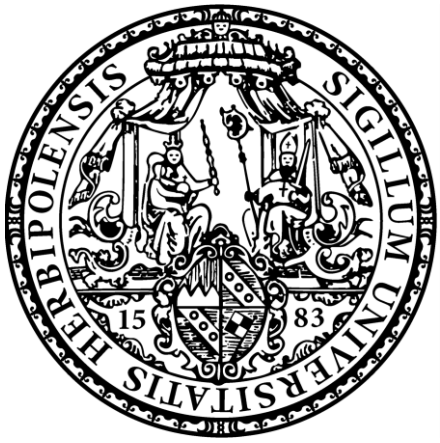

Parkinson's disease revisited: multiple circuitopathies

Neuinterpretation des Morbus Parkinson als multiple Netzwerkerkrankung

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Nicoló Gabriele Pozzi

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Mitglieder des Promotionskomitees:

Vorsitzende/r: Prof. Dr. Paul Pauli

1. Betreuer: Prof. Dr. Dr. Ioannis Ugo Isaias

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3. Betreuer: Prof. Dr. Esther Asan

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Abstract

Parkinson's disease (PD) is among the most common neurodegenerative conditions, and it is characterized by the progressive loss of dopaminergic neurons and a great variability in clinical expression. Despite several effective medications, it still causes disability as all patients show treatment-resistant symptoms and complications.
A possible reason for this therapeutic-burden and great clinical variability lies in a probable misconception about its pathophysiology, one that focuses on neurodegeneration, while largely neglecting its functional consequences and the related compensatory changes. In this thesis, I expand on the hypothesis that some PD symptoms have a dysfunctional origin and reflect derangements of neural network dynamics, the means by which brain coordination supports any motor behaviour. In particular, I have investigated resting tremor and freezing of gait, two common symptoms with an enigmatic mechanism and suboptimal management.
In the case of tremor, I predicted a pathological change in response to dopamine loss, which included the activation of noradrenergic (NA) neurons of the locus coeruleus (LC) projecting to the cerebellum. This compensatory LC activation that supports dopaminergic neurons might indeed come at the expense of tremor development. To assess the role of LC-NA in tremor development, I recorded tremor occurrence in the reserpinized rat model of PD, one of very few showing tremor, after selective lesioning (with the neurotoxin DSP-4) of the LC-NA terminal axons. DSP-4 induced a severe reduction of LC-NA terminal axons in the cerebellar cortex and this was associated with a significant reduction in tremor development. Unlike its development, tremor frequency and the akinetic rigid signs did not differ between the groups, thus suggesting a dopaminergic dependency. These findings suggest that the LC-NA innervation of the cerebellum has a critical role for PD tremor, possibly by exerting a network effect, which gates the cerebello-thalamic-cortical circuit into pathological oscillations upon a dopaminergic loss in the basal ganglia.
In contrast, for the study of freezing of gait, I worked with human PD subjects and deep brain stimulation, a therapeutic neuromodulation device that in some prototypes also allows the recording of neural activity in freely-moving subjects. Gait freezing is a disabling PD symptom that suddenly impairs effective stepping, thus causing falls and disability. Also in this study, I hypothesized that the underlying pathophysiology may be represented by dysfunctional neural network dynamics that abruptly impair locomotor control by affecting the communication in the supraspinal locomotor network. To test this hypothesis, I investigated the coupling between the cortex and the subthalamic nucleus, two main nodes of the supraspinal locomotor network, in freely-moving subjects PD patients and also performed molecular brain imaging of striatal dopamine receptor density and kinematic measurements. I found that in PD patients, walking is associated with cortical-subthalamic stable coupling in a low-frequency band (i.e. θ-α rhythms). In contrast, these structures decoupled when gait freezing occurred in the brain hemisphere with less dopaminergic innervation. These findings suggest that freezing of gait is a "circuitopathy", with dysfunctional cortical-subcortical communication.
Altogether the results of my experiments support the hypothesis that the pathophysiology of PD goes beyond neurodegenerative (loss-of-function) processes and that derangement of neural network dynamics coincides with some disabling PD symptoms, thus suggesting that PD can be interpreted as the combination of multiple circuitopathies.

Zusammenfassung

Die Parkinson-Krankheit ist eine neurodegenerative Erkrankung mit einem progressiven Verlust dopaminerger Neurone, die trotz wirksamer Medikamente zur Einschränkung in der Lebensqualität führen kann.
Eine mögliche Ursache für diese unzureichende Behandlung der Symptome liegt in einem möglichen Missverständnis über die Pathophysiologie der Krankheit, die sich auf die Neurodegeneration konzentriert. Bei der Parkinson-Krankheit können jedoch funktionelle Veränderungen aufgrund der Neurodegeneration sowie die damit verbundenen kompensatorischen Modifikationen sehr wichtig sein. Der Fokus meiner Dissertation liegt in der Bearbeitung der Hypothese, dass einige Symptome der Parkinson-Krankheit einen dysfunktionellen Ursprung haben können. Insbesodere habe ich den Ruhetremor und das Freezing-Phänomen, das eine Blockade des Gehens bedeutet, untersucht, um zu erklären, ob ein Störung der neuronalen Netzwerkdynamik diese Symptome verursachen kann.

In dieser Arbeit wurde zuerst die Entwicklung des Ruhetremors bei der Parkinson-Krankheit untersucht. Meine Hypothese war, dass eine Aktivierung von projizierenden noradrenergen Fasern des Locus-Coeruleus zum Cerebellum das Auftreten des Tremors verursachen kann, welches durch den Verlust dopaminerger Neurone verursacht wird. Da die Aktivität des Locus-Coeruleus bei Patienten mit Parkisnon-Krankheit nicht messbar ist, wurde dies in einem Parkinson-Rattenmodell untersucht. Die Ratten wurden etweder mit Reserpin oder mit Reserpine plus eine Neurotoxin gegen noradrenerger Neuronen (DSP-4) behandelt. Diese Behandlung mit DSP-4 führte zur Degeneration noradrenerger Terminalen im Locus-Coeruleus. Das Auftreten von Tremor zwischen die beiden Gruppen von Ratten war unterschiedlich. Insbesondere entwickelten DSP-4 behandelte Ratten einen niedrigen Ruhetremor. Dieses Ergebnis deutet darauf hin, dass die noradrenerge Innervation des Cerebellums vom Locus-Ceruleus für das Auftreten des Ruhetremors eine große Rolle spielt. In der Frequenz des Tremors sowie in den akinetischen Symptomen konnte kein Unterschied zwischen den Gruppen festgestellt werden. Das zeigt, dass diese akinetischen Symptome vom Dopaminverlust abhängig sind. Die Kombination von Tremor und akinetischen Symptomen kann aufgrund eines patologischen Netzwerkeffekts entstehen, welche vom Verlust dopaminerger Neurone in den Basalganglien im Zusammenspiel mit der kompensatorischen Aktivierung noradrenerger Neurone des Locus-Coeruleus verursacht werden kann.

Des Weiteren wurde der Ursprung des Freezing-Phänomens bei Patienten, die an der Parkinson-Krankheit leiden und eine therapeutische Behandlung mittels Tiefer Hirnstimulation (THS) bekommen haben, untersucht. Insbesodere konnten mittels neuer THS-Prototypen Messungen neuronaler Aktivität von Bewegungen durchgeführt werden. In dieser Studie stellte ich die Hypothese auf, dass die Pathophysiologie des Freezings durch eine fehlerhafte neuronale Dynamik der Bewegungsnetzwerke erklärt werden kann. Um dies zu testen, wurde die Kommunikation zwischen den zwei Hauptknoten des Bewegungsnetzwerkes, dem Kortex und dem Nucleus Subthalamicus, bei THS behandelten Parkinson-Patienten während des Gehens und den Freezing-Episoden untersucht. Zudem wurde bei diesen Patienten eine molekulare Darstellung der dopaminergen Rezeptoren in den Basalganglien durchgeführt. Zusätzlich wurden kinematischen Messungen der Bewegungen vorgenommen, die eine präzise Beschreibung des Freezings ermöglichen. Es konnte gezeigt werden, dass bei Patienten mit der Parkinson-Krankheit ein Zusammenhang von stabiler Kommunikation zwischen dem Kortex und dem Nucleus Subthalamicus bei einer bestimten Frequenz (d.h. θ-α-Rhythmen) beim Gehen besteht. Beim Auftreten des Freezing-Phänomens konnte diese Kommunikation in der Gehirnhemisphäre mit weniger dopaminerger Innervation nicht mehr nachgewiesen werden. Diese Ergebnisse deuten darauf hin, dass das Freezing-Phänomen eine „Circuitopathie“ ist, in der eine fehlerhafte Kommunikation zwischen kortikalen und subkortikalen Arealen zur Bewegungsblockade führen kann.

