> =0.07 (-0.12, 3.2) <i>d</i> =-0.72	<i>P</i> =0.13 (-0.3, 3.2) <i>d</i> =-0.75
Duration (years)	9.3±3.9	9.5±5.1	10.1±5.7	F(2,53)=0.12 <i>P</i> =0.89	<i>P</i> =0.99 (-4.0, 3.6) <i>d</i> =-0.04	<i>P</i> =0.88 (-4.5, 3.0) <i>d</i> =-0.16	<i>P</i> =0.94 (-4.6, 3.5) <i>d</i> =-0.11
HY stage	1.8±0.4	2.3±0.7	2.6±0.7	F(2,53)=8.4 <i>P</i> =0.007	<i>P</i> =0.04 (-0.95, -0.02) <i>d</i> =-0.90	<i>P</i> < 0.01 (-1.22, -0.30) <i>d</i> =-1.39	<i>P</i> =0.39 (-0.78, 0.23) <i>d</i> =-0.39
UPDRS Part III	16.1±10.3	22.7±9.3	28.1±12.4	F(2,53)=6.22 <i>P</i> =0.004	<i>P</i> =0.15 (-15.0, 1.8) <i>d</i> =-0.67	<i>P</i> < 0.01 (-20.2, -3.7) <i>d</i> =-1.06	<i>P</i> =0.33 (-14.4, -3.6) <i>d</i> =-0.49
PIGD score	3.7±2.7	4.8±3.4	7.5±3.4	F(2,53)=7.37 <i>P</i> =0.002	<i>P</i> =0.59 (-3.5, 1.4) <i>d</i> =-0.34	<i>P</i> < 0.01 (-6.2, -1.4) <i>d</i> =-1.25	<i>P</i> =0.04 (-5.4, -0.2) <i>d</i> =-0.82
Fallers (≥ 1 fall)†	10/23 (43.5%)	11/16 (68.8%)	15/17 (88.2%)	<i>P</i> =0.01 V=0.39	<i>P</i> =0.38 V=0.13	<i>P</i> < 0.05 V=0.46	<i>P</i> =0.67 V=0.36
LEDD (mg)	834±461	469±317	633±306	F(2,53)=4.44 <i>P</i> =0.02	<i>P</i> < 0.05 [66, 664] <i>d</i> =0.89	<i>P</i> =0.23 (-93, 495) <i>d</i> =0.50	<i>P</i> =0.44 (-484, 156) <i>d</i> =-0.53

Pairwise post-hoc group comparisons were conducted using the Tukey–Kramer test, except for the percentage of fallers.

†For post-hoc group comparisons in the percentage of fallers, *P*-values of Fisher's exact test were multiplied by 3 to apply Bonferroni adjustment. V, Cramer's V;

HY, Hoehn–Yahr classification; LEDD, levodopa equivalent daily dose; PI GD, postural instability gait disturbance; UPDRS, Unified Parkinson's Disease Rating Scale.

Table 2. Cognitive performance of subpopulations of patients with Parkinson's disease classified according to quantitative gait data analysis

						Post-hoc <i>P</i> -value (95% CI) and Cohen's <i>d</i>		
		Preserved gait	Mildly impaired gait	Severely impaired gait		Preserved vs. Mildly impaired gait	Preserved vs. Severely impaired gait	Mildly impaired gait vs. Severely impaired gait
Global	MMSE	29.5±0.8	29.1±1.4	27.3±2.5	F(2,52)=7.80	<i>P</i> =0.72 (-0.88, 1.72)	<i>P</i> <0.001 (0.80, 3.47)	<i>P</i> <0.05 (0.27, 3.17)
					<i>P</i> =0.001	<i>d</i> =0.38	<i>d</i> =1.26	<i>d</i> =0.88
	MoCA	25.5±2.4	26.5±3.8	23.1±3.5	F(2,52)=3.07	<i>P</i> =0.60 (-3.34, 1.43)	<i>P</i> =0.21 (-0.72, 4.20)	<i>P</i> <0.05 (0.04, 5.36)
					<i>P</i> =0.06	<i>d</i> =-0.32	<i>d</i> =0.85	<i>d</i> =0.95
Attention	TMT-A	34.2±9.9	35.5±10.3	65.5±38.9	F(2,52)=6.85	<i>P</i> =0.98 (-18.37, 15.46)	<i>P</i> <0.01 (-42.49, -7.68)	<i>P</i> <0.05 (-42.50, -4.77)
					<i>P</i> =0.002	<i>d</i> =-0.17	<i>d</i> =-1.18	<i>d</i> =-1.04
	SDMT	42.6±8.7	44.7±9.8	29.4±11.1	F(2,52)=8.40	<i>P</i> =0.72 (-8.19, 4.18)	<i>P</i> <0.01 (2.57, 15.3)	<i>P</i> <0.001 (4.04, 17.9)
					<i>P</i> <0.001	<i>d</i> =-0.23	<i>d</i> =1.34	<i>d</i> =1.47
Executive	CDT	14.4±2.1	14.7±0.8	13.7±2.0	F(2,52)=1.02	<i>P</i> =0.84 (-1.73, 1.08)	<i>P</i> =0.59 (-0.85, 2.04)	<i>P</i> =0.34 (-0.65, 2.49)
					<i>P</i> =0.37	<i>d</i> =-0.20	<i>d</i> =0.32	<i>d</i> =0.65
	TMT-B	102.4±60.0	90.7±62.4	181.9±86.3	F(2,52)=5.11	<i>P</i> =0.85 (-37.68, 59.72)	<i>P</i> <0.05 (-105.75, -5.51)	<i>P</i> <0.05 (-120.98, -12.33)
					<i>P</i> <0.01	<i>d</i> =0.19	<i>d</i> =-1.10	<i>d</i> =-1.20
Language	CFT	20.3±6.0	18.8±4.9	15.4±4.2	F(2,52)=2.39	<i>P</i> =0.57 (-2.20, 5.39)	<i>P</i> =0.08 (-0.38, 7.44)	<i>P</i> =0.52 (-2.30, 6.17)
					<i>P</i> =0.10	<i>d</i> =0.28	<i>d</i> =0.94	<i>d</i> =0.75
	Similarities	24.6±6.4	23.2±6.4	20.1±4.4	F(2,52)=1.15	<i>P</i> =0.70 (-2.84, 5.69)	<i>P</i> =0.30 (-1.66, 7.12)	<i>P</i> =0.78 (-3.46, 6.06)
					<i>P</i> =0.15	<i>d</i> =0.21	<i>d</i> =0.79	<i>d</i> =0.56
Memory	HVLT delayed recall	7.1±2.2	7.6±3.1	5.1±2.4	F(2,52)=2.45	<i>P</i> =0.83 (-2.32, 1.41)	<i>P</i> =0.21 (-0.55, 3.29)	<i>P</i> =0.10 (-0.25, 3.91)
					<i>P</i> =0.10	<i>d</i> =-0.18	<i>d</i> =0.90	<i>d</i> =0.91
	RCFT delayed recall	18.9±6.7	17.9±8.2	12.1±6.4	F(2,52)=2.61	<i>P</i> =0.87 (-4.18, 6.34)	<i>P</i> =0.07 (-0.37, 10.46)	<i>P</i> =0.24 (-1.90, 9.83)
					<i>P</i> =0.08	<i>d</i> =0.14	<i>d</i> =1.04	<i>d</i> =0.79
Visuospatial	CPT	10.0±0.0	10.0±0.0	9.9±0.2	F(2,52)=0.78	<i>P</i> =1.00 (-0.11, 0.11)	<i>P</i> =0.49 (-0.06, 0.16)	<i>P</i> =0.54 (-0.07, 0.17)
					<i>P</i> =0.46	<i>d</i> = N/A	<i>d</i> =0.37	<i>d</i> =0.34

