Bohnen Nicolaas Ida (Orcid ID: 0000-0003-0352-6414)
Hallett Mark (Orcid ID: 0000-0002-3180-6811)
Nieuwboer Alice (Orcid ID: 0000-0003-1193-6229)
Nutt John (Orcid ID: 0000-0003-2759-1106)
Kang Un Jung (Orcid ID: 0000-0002-5970-6839)

Discussion of Research Priorities for Gait Disorders in Parkinson's Disease

Nicolaas I. Bohnen MD, PhD[1], Rui M. Costa DVM, PhD[2], William T. Dauer MD[3], Stewart A. Factor DO[4], Nir Giladi MD[5], Mark Hallett MD, DM(hon)[6], Simon J. G. Lewis MBBCh, BSc, FRCP, FRACP, MD[7], Alice Nieuwboer PhD[8], John G. Nutt MD[9], Kaoru Takakusaki MD, PhD[10], and Un Jung Kang MD[11], Serge Przedborski MD, PhD[12], Stella M. Papa MD[13*] and other members of the MDS-Scientific Issues Committee.

[1] Departments of Radiology & Neurology, University of Michigan and VA Ann Arbor Healthcare system, Ann Arbor, MI, USA.
[2] Departments of Neuroscience and Neurology, Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY, USA.
[3] Departments of Neurology and Neuroscience, and the Peter O'Donnell Jr. Brain Institute, UT Southwestern, Dallas TX USA.
[4] Jean and Paul Amos Parkinson's disease and Movement Disorders Program, Emory University School of Medicine, Atlanta, GA USA.
[5] Movement Disorders Unit, Department of Neurology, Tel-Aviv Sourasky Medical Center, Sackler School of Medicine and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel.
[6] Human Motor Control Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA.
[7] ForeFront Parkinson's Disease Research Clinic, Brain and Mind Centre, School of Medical Sciences, University of Sydney, NSW, Australia.
[8] Department of Rehabilitation Sciences, KU Leuven, Belgium.
[9] Movement Disorder Section, Department of Neurology, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97034, USA.
[10] Department of Physiology, Section of Neuroscience, Asahikawa Medical University, 2-1-1-1, Midorigaoka-Higashi, Asahikawa, Hokkaido, 078-8510, Japan.
[11]Departments of Neurology, Neuroscience and Physiology, Neuroscience Institute, The Marlene and Paolo Fresco Institute for Parkinson's and Movement Disorders, The Parekh Center for Interdisciplinary Neurology, New York University Grossman School of Medicine, New York, NY, USA.
[12]Departments of Pathology & Cell Biology, Neurology, and Neuroscience, Columbia University, New York, NY, USA.
[13]Department of Neurology, School of Medicine, and Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA.

[*]Correspondence: Dr. Papa, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA; spapa@emory.edu

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Abstract

Gait and balance abnormalities develop commonly in Parkinson's disease and are among the motor symptoms most disabling and refractory to dopaminergic or other treatments including DBS. Efforts to develop effective therapies are challenged by limited understanding of these complex disorders. There is a major need for novel and appropriately targeted research to expedite progress in this area. The Scientific Issues Committee of the International Parkinson and Movement Disorder Society has charged a panel of experts in the field to consider the current knowledge gaps and determine the research routes with highest potential to generate groundbreaking data.

Introduction

Gait and balance disturbances play an important role in the quality of life, morbidity, and mortality of patients with Parkinson's disease (PD). The current pharmacological and surgical treatments, albeit helpful with other aspects of PD, provide modest benefit, leaving patients with gait impairment, postural instability and frequent falls[1-4]. Understanding these complex disorders has long stimulated research giving rise to a large collection of original studies and reviews. Modern imaging technologies and experimental models have contributed considerable information to analyzing the function of neural circuits in the control of posture and the generation of locomotion patterns[5, 6]. However, review of the prolific literature also reveals a series of shortcomings in the analysis of any type of data, i.e., demographics, imaging, kinematics, and drug or surgical trials. Most studies suffer from variability in the definition or classification criteria of freezing or other gait and postural abnormalities[7-9]. In addition, the characteristics of these disorders, some with episodic presentation or varied features, pose significant challenges for standardized assessment in clinical studies. In the experimental field, these disorders have not been reproduced well in Parkinson models, clearly a major limiting factor for research progress. As a result, our understanding of the underlying biology of gait and balance abnormalities in PD, a significant source of disability for the majority of patients, remains insufficient to develop effective therapeutic approaches.

The loss of postural control and altered locomotion patterns in PD typically cause impaired balance, instability, falls, slow walking with reduced step length, en bloc turns, festination, and freezing of gait (FOG) (see reviews in[10-12]). Festination is defined as accelerated stepping, which can be related to inability to adjust the size of steps to the progressively increased body inclination along with loss of control of body position during walking. FOG is defined as inability to progress despite attempts to walk. FOG is characterized by a brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk. Impaired balance is the consequence of abnormal reactive and anticipatory postural responses producing instability and propensity to fall. Anticipatory postural adjustments (APA) are also thought to impact locomotion patterns, and plausibly play a role in freezing, although APA in the context of FOG may have different biological substrates than in postural instability related to falls. However, the present discussion will be centered on FOG and impaired postural control in its multiple dimensions as the major gait disorders in PD, and they will be treated together for the purpose of identifying common research directions. Numerous studies have proposed different mechanisms that could presumably be involved in such gait disorders. However, most of the clinical data are correlational, and the scarce non-human experimental data have only made incremental progress. Therefore, the focus of this panel discussion is on research directions to address the critical knowledge gap–the "pathophysiology" of postural control and FOG in PD. We begin by summarizing the neural substrates thought to play a mechanistic role in order to discuss research strategies to advance our current knowledge. Subsequently, such strategies will be discussed with examples of potential experimental approaches.

