Dynamic balance and gait impairments in Parkinson's disease: novel cholinergic patterns

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Abstract

The cholinergic system has been implicated in postural deficits, in particular falls, in Parkinson's disease. Falls and freezing of gait typically occur during dynamic and challenging balance and gait conditions, such as when initiating gait, experiencing postural perturbations, or making turns. However, the precise cholinergic neural substrate underlying dynamic postural and gait changes remains poorly understood. The aim of this study was to investigate whether brain vesicular acetylcholine transporter binding, as measured with [^{18}F]-fluoroethoxybenzovesamicolbinding PET, correlates with dynamic gait and balance impairments in 125 patients with Parkinson's disease (mean age 66.89±7.71 years) using the abbreviated Balance Evaluation Systems Test total and its four functional domain sub-scores (anticipatory postural control, reactive postural control, dynamic gait, and sensory integration). Whole brain false discovery-corrected ($P < 0.05$) correlations for total abbreviated Balance Evaluation Systems Test scores included the following bilateral or asymmetric hemispheric regions: gyrus rectus, orbitofrontal cortex, anterior part of the dorsomedial prefrontal cortex, dorsolateral prefrontal cortex, cingulum, frontotemporal opercula, insula, fimbria, right temporal pole, mesiotemporal, parietal and visual cortices, caudate nucleus, lateral and medial geniculate bodies, thalamus, lingual gyrus, cerebellar hemisphere lobule VI, left cerebellar crus I, superior cerebellar peduncles, flocculus, and nodulus. No significant correlations were found for the putamen or anteroventral putamen. The four domain-specific sub-scores demonstrated overlapping cholinergic topography in the metathalamus, fimbria, thalamus proper, and prefrontal cortices but also showed distinct topographic variations. For example, reactive postural control functions involved the right flocculus but not the upper brainstem regions. The anterior cingulum associated with reactive postural control whereas the posterior cingulum correlated with anticipatory control. The spatial extent of associated cholinergic system changes were least for dynamic gait and sensory orientation functional domains compared to the anticipatory and reactive postural control functions. We conclude that specific aspects of dynamic balance and gait deficits in Parkinson's disease associate with overlapping but also distinct patterns of cerebral cholinergic system changes in numerous brain regions. Our study also presents novel evidence of cholinergic topography involved in dynamic balance and gait in Parkinson's disease that have not been typically associated with mobility disturbances, such as the right anterior temporal pole, right anterior part of the dorsomedial prefrontal cortex, gyrus rectus, fimbria, lingual gyrus, flocculus, nodulus and right cerebellar hemisphere lobules VI and left crus I.

Keywords: Parkinson's disease; dynamic balance; cholinergic; PET

Introduction

Gait impairments, balance issues, and falls stand as significant hallmarks of Parkinson's disease (PD), markedly reducing quality of life, escalating healthcare expenses, and amplifying the burden on caregivers.[1,2] As the condition progresses, debilitating postural instability and gait difficulties become increasingly prevalent.[3] These motor impairments pose considerable therapeutic challenges, typically showing increasing refractoriness to dopaminergic treatments.[4-6] This indicates that other neurotransmitter systems may also play a pivotal role in regulating gait and balance changes in PD besides the dopaminergic system. In line with this understanding, our previous imaging studies revealed alterations in the cholinergic system associated with both freezing of gait and falls in PD.[7,8] Specifically, falls were associated with cholinergic deficits in the thalamus, primarily the right lateral geniculate nucleus, whereas freezing of gait was associated with reduced vesicular acetylcholine transporter binding in striatal cholinergic interneurons and the limbic archicortex.[8] Our recent work also demonstrated that loss of cholinergic nerve terminals in the medial geniculate nucleus, an important multisensory (particularly auditory and vestibular) processing metathalamic relay station, associates robustly with ratings of non-episodic balance impairments.[9] In our previous acetylcholinesterase PET studies, we established a correlation between the pedunculopontine nucleus-thalamic binding of [11C]-methylpiperidin-4-yl propionate ([11C]-PMP) and postural sensory integration, an important function of postural control.[10] We also demonstrated that the right hemispheric vestibular cortical cholinergic system plays a role in maintaining balance, especially during visual-vestibular integration tasks.[11] However, both studies had limitations due to the constraints of the [11C]-PMP radioligand, precluding the examination of high-binding brain areas, such as the striatum and cerebellum. The introduction of vesicular acetylcholine transporter [18F]-fluoroethoxybenzovesamicol ([18F]-FEOBV) PET has opened new avenues for in vivo quantification of the presynaptic cholinergic system. This radiotracer provides highly detailed and specific imaging of cholinergic neurons, enabling precise estimation of ligand binding in regions densely populated with cholinergic terminals, such as the subcortical gray matter regions.[12]

Episodic mobility disturbances, such as falls and freezing of gait, typically occur during dynamic and challenging balance and gait conditions, such as when initiating gait, experiencing postural perturbations, making turns, walking in dim light, or on uneven surface. However, the precise cholinergic neural substrate underlying dynamic postural and gait remains poorly understood. Therefore, the main purpose of the present study was to explore whether regional cerebral [18F]-FEOBV binding associates with specific impairments of gait and balance, as assessed by the abbreviated Balance Evaluation Systems Test (Mini-BESTest), in patients with PD. The Mini-BESTest incorporates four sub-categories of dynamic

balance and gait conditions: anticipatory postural control, reactive postural control, dynamic gait, and sensory orientation. Previous research has demonstrated the high interrater reliability, construct validity, and test/retest reliability of the MiniBESTest within a PD cohort.[13–15] The MiniBESTest has also been found to independently predict falls and provides a robust measurement of dynamic balance and gait in PD populations.[16,17] For this study, we performed whole-brain voxel-based [18F]-FEOBV PET correlation analysis with the total summed score as primary analysis and the four functional domain sub-conditions as post-hoc exploratory analyses. Our overall hypothesis was that cholinergic system changes underlying greater difficulty on dynamic balance and gait conditions involve more widespread supra- and infratentorial brain regions that may partially overlap but also may be topographically distinct among the four functional MiniBESTest conditions.

Materials and methods

Subjects

A sample of 125 predominantly male (males=97, females=28) PD patients participated in the present cross-sectional study. On average, the participants were 66.89±7.71 years old, and have had motor symptoms for 6.12±4.77 years. All participants in the present study satisfied the UK Parkinson's Disease Society Brain Bank criteria for clinical diagnosis of Parkinson's disease.[18] Based on Hoehn & Yahr (HY) staging, most participants in this study had moderate disease severity (HY_{2-3}=108), and relatively few participants presented with mild ($HY_{<2}$=12) or severe disease ($HY_{>3}$=5). Group averaged HY stage was 2.43±0.60. Sixty-seven participants were treated with carbidopa-levodopa alone, 38 were on a combined regiment of carbidopa-levodopa and dopamine agonists, 9 participants were only taking dopamine agonists with no carbidopa-levodopa, and the remaining 11 participants were receiving neither carbidopa-levodopa nor dopamine receptor agonists. None of the participants in the present study were prescribed anti-cholinergic or cholinesterase inhibitor drugs. Anatomical imaging evidence of major intracranial lesion including large vessel strokes were exclusionary to participation in the study. Correlational findings pertaining to total axial motor deficit ratings and regional cholinergic nerve terminal integrity were obtained from an overlapping participant cohort and published on previously.[9]

Clinical assessments

The Movement Disorder Society-revised Unified Parkinson's Disease Rating Scale motor examination was administered to study participants in the morning, before they took their regularly prescribed

dopaminergic medications ('off' state).[19] The mean motor examination score on the Movement Disorder Society-revised Unified Parkinson's Disease Rating Scale part three was 35.69±13.31 (range 9-76). Subjects completed the Montreal Cognitive Assessment.[20] Mean Montreal Cognitive Assessment scores were 26.26±2.83. The levodopa equivalent dose was computed as described previously.[21]

Abbreviated balance evaluation systems test (Mini-BESTest)

The Mini-BESTest assesses dynamic balance and gait functions and includes fourteen items scored from zero to two, providing sub-scores for anticipatory postural control, reactive postural control, dynamic gait, and sensory orientation.[22] The Mini-BESTest has a minimum score of zero and a maximum score of twenty-eight, with a lower score reflecting balance and gait difficulties. The Mini-BESTest was performed in the dopaminergic medication 'off' state. See **Figure 1**.