Insgesamt stützen die Ergebnisse meiner Experimente die Hypothese, dass die Pathophysiologie der Parkinson-Krankheit sowohl über neurodegenerative Prozesse (Zellverlust) als auch über Störungen der neuronalen Netzwerkdynamik (Funktionsverlust) hinausgeht. Das deutet darauf hin, dass die Parkison-Krankheit als „Circuitopathie“ interpretiert werden kann.

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I. Outline of the Thesis

Since the beginning of my clinical practice in neurology, I have been captivated by the many clinical shades of Movement Disorders. The various symptoms of these conditions truly illustrate the permanent mystery of basal ganglia function[1] and the complexity of neural control of motor behaviours.[2,3]
Decoding neural activities and understanding how neurons generate complex volitional acts are at the core of neuroscience and define the study of motor control.[3,4] This also represents an unmet need in the care of patients with movement disorders, which still only relies on symptomatic treatments.[5] Even if the aetiology of the disease is unknown, the correction of its pathophysiological derangements can improve patient symptoms and ameliorate quality of life.

A pragmatic example of this is Parkinson's disease, a chronic neurodegenerative movement disorder characterized by falling dopamine levels in the basal ganglia.[5] The cause of the neurodegeneration of nigral dopaminergic cells is unknown. Patients are efficiently treated with dopaminergic replacement therapies that restore the motor processing, alleviate the symptomatology, reduce mortality and improve quality of life for both patients and care-givers.[5]
Sadly, not all symptoms respond equally to dopaminergic treatments. In particular, rest tremor and gait impairments, such as freezing of gait[6], respond poorly to this treatment and thus still create a major source of social distress and motor disability as well as a primary cause of isolation, institutionalization and mortality.[7,8]
These variations in treatment response are supposed to reflect the individual physiopathological patterns of neurodegeneration, which progressively impair motor processing.[9] Although conceivable, this idea reduces Parkinson's disease to a deficiency condition, neglecting the role of concurrent functional changes possibly triggered by largely independent forms of neurodegeneration. Compensatory or dysfunctional neuronal activities in preserved and functionally connected brain areas may underpin distinctive symptoms, a mechanism known as dynamic diaschisis.[10]
Hence, I started to consider whether tremor and freezing of gait in Parkinson's disease may represent purely functional alterations of a diffuse neural system, which go beyond the basal ganglia and rely on several other non-dopaminergic neurotransmitters. This subtle difference is not insignificant, as might hold the key to proper (comprehensive) treatment.

The first evidence of a distinctive mechanism for tremor and gait freezing in Parkinson's disease came from their peculiar clinical features that will be discussed in the 'Introduction' of this thesis.
The description of the clinical aspects of Parkinson's disease will be followed by a brief discussion of principles of anatomy of the motor system. This will cover the organization and function of the cortex, thalamus, cerebellum, some brainstem nuclei and the basal ganglia, which will be discussed more in depth.
These concepts are an essential introduction to the review of current theories of Parkinson's disease pathophysiology. Each theory will be discussed detailing the evidence, pros and cons. The introduction will be closed with a description of the purpose of this work, which originated from the following questions:
Why is Parkinson's disease so clinically diverse? Why are tremor and gait freezing so different from the other symptoms? Which are their neural mechanisms and triggers? And, ultimately, do we need to revisit the current interpretation of Parkinson's disease pathophysiology?
The 'Studies' section will provide some answers to these questions and focus on the core experiments of my PhD. In particular, I will first discuss the limits of the current understanding of these symptoms, provide a description of my working hypothesis that guided the design of the experiments and, after presenting the methodology used, I will describe the results and their possible interpretation.
In the 'Discussion', I will provide a clinical application for these findings and explain the translational relevance of the studies. Moreover, I will propose a refined hypothesis of tremor and gait freezing pathophysiology in Parkinson's disease. This speculative section will be integral to the development of the future research projects that will help refine this theory. The thesis will end with the 'Bibliography' of the evidence and 'Acknowledgments'.

II. Introduction

2.1 Parkinson's disease: a clinical overview

Parkinson's disease (PD) affects more than 6 million people worldwide and is (together with essential tremor, ET) the most common movement disorder and the second most common neurodegenerative condition after Alzheimer's Disease[11,12].
The prevalence of PD in Europe ranges between 65.6 and 12,500 per 100,000 and the annual incidence rate between 5 and 346 per 100,000[11,12]. It presents a lifetime risk of 1.5% and incidence rises sharply with age, with a median age of onset of 60 years[11,12]. Up to 15% of the cases are, however, juvenile forms (young-onset PD) that develop before the age of 41[13,14].
PD is a chronic disease, with a mortality ratio of 2 to 1.5 and a mean duration of 15 years[11,12], in which it progressively impairs motor behaviours, determines an increasing social disability and ruins the quality of life of patients and families[15].

The causes of the disease remain unknown.
The vast majority of PD cases are sporadic and only ~10% of patients report a positive family history[16].
Six genes have been unequivocally linked to heritable, monogenic PD forms: SNCA (PARK1) and LRRK2 (PARK8) mutations are responsible for autosomal-dominant PD forms, whereas mutations in Parkin (PARK2), PINK1 (PARK6), DJ-1 (PARK7), and ATP13A2 (PARK9) cause autosomal recessive PD[16].
Polymorphic length and SNP variations in SNCA and p.G2385R and p.R1628P missense SNPs in the LRRK2 gene as well as variants in the β-glucocerebrosidase gene (GBA) represent the most significant genetic risk factors for PD[16]. Recently, also mitochondrial and transport pathways to lysosomal genes have been suspected to play a role in PD development[17].
Men are about 1.5 times more likely than women to develop PD, but the reasons for this gender association remain unclear. PD is also not related to either race or creed and, given the reports prior to 1817, year of the publication of "*Essay on shaking palsy*" by Sir James Parkinson, it cannot be considered a post-industrial condition[12].

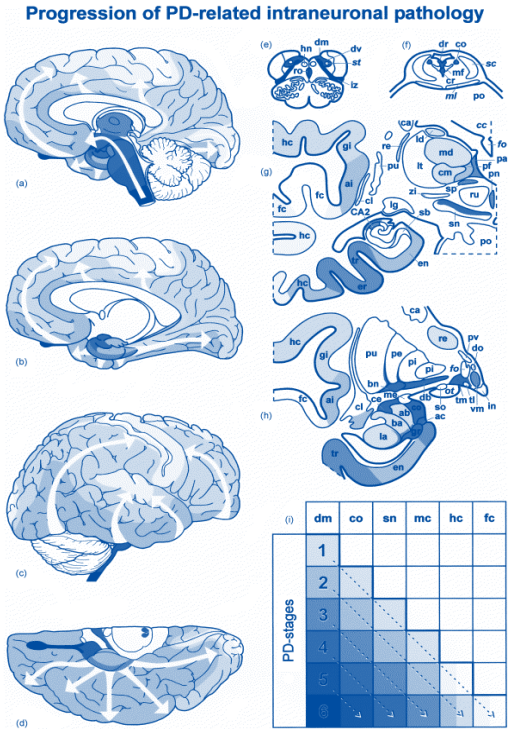

Figure 1 - Detailed description of the Braak hypothesis. (modified from Braak et al. [350]) Neurodegenerative processes are proposed to start in the midbrain and in the olfactory bulb to spread over time in the midbrain and telencephalon, thus affecting the entire brain with a bottom-up and rostro-caudal diffusion.