Discussion of the current knowledge on pathophysiology

A large network of cortical, basal ganglia, thalamic, cerebellar, brainstem, and spinal circuits participates in the mechanisms underlying gait disorders in PD[13-16]. Whether symptoms are related to a generalized breakdown in this large network or a localized dysfunction affecting a particular circuit is not clear. Figures 1 and 2 show a simplified representation of this multiple circuit network.

In our discussion of the functional anatomy of locomotion in humans, we begin with the central pattern generator (CPG) circuits in the spinal cord, which, as reminded by Griller and El Manira[17], are essential not only for the propulsive component of locomotion, but also for a variety of other aspects of this complex motor behavior, including its rhythmicity and coordination. These spinal cord CPG circuits are not merely segmental reflex loops made of proprioceptive afferents synapsing on alpha-motor neurons, but real processing interface hubs that control the timing, speed and strength of different muscle contractions and integrate the sensory feedback and instructive signals from a variety of supraspinal neural pathways localized in the brainstem and above to enable initiation, termination and direction of locomotion as well as to set the posture to follow the movements[17]. Moreover, once locomotion is initiated, spinal cord CPG circuits signal to the brainstem and cerebellum, which, in turn, send information back to the spinal cord to allow rapid corrections of locomotion and posture[17]. The picture that emerges from this succinct description is that the spinal cord CPG circuits play a key role in converting non-patterned commands originating from supraspinal locomotor centers into well-coordinated, rhythmic movements and participate into reciprocal neural circuits that enable real-time adjustments of the movements and posture to perturbations occurring during locomotion.

Among key supraspinal nodes is the ***mesencephalic locomotor region*** *(MLR),* which has multiple connections to other significant nodes in this network. The MLR includes the pedunculopontine nucleus (PPN), the lateral cuneiform nucleus and the surrounding midbrain area. It is important to recognize the complexity of the MLR anatomy and connectivity, and that different cell types and circuits in this region likely have distinct functions. Although not fully elucidated, the MLR may play a role in initiating and controlling locomotion via descending projections modulating brainstem nuclei involved in locomotion and lower spinal circuits, but also ascending projections controlling dopaminergic systems and arousal[18]. For example, in mouse models, cuneiform glutamatergic neurons seem to be involved in high-speed locomotion, as are glutamatergic neurons in the caudal medulla (lateral paragigantocellular nucleus)[19, 20]. However, the mechanisms of exploratory and escape locomotion seem to be controlled by distinct neuronal populations of MLR areas[19]. Studies of MLR stimulation have been associated with activation, modulation or inhibition of locomotion[21-23] depending on the specificity of the targeted area. More recently, studies of PPN neurons with high cell resolution revealed that activation of glutamatergic neurons controls muscle tone and posture, and disrupts gait[18, 23]. Of note, PPN DBS has been reported to improve FOG in patients with PD, although data remain controversial[24, 25]. Glutamatergic and cholinergic PPN neurons (ChNs) influence multiple spinal and cerebellar regions[26]. Glutamatergic PPN neurons project downstream to other brainstem motor centers but also project to SNc dopamine neurons and can modulate the phasic activity of dopamine neurons and DA levels[27-29]. ChNs project to vestibular cerebellar circuits (medial vestibular nucleus), thalamic nuclei, and dopaminergic neurons in the midbrain (both SNc and VTA), and to the medioventral medulla, which projects into the reticulospinal tract [30]. In turn, the PPN is innervated by basal ganglia output nuclei,

which are GABAergic, namely internal pallidum and substantia nigra pars reticulata. This suggests that PPN disinhibition is involved in locomotion, but the PPN also receives excitatory inputs from other regions (STN, cortex, midbrain) and the functional heterogeneity of PPN neurons complicate the interpretation of its control of locomotion. In addition to locomotion, the PPN is also involved in the arousal attention process in animal behavior, and has oscillatory activity in the gamma band frequency that correlates with both stepping and arousal [31]. On the other hand, the reported morphological (ChN loss) or physiological changes of PPN/MLR in human postmortem studies and PD models have been largely variable and inconclusive[32-34].

Basal ganglia contribute to mechanisms of gait disorders through the modulation of descending midbrain and brainstem circuits but also through the modulation of thalamic and cortical circuits (Figure 1). However, a significant amount of clinical data is in conflict with a primary role of traditionally postulated basal ganglia mechanisms in gait disorders of PD. DBS of the subthalamic nucleus (STN) has shown variable effects on gait disorders [13,35], although various studies reported FOG improvement (see data review in[36]). Global motor effects of STN DBS including the reduction of "off" periods likely play a role in FOG changes[37]. While the occurrence of FOG is typically reduced by dopaminergic drugs, in many cases they do not help, and in a minority of patients these drugs worsen FOG[38]. Notably though, studies of the biomechanical components of anticipatory postural adjustments (APA) in gait initiation show changes that correlate with dopamine depletion in the striatum [39-41]. Nevertheless, abnormalities of APAs in FOG have not consistently been established across clinical laboratories[42]. These clinical observations thus suggest that the nigrostriatal dopamine system, albeit a major contributor, may not be the only system involved [16]. This is also supported by the

occurrence of similar gait disturbances in neurological disorders with spared dopamine system. Dopamine projections to various basal ganglia nuclei, and even to brainstem circuits could play a role, but a relation to non-dopaminergic mechanisms may be highly relevant as they become further compromised with disease progression [43]. Postural adjustments, the sequence effect, and small steps are particularly important for various walking conditions, such as turning, and frequently trigger FOG. There is evidence from animal studies that striatal neurons participate in the control of both locomotion and turning [44]. It is important to consider that abnormal patterns of activity from basal ganglia output to brainstem regions could play a key role in FOG mechanisms. In PD, neuronal activities in the internal pallidum/substantia nigra pars reticulata undergo significant changes including excessive synchronization with pathological (beta band) oscillations[45]. Such changes in these inhibitory projections to the brainstem locomotor areas may disrupt locomotion patterns and postural adjustments and, depending on the behavioral context, ultimately cause stepping blocks (FOG).