Imaging and statistical analysis

Study participants underwent a brain MRI scan in a 3 Tesla Philips Achieva system (Philips, Best, The Netherlands). Biograph 6 TruPoint PET/CT scanner (Siemens Molecular Imaging, Inc., Knoxville, TN) in 3D imaging mode was used to administer the PET imaging protocol.[9] [18F]-FEOBV preparation was done as previously described.[23] Six delayed dynamic imaging frames (5-minute frames, 30 minutes total) were acquired 3 hours after an intravenous bolus dose injection of 8 mCi [18F]-FEOBV.[24] Scatter and motion artifact correction was applied to the resulting dynamic PET images.[9] Distribution volume ratio (DVR) parametric [18F]-FEOBV PET images were obtained using a non-invasive supratentorial white matter reference tissue approach.[25,26] DVRs were calculated by summing the dynamic imaging frames across time and dividing the resulting summed image by the mean of the voxelwise signal observed in the reference tissue.[26] Statistical parametric mapping (SPM) software (SPM12; Welcome Trust Centre for Neuroimaging, University College, London, England [https://www.fil.ion.ucl.ac.uk/spm/software/spm12/]) was used to perform MRI-PET registration and partial volume correction on the resulting parametric PET images.[9]

Whole-brain voxel-based [^{18}F]-FEOBV PET and Mini-BESTest total score and sub-scores correlational analyses were performed using parametric SPM12 or non-parametric (SnPM) software after adjustment for levodopa equivalent dose dependent on the data distribution. Cluster peak analyses were also performed on clusters with a minimum of 50 voxels with location of the peak voxel in Montreal Neurological Institute (MNI X-Y-Z space), peak voxel (pseudo) t-score, applicable Brodmann areas (BA), and the associated network regions reported for each valid cluster. For both the whole brain voxel-based and the peak cluster analysis significance thresholds after false discovery (FDR) correction were set at $P < 0.05$. Trending correlations which fail to survive FDR correction are presented at uncorrected threshold of $P < 0.001$.

To assess the extent of shared clinicometric variance between the MiniBEST subscores, a partial correlation analysis was performed. For each MiniBEST subscore, a multiple linear regression model was fitted predicting that subscore from all the other subscores. Proportion of variance in each MiniBEST subscore shared with other subscores was evaluated using the coefficient of determination (R^2) of the resulting model. Correlational structure of the MiniBEST subscores was examined by tabulating the standardized regression coefficients (β) of the resulting multiple regression models as a partial correlation matrix.

Standard protocol approval, registration, and subjects' consent

This study (ClinicalTrials.gov Identifiers: NCT02458430 & NCT01754168) was approved by the Institutional Review Boards of the University of Michigan and the Ann Arbor Veterans Affairs Healthcare System. All participants provided written informed consent.

Results

MiniBEST subscore partial correlation analysis

The tabulated partial correlation matrix along with model R^2 values corresponding to each of the MiniBESTest subscores can be found in Table 1. About 30-50% of variance in each subscore appears shared with the other subscores, meaning that at least about half of the variance in each subscore is unique to that subscore. The partial correlation structure of MiniBESTest subcores suggests that the anticipatory postural control subscore is most strongly related to the MiniBEST sensory subscore, but also demonstrates significant association with all the other subscores. In contrast, the reactive postural control

subscore appears to significantly associate only with the anticipatory subscore. Dynamic gait subscore associates most strongly with the sensory but also significantly with anticipatory subscore, while sensory subscore associates with both dynamic gait and anticipatory subscore to an approximately equal extent.

Whole brain vesicular acetylcholine transporter PET voxel-based correlations with total Mini-BESTest scores

The whole brain voxel-based correlation analysis (FDR-corrected, $P < 0.05$) unveiled significant correlations between vesicular acetylcholine transporter binding and total Mini-BESTest scores across distinct brain clusters. These correlations encompassed specific brain regions, including frontotemporal areas such as the gyrus rectus, orbitofrontal regions, right anterior dorsomedial prefrontal cortex (upper medial BA 10 and BA 9), bilateral dorsolateral prefrontal cortex, all segments of the cingulum, bilateral insula, right more than left frontotemporal operculum, bilateral mesiotemporal cortex, left mid posterior temporal cortex, right more than left fimbriae/isthmus cingulum, right temporal pole, left more than right peri-central cortex. Subcortical areas involved bilateral caudate nuclei, metathalami (lateral and medial geniculate bodies) and thalami proper. Additionally, correlations extended to the occipito-parietal cortices, including the right parietal cortex, occipital cortex, right more than left lingual gyri. Furthermore, distinctive correlations were evident in the cerebellum, particularly the right cerebellar hemisphere lobule VI, left cerebellar crus I, bilateral superior cerebellar peduncles, nodulus, right flocculus, and upper dorsal vermis. No significant correlations were present for the putamen and anteroventral striatum (nucleus accumbens; Figure 2, Table 2). Peak cluster analysis findings (FDR significant $P < 0.05$) are shown in Supplemental Table 1. Most extensive clusters were centered around the right hippocampus, right superior frontal gyrus, right inferior frontal gyrus triangular part, and left more than right precentral cortices. The same analysis was repeated with a stricter voxelwise statistical threshold (FDR-corrected, $P < 0.01$), see supplementary figure 1.

Whole brain vesicular acetylcholine transporter PET voxel-based correlations with anticipatory postural control subscore

Similar to the findings observed for the Mini-BESTest total score, the whole brain voxel-based correlation analysis (FDR-corrected, $P < 0.05$) revealed a significant correlation between the anticipatory postural control subtest and regional vesicular acetylcholine transporter binding. These correlations were identified

in brain clusters topographically situated across frontotemporal cortices, subcortical regions, occipito-parietal cortices, and the cerebellum. While the frontotemporal correlation was less extensive compared to the total score, it notably involved similar brain regions: bilateral gyrus rectus, bilateral orbitofrontal regions, anterior cingulum, bilateral dorsolateral prefrontal cortex (BA 8 and 9), right more than left frontotemporal operculum, right insula, bilateral fimbriae/isthmus cingulum, and right temporal pole. The lower vesicular acetylcholine transporter binding observed in bilateral caudate nuclei, lateral and medial geniculate bodies, and thalami proper exhibited a significant correlation with lower anticipatory postural control subtest scores, aligning with the correlation found in the Mini-BESTest total score. Additionally, significant correlations were identified between anticipatory postural control scores and vesicular acetylcholine transporter binding in occipito-parietal cortices and cerebellar areas, consistent with the findings outlined in the total Mini-BESTest scores section (Figure 3, Table 2). Notably, the right parietal cortex and flocculus exhibited no significant correlation in this case. The anticipatory postural control subset scores exhibited a positive correlation with the brainstem (mesencephalic tectum). There were no significant correlations observed for the putamen and anteroventral striatum. Peak cluster analysis findings (FDR significant $P < 0.05$) are shown in Supplemental Table 2. Most extensive clusters were centered around the left thalamus, right insula, left medial superior frontal gyrus, and left precentral cortex regions.

Whole brain vesicular acetylcholine transporter PET voxel-based correlations with reactive postural control subscore

The whole brain voxel-based correlation analysis (FDR-corrected, $P < 0.05$) revealed extensive correlations between reactive postural control subtest scores and regional vesicular acetylcholine transporter binding within frontotemporal regions, including bilateral gyrus rectus, bilateral orbitofrontal cortex, predominantly the right anterior parts of the dorsomedial prefrontal cortex (upper medial BA 10 and BA 9), bilateral dorsolateral prefrontal cortex (BA 8 and 9), anterior cingulum, left-dominant frontotemporal operculum, bilateral insula, bilateral mesiotemporal lobes, bilateral fimbriae/isthmus cingulum, left peri-central cortex, right temporal pole, inferior temporal lobes, and left mid and posterior temporal lobe. Additionally, significant correlations emerged in subcortical structures, notably involving bilateral caudate nuclei, lateral and medial geniculate nuclei, and the left pulvinar. Significant correlations were observed also in parietal and cerebellar regions, albeit more limited compared to the Mini-BESTest total and anticipatory postural control scores, encompassing the right parietal cortex, right flocculus, nodulus, and bilateral superior cerebellar peduncles (Figure 4, Table 2). Notably absent were the putamen,

anteroventral striatum, right cerebellar lobule VI, brainstem, and visual cortex. Peak cluster analysis findings (FDR significant $P < 0.05$) are shown in Supplemental Table 3. Most extensive clusters were centered around the right superior frontal gyrus, left precentral, left postcentral, right inferior frontal gyrus triangular part, and left inferior temporal gyrus regions.