At its core, PD is a neurodegenerative disease. It is characterized by a variety of symptoms that reflect an altered functioning of the basal ganglia, which always show a striatal dopaminergic innervation loss[18–20]. PD pathology is typical and yet puzzling as it fails to explain the many aspects of its clinical spectrum. The main alteration of PD is the loss of dopaminergic neurons from the substantia nigra pars compacta (field A9), which gives rise to dopaminergic striatal projections[19,20]. Neurodegeneration is accompanied by the development of Lewy bodies that are confined aggregations of misfolded (insoluble) α-synuclein, which localize in the soma and processes of surviving neurons[19,20]. Of note, Lewy pathology is not limited to the substantia nigra but affects other brain areas as well as peripheral nerves like the vagus nerve and the enteric nervous system[21–23]. Assessing Lewy bodies distribution in pathologically confirmed cases of PD, Braak and colleagues suggested that Lewy pathology marked the disease progression and identified six different stages with caudal-to-rostral evolution (Figure 1)[24].

While several pathological studies correlate Lewy pathology (stage 4-6) with PD dementia, further studies are needed to confirm the association with other symptoms[25]. Moreover, Lewy pathology is not the only alteration present in PD and α-synuclein can generate a variety of other aggregates that together with inclusions of β-amyloid plaques and tau-containing neurofibrillary tangles, which can be found in up to 50% of PD cases, exert a toxic and inflammatory action[19,20].
Neuro-inflammation is followed by reactive gliosis and microgliosis processes that also affect non-dopaminergic pathways, altering cholinergic, serotonergic, adrenergic, glutamatergic, GABAergic and adenosine-mediated neurotransmission, thus possibly inducing different symptoms[19,20].

PD symptomatology is incredibly rich and varies from patient to patient as well as with disease progression (Figure 2)[19,20]. Symptoms can be divided into motor and non-motor.

Motor symptoms	Non-motor symptoms
Tremor, bradykinesia, rigidity, postural instability	Cognitive impairment, bradyphrenia, tip-of-the-tongue (word finding) phenomenon
Hypomimia, dysarthria, dysphagia, sialorrhoea	Depression, apathy, anhedonia, fatigue, other behavioural and psychiatric problems
Decreased arm swing, shuffling gait, festination difficulty arising from chair, turning in bed	Sensory symptoms: anosmia, ageusia, pain (shoulder, back), paresthesias
Micrographia, cutting food, feeding, hygiene, slow activities of daily living	Dysautonomia (orthostatic hypotension, constipation, urinary and sexual dysfunction, abnormal sweating, seborrhoea), weight loss
Glabellar reflex, blepharospasm, dystonia, striatal deformity, scoliosis, camptocormia	Sleep disorders (REM behaviour disorder, vivid dreams, daytime drowsiness, sleep fragmentation, restless legs syndrome)

Figure 2 – Summary of the motor and non-motor features of Parkinson's disease

Non-motor symptoms are common and nonspecific but can precede the motor onset, thus marking a premotor phase of the disease (Figure 3)[19,26].
This phase of PD remains largely unexplored but it is likely characterized by combined changes in many neurotransmitters and different regions in and outside the brain[19,26]. Increasing evidence supports the role of peripheral neurodegeneration, possibly starting in the GI[21] and then proceeding to the central nervous system through the cranial nerves, especially the vagus nerve[22]. This nerve presents Lewy body pathology before the onset of the motor symptoms, and results from a recent pathological study show that dissection of this nerve in cardiac care is associated with a reduced rate of PD development[27]. Despite being interesting, the evidence for a distinctive premotor phase in PD remains insufficient and nonspecific and the presence of motor onset is currently still required for a diagnosis of PD[19,20,28,29].

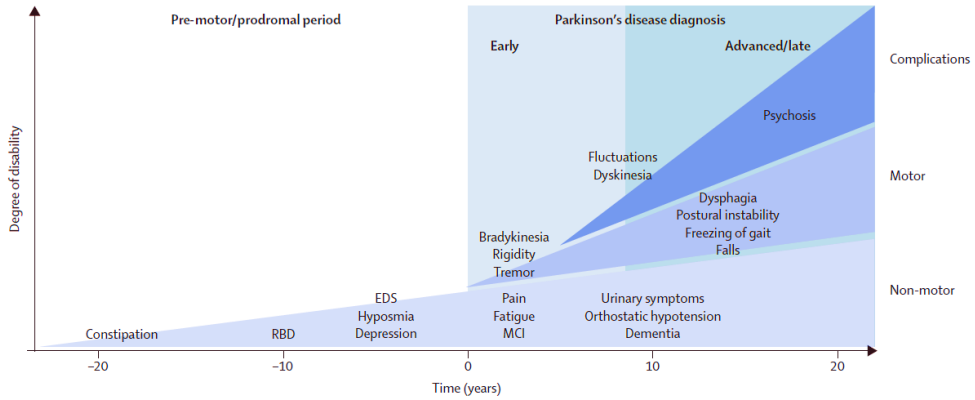

Figure 3 – Schematic description of the natural history of Parkinson's disease (modified from Kalia et al [19]).
A premotor period in which neurodegenerative processes are not associated with any motor impairment is followed by the development of motor symptoms that progressively aggravates and determines the onset of complication and disability

The motor symptoms of PD include bradykinesia, muscular rigidity, rest tremor, and postural and gait impairment, of which freezing of gait (FOG) is its prototypical example (Figure 2)[28,29].

The diagnosis of PD cannot be made without the detection of unequivocal bradykinesia, which is defined by slowness, and a progressive reduction of speed and amplitude on sequential motor tasks[28,29].
Repetitive fine movements of hands and feet are affected. Of note, reduction can be unilateral in an early phase. Bradykinesia may also limit facial expression and the ability to show emotions, impairing normal speech that might sound slow, quiet, and lacking in rhythm and melody[28,29].
Most PD patients also exhibit muscular rigidity, characterized by a steady resistance to passive movement that is independent of velocity[28,29]. Rigidity in PD is present to the same extent over the whole range of movement and can affect both limb and axial muscles[28,29].
Tremor is the third cardinal motor feature of PD and it represents the only hyperkinetic feature of an essentially hypokinetic movement disorder; together with gait disturbances and postural instability (the fourth cardinal features of PD) which will be discussed in detail later.
Based on the predominant symptoms presented by the patient, the disease can be clinically classified as: i) tremor-dominant, ii) akinetic rigid, iii) with primary postural instability and gait disturbances (PIGD) or iv) mixed phenotype[30].
Although a consensus classification is missing and most of the patients present a mixed phenotype, this phenomenological classification is not trivial as the course and prognosis of PD differ between subtypes[15,30]. In particular, tremor-dominant patients present a slow rate of progression and less disability, whereas PIGD patients suffer from high disability and increased mortality[15,30].
Longitudinal studies similarly show that tremor and FOG are identifiable conditions with clear patterns of disease progression, anticipating low- and high-disability, respectively[30]. Interestingly, both these symptoms are not associated with severity of bradykinesia or rigidity[7,8]. Moreover, they both are episodic in nature and triggered by cognitive or emotional stress, thus suggesting a complex and distinctive pathophysiology[7,8].