Dysfunction in various nodes of the large network controlling posture and locomotion could be compensated by processing of sensory inputs through the ***thalamus***, particularly visual, vestibular and proprioceptive information during postural changes. Molecular imaging studies have shown thalamic and metathalamic changes in patients with PD that correlate with falls and FOG[46]. Therefore, thalamic circuits participating in the control of body posture may underlie inadequate postural adjustments that trigger FOG in PD, for example during turning[47]. It is important to note that these mechanisms may play a role in locomotor paradigms that are typically triggering FOG, i.e., gait initiation, turning, doorway, etc. Connections of the medial thalamic nuclei with cortical associative and limbic areas are enhanced with the appearance of

FOG in patients with PD[48]. The increased coupling to those non-motor cortical areas has been interpreted as compensation to deficient sensorimotor integration or reinforcement of malfunctioning circuits[49]. The ***cerebellum,*** a major player in postural control, may also be involved in FOG mechanisms and FOG triggering. Turns are a common trigger of FOG, and clearly, the postural control of turning could be a lead mechanism[50]. In particular, the vestibular system, including the cholinergic vestibular projections to the cerebellum (vermis, flocculonodular lobules) are thought to be a significant contributor based on PET imaging studies in patients with PD where the onset of gait disorders was associated with cerebellar and metathalamic cholinergic changes, including the medial geniculate body[51]. The medial geniculate body has intrinsic involvement with processing of ponto-cerebellar vestibular information that is critical for postural control and navigation. Using a vesicular acetylcholine transporter ligand (VAChT; ^{18}F-FEOBV) for PET, it is now possible to visualize distinct and prominent uptake in the flocculus, nodulus and vermis regions[52]. The flocculo-nodular lobules also known as the vestibular cerebellum (also including part of the vermis) are the regions of the cerebellum that receive vestibular and visual information. The flocculo-nodular lobules are involved in vestibular reflexes, eye movements and balance. For example, the cerebellar flocculus is intimately related to the control of compensatory eye movements, providing stabilization of the retinal image during involuntary head rotation[53]. The cholinergic system in the cerebellar flocculus exerts a neuromodulatory effect on vestibular-ocular reflexes, and may be involved with vestibular compensation[54, 55]. Animal studies have identified that cerebellar cholinergic projections may originate in the medial vestibular nucleus, directly innervating both the cerebellar cortex and cerebellar nuclei[53]. However, in spite of the strong rationale and the

reported connectivity changes of thalamic[56] and cerebellar[57] regions in PD, data to ascertain the role of these network nodes in FOG mechanisms are lacking.

Breakdown of ***cortical*** circuits could cause decoupling of the nodes executing the gait program when the subject tries to initiate gait or during walking. Frontal regions including the SMA and PMA may also participate in the generation of automatic motor patterns of gait [58]. The fact that dual tasking is another typical trigger of FOG[9, 59, 60] suggests that deficits in associative cognitive areas contribute to disrupting gait automation[61, 62]. Altered cognitive management of tasks and automation could thus cause FOG in various behavioral contexts. Notably, attentional and emotional factors that may cause disengagement of motor cortical circuits are known to precipitate freezing episodes. Likely, the '*limbic system'* and its multiple cortical connections influence the coupling and uncoupling of automated programs. Neuroimaging studies have shown changes in limbic areas in patients with PD and FOG[63, 64]. Clinical data supporting cortical involvement in FOG also derive from studies using transcranial magnetic stimulation (TMS) and electrophysiology[65, 66]. The TMS studies particularly implicate the SMA[67] and the prefrontal cortex[68] as relevant for understanding and treating FOG[69].

Postural instability, a cardinal symptom of PD that may also develop as part of the brain aging process, may evolve to significant balance dysfunction as disease progresses. Both cortical (various areas) and subcortical circuits may contribute to loss of balance, particularly in aging patients. Experimental lesions of cortical cholinergic terminals that induce '*attention deficits*' can increase the propensity to fall in rodents with combined lesions of striatal dopaminergic terminals [70]. Freezing with the same characteristics as in patients with PD has not been

observed in these animals, but stance and walking instability frequently coexist with FOG in patients with PD, which may result from different or common underlying mechanisms. Patients with frontal lesions may have a "frontal gait disorder" that includes FOG [71-73]. Likely, multiple cortical circuits involving attention, emotion, and associative functions play a role in both postural control and locomotion automation processes (Figure 2). Cognitive decline has been largely implicated in gait disorders mostly based on correlation data in clinical studies [74, 75], but experimental tests of disrupted frontal circuits are lacking.

Finally, it is critical to consider that the aforementioned nodes involving different brain regions may be partial contributors to the altered mechanisms of postural control and locomotion. A major consideration should be given to the robust ***basal ganglia output*** to brainstem regions that control locomotion[76], as an essential part of the network affected in PD. Presumably, the altered pattern of activity of the basal ganglia output nuclei in PD may also affect projections to the MLR/PPN creating functional changes in the core control of gait and posture. Such changes could be compensated by multiple mechanisms in the network that also influence these brainstem regions[77], but those mechanisms may become compromised as the disease evolves. Therefore, FOG and loss of balance may be governed by dopamine depletion with the addition of dysfunction in glutamate, GABA and other signaling mechanisms participating in the large network of posture-gait control (Figure 2).