Whole brain VAChT PET voxel-based correlations with dynamic gait subscore

Whole brain voxel-based correlation analysis (non-corrected at $P = 0.001$) showed correlations between the DG subtest scores and regional vesicular acetylcholine transporter binding in the following regions: dorsolateral prefrontal cortex (BA 8 and 9), right insula, bilateral mesiotemporal, bilateral mid posterior temporal cortex, right fimbriae/isthmus cingulum, right lateral geniculate nucleus, bilateral superior thalami, brainstem (pedunculopontine nucleus - laterodorsal tegmentum (PPN/LTD) and tectum), and right cerebellar hemisphere lobule VI (Figure 5, Table 2). Absent were the gyrus rectus, orbitofrontal regions, cingulum, anteromesial frontal regions, right temporal pole, caudate nuclei, The extent of involved topography was substantially less compared to the anticipatory postural control and reactive postural control Mini-BESTest sub-score correlations. Peak cluster analysis findings (non-corrected at $P < 0.001$) are shown in Supplemental Table 4. Most extensive clusters were centered around the bilateral thalamic complexes, bilateral superior frontal gyri, right hippocampus and left para-hippocampal gyrus regions.

Whole brain vesicular acetylcholine transporter PET voxel-based correlations with sensory orientation subscore

Voxel-based correlation analysis (non-corrected at $P = 0.001$) showed correlations between the sensory orientation subtest scores and regional vesicular acetylcholine transporter binding in the left anterior dorsomedial prefrontal cortex (upper medial BA 10 and BA 9), right more than left dorsolateral prefrontal cortex (BA 8 & 9), bilateral hippocampi, bilateral fimbriae/isthmus cingulum, left caudate nucleus, right more than left lateral geniculate nuclei, left medial geniculate nucleus, left dorsomedial thalamus, right more than left lingual gyrus, brainstem (PPN/LTD and tectum), left cerebellar hemisphere lobule VI, left cerebellar crus I, left more than right superior and left middle cerebellar peduncles, and nodulus (Figure 6, Table 2). Absent were the gyrus rectus, cingulum, operculum, insula, inferior temporal lobes, parietal and visual cortex, flocculus, vermis, and flocculus. The extent of correlated topography was substantially

less compared to the anticipatory postural control and reactive postural control sub-score correlations. Peak cluster analysis findings (non-corrected at $P < 0.001$) are shown in Supplemental Table 5. Most extensive clusters were centered around the bilateral hippocampi, left caudate nucleus and right thalamus regions.

Discussion

Our analysis of four MiniBESTest sub-scores demonstrated shared and unique variance. Whereas the MiniBESTest subscores share a portion of variance with each other, each subscore still carries a distinct portion of variance that is not explained by the others. Our partial correlation analysis of the MiniBESTest domain subscores appears to suggest that anticipatory postural control plays a more central role in dynamic balance and gait impairments in PD than previously recognized, as it was the only domain which was independently predictive of every other MiniBESTest domain in our sample of patients. A prior longitudinal study of gait and balance deficit progression in PD demonstrated that the most pronounced longitudinal decline in MiniBESTest total scores was observed among patients with freezing of gait and was largely driven by decreases in the anticipatory postural control domain subscore.[27] Longitudinal emergence of freezing of gait, on the other hand, was associated with specific declines in sensory and dynamic gait subscores,[27] which we observed were significantly associated with each another and the anticipatory domain subscore, but not the reactive domain subscore. The results of our partial correlation analysis in conjunction with prior work on longitudinal progression of MiniBESTest scores suggest that anticipatory postural control impairments might represent an advanced stage of gait and balance decline in PD patients, which would explain why patients with lower anticipatory domain subscores tended to have a more severe impairment in all the other domains also.

We investigated the uncharted territory of cholinergic system changes associating with impairments on dynamic gait and balance in individuals with PD, assessed using the MiniBESTest. Leveraging the precision of [^{18}F]-FEOBV, a highly selective radiotracer, we uncovered extensive multi-regional correlations between [^{18}F]-FEOBV binding and both the overall Mini-BESTest scores and its functional domain sub-scores. Our findings signify a broader neural network involvement beyond the traditionally established regions associated solely with postural control and gait dynamics. Intriguingly, we observed correlations within brain regions not conventionally linked to these functions, including the right antero-mesofrontal regions, right temporal pole, bilateral fimbria, insula, right cerebellar hemisphere lobule VI, and left cerebellar crus I. Such diverse correlations suggest a more intricate interplay

contributing to the comprehensive motor impairments observed in PD. The following discussion will comprehensively detail both the shared and distinct correlations across total Mini-BESTest scores and functional domain sub-scores, contextualizing these findings within the landscape of previous studies.

Some regional correlations were found across most of the Mini-BESTest subtests, including medial prefrontal cortex, cingulum, gyrus rectus, insula, right frontotemporal operculum, bilateral mesiotemporal lobes, right temp pole, caudate nucleus, metathalami, thalami proper, brainstem, and some cerebellar regions, such as the right cerebellar hemispheric lobule VI, right flocculus, nodulus, and superior cerebellar peduncles. This pattern is enriched in limbocortical and paralimbic cortical regions, caudate nuclei, metathalami, thalami, and specific cerebellar regions. This pattern also implicates degeneration and/or dysfunction of virtually all brain cholinergic systems in dynamic balance and deficits in PD and further highlights the use of the MiniBESTest as a unidimensional assessment for dynamic gait. The limbic and paralimbic deficits may explain the role of the cholinergic forebrain in modulating incoming sensory information related to dynamic postural control and gait functions,[28] since these regions are known to receive extensive cholinergic projections from the basal forebrain Ch4 complex, while also being a major source of reciprocal projections back to the nucleus basalis of Meynert.[29] Striatal cholinergic innervation is dominated by striatal cholinergic interneurons, critical for integrating cortically derived sensory information.[30] Thalamus and metathalamus receive cholinergic inputs from the pedunculopontine - laterodorsal tegmental complex,[31] which may serve as a rapid, alerting channel for salient stimuli,[32] and cerebellar cholinergic innervation derives heavily from the vestibular complex,[33] another critical node for dynamic postural and gait functions.

These results are consistent with prior work. We previously showed that cholinergic deficits of the metathalamus (lateral geniculate nucleus and medial geniculate nucleus) associate with non-episodic postural control deficits (postural imbalance) as well as episodic mobility disturbances, such as falls and freezing of gait.[8,9,34] Cholinergic terminal deficits in the cingulum correlated robustly with anticipatory postural control (more posterior cingulum) and reactive postural control (more anterior cingulum). The cingulum is part of a centro-cingulate network that we previously described associated with cognitive deficits in early PD.[35] Our cumulative data suggest that cingulum cholinergic afferents may play a role in rapid information exchange in both inter-hemispheric and anterior-to-posterior brain communications that are important in neural circuitry underlying anticipatory postural control and reactive postural control functions in PD. The mesiotemporal limbic cortex, including the hippocampus and its fimbria, is known for its role in spatial navigation - crucial for ambulation.[36] We previously also reported a robust association between cholinergic deficits in entorhinal cortex and postural control deficits in PD.[9]

The insular cortex is an important hub for integration of visceral, emotional, motor, and cognitive information.[37] Tinaz *et al*. argued that the insular cortex is an important node for integrating limbic circuit information with basal ganglia motor circuitry.[37] Our findings are consistent with this model. The anterior insula also participates in the salience network function where it contributes to bottom-up control of attentional function and flexible transitioning between brain network states [38] – cognitive faculties which underpin the adaptive control of behavior in uncertain and constantly changing environments.

Consistent with our prior studies of falls and non-episodic imbalance functions in PD,[8,9] we found that the cholinergic terminal deficits of the caudate nucleus, as opposed to the putamen, correlated with all Mini-BESTest sub-tests except dynamic gait in this study. Microdyalisis work in rats suggests that muscarinic cholinergic signalling in the caudate nucleus is necessary for suppression of old stimulus-response contingencies while new ones are being acquired, a cognitive faculty referred to as "reversal learning".[39] Impaired reversal learing under probabilistic-reward behavioral paradigm has been previously reported in PD patients.[40] If adaptation of postural control strategy to novel, challenging environmental conditions depends on a process of suppressing old sensory-motor representations while new ones are being actively acquired and engaged (essentially reversal learning), then it might explain why loss of cholinergic signalling in the caudate nucleus appears to play a central role in postural control impairments of PD.