Tremor is present in up to 75% of the patients with PD[31]. It is pragmatically defined as a rhythmic, mechanical oscillation of at least one body region, but it can manifest in many different ways, so that its definition depends on the conditions activating it[7,28,31,32].
Resting tremor is a peculiar feature of PD; it occurs in a body part that is not voluntarily activated and is completely supported against gravity. The amplitude of tremor can increase during stress, diminish or disappear during the active movement of the limb[32,33], and reoccur after its termination[34].
Action tremor is less common in PD and it is defined as any tremor occurring on voluntary contraction of a muscle, being so subdivided in postural, isometric and kinetic tremor.
Postural tremor is present during voluntary maintenance of position against gravity. Isometric tremor occurs as a result of isometric muscle contraction (i.e. muscular contraction without movement).
Kinetic tremor occurs during any voluntary (non-goal-directed and goal-directed) movement. Intention tremor is a subtype of kinetic tremor and identifies a condition in which tremor amplitude increases and movement velocity fluctuates when approaching the target[32,33].
Tremor can affect any side of the body (e.g. head, chin, jaw, vocal cords, upper/lower extremity) and present in a variety of frequencies, which are usually classified as: low: < 4 Hz, medium: 4-7 Hz, high: > 7 Hz[32,33].
Subjects with PD can present with any kind of tremor[35], but it must be accompanied by at least bradykinesia[28,29].
The pill-rolling, resting tremor is typical, but up to 60% of PD patients have different forms of postural- and action tremor[31]. Three main clinical patterns of tremor can be recognized in PD: a) type I “classical” PD tremor that is a pure resting tremor within a 4-6 Hz frequency, which can be higher in the early stage of the disease; b) type II PD tremor that combines resting tremor with kinetic tremor, which presents with the same frequency but pauses when a voluntary movement is initiated and reoccurs after a few seconds with the hands outstretched; and c) type III PD tremor that is an isolated postural and action tremor [36], which happens rarely and in ~20% of the cases is misdiagnosed as ET variant [33,34].
Of note, the onset of tremor can precede the onset of PD (i.e. the development of the other cardinal features) and, when it dominates the clinical symptomatology, the progression of the disease is usually benign[30]. In particular, tremor-dominant forms do not exhibit FOG or other axial symptoms for many years[30]. However, tremor can be a source of disability itself, being often perceived as a social stigma[37,38].
Tremor is common in juvenile PD forms, thus affecting active people who can be severely limited in their job and social relations[30,31]. Moreover, tremor is not consistently controlled with available therapies for PD[7,28,31].
The mainstay treatment of PD is the dopaminergic replacement therapy, which has been proven safe and effective in controlling most of the symptoms in an early phase of the disease[39]. However, these treatments have limitations

as they only partially ameliorate non-dopaminergic symptoms (such as tremor[6]) and do not influence the neurodegenerative processes of PD, which progressively worsen the clinical symptoms[15].

Disease progression is also accompanied by the development of treatment-related complications, namely motor fluctuation and dyskinesia[40]. Dyskinesias most commonly occur at peak dose and typically alternate with the wearing-off state, which reflect an early deterioration of the effect of the medication[40].
Late-stage PD is also complicated by treatment-resistant motor features, which include dysphagia, postural instability, gait freezing and falls[41]. These conditions are only marginally improved by dopaminergic replacement therapy that may, in some cases, even be detrimental[42,43]. They are common issues as ~50% of advanced PD patients report choking and up to 80% develop FOG[41]. Most relevant is the fact that these symptoms are the major cause of death in patients with PD[41].

Freezing of gait, in particular, is a dangerous and disabling symptom of PD that severely limits patient mobility, independence and social interaction. It also increases the risk of falling, and so, the mortality of PD[8].
It is characterized by sudden, unpredictable episodes of an inability to produce effective forward stepping that may occur during gait, gait-initiation, turning or gait-brakes[8]. Interestingly, it only affects forward walking, as patients with FOG can easily walk backwards or climb stairs.
The mechanism of FOG is still unclear but risk factors have been recognised in the absence of tremor and the presence of a gait disorder. FOG does not correlate with bradykinesia or rigidity, thus suggesting that it is an independent feature of PD[44].
FOG episodes are dangerous and can trigger falls as it fragments lower- and upper-body movements during walking, thus provoking forward and lateral postural imbalance. FOG dynamics is typical: when an adjustment of an ongoing movement (either walking or standing) is required, FOG impairs step execution, so that the foot remains ‘stuck’ to the ground while the rest of the body progresses in the planned movement. This creates an imbalanced condition that results in either a fall or a recovery of the baseline posture[45].
Three main clinical patterns of FOG can be recognized: (i) shuffling forward, with very short, shuffling steps, (ii) trembling in place, with alternating rapid knees movements (knee trembling), and (iii) complete (or total) akinesia, with no limb or trunk movement[46].
Independently from their type, FOG episodes are mostly unpredictable[8]. Several daily-life situations can trigger their occurrence, such as turning while walking to avoid an obstacle on the walking path, gait initiation, narrow-passing through obstacles (e.g. doorways) and gait-brake before reaching a destination[47]. Time pressure severely worsens the tendency to freeze. Examples are, attempting to cross a busy street before the traffic signal changes or reaching the door after a doorbell[47]. Therefore, the home-environment is particularly dangerous for patients with FOG[8].
Another triggering factor is represented by the cognitive load[48]. Dual-tasking is normally impaired in PD patients with FOG and walking while performing cognitive exercises can result in FOG development[48].
Emotional states like anxiety[49], depression and stress can also concur in FOG development, whereas engagement and focused attention can relieve FOG[48]. The use of verbal or auditory stimuli can improve as well as aggravate FOG episodes mostly in an individual fashion[48].
Altogether these FOG characteristics highlight a strict relation with cortical cognitive processes that might compensate for the loss of motor automaticity induced by the dopaminergic loss occurring in PD[8].
Dopamine replacement therapy is a double-edged sword in FOG management[50]; FOG is more likely to occur during the wearing-off phase, but can also happen under medication (meds-on)[43].

Dopaminergic treatments are also poorly effective in the treatment of non-motor symptoms that complicate the late-disease course[41]. Cognitive impairment is particularly relevant as it occurs in 83% of PD subjects with more than 20 years of disease duration and it associates with FOG[30]. The combination of these late-stage symptoms increase mortality in PD and likely reflects both the spread of neurodegeneration to cortical brain areas and the failure of all compensatory mechanisms.

2.2 The motor system: principles of anatomy

An overview of the anatomical organization of the motor system is essential to discuss the pathophysiological alterations determining PD symptomatology and the interpretations of the experiments presented in this thesis. The neural anatomy of the motor systems includes several different structures that extend from the cortex to the motor end-plates in the muscles. These structures are diverse, but they work together in a functional ensemble to allow timely, smooth and (apparently) effortless execution of every volitional movement. Hence, they will be described here as a component of a single functional system with less detail about their peculiar structural characteristics and more emphasis on their function and connectivity. Since PD mainly impairs volitional movement, with little alteration of reflex motor activity, I will focus on supraspinal motor control, discussing cortical, thalamic, basal ganglia, cerebellar and brainstem motor centres (information on the spinal control of movements can be found in [4,51,52]).

Motor control is a learned skill and requires the coordinated interaction of distributed brain areas.
Volitional movements are ruled by descending neural drives from motor cortical centres, which through the cortico-spinal, cortico-bulbar and cortico-reticulo-spinal tracts modulate the activity of the neurons in the brainstem and in the spine.
The motor pathways can be functionally divided into: descending pathways, which control the motor drive; and re-entrant circuits, which refine motor commands through complex neural interactions[5,53].

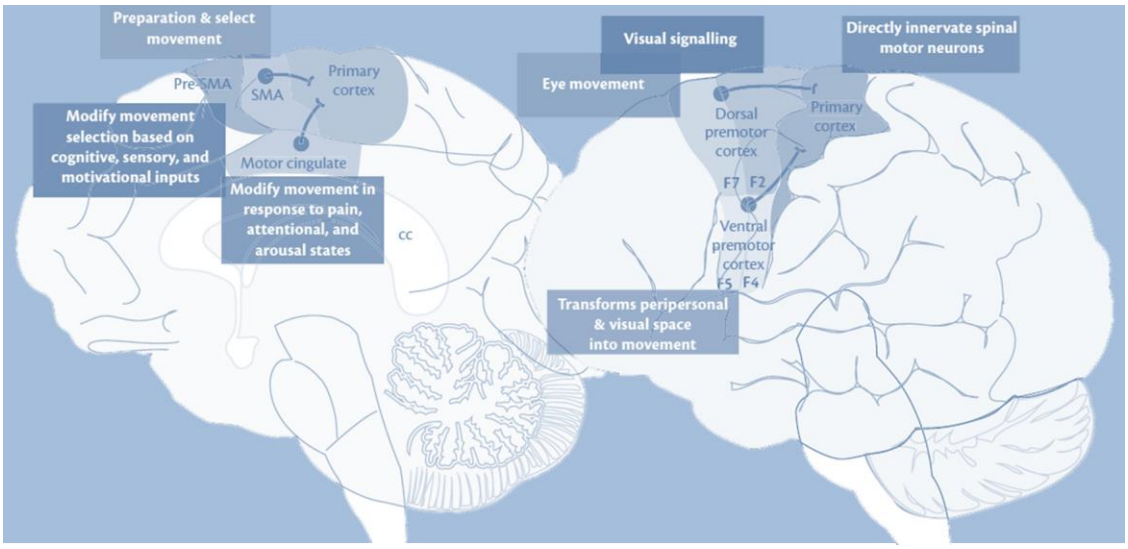

Figure 4 - Cortical regions involved in motor control and their functions (modified from [5]).
Cortico-cortical excitatory projections to the primary motor cortex are indicated in dark grey.