JLO	22.3±5.2	22.6±5.6	20.9±6.0	F(2,52)=0.02 P=0.98	P=0.99 (-4.19, 3.77) d=-0.05	P=0.98 (-4.45, 3.74) d=0.35	P=1.00 (-4.59, 4.29) d=0.29
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Pairwise post-hoc group comparisons were conducted using the Tukey–Kramer test.

CDT, clock drawing test; CFT, category fluency test; CPT, copying pentagons test; HVLT, Hopkins verbal learning test–revised; JLO, judgment of line orientation; MMSE, Mini-Mental State Examination; MoCA, Montreal cognitive assessment; RCFT, N/A; not applicable, Rey–Osterrieth complex figure test; SDMT, symbol digit modalities test; TMT-A, trail-making test-A; TMT-B, trail-making test-B; CI, confidence interval.

Table 3. The difference in functional connectivity between the preserved gait and severely impaired gait subgroups

Network functional connectivity (1st level)			FDR corrected <i>P</i> -value
Striatum	Frontoparietal network	F(2,51)=9.37	0.01
ROI functional connectivity (2nd level)			<i>P</i> -value
Caudate	Lt Frontoparietal network	T(52)=3.91	<i>P</i> <0.001
			d=1.21
Posterior putamen	Lt Frontoparietal network	T(52)=3.08	<i>P</i> =0.003
			d=0.98
Caudate	Rt Frontoparietal network	T(52)=2.98	<i>P</i> =0.004
			d=1.05

FDR, false discovery rate; ROI, region of interest; Lt, left; Rt, right.

FIGURE LEGENDS

Figure 1. **Key network nodes specified using group-level spatial independent component analysis (ICA).** Sixteen ICA maps were identified as regions of interest (ROIs) for functional connectivity analysis. DAN, dorsal attention network; DMN, default mode network; FPN, frontoparietal network; LIM, limbic network; SMN, sensorimotor network; VAN, ventral attention network; VIS, visual network.

Figure 2. **Clustering of quantitative gait data in patients with Parkinson's disease (PD) after t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction.** Each point represents a patient with PD, and the distance between the points corresponds to the difference in gait performances with and without a secondary cognitive task. Using cluster analysis, the patients were classified into three subgroups (blue, preserved gait subgroup [$n=23$]; red, mildly impaired gait subgroup [$n=16$]; and yellow, severely impaired gait subgroup [$n = 23$]).

Figure 3. **Functional connectivity presented as a connectome ring showing key network nodes with significant positive (red) and negative (blue) functional connectivity in subpopulations of patients with Parkinson's disease.** Comparisons among the subgroups demonstrated higher functional connectivity between the FPN.Lt, FPN.Rt, and caudate and between the FPN.Lt and posterior putamen in the preserved gait subgroup than in the severely impaired gait subgroup. No significant difference was found between the preserved gait and mildly impaired gait subgroups or between the mildly impaired gait and severely impaired gait subgroups.
FPN.Lt: left frontoparietal network; FPN.Rt: right frontoparietal network.

Figure 4. **Correlation between decline in functional connectivity and reduction in gray matter density of Ch4.** Functional connectivity and gray matter density of Ch4 were adjusted for the effects of age and total intracranial volumes. Regression lines are presented when statistically significant.