Our study showed evidence of some cholinergic deficits in brainstem subregions, notably the PPN-LDT and tectum. Superior collicular and inferior collicular deficits may reflect degeneration of highly collateralized PPN-LDT projections also innervating the lateral geniculate nucleus and medial geniculate nucleus, respectively.[31]

Our analysis revealed that the regional correlations observed in Mini-BESTest subtest scores were essentially embedded within the broader pattern of correlated deficits identified in the correlation map associated with the Mini-BESTest total score. Interestingly, several regions correlating with specific subtests had been previously recognized as crucial components within the circuits governing various facets of dynamic gait and postural control. For instance, we found a notable association between lower cholinergic nerve terminal integrity in the anterior section of the dorsomedial prefrontal cortex (specifically, upper medial BA 10 and BA 9) and the reactive postural control and sensory orientation subtests. Notably, the medial prefrontal cortex serves as a central hub within significant large-scale neural networks, such as the default mode network, of which the cholinergic basal forebrain is another more recently recognized hub.[41] The topography of gait and balance related cholinergic deficits observed in the present work appears to overlap with two distinct, but closely related, patterns of cerebello-frontal connectivity previously described in the literature: the motor loop, which links together motor cortex,

thalamus, and anterior cerebellum, and the executive loop, which connects prefrontal cortex, pons, and posterior cerebellum.[42]

Anticipatory postural control

Anticipatory postural adjustments are important components of postural control that prepare for subsequent potentially destabilizing voluntary movements, such as gait initiation. This preparatory phase entails a lateral and posterior shift of the center of mass towards the stance leg followed by a forward shift towards the intended direction of movement,[43] and is thought to be mediated by an interaction of primary and supplementary motor cortices with brainstem locomotor centers, including the mesencephalic locomotor region.[44] Anticipatory postural adjustment modulation is linked to the descending mesencephalic locomotor region pathways to the spinal central pattern generators that are in part controlled by the striatum and cortex.[44] Our findings align with this understanding, revealing a correlation between brainstem activity and anticipatory postural control sub-scores.

Reactive postural control

Reactive postural control is the ability to recover from an unexpected external perturbation to a stable position. Ultimately, reactive postural control determines whether a person will recover from a perturbation or will fall. Bilateral putamina, cerebellum, including the upper anterior cerebellar lobe, vermis and lobule VI, thalamus, and cortical regions (especially the supplemental motor area, paracentral lobule, motor cortex, superior temporal gyrus, and to variable degrees the inferior frontal cortex, anterior prefrontal cortex, insulae, visual cortex, anterior and mid cingulum, fusiform gyrus have been implicated in reactive postural control in fMRI studies.[45]

Dynamic gait

The MiniBESTest dynamic gait score includes various challenged gait conditions, including changes in gait speed while walking, with head turn, walk with pivot turns, step over obstacles and single and dual Timed Up & Go tests.[22] MRI and PET activations of dynamic balance conditions have shown variable involvement of the cerebellum, including the anterior and posterior, vermis, midbrain (red nucleus, mesencephalic locomotor region and pons), thalamus, caudate, putamen, insula, and supplemental motor area, dorsal promotor cortex, and mid cingulum for dynamic balance conditions, including tandem and unipedal stance.[45]

Sensory orientation

Adaptive control of posture and gait relies on effective multisensory integration of afferent sensory signals originating from visual, somatosensory, and vestibular systems (among other), which occurs across multiple levels of the sensory-motor neural processing hierarchy.[46] Relatively more automatic walking is less involved with supratentorial locomotor circuitry and depends largely on descending projections from the brainstem to the spinal cord, which include reticulospinal pathways originating from the lateral part of the mesopontine tegmentum.[44] In contrast, walking in unfamiliar or environmentally more challenging conditions require more cognitive processing of postural control and gait, which depends on knowledge of self-body, such as body schema and body motion in space.[44] Though the thalamus is primarily known for its role in the relay of sensory information to downstream structures, its contributions to early multi-sensory integration of posture related signals are becoming increasingly recognized.[10,46,47] Structural integrity of the hippocampus in healthy older adults has been associated with effective postural control under conditions which depend on effective vestibular-proprioceptive integration.[48] A study on motor imagery of stance was performed on blind and vestibularly-deprived participants, and appeared to suggest the differential involvement of the anterior hippocampal activity in processing of posture-related vestibular information while parahippocampal and fusiform gyrus activity was more strongly implicated in the processing of posture-related visual information.[49] The hippocampus thus seems to play a role in neural processes related to multisensory balance control. Lastly, higher-order sensory integration mechanisms which support the processing of self-location and first-person perspective appear to depend on multi-modal temporo-parietal cortices.[50] The sensory orientation sub-test does involve elements of vestibular sensory conflict tested that previously had robust correlations with cholinergic losses in the medial geniculate nucleus.[51] There is recent evidence that projections of the inferior colliculus in the tectum to the medial geniculate nucleus (and other regions) may play a role in alternative relatively spared motor resilience pathways in PD, such as can be seen with paradoxical akinesia.[52] Other prominently correlated brain regions associated with sensory orientation included the left anterior part of the dorsomedial prefrontal cortex (upper medial BA 10 and BA 9), right more than left dorsolateral prefrontal cortex (BA 8 *and* 9), bilateral hippocampi, fimbriae, left caudate nucleus, right more than left lateral geniculate nucleus and left medial geniculate nucleus, left dorsomedial thalamus, right more than left lingual gyrus, brainstem, nodulus, left cerebellar lobule VI and left crus I, left more than right superior cerebellar peduncles and left middle cerebellar peduncle.

Our findings present compelling evidence indicating cholinergic deficits in brain regions not conventionally linked to postural control and dynamic gait functions. Specifically, we observed

correlation with the right antero-mesofrontal regions, right temporal pole, bilateral fimbria, insula, right cerebellar hemisphere lobule VI, and left cerebellar crus I. These areas, although not traditionally associated with gait and balance, might play a significant role in orchestrating motor functions within the broader spectrum of PD. The involvement of these seemingly unrelated regions underscores the intricate and widespread impact of cholinergic deficits on motor control in PD. This suggests a broader neural network involvement beyond the established regions directly linked to postural control and gait dynamics. The aberrations detected in these regions might contribute to the comprehensive motor impairments witnessed in PD, shedding new light on the multifaceted nature of motor dysfunction in this condition. Plus, these results underscore the importance of employing highly specific radiotracers, like [^{18}F]-FEOBV. Coupled with a comprehensive evaluation of gait and balance, the use of such precise imaging tools becomes imperative to unravel the complex and nuanced role of cholinergic dysfunction in these clinical manifestations within PD. The involvement of these seemingly unrelated regions highlights the need for a more holistic understanding of the neural underpinnings of motor dysfunction in PD, urging a broader exploration beyond established motor control regions.

An unexpected finding in our study was that cholinergic deficits of the right temporal pole were robustly associated with anticipatory postural and reactive postural control. It is possible that connectivity with the hippocampus and processing of emotional information may play a role.[53]

The vestibular system and its cerebellar connections play critical roles in dynamic gait and postural functions. Medial vestibular cholinergic neurons are important inputs to the vestibular cerebellum.[33] Cerebellar cholinergic deficits in this study were variable across the spectrum of the vestibular cerebellum (flocculus, nodulus) with correlated impairments in anticipatory postural control, reactive postural control and sensory orientation. We also found evidence of correlated cholinergic deficits in cerebellar cortical regions. Cerebellar regions included the flocculus, upper vermis and the hemispheric regions. The involvement flocculus and vermis may reflect vestibular and proprioceptive sensory information processing, respectively.[54] The flocculus is known to contribute to the vestibulo-ocular reflex which stabilizes gaze during head rotation.[55] We found that the right flocculus had significant association with reactive postural control alone. The orienting eye velocity of the slow component of the angular vestibulo-ocular reflex also crucially depends on the nodulus and rostral-ventral uvula of the vestibulo-cerebellum function.[56]

Associations with the upper vermis region were present for anticipatory postural control and reactive postural control. This may be in part associated with the cerebellar locomotor region.[44] Cerebellar lobule VI has been implicated as part of neural circuitry involved in spatial and sensorimotor processing.[57] We found evidence that cholinergic lobule VI changes are associated with reactive postural control,

dynamic gait, and sensory orientation in the PD population. Cerebellar lobule VI and crus I may also play a role in visual divergent thinking.[58] Crus I has also been found to be involved with emotional processing implicating cerebellar-limbic circuitry that may be activated with fear of falling in persons with PD and falls.[59]

The wide range of correlated cholinergic deficits, involving all major brain cholinergic systems, is intriguing. It is possible that this reflects redundancy in brain systems underpinning dynamic balance functions. Dysfunction of multiple systems-regions may be needed to markedly disrupt these important functions. Our results imply potential avenues for pharmacologic therapies targeting cholinergic deficits, offering promise for future intervention strategies. Our findings may also support research into novel approaches for the management of dynamic balance and gait changes in PD, including targeting novel cholinergic regions involved in dynamic balance and gait with invasive or non-invasive neurostimulation techniques.