SMA, supplementary motor cortex; cc, corpus callosum.

Descending pathways originate from the cerebral cortex and the brain stem nuclei (i.e. the red nucleus, vestibular nuclei, superior colliculus) and terminate onto spinal and brainstem neurons.
The main descending pathway is the cortico-spinal path, which control voluntary body movements. The neurons of this pathway are located in several cortical regions including the primary motor cortices (Brodmann's area 4; M1), sensory cortices (BA3; S1), anterior cingulate motor area (BA 24; CMA) and the premotor cortices (medial and lateral BA 6).
The M1 controls movement activation and direction[5]. It contains several functional fields (e.g. F1 and F2) and a complete representation of the body muscles, but it is organized in maps of organized movements (Figure 4). M1 neural clusters can simultaneously excite and inhibit different muscles, thus permitting the proper execution of

fine movements. Movement modulation in response to pain, attentional, and arousal states is sustained by the CMA, which presents direct projections to the sensorimotor, limbic, and executive systems[5].
The premotor cortex, instead, performs distinctive motor functions in its lateral and medial part. The lateral premotor cortex (LPMC) transforms peripersonal and visual space information into motor commands, thus supporting reaching and visual signalling and eye movements[54]. The medial premotor cortex, which correspond to the SMA proper (F3) and pre-supplementary motor area (pre-SMA, F6) is involved in preparation and selection of movement as well as in movement modulation according to cognitive, sensory, and motivational signals (Figure 4)[5].

Cortical commands influence the activity of the motor neurons as well as those of the brainstem and spinal central pattern generators (CPGs)[5,52]. These are neural clusters that spontaneously generate rhythmic and stereotyped motor patterns, such as stepping or breathing[52]. These rhythmic movements also require proper muscle tone[55], which is not under complete voluntary control, but relies on excitatory monoaminergic reticulospinal and inhibitory cholinergic pontine reticular formation inputs.
Spinal CPGs are essential for human locomotion and are regulated by direct corticospinal input as well as by the mesencephalic locomotor region (MLR) in the brainstem via both direct and medullary reticulospinal connections[51,52,55]. Sensory afferents from proprioceptive and skin receptors, reticulospinal and vestibulospinal inputs can activate and modify CPGs activity which adapts the tone, speed and quality of patterns for locomotion[55].
The MLR and medullary reticular formation receive inputs from the basal ganglia and cerebellum, especially from the subthalamic and cerebellar locomotor regions (SLR and CLR)[55]. These regions were demonstrated in recent imaging studies in humans, which suggested that this complex coordination is required for the voluntary adaption of motor patterns (e.g. when circumventing obstacles) and ensures proper locomotion[51,52,55].

Re-entrant circuits consist mainly of two subcortical structures: the cerebellum and the basal ganglia, which regulate integration, modulation and adaptation of motor behaviours[5,53,55]. Both systems comprise a large number of integrated regions that impact motor control through the modulation of reciprocal, thalamic and brainstem (e.g. MLR) neural activities[5,51,53].

The thalamus (50–60 nuclei) is located in the diencephalon and is a relay centre for sensory and motor mechanisms (Figure 5)[56]. Thalamic regions participating in motor control include ventral, posterior and intralaminar nuclei, which contain aspiny glutamatergic excitatory neurons conducting short-latency excitatory responses[5,56]. Thalamocortical neurons express tonic and burst-firing activities[57], with tonic firing sustaining transmission of afferent signals to cortex and bursting inhibiting cortico-cortical communication[58].
Each thalamic nucleus projects to well-defined cortical areas, so that multiple cortical areas receive afferents from a single thalamic nucleus and send information back to the same and/or different nuclei (Figure 6)[5,56]. These reciprocal and non-reciprocal connections regulate cortical activity and allow the synchronization of thalamocortical oscillations, thus sustaining the information flow across functionally related cortical fields[5,56]. For example, cortical regions associated with executive function (such as the dorsolateral prefrontal cortex, DLPC) have non-reciprocal connections to thalamic regions projecting to premotor cortices (ventral anterior thalamus, VA), while LPMC and SMA have non-reciprocal

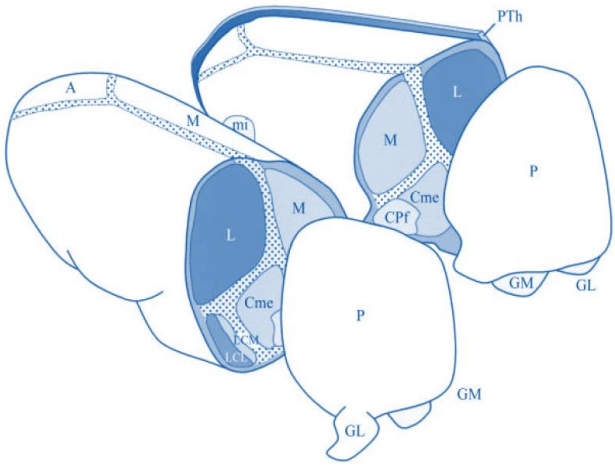

Figure 5 - **Diagrammatic view of the internal structure of the dorsal thalamus (modified from Herrero et al [56]).**
The figure represents a posterior view of right and left sectioned thalamus. The internal medullary lamina separates the regions

A region superior, *Cme* nucleus centralis medius, *CPf* nucleus centralis parafascicularis, *GL* nucleus geniculatus lateralis, *GM* nucleus geniculatus medialis, *dotted areas* internal medullary lamina, *LCL* nucleus ventralis caudalis lateralis, *LCM* nucleus ventralis caudalis medialis, *L* regio lateralis, *M* regio medialis, *mi* massa intermedia, *P* regio posterior, *PTh* nucleus perithalamicus)

connections with thalamic regions projecting to M1 (ventrolateral thalamus) (Figure 6)[59].
The thalamus also projects to the basal ganglia, especially the parafascicular nucleus (Pf) and the centromedian nucleus (CM), which receive topographically organized input from the basal ganglia and projects back to the striatum, bilaterally[60].
The basal ganglia projections to the thalamus reach the ventral anterior, anterior ventrolateral, and caudal intralaminar nuclei and exert a strong inhibition on thalamocortical activity[60]. To the contrary, the output nuclei of the cerebellum excite thalamocortical activity acting on the ventrolateral posterior and ventro-intermedial nuclei[5,60].

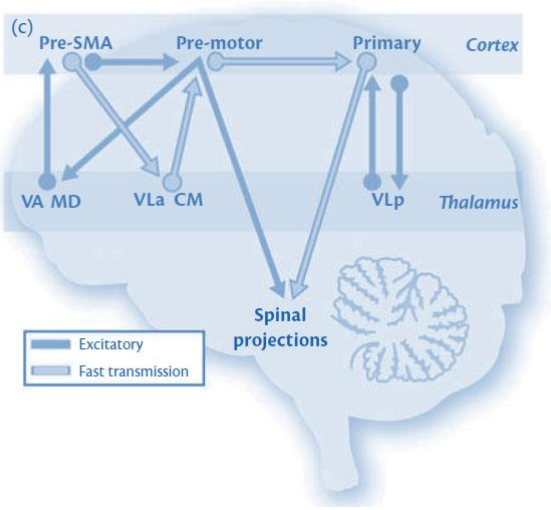

Figure 6 - **Thalamic motor areas and their involvement in motor control (modified from [5]).**
The diagram shows the main cortico-thalamic-cortical pathways involved in movement.