Filling the Gaps, Research Strategies

As discussed above, the large motor network controlling posture and locomotion spans from the spinal cord to the cortex including several midbrain and subcortical circuits, and is also influenced by other networks (non-motor systems), rendering the study of underlying mechanisms of gait disorders highly complex. Furthermore, different components of this network and multiple systems could be affected due to the extended neurodegenerative process or functional changes in PD. The available clinical and experimental data support a combination of dysfunctional circuits rather than a single node mechanism disrupting the whole network [12, 13, 78]. However, this notion as well as further data interpretations into underlying pathophysiology are still untested hypotheses. After discussing the types of data generated thus far, it became clear that there is a major need for progress from the past associative/observational studies to novel *"functional/operative"* studies. *It is necessary to design experimental models with dynamic operating features to test circuit activity and interactions inside and outside the motor network in different behavioral contexts.* These types of models rely on current technological advances, which provide a variety of tools to selectively challenge a circuit or cellular population with high resolution and even microscale time precision [79-81]. In addition, a combination of different probes (biochemical, electro-physiological, genetic, imaging) should be applied to studies in experimental models for rigorous testing of candidate mechanisms. Also important, inferences derived from these experimental models require further analysis in patients with gait disorders. In the clinical phase, the design of appropriate tests in patients is key to reveal the mechanisms analogous to human motor behavior. *Therefore, our analysis favors research strategies that are based on developing hypothesis-driven, dynamic studies to manipulate circuits in the posture/gait network and their connections to other networks*–a *systems neuroscience strategy*. *A sequential*

approach from experimental to clinical tests should also be taken in order to extrapolate model findings to disease dysfunction.

Such "*multi-scale approach"* has two components, a preclinical modeling/testing and a clinical analysis, that are interdependent. Modeling can be refined based on the analysis of clinical information (reversed translational approach[82-84]), and discoveries from animal models need to go back to human testing for validation. A key aspect for this scheme is to apply modern strategies of systems biology[85] analyzing big datasets to study gait disorders in patients with PD. *The clinical component of this multi-scale approach, thus, needs to innovate with a "systems biology strategy", which applies "big data" analyses from a variety of sources (transcriptomics, metabolomics, proteomics, etc.) combined with physiological and imaging data to draw inferences from multiple interacting systems.* Such analyses may identify pathway/node/system associations by common indicators from different data sources in a way that is similar to multivariate principal component analyses–PCA based algorithms used in many applications to analyze covariance in big datasets[86]. These new insights from systems biology (clinical analyses) could be a powerful tool to refine modeling and advance the preclinical assessment of hypotheses (systems neuroscience). The ultimate dissection of a mechanism will also require a bidirectional approach, which includes a clinical test/validation of the model findings. Summarizing, we therefore propose to prioritize the following path to advance our understanding of gait disorders in PD:

- A multi-scale approach based on systems biology strategies in clinical studies and associated experimental modeling.
- Systems neuroscience strategies to develop testable experimental models of the interacting circuits and networks that control gait and postural balance and may be

functionally impacted in PD. These strategies should use advanced technologies and analysis of multiple measures to address discrete mechanistic hypotheses.

- A human testing phase related to the designed modeling studies consisting of adaptive paradigms to investigate model findings in patients and validate them as pathophysiologic mechanisms in PD.

There are many examples of the potential of systems biology strategies to shed light on molecular, cellular and circuit pathways involved in neuropsychiatric disorders. To provide an overview of the potential of the systems neuroscience strategy, next we will exemplify its application for addressing two known hypotheses on the mechanisms of gait disorders in PD, i.e.: (I) lack of motor automation associated with cognitive and attentional deficits, and (II) altered postural control and sensorimotor integration

A. Motor automation with cognition/attentional deficits.

- ***Phase 1, Modeling.*** Dopamine depletion in PD may alter the execution of complex motor programs, and likely those that are automated such as locomotion[87]. It is critical that we understand the descending circuits controlling locomotion, and how basal ganglia output can control these circuits. It is also critical to determine where in the brain dopamine depletion causes problems in locomotion, if at all. Deficiencies in execution could be compensated by attentional reinforcement of the program execution, but such compensation may fail due to co-existence of impaired attention [88, 89]. Therefore, gait abnormalities and possibly freezing could also result from disengagement of automation by shortage of supportive associative (cortical) circuits that are involved in behavior-directed attention. A strategy to address this hypothesis would be to develop a model where the interaction between these systems could be

specifically tested, i.e., manipulation of activity in attentional circuits in conditions of intact or depleted dopamine while assessing gait in various behavioral contexts. A distinction should be made between self-paced gait and cued gait in these tests because the underlying mechanisms of locomotion control are likely different. Cued gait that may not rely on the automated process as much as on sensory information may be intact in models of dopamine depletion and may not be sensitive to attentional states. In addition, cued gait may not be associated with postural or other abnormalities in the animal model that are compatible with FOG.

Neurons of the nucleus Basalis of Meynert and Substantia Innominata provide cortical cholinergic innervation, which participates in mechanisms of attention, and lesions of these neurons (ChNs) in rodents with dopamine depletion lead to impaired attention and increase the frequency of falls [44, 47, 90]. The activity of basal forebrain ChNs could be selectively excited and suppressed using optogenetics to assess specific, time-linked effects on motor performance. Therefore, the premise in this example that automated locomotion in PD is affected by a shortage of attention could be tested by optical driving of basal ChN activity in animals with dopamine depletion while performing different motor tasks during walking. Both neuron excitation and inhibition could be tested in this model to confirm the specific effects of attentional circuits. In addition to this direct assessment of the circuit function, complementary analyses of morphology, signaling molecules, and network activities (cellular and field potential recordings, fiber photometry, or calcium imaging) would add supporting information to characterize the pathophysiologic mechanism[91, 92].