We also found that the extent of involved cholinergic topography was substantially less pronounced for the sensory orientation sub-test compared to the anticipatory and reactive postural control Mini-BESTest sub-scores and did not survive whole brain correction for multiple testing. We conclude that specific dynamic balance and gait deficits are associated with overlapping but also distinct patterns of cerebral cholinergic system changes in widespread brain regions in PD. This is a unique study as this study shows evidence *of novel patterns* of cholinergic deficits involved in dynamic balance and gait disruption in PD, including the right temporal pole, right anterior part of the dorsomedial prefrontal cortex, gyrus rectus, fimbriae, lingual gyrus, cerebellar peduncles, right lobules VI and left crus I, flocculus, and nodulus. Despite substantial cholinergic topographic overlap between anticipatory and reactive postural control subscres our clinicometric analysis demonstrated that ancipatory but not reactive postural control sub-score is a major determinant of dynamic balance and gait in Parkinson's disease.

There are several limitations of this study including its cross-sectional analysis design, which precludes us from making inferences about the relationship between the trajectory of cholinergic system decline and disease progression. PET imaging was performed in the dopaminergic medication 'on' state, which arguably might have influenced our result, though our prior work shows that dopamine D2 receptor agonist intake, which was also associated with ~500 mg higher levodopa equivalent dose on average, did not appear to correlate significantly with [^{18}F]-FEOBV binding in PD patients scanned in 'on' state.[60] Furthermore, the statistical parametric mapping approaches we applied herein included levodopa equivalent dose as a covariate, to control for its possible influence on the FEOBV signal. Lastly, due to the limitations of the [^{18}F]-FEOBV, we can only make inference about the density of pre-synaptic nerve terminals, and not about any specific muscarinic/nicotinic post-synaptic cholinergic signalling targets

engaged by these nerve terminals, which would require other radioligands or pharmaceutical interventions specifically targeting these receptors.

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Data availability

All data are available upon reasonable request.

Competing Interests

The authors report no competing interests and that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure legends

Figure 1. Summary of Mini-BESTest assessments

Figure 2. Whole brain vesicular acetylcholine transporter PET voxel-based correlation analysis and total Mini-BESTest scores.

Whole brain voxel-based correlation analysis t-value statistic image (N = 125, FDR-corrected, P < 0.05) for total Mini-BESTest scores included the following vesicular acetylcholine transporter binding regions: gyrus rectus, orbitofrontal regions, right anterior dorsomedial prefrontal cortex (upper medial BA 10 and BA 9), bilateral dorsolateral prefrontal cortex, all segments of the cingulum, bilateral insula, right more than left frontotemporal operculum, bilateral mesiotemporal cortex, left mid posterior temporal cortex, right more than left fimbriae/isthmus cingulum, right temporal pole, bilateral caudate nuclei, metathalami (lateral and medial geniculate bodies), thalami proper, right parietal cortex, left more than right peri-central cortex, occipital cortex, right more than left lingual gyri, right cerebellar hemisphere lobule VI, left cerebellar crus I, bilateral superior cerebellar peduncles, nodulus, right flocculus, and upper dorsal vermis. No significant correlations were found for the putamen and anteroventral striatum.

Figure 3. Whole brain vesicular acetylcholine transporter PET voxel-based correlations and anticipatory postural control.

Whole brain statistical non-parametric mapping (SnPM) voxel-based analysis pseudo t-value statistic image showed regionally significant false discovery rate (FDR)-corrected (N = 125, P < 0.05) correlations between the Mini-BESTest and anticipatory postural control sub-test scores were seen in the following vesicular acetylcholine transporter binding regions: bilateral gyrus rectus, bilateral orbitofrontal regions, anterior cingulum, bilateral dorsolateral prefrontal cortex (BA 8 & 9), right more than left frontotemporal operculum, right insula, bilateral fimbriae/isthmus cingulum, right temporal pole, bilateral caudate nuclei, lateral and medial geniculate bodies, thalami proper, bilateral occipital cortex, right more than left lingual gyri, left precentral cortex, brainstem (mesencephalic tectum), right cerebellar hemisphere lobule VI, bilateral superior cerebellar peduncles, nodulus, and upper dorsal vermis. No significant correlations were seen for the putamen, anteroventral striatum, right parietal cortex, and the flocculus.

Figure 4. Whole brain vesicular acetylcholine transporter PET voxel-based correlations and reactive postural control.

Whole brain statistical non-parametric mapping (SnPM) voxel-based analysis pseudo t-value statistic image showed regionally significant FDR-corrected (N = 125, $P < 0.05$) correlations between vesicular acetylcholine transporter PET and the reactive postural control sub-tests scores in the bilateral gyrus rectus, bilateral orbitofrontal cortex, right more than left anterior parts of the dorsomedial prefrontal cortex (upper medial BA 10 and BA 9), bilateral dorsolateral prefrontal cortex (BA 8 & 9), anterior cingulum, left more than right frontotemporal operculum, bilateral insula, bilateral mesiotemporal lobes, bilateral fimbriae/isthmus cingulum, left peri-central cortex, right temporal pole, inferior temporal lobes, left mid and posterior temporal lobe, bilateral caudate nuclei, lateral and medial geniculate nuclei, left pulvinar, right parietal cortex, right flocculus, nodulus, bilateral superior cerebellar peduncles. Notably absent were the putamen, anteroventral striatum, right cerebellar lobule VI, brainstem, and visual cortex.

Figure 5. Dynamic gait scores.

Exploratory statistical non-parametric mapping (SnPM) whole brain voxel-based analysis pseudo t-value statistic image (N = 125, non-corrected at $P = 0.001$) showed correlations between the dynamic gait scores and vesicular acetylcholine transporter expression in the right dorsolateral prefrontal cortex (BA 8 & 9), right insula, bilateral mesiotemporal, bilateral mid posterior temporal cortex, right fimbriae/isthmus cingulum, right lateral geniculate nucleus, bilateral superior thalami, brainstem (PPN/LTD and tectum), and right cerebellar hemisphere lobule VI. Notably absent were the gyrus rectus, orbitofrontal regions, cingulum, anteromesial frontal regions, right temporal pole, caudate nuclei, The extent of involved topography was substantially less compared to the anticipatory and reactive postural control Mini-BESTest sub-scores.
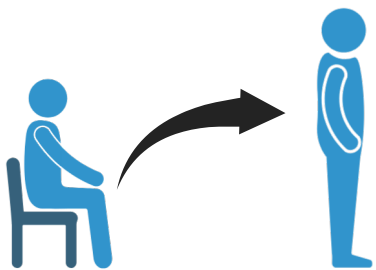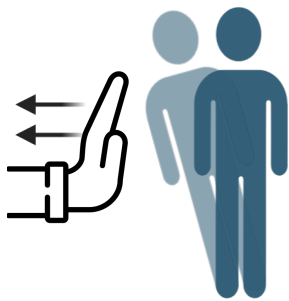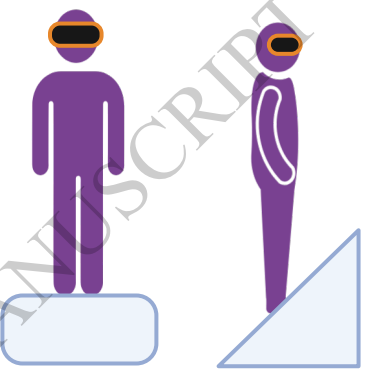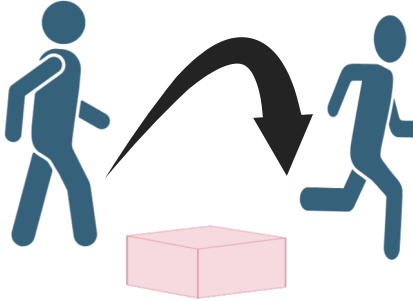
Figure 6. Sensory orientation.

Exploratory whole brain statistical non-parametric mapping (SnPM) voxel-based analysis pseudo t-value statistic image (N = 125, non-corrected at $P = 0.001$) showed regional correlations between the sensory orientation scores and vesicular acetylcholine transporter expression in the left anterior part of the dorsomedial prefrontal cortex (upper medial BA 10 and BA 9), right more than left

dorsolateral prefrontal cortex (BA 8 & 9), bilateral hippocampi, bilateral fimbriae/isthmus cingulum, left caudate nucleus, right more than left lateral geniculate nuclei, left medial geniculate nucleus, left dorsomedial thalamus, right more than left lingual gyrus, brainstem (PPN/LTD and tectum), left cerebellar hemisphere lobule VI, left cerebellar crus I, left more than right superior and left middle cerebellar peduncles and nodulus. Notably absent were the gyrus rectus, cingulum, operculum, insula, inferior temporal lobes, parietal and visual cortex, flocculus, vermis, and flocculus.