CM, central median; pre-SMA, pre-supplementary motor area; MD, medial dorsal thalamus; VA, ventral anterior thalamus; VLa, ventral lateral anterior thalamus; VLp, ventral lateral posterior thalamus.

The cerebellum plays an important role in movement coordinated integration, postural control and motor learning[5,53,61,62].
In humans, this structure consists of three deep central nuclei (dentate, interposed and fastigial nucleus) surrounded by a cerebellar cortex, which is organized in three layers: the deep layer of granule cells, the intermediate layer of Purkinje cells, and the superficial layer of Golgi, stellate, and basket cells. The deep cerebellar nuclei are inhibited by the Purkinje cells, which are activated by climbing and mossy fibres originating in the inferior olivary nuclear complex, pons, brainstem, and spinal cord relay nuclei. The cerebellum also receives inputs from motor and sensory cortical regions[5,61,62].
Based on the type of motor input, the cerebellum can be functionally divided into spinocerebellum, vestibulocerebellum and cerebrocerebellum[5,61,62].
The spinocerebellum compares motor commands with ongoing sensory information and adjusts motor programs to improve accuracy between intent and performed movements. Spinocerebellar pathways synapse on neurons in the fastigial and interposed deep nuclei, as well as the cerebellar cortex.
The vestibulocerebellum plays a role in postural control and in coordinating head and eye movements via the medial vestibulospinal tract, as well as eye movement control via fibres in the medial longitudinal fasciculus to extraocular motor nuclei.
The cerebrocerebellum is involved in motor learning and movement coordination; it receives input directly from the pontine nuclei through the middle cerebellar peduncle[5,61,62].
Output from cerebellum terminates mostly in the red nucleus, in the midbrain and in ventrolateral posterior and ventro-intermedial nuclei of the thalamus. These thalamic nuclei project to several cortical motor regions, thus closing a loop between cerebellum and cortex and establishing the cerebello-thalamic-cortical circuit[5,61,62].
The cerebellum is also indirectly connected with the basal ganglia via a disynaptic pathway. This projection from the deep cerebellar nuclei to the striatum via the centrolateral nucleus of the thalamus was indeed demonstrated in both mice and primates[5,63,64]. There is also evidence that the striatum receives inputs from portions of the ventral thalamus that are related to the cerebellum [5,65].

The basal ganglia (BG) comprise a functional ensemble of sparse and interconnected subcortical nuclei involved in motor control and learning, emotional and reward processing, as well as, associative and cognitive functions (Figure 7)[53,66–69].

The BG nuclei encompass the caudate nucleus, putamen, the internal and external segment of the globus pallidus (GPi and GPe, respectively), subthalamic nucleus (STN) and the substantia nigra (SN). The pedunculopontine nucleus (PPN), nucleus accumbens and the olfactory tubercle can be considered associated BG nuclei[66].
The caudate nucleus and the putamen share similar structure and function and constitute the Striatum.
The striatum, as the other BG nuclei, can be functionally distinct in dorsal and ventral systems. The ventral striatum with the nucleus accumbens, basal nucleus of Meynert, olfactory tubercle, the substantia innominate, ventral tegmental area (VTA) and the amygdala is part of the limbic system that control emotional, volitional and motivational processes as well as long-term memory and olfaction[5,66]. Instead, the dorsal striatum with the GPi and GPe, PPN, STN and SN are responsible for motor control[5,67] and will be discussed further.

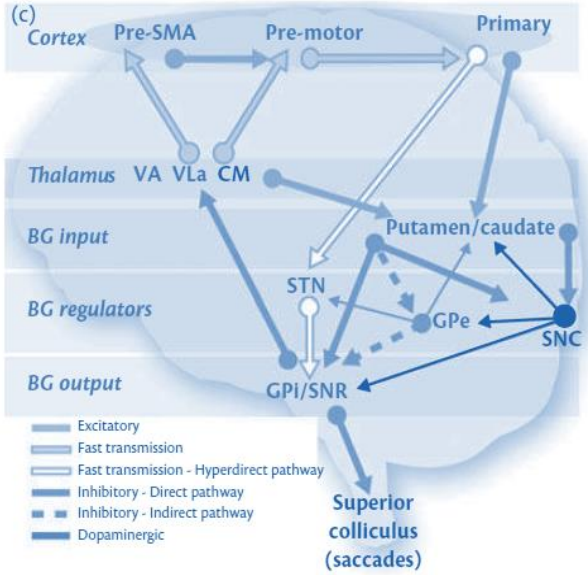

Figure 7 Anatomy of the basal ganglia and their regulation of motor cortices (modified from [5]).
The diagram shows the main anatomical projections and regulatory interconnections involving the basal ganglia. The basal ganglia can be subdivided, based on their interactions, into input, output, and regulator nuclei.

BG, basal ganglia; CM, central median; pre-SMA, pre-supplementary motor area; MD, medial dorsal thalamus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VA, ventral anterior thalamus; VLa, ventral lateral anterior thalamus; VLp, ventral lateral posterior thalamus.

The Striatum

The striatum is the largest subcortical brain structure and it functionally represents the gate of the BG[67].
It is composed of 90% projection neurons, which show GABAergic inhibitory axons and are known as medium-sized spiny neurons (MSNs)[66,68]. MSNs are divided further according to the expression of the dopamine receptor family into DR1- and DR2-MSNs[66,68].
In addition to MSNs, the striatum contains two types of interneurons, which are classified according to their neurochemical profiles[66,68]. The main population is composed of GABAergic fast-spiking interneurons (FSIs), which receive direct cortical input and can inhibit MSNs[70]. The second type of striatal interneurons are cholinergic tonically-active neurons (TANs), which are strongly driven by thalamostriatal inputs. TANs and FSIs modulate the activity of MSNs and like them are influenced by dopaminergic inputs, thus forming an intra-striatal micro-circuit that perform a first-level integration of the input conveying to the BG[70].
The largest striatal input comes from the cortex, with both ipsi- and contralateral cortices sending glutamatergic projections to MSNs in a topographically organized fashion[66,68].
Thalamostriatal projections are also a source of glutamate, with the midline, intralaminar and ventral (motor) thalamic nuclei forming definite bundles[66,68]. However, the functionally most relevant input to the striatum is the dopaminergic nigrostriatal projection[66,68].
This system originates from the SNc (A9 neurons[71]) and is essential for proper striatal (and BG) functioning, and also represents the key structure of PD neurodegeneration. Nigrostriatal axons synapse with both types of striatal MSNs (i.e. DR1- and DR2-MSNs) and exert an opposite action, with dopamine release facilitating D1R-MSNs firing while inhibiting D2R-MSNs activity[66,67].
Recent evidence also showed that striatal dopamine release influences the spatial distribution of MSNs activation, thus shaping the activity of functionally specific neuronal clusters[72]. This finely-tuned striatal regulation might be also partially exerted by the serotonergic projections originating from the raphe nuclei and is reinforced by the discrete organization of striatal output, which present DR1-MSNs mainly projecting to the GPi and SNr and DR2-MSNs mainly targeting the GPe nucleus[73].

Internal segment of Globus Pallidus (GPi) and Substantia Nigra Pars Reticulata (SNr)

The GPi and the SNr are the output nuclei of the BG and represent the main target for GABAergic inhibiting striatal DR1-MSNs, and glutamatergic excitatory axons from the STN, and afferents from thalamic CM/Pf also exist[66,74].

Although different anatomical structures, the GPi and the SNr share several structural features, both presenting inhibitory GABAergic projections with a high rate of tonic discharge[66]. This firing is spontaneous during wakefulness and does not present the pauses that characterize GPe neurons[70]. GPi and SNr neurons are capable of bursting at very high rates, which increases in cases of dopamine depletion[70], possibly because of both an altered afference and changes in their intrinsic activity[70].

The GPi and SNr innervate thalamic and brainstem targets (i.e. superior colliculus and the PPN), thus inhibiting cortical and spinal neural activity[75].

External segment of the Globus Pallidus (GPe)

The GPe is the main target of GABAergic inhibiting striatal DR2-MSNs and also receives a strong glutamatergic excitatory input from thalamic and CM/Pf nuclei and the STN, which is also GPe primary target[66,69].

This anatomy suggests a central role for the GPe in coordinating BG-wide activity.