- ***Phase 2, Human studies.*** Loss of attentional compensation resulting from involvement of the cholinergic system may occur later in the time course of the disease, which can explain variable therapeutic responses to dopaminergic treatments. Therefore, it may be important to analyze patients longitudinally at different disease stages to assess the timing for a cholinergic participation, likely with an attention deficit. Studies in patients with PD can use different methods to manipulate attention while walking, a typical example is the use of dual tasks. The key to testing the role of the basal cholinergic circuit lies in imaging technologies that can evidence significant activity changes, such as fMRI or FDG-PET, comparing patients with and without FOG, and normal subjects. However, to study gait and balance disorders there has been a critical limitation of imaging procedures that require decubitus position of the subject. Some recent advances to achieve real-life in vivo imaging could overcome this problem. A recently developed PET "helmet" may soon be used for imaging during real performance of the gait task with attentional distractions. This FDG-PET analysis may provide the data to analyze activity in the basal cholinergic circuit during the interference of attention with gait automation leading to FOG in patients, and thereby validating or rejecting the experimental model findings on the role of this circuit. Human studies should always try to surpass the correlational analyses done in the past by including, if suitable, pharmacological tests or other interventions in order to manipulate the system or circuit under study. In this example, the repetition of the PET-behavioral protocol under the effect of cholinergic drugs could be used. In addition, the combination of FOG assessment methods can provide timely association of FOG to the circuit activity changes as measured by PET. For example, kinematic sensor signals may be used to

identify the onset of a freezing episode. This information allows the investigator to compare the average regional cerebral glucose metabolism preceding and during/following the freezing episode. Also important, EMG data could be used to document whether or not the observed freeze was associated with leg trembling since the pathophysiology may differ. Tests in the "off" and "on" states could determine the contribution of dopamine. Obviously, the design of adaptive paradigms to test model findings in human studies will often depend on technological advances, of which the PET helmet is a good example. Other approaches for human studies are provided in the next example.

B. Postural control and sensorimotor integration.

- ***Phase 1, Modeling.*** Processing of visual, vestibular and proprioceptive information to control body posture during motion is altered in PD. As discussed above, impaired balance during postural adjustments may disrupt locomotion, and this could underlie FOG episodes and falls. Small steps and execution of movement sequences may require such adjustments especially in certain walking conditions that commonly trigger FOG, for example with turning or passing through a doorway [93, 94]. Freezing has been difficult to reproduce in rodents, but gait abnormalities, postural instability and falls have been reported in various models, including following peripheral sensory (vestibular) lesions[95]. There may be species differences in postural control of locomotion, but FOG, which is not seen with peripheral sensory lesions in humans, is presumably related to central mechanisms, principally the major role of the basal ganglia modulation of brainstem regions controlling posture and locomotion, and potentially the contribution of

integrating sensorimotor information (visual, vestibular, proprioceptive) at the cortical level. In this example of a systems neuroscience research strategy, we will focus on the integration of sensorimotor information. Several thalamic areas including the VPL nucleus, geniculate nuclei (metathalamus), colliculi and pulvinar (visual and auditory signals), as well as the entorhinal cortex (spatial navigation) participate in processing sensory information. For the purpose of this example, we will focus on the thalamus, cerebellum and basal ganglia, as primary sensorimotor processing circuits that transfer information to various cortical areas including frontal associative areas. The cerebellar and basal ganglia projections, likely with distinct behavioral roles[96], use different thalamic relays, mostly the ventral posterior lateral nucleus pars oralis (VPLo) and the ventral lateral nucleus pars oralis (VLo), respectively. This configuration creates a favorable scenario to separately analyze the signals from those nodes.

A possible strategy to study the potential contribution of sensorimotor integration to the mechanisms of posture control and FOG could be based on stepwise studies, focusing first on thalamocortical circuits. To dissect the role of these thalamic circuits in deficient postural balance, recordings of their activities need to be analyzed together with body posture adjustments in behaving animals. Combined expression of calcium indicators in neurons of the VPL, VPLo and VLo could be used to assess the global function of these regions (multifiber photometry) in relation to postural changes with different turning tasks comparing normal with dopamine-depleted states in rodents. These data could then be paired with analysis of neuronal firing in the connected associative cortical area using the same turning tasks. Results of these studies may indicate whether sensorimotor integration is disrupted at the afferent level (e.g., the

cerebellar or basal ganglia circuit) or the cortical processing of the information itself. The subsequent study would consist of operative tests using optic or chemogenetics to perturb negatively and positively the neuronal activity in selective circuits and generate confirmatory functional (behavioral) data. This challenging part may require assessment of different technologies and animal models since there are gait differences across species that may create problems to reproduce the gait abnormalities seen in patients. While postural instability and falls are well reproduced in rodent models of PD, FOG has been more difficult. Freezing episodes have been occasionally reported in some models, e.g.: PPN activation[23]. Nevertheless, non-human primates with severe toxin-induced parkinsonism develop FOG and are especially useful to assess specifically freezing episodes during turning or other conditions of postural adjustments [16, 97]. These studies could be designed with modern electrophysiologic and optogenetic technologies for animal research, such as chronic implants of electrode arrays and fiber optics, as well as minimally invasive optogenetic stimulation[79].

- ***Phase 2, Human studies.*** In this example of human studies to investigate the role of postural control and sensorimotor integration it is important to discuss first the pathophysiology of different types of FOG. As noted earlier, there are at least two prominent types of FOG, failure to have any movement and rapid alternating trembling of the legs. The first maybe the failure to initiate a motor program, but the nature of the second is obscure. The similarities and differences need to be explored clinically and physiologically. Neural activity of the brain can be monitored while walking with EEG, which can now be supplemented with deep brain recording using the new DBS recording systems with enhanced resolution for the STN and its surroundings including

the SNr, plus LFP recordings of some cortical areas. FDG PET or rCBF SPECT studies could be performed with "ictal" radioligand injection, i.e.: during the freezing episode[98-100]. The tracers are trapped in the brain during the episode and the supine imaging taken after the episode can thus reveal changes related to FOG subtypes. Another point mentioned above is the pathophysiology of sequence effect, the serial reduction in size or speed of movements with every step in a sequence (e.g.: while the patient is writing or walking). This has long been recognized as an important component of Parkinson bradykinesia[101], and progressive shortening of steps can trigger a freeze albeit not consistently. The sequence effect is less responsive to dopamine than is the slowness, and this may be one of the reasons that FOG is not very dopamine responsive. The physiology of the sequence effect has hardly ever been investigated, and, since the sequence effect can be seen in hand movements as well as walking, it should be relatively accessible to study. Studies with neuroimaging, EEG, and brain excitability using transcranial magnetic stimulation could be undertaken to assess the role of the network nodes in the sequence effect[102]. Ambulatory EEG may be more problematic due to contaminating movement artifacts[103, 104]; however, when properly dealt with EEG shows an increase in cortical synchronization with FOG[105]. Understanding these physiological aspects is important to interpret data obtained in circuit studies that may vary depending on the particular postural control.