Anticipatory postural adjustments Scored: 0-6	Reactive Postural Control Scored: 0-6	Sensory Orientation Scored: 0-6	Dynamic Gait Scored: 0-10
Self-initiated change in base of support	Reaction to unexpected perturbation caused by removal of external support	Maintaining balance under different sensory conditions	Maintaining balance during various gait conditions
1) Sit to stand (0-2) 2) Rise to toes (0-2) 3) Stand on one leg left and right (0-2)*	1) Compensatory Stepping Correction Forward (0-2) 2) Compensatory Stepping Correction Backwards (0-2) 3) Compensatory stepping correction lateral (0-2)*	1) Eyes Open Firm Surface (0-2) 2) Eyes Closed Foam Surface(0-2) 3) Eyes Closed Incline (0-2)	1) Change in Gait Speed (0-2) 2) Walk With Head Turns Horizontal (0-2) 3) Walk With Pivot Turns (0-2) 4) Step Over Obstacles 5) Timed Up and Go with Dual Task (3 Meter walk) (0-2)

Score determination:
2= Normal
1= Moderate
0= Severe

*Scores are calculated by taking the side left or right with the lowest numerical score

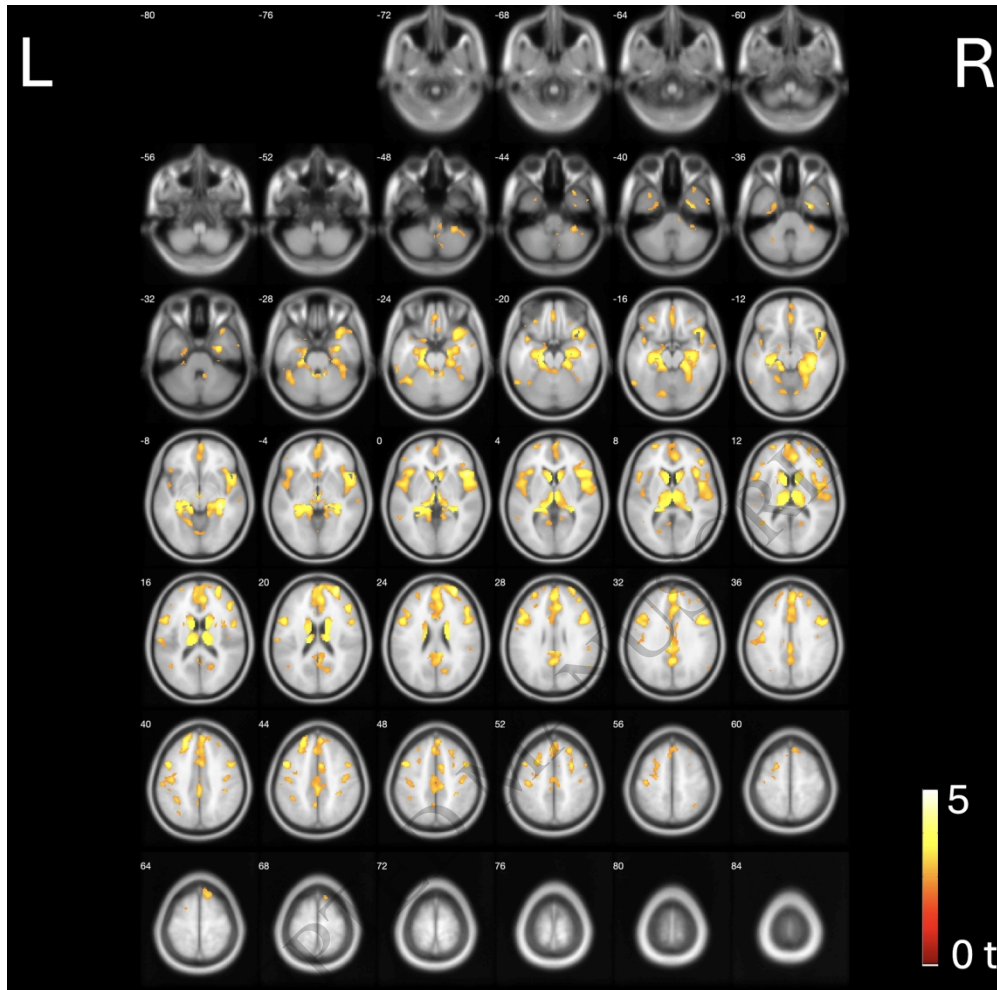FIGURE 2: Mini-BESTest Total FDR < 0.05

278x274mm (300 x 300 DPI)
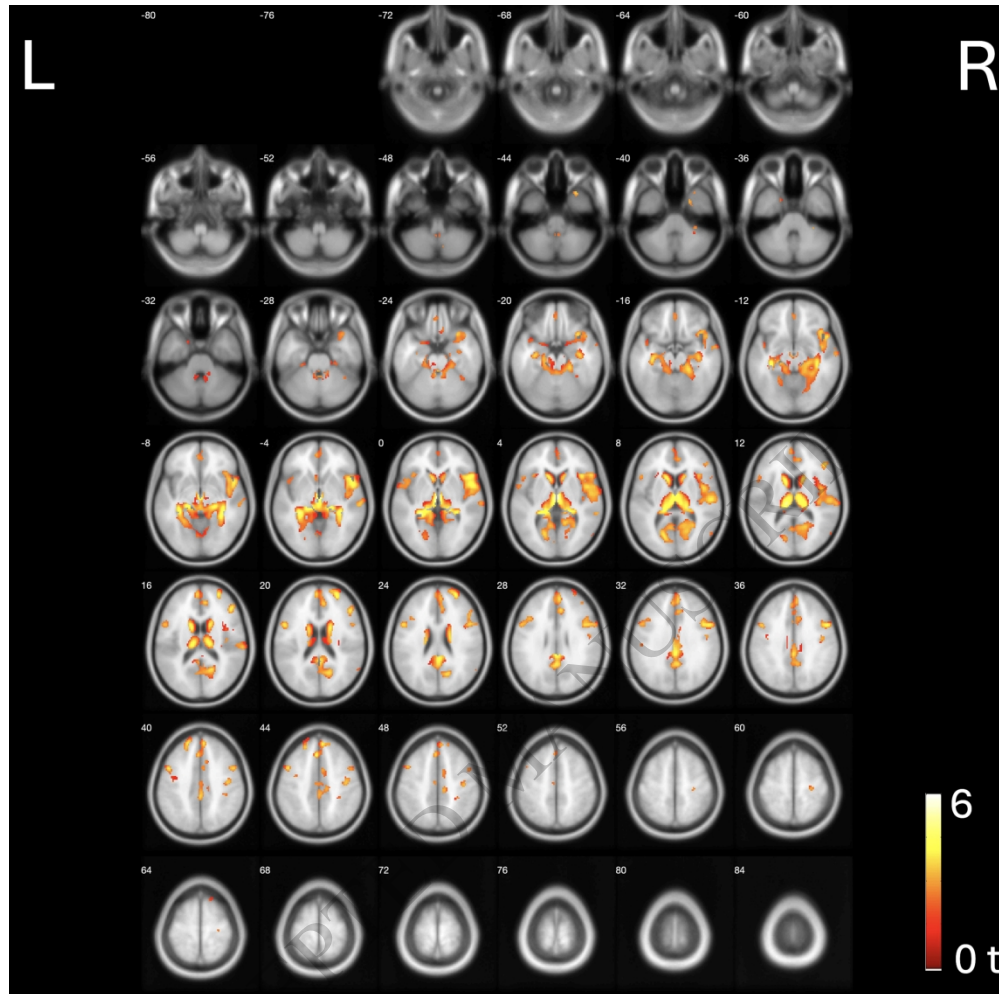
FIGURE 3: Mini-BESTest Anticipatory Postural Control (APC) FDR < 0.05