The GPe and GPi are reciprocally interconnected and share several structural features, being the GPe also composed of sparsely distributed GABAergic neurons[66,69]. *In vivo* recordings consistently showed that most GPe neurons fire at high rates with occasional pauses, while a minor group fires sparsely with intermittent bursts[70]. The many GPe-STN interconnections create a tight microcircuit that is supposed to work as a functional pacemaker for the BG ensemble[76].

The Subthalamic Nucleus (STN)

The STN is the only excitatory structure of the BG and shows a rich afferent innervation that encompasses (i) GPe GABAergic inhibitory projections, (ii) direct cortical glutamatergic excitatory projections from M1 and premotor cortices (mainly SMA), (iii) bilateral thalamic glutamatergic excitatory projections from the caudal intralaminar nuclei and (iv) sparse dopaminergic projections from the SNc[66,69]. Of relevance, the cortical projections are monosynaptic and somatotopically-organized, thus representing a striatal by-pass[77].

These rich afferences are essential for regulating the STN neural firing, which depends on the interplay between its intrinsic electrophysiological properties and glutamatergic, GABAergic and dopaminergic inputs. STN neurons are spontaneously active and show a tonic firing pattern with a firing rate in the 10–30 Hz range in (healthy) nonhuman primates[70]. They express T-type (Cav 3) calcium channels, which prompt a rebound burst firing, and the likelihood of burst seems mainly influenced by dopamine[70].

The STN glutamatergic projections reach (i) the GPi and the SNr and (ii) to the GPe with highly branched neuronal processes, which allows a simultaneous innervation of these three nuclei[66,69]. It also influences the spinal CPGs acting on the PPN, which increases the activity of the nucleus reticularis gigantocellularis of the reticulospinal tract[55,78,79].

Substantia Nigra pars compacta (SNc)

The SNc (A9 neurons) supplies the BG with dopamine[71] and it mainly targets striatal MSNs, thus forming nigrostriatal projections[66]. The SNc neurons are rich in neuromelanin, a dopamine precursor that paints this structure black[80].

They show intrinsic pacemaker activity, firing below 10 Hz *in vivo* to maintain tonic release of dopamine in both striatal and extra-striatal BG nuclei, thus strongly influencing the activity and firing patterns of the whole BG ensemble[70]. In addition to tonic dopamine release, SNc neurons provide phasic release of dopamine that have been shown to regulate the strength of cortico-striatal synapses, thus facilitating reward-based learning[70].

Nigrostriatal projection degenerates in PD and causes a severe dopamine loss in the striatum of PD patients. Lack of dopamine in the striatum may alter the morphology of dendritic spines of MSNs, which appear fewer and altered in patients and animal models of PD[5,81].

The brainstem also hosts two other dopaminergic neuron ensembles that mainly target extra-BG nuclei: the ventral tegmental area (VTA, A10 neurons) and the retrorubral filed (A8 neurons)[66,80].

The VTA belongs to the ventral BG and innervates the nucleus accumbens and other territories of the limbic system as well as the locus coeruleus (LC), the main source of norepinephrine of the brain[82]. This nucleus was showed to react to dopaminergic denervation by increasing its firing, thus modifying SNc activity and possibly

compensating for initial neurodegeneration. Indeed, VTA neurons degenerate only marginally as compared to SNc[80] and may trigger early compensatory attempts. The A8 instead are a small proportion of dopaminergic neurons (≈1000 cells) that are located dorsally and caudally to the SNc. Like VTA neurons, the A8 neurons also project to the nucleus accumbens and the limbic cerebral cortex, thus being part of the limbic system [66,80].

2.3 Functional anatomy: from structure to function

The many BG nuclei can be considered a functional ensemble, which represent a common station for parallel neural loops that start from the cortex, traverse BG and thalamus, and return to the cortical area of origin [5,83–85], thus defining the cortical-striato-thalamic circuit.
This circuit processes four different functions in the 'motor', 'oculomotor', 'associative', and 'limbic' loops (Figure 8)[84]. Although there is a degree of convergence[5], these loops are largely functionally distinct and show an elevated topographic organization that allows simultaneous processing of different information[5,83–85].

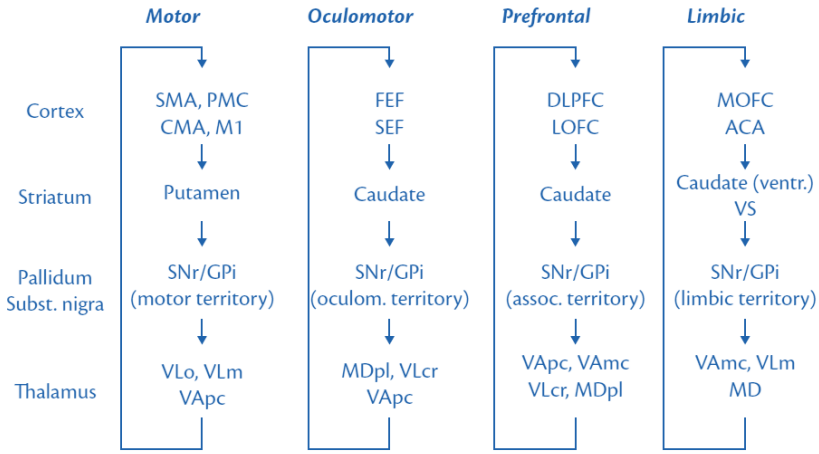

Figure 8 - Circuit anatomy of the cortex–basal ganglia–thalamocortical circuits (modified from [5]).
The basal ganglia are part of multiple segregated circuits that involve specific territories in the basal ganglia and associated areas of thalamus and cortex.

ACA, anterior cingulate area; CMA, cingulate motor area; DLPFC, dorsolateral prefrontal cortex; FEF, frontal eye fields; LOFC, lateral orbitofrontal cortex; M1, primary motor cortex; MD, mediodorsal nucleus of the thalamus; MDpl, mediodorsal nucleus of thalamus, pars lateralis; MOFC, medial orbitofrontal cortex; PMC, pre-motor cortex; SMA, supplementary motor area; SEF, supplementary eye field; VApc, ventral anterior nucleus of thalamus, pars parvocellularis; VAmc, ventral anterior nucleus of thalamus, pars magnocellularis; VLm, ventrolateral nucleus of thalamus, pars medialis; VLo, ventrolateral nucleus of thalamus, pars oralis; VLcr, ventrolateral nucleus of thalamus, pars caudalis, rostral division.

This functional organization is maintained also within the BG, which present a functional subdivision in distinctive territories that goes beyond the anatomical structures[5,86–88].
The BG gates cortical inputs with the striatum and the STN[66,77]. In particular, striatal MSNs organize cortical inputs onto two different pathways: (i) D1R-MSNs form a *direct* (monosynaptic) GABAergic inhibitory pathway that directly reaches the GPi and SNr, and (ii) D2R-MSNs form an *indirect* (polysynaptic) pathway that targets the GPe, which sends GABAergic inhibitory projections to STN that finally reaches the GPi and SNr with glutamatergic excitatory axons (Figure 9)[67].
These pathway distinctions are based on anatomical tract-tracing and immunohistochemical studies, which showed that the MSNs of the *indirect* pathway express encephalin while the *direct* pathway MSNs express substance P[67]. Studies in transgenic mice also confirmed the lack of overlap between the D1R- and D2R-MSNs composing the two pathways[5,67], but were not confirmed by single-cell tracing studies in both rodents and monkeys[5,89].
Although questioned[73], this discrete segregation holds a functional relevance as some models for BG function are built around the notion that activation of the *direct* and *indirect* pathways have opposite effects on motor control[5,67]. Specifically, it is thought that the *direct* pathway by directly inhibiting GPi and SNr GABAergic output leads to activation of thalamic and cortical activities, thus facilitating movements[5,67]. To the contrary, the activation of the

indirect pathway results in inhibition of GPe GABAergic neurons, which release STN glutamatergic firing, thus increasing the GPi and SNr inhibition of thalamus and cortex and reducing the drive to movement[5,67].