Analysis of differences in the activities of the thalamic targets between patients with and without FOG, and normal subjects may be approached with PET-ligand studies using GABA ligands, such as ^{11}C-flumazenil (basal ganglia projections) and newly developed ligands for ionotropic glutamate (NMDAR and AMPAR) receptors (cerebellar

projections)[106]. Comparison of L-Dopa off and on in these PET studies can assess the impact of dopamine replacement also in the cerebellar circuit by network effects. The correlation of differences induced by L-Dopa with dopamine-responsive FOG may confirm the PET-ligand binding results. Although, as mentioned above, it is important to design pharmacological assessments, in this case the use of GABAergic and glutamatergic drugs will have diffuse effects in the gait network. If results in the model revealed that sensorimotor integration was disrupted at the cortical process, human studies to test such findings may also be designed. Transcranial magnetic stimulation can be used to assess the role of connected associative cortical areas during postural adjustments while walking comparing patients with and without FOG[107]. These data can be paired with EEG analysis during the performance of the same tasks[108].

Conclusions

This panel has held a critical discussion of the current knowledge gaps about gait abnormalities in PD, chiefly FOG and balance dysfunction, to determine the top research priorities. It was clear that from phenomenology to causality and to therapeutics, in each area there are many unanswered questions, poor definitions, and conflicted, inconclusive or lacking data. Nevertheless, it stood out that a major barrier to advance the field was our poor understanding of the complex underlying pathophysiology with its diverse anatomical and physiological facets. The panel consensus then was that pathophysiology of FOG and balance dysfunction should be the targeted topic to set research priorities.

Various approaches have been taken in the previous research, but multidimensional strategies that could examine interacting systems have been seldom used. The pathophysiological mechanisms of FOG and postural control seem to involve a large brain network where motor, sensory, and cognitive/emotional systems intersect. The perspective view of the panel was that to unravel such mechanisms, it is necessary to develop '***systems biology'*** strategies taking a multi-scale approach for clinical analysis and experimental modeling, the latter beginning with a ***'systems neuroscience'*** strategy to study the circuits/networks involved.

Therefore, the panel agreed to the following recommendations as top research priorities:

- The application of "Multi-scale, Systems Biology" strategies, which transitions in both directions between clinical to modeling analyses.
- The application of "Systems Neuroscience" strategies to develop hypothesis-driven dynamic studies of circuit function in posture/gait network and extra-network connections, whose dysfunction may become relevant after loss of adequate control by the basal ganglia output in PD.
- The application of such research strategies with the goal to address *discrete hypotheses* on pathophysiologic mechanisms and model FOG and balance dysfunction of PD.
- The application of research approaches that use testable animal models of PD, particularly refining the existing models, such as the rodents and non-human primates that can reproduce parkinsonian FOG and balance disturbances.
- A broad, multidimensional approach that includes the analyses of biochemical, electrophysiological, genetic, imaging, and other data to address the hypothesis.

- A sequential experimental design that transitions from the modeling phase to a human testing phase based on developing adaptive paradigms for studies in patients.
- The application of the latest advanced technologies that can serve better to test the hypotheses in both modeling and clinical phases.

This panel discussion also led to the conclusion that there are multiple points to address related to refining the characterization, detection, and quantification of gait disorders in patients with PD. These points are key to design protocols in a variety of clinical studies aiming at investigating mechanisms as well as evaluating therapies. Furthermore, they are critical for the application of systems biology strategies, as discussed here. Some important clinical queries also need to be answered; for example, the development and progression of FOG and balance dysfunction, whose patterns in PD remain unclear. A detailed analysis of these clinical issues and the research approaches to address them require further discussion, and the MDS-SIC has undertaken such analysis with the preparation of a Viewpoint article[109].

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Authors' contributions:

All authors have contributed equally to drafting the manuscript according to the panel discussions. All authors have also contributed equally to reviewing and editing the manuscript.

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Figure Legends

Figure 1. Motor network of posture/gait control. A fundamental circuitry between the brainstem (including the PPN/MLR) and the spinal cord via the brainstem-spinal cord pathways contributes to the generation of locomotor rhythms and the regulation of postural muscle tone. Signals from motor cortex conducted through the lateral corticospinal tract (CST) to the brainstem and spinal cord contribute to volitional gait control. The regulation of appropriate postural balance and gait mediated by the basal ganglia and the cerebellum is conducted downwards through their connections with the brainstem and upwards to the cortex via the thalamus. CPG: central pattern generator; BS-SC: brainstem-spinal cord pathways; PPN/MLR: pedunculopontine nucleus/mesencephalic locomotor region.

Figure 2. Multiple systems network in posture/gait control. Cognitive, emotional, and sensory (**v**isual, auditory, vestibular, and proprioceptive) signals influence various nodes of the motor network (cortex, basal ganglia, cerebellum, brainstem). In addition, multiple interactions between the sensory, limbic, and cognitive systems modulate motor behaviors including gait and postural control. For example, the limbic system has dense connections with the prefrontal cortex (CTX) and the basal ganglia. The parietal, temporal, and occipital association cortices connect with the prefrontal cortex (cognitive system) via dorsal and ventral pathways. Descending motor signals are further integrated with proprioceptive sensory signals at the level of the spinal cord. CPG: central pattern generator; BS-SC: brainstem-spinal cord; CST: corticospinal tract