278x276mm (300 x 300 DPI)
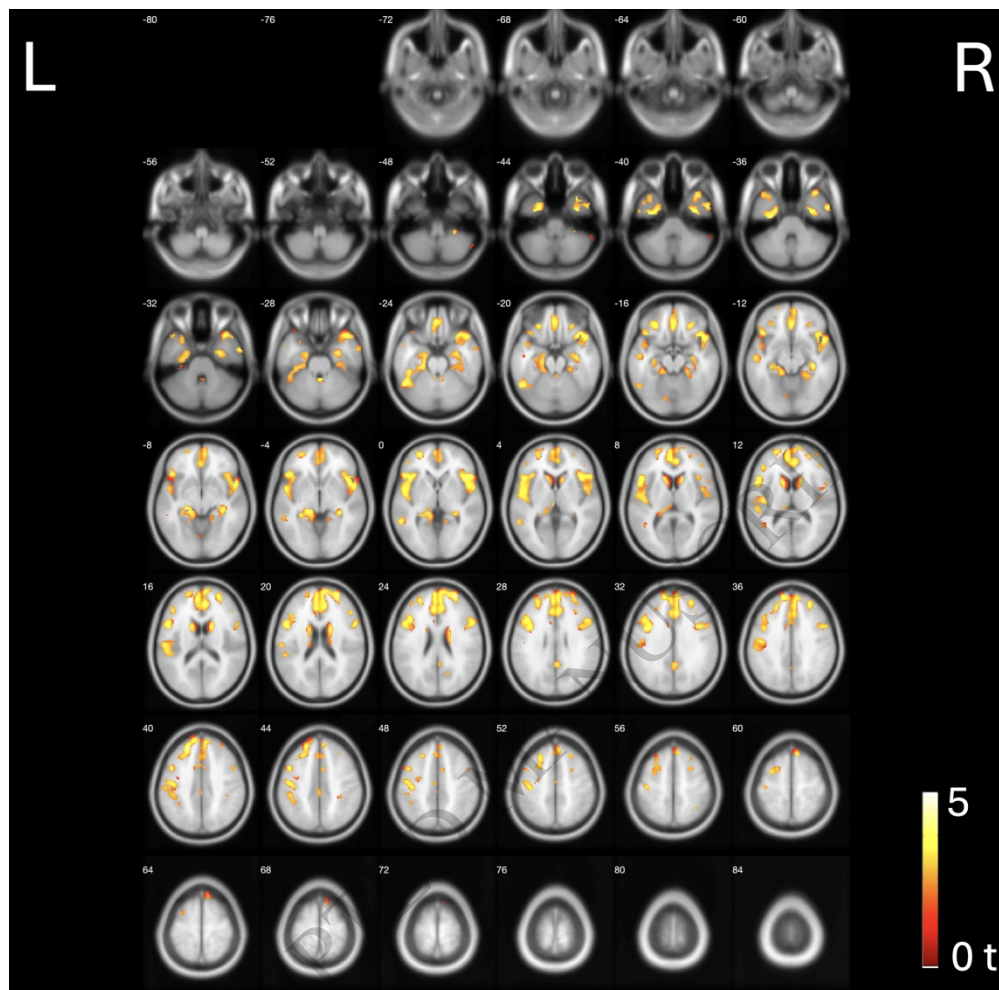
FIGURE 4: Mini-BESTest Reactive Postural Control (RPC) SnPM FDR < 0.05

278x274mm (300 x 300 DPI)
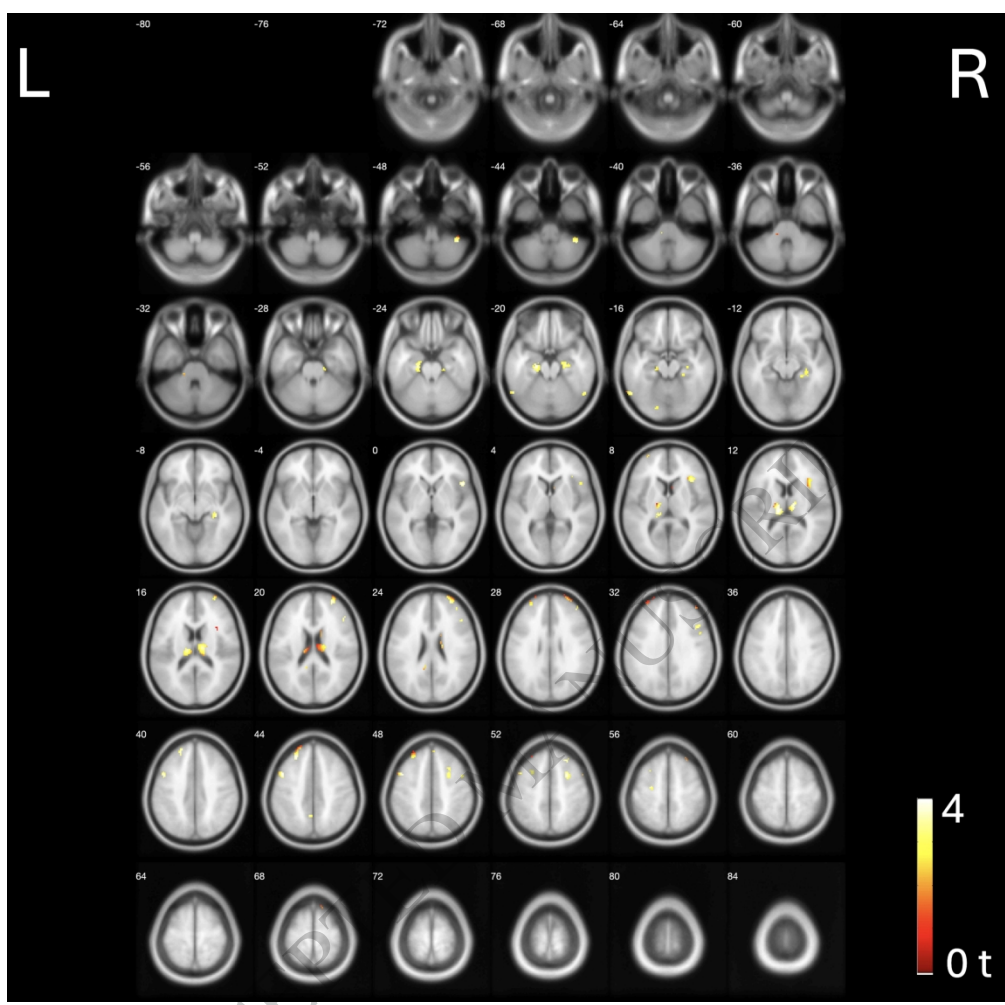
FIGURE 5: Mini-BESTest Dynamic Gait Uncorrected P=0.001

278x275mm (300 x 300 DPI)
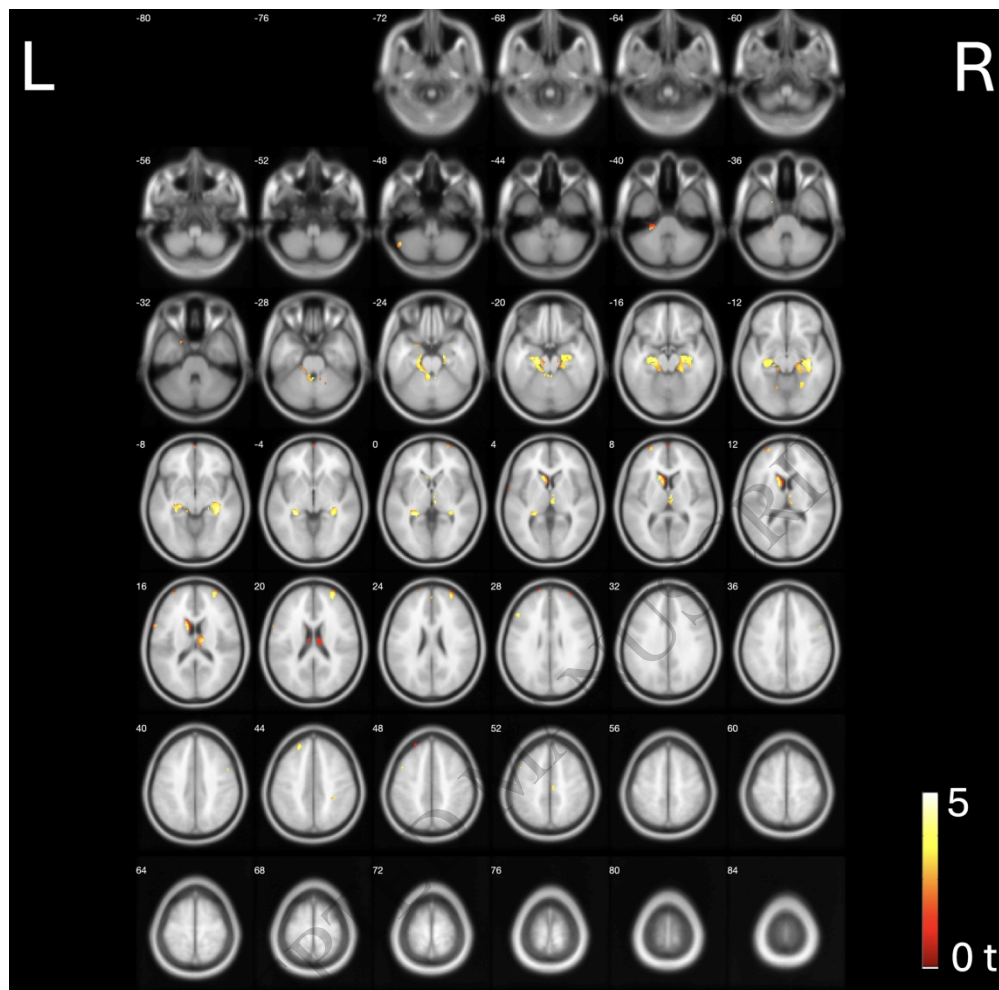
FIGURE 6: Mini-BESTest Sensory Orientation Uncorrected P=0.001