Figure 1

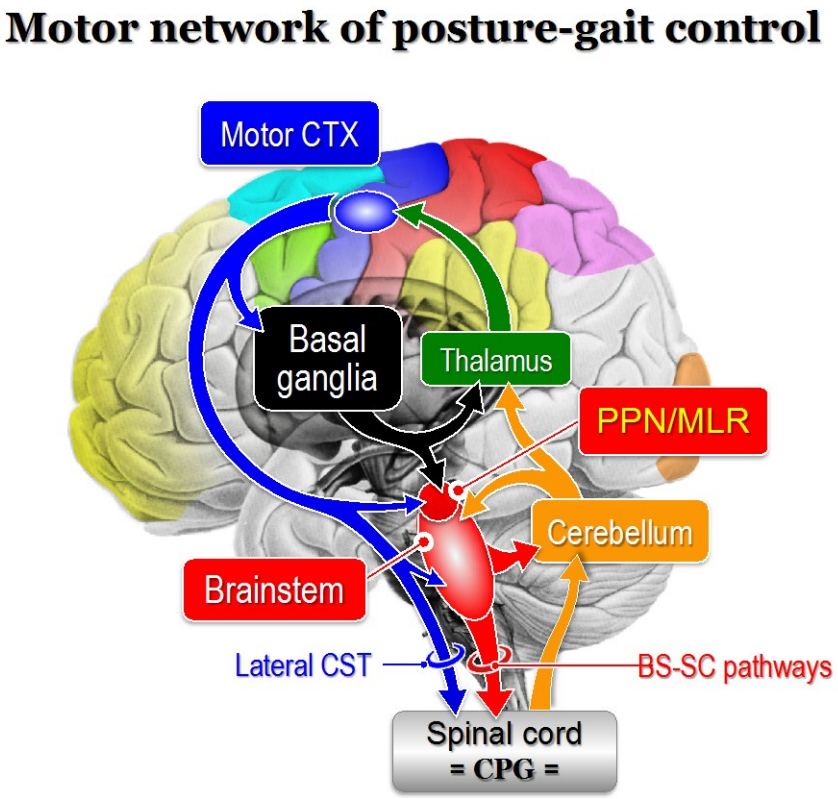
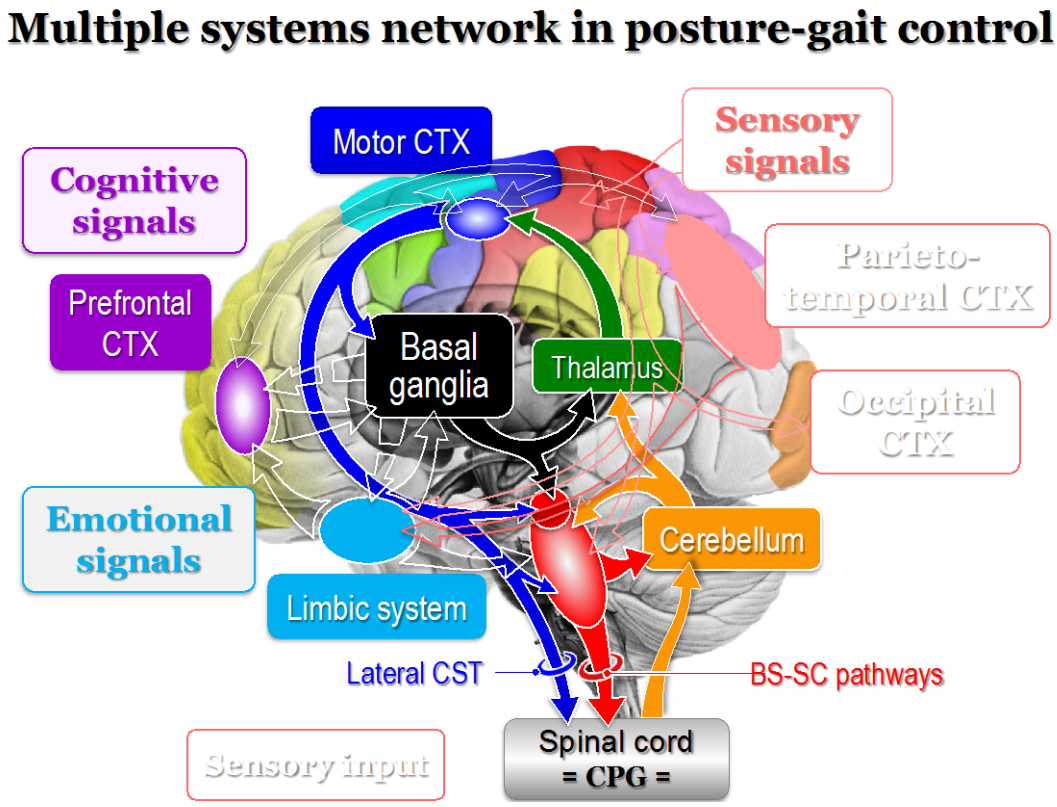
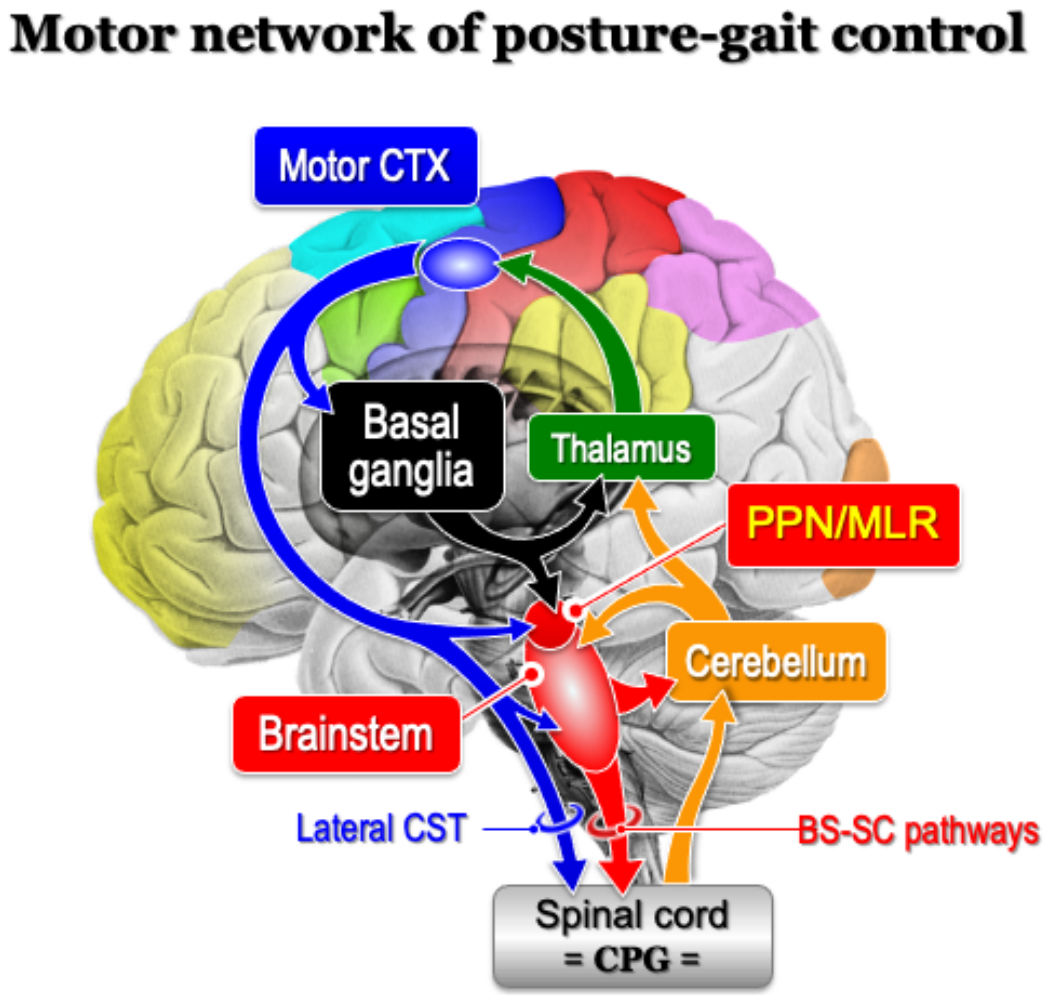
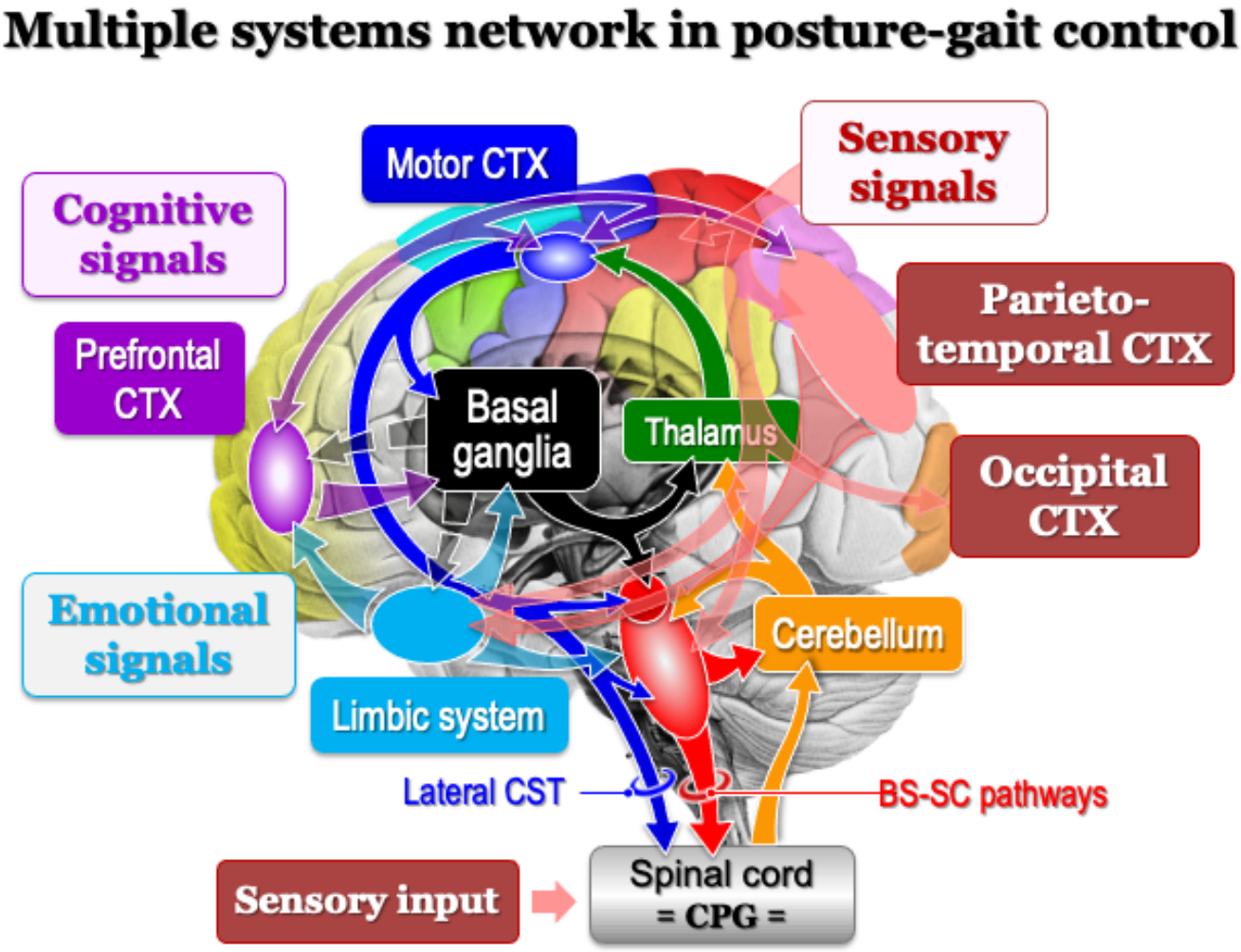


Figure 2





MDS_28883_Figure 1.tiff



MDS_28883_Figure 2.tiff