278x274mm (300 x 300 DPI)

Table 1. MiniBEST subscore partial correlation matrix table with proportion of variance captured in each subscore by the other subscores

MiniBEST Subscore	R^2	$\beta_{Anticipatory}$	$\beta_{Reactive}$	$\beta_{DynamicGait}$	$\beta_{Sensory}$
Anticipatory	0.481		0.214* [0.064,0.364]	0.214* [0.049,0.378]	0.407* [0.239,0.575]
Reactive	0.303	0.287* [0.085,0.49]		0.156 [-0.037,0.35]	0.197 [-0.013,0.407]
Dynamic Gait	0.41	0.243* [0.056,0.43]	0.132 [-0.032,0.296]		0.374* [0.19,0.557]
Sensory	0.501	0.391* [0.23,0.553]	0.141 [-0.009,0.291]	0.316* [0.16,0.471]	

Standardized regression coefficients for which the difference from 0 is statistically significant (as defined by coefficient P < 0.05) are bolded and marked with an asterisk.
Abbreviations: R^2 = coefficient of determination; β = standardized regression coefficient
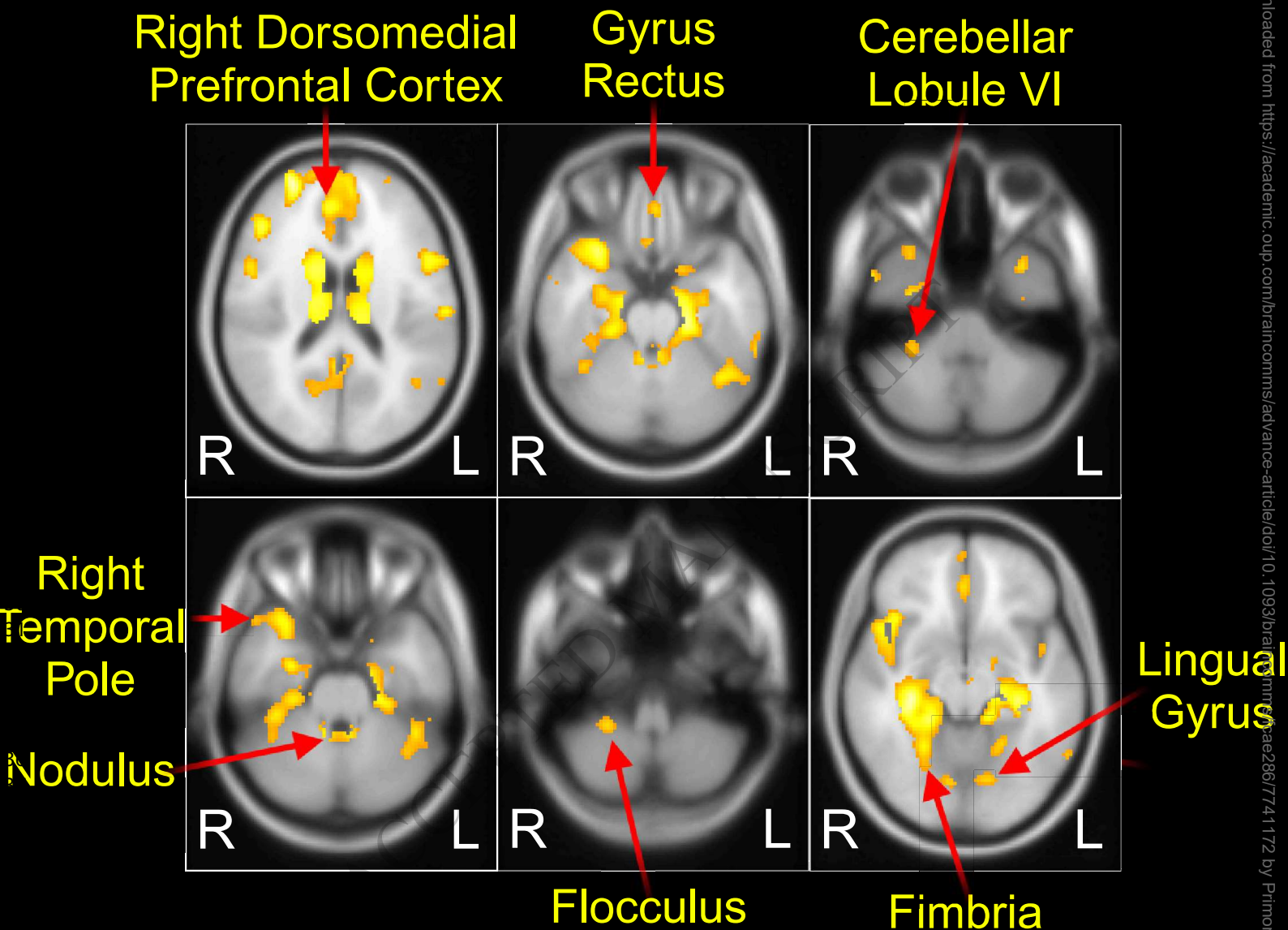
Table 2. Summary overview of topographic system changes associating with Mini-BESTest total and sub-test scores.

FEOBV PET region	Total Mini-BESTest	APC	RPC	Dynamic gait	Sensory orientation
Gyrus rectus/orbitofrontal	B	B	B	-	-
Anterior part of the dorsomedial prefrontal cortex (upper medial BA 10 and BA 9)	R	B	R>L	-	L
Dorsolateral prefrontal cortex (BA 8 & 9)	R>L	R>L	B	R	R>L
Cingulum	all segments esp. R>L anterior and anterior mid cingulum	esp. posterior & retrosplenial segments	mainly anterior segments	equivocal posterior	-
Frontotemporal operculum	R>L	R>L	L>R	-	-
Insula	R>L	R>L	B	R	-
Mesiotemporal lobe	B	R	B	B	B (esp. hippocampi)
Fimbria	R>L	B	B	R	B
Temporal pole	R	R	R	-	-
Inferior temporal lobe	-		B	-	-
Mid posterior temporal lobe	L	R	L	B	
Caudate nucleus	B	B	B	-	L
Anteroventral striatum	-	-	-	-	-
Putamen	-	-	-	-	-
LGN & MGN	B	B	B	R LGN	R > L LGN & L MGN
Thalamus proper	B	B	L pulvinar	B superior	L dorsomedial
Parietal cortex	R	-	+	-	-
Visual cortex	B	R>L	-	-	-
Lingual gyrus	R>L	R>L	-		R>L
Brainstem (pedunculopontine nucleus/tectum)	-	+	-	+	+
Cerebellum flocculus	R +	-	R	-	-
Cerebellum nodulus	+	+	+	-	+
Vermis	dorsal upper vermis	dorsal upper vermis	left dorsal upper	-	-
Cerebellum hemispheres	R VI +, L crus I	R VI +	Equivocal	R VI +	L VI + L crus I
Cerebellar peduncles	B superior	B superior	B superior	-	L>R superior & L middle

[a]Abbreviations: APC = anticipatory postural control; BA = Brodmann area; B= bilateral, MGN = medial geniculate nucleus; L= left, LGN = lateral geniculate nucleus; R= right, RPC= reactive postural control.

Right Dorsomedial Prefrontal Cortex

Gyrus Rectus

Cerebellar Lobule VI

Right Temporal Pole

Nodulus

Flocculus

Fimbria

Lingual Gyrus

NEWLY RECOGNIZED CHOLINERGIC REGIONS ASSOCIATING WITH DYNAMIC BALANCE & GAIT IN PARKINSON'S DISEASE