This dualistic effect is the core of the 'scaling' hypothesis of BG function, which states that the *direct* and *indirect* pathways regulate movement amplitude and speed (Figure 9)[5,67]. According to this model, when a movement needs to be executed, the cortical inputs activate the *direct* pathway that facilitates movement by disinhibiting thalamo-cortical neurons. The facilitated movement is corrected or terminated by a following inhibitory activation of the *indirect* pathway, which leads to a disinhibition of BG output nuclei (increased BG inhibition of thalamo-cortical neurons)[5,67]. Evidence for this model comes from studies of the activity of pallidal neurons in monkeys trained to perform movements with different amplitudes[5,90].
An alternative hypothesis proposed a role for BG in 'action selection' through a centre-surrounding mechanism (Figure 9)[91]. In this view, the activation of the *direct* pathway facilitates intended movements, while unintended movements are contextually suppressed by the *indirect* pathway[5,91]. To this end, either *direct* and *indirect* pathway MSNs receive detailed information about intended and unintended movements, respectively, or the FSIs (striatal GABAergic fast-spiking interneurons) self generates these opposing activation patterns through lateral inhibition mechanisms (Figure 9)[5,91].

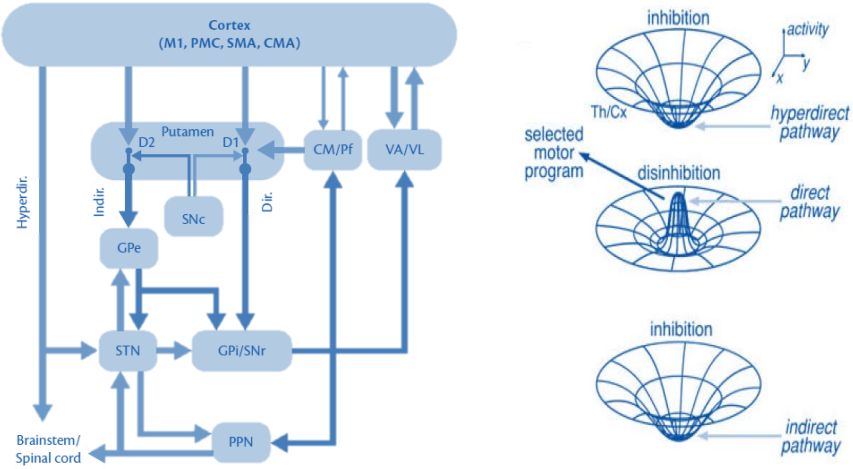

Figure 9 – Conceptualization of the circuits and functions of the cortical-striato-thalamic circuit (modified from [5] and [92]).
On the left, the connections between cortical and basal ganglia nuclei, thalamus, and cortex are represented. This is a detailed representation of the 'motor circuit' of the basal ganglia. Blue arrows indicate inhibitory connections; grey arrows indicate excitatory connections.
On the right, the dynamic model of basal ganglia function is reported. This model explains the activity changes in thalamus and/or cortex (Th/Cx) caused by sequential inputs through the hyperdirect (top), direct (middle), and indirect (bottom) pathways. Time (t) proceeds from top to bottom.

Main abbreviations: CM, centromedian nucleus of thalamus; CMA, cingulate motor area; Dir., direct pathway; D1, D2, dopamine receptor subtypes; Indir., indirect pathway; M1, primary motor cortex; Pf, parafascicular nucleus of the thalamus; PMC, pre-motor cortex; PPN, pedunculopontine nucleus; SMA, supplementary motor area.

Although fascinating, both the 'scaling' and 'action selection' hypotheses are questioned because of the excessively long latencies of BG firing activity, which occurs too slowly[5]. Moreover, recent evidence casts serious doubts on the functional activation of *direct* and *indirect* MSNs[73]. By the use of a fluorescence micro-endoscopy in freely behaving wild-type or parkinsonian mice to simultaneously monitor activity and spatial arrangements of genetically engineered MSNs, which became fluorescent when calcium entered the cell (i.e. spiking), Parker et al. recently reported that similar clusters of *direct* and *indirect* MSNs are simultaneously activated during movement, thus contradicting both the 'scaling' model (under which *direct* MSNs should fire before *indirect* MSNs) as well as the 'action selection' model (which predicts that clusters of *indirect* MSNs should be larger than *direct* MSNs)[72]. Taken together, these findings suggest the basic execution of movement (i.e. movement scaling and selection) largely relies on cortical processing, while the BG may possibly regulate their dynamic shaping and *vigour*[92].

The BG also present a 'hyperdirect' pathway that links premotor cortices and the STN with a monosynaptic glutamatergic excitatory connection[77], thus bypassing the striatum and allowing an occasional fast cortical increase of BG output[93].
This pathway may support the inhibition of ongoing motor programs under behavioural situations that require 'set-shifting' (i.e. the ability to unconsciously shift attention)[5].
Imaging findings showed that the neural network controlling the response inhibition includes frontal and prefrontal regions (particularly the right pre-SMA), which are directly connected to the BG and the STN, directly[5]. Accordingly, lesion of the BG and, the STN in particular, have been associated with deficits in stop-signal reaction time tasks, particularly in situations of behavioural conflict[5,94,95]. STN lesions have also been shown to affect the 'oculomotor' loop, inhibiting automatic eye movements when switching to voluntary ones[96].

The different functional loops also show a preferential organization of output.
The 'motor' and 'oculomotor' loops mainly relay on the GPi, which projects to the thalamus in a topographically specific manner, reaching the ventrolateral nucleus of the thalamus (VL) [5,67].
The 'associative' and 'limbic' (non-motor) loops mostly rely on the SNr output, which reaches the ventral anterior nucleus (VA). The cortico-striato-thalamic circuit is then closed by thalamic projections from the VL and VA nuclei to motor and non-motor cortical areas, respectively[5,67].
Other relevant BG output projections are directed at the brainstem, including prominent reciprocal connections to the PPN, which may then give rise to descending projections for regulation of locomotion[55,78,79].
Saccades movements are regulated by combined SNr and GPi input, which regulate the activity of the superior colliculus[96].

2.4 Pathophysiology of parkinsonism: current hypothesis

The complex BG organization supports the unconscious and simultaneous processing of different (motor, cognitive and emotional) signals that shape normal behaviours. This functional organization breaks down with the dopamine loss occurring in PD[70,97].
Neurons in the BG and thalamus show pathological activities that have been associated with motor impairments in patients and animal models of PD, thus encouraging the formulation of many pathophysiological theories.
These functional models focus on distinctive alterations occurring in PD, such as abnormal firing rates and burst patterns or exaggerated oscillations and neuronal synchrony[70,97]. However, it is important to stress that these abnormalities are not isolated, but combined and influencing each other.

The 'rate model' of PD
This model is based on the observation that dopamine loss alters the firing rate of BG neurons[98].
Recordings in a 6-hydroxydopamine (6-OHDA) rodent model of PD have shown that the activity of *direct* pathway MSNs is reduced, while the spontaneous discharge and responses to cortical stimulation of *indirect* pathway MSNs are enhanced[99].
These changes in *direct* and *indirect* MSNs match the increased STN and GPi activities and the reduced GPe firing found in non-human primates treated with 1- methyl- 4- phenyl- 1,2,5,6- tetrahydropyridine (MPTP)[97,100]. Moreover, the alterations of STN and GPi activity mirror the recordings in PD patients undergoing neurosurgical interventions[97,101–103]. Notably, these changes were characteristic of PD, lacking in subjects with essential tremor[104].

The 'rate model' explains these abnormal STN, GPe and GPi firing as consequences of the unbalanced activity between *direct* and *indirect* pathways that follow striatal dopamine loss (Figure 10). In particular, the loss of dopaminergic inhibition onto the GABAergic inhibitory D2R-MSNs of the *indirect* pathway would suppress the inhibitory GPe action on the STN, thus realising STN firing and increasing the inhibitory activity of GPi and SNr neurons.
In addition, the dopamine loss would decrease the activation of striatal D1R-MSNs of the *direct* pathway, thus further supporting GPi and SNr firing. The resulting increased GPi and SNr GABAergic inhibitory activity supresses thalamic and brainstem neural activity, possibly impairing normal movements (Figure 10)[67,105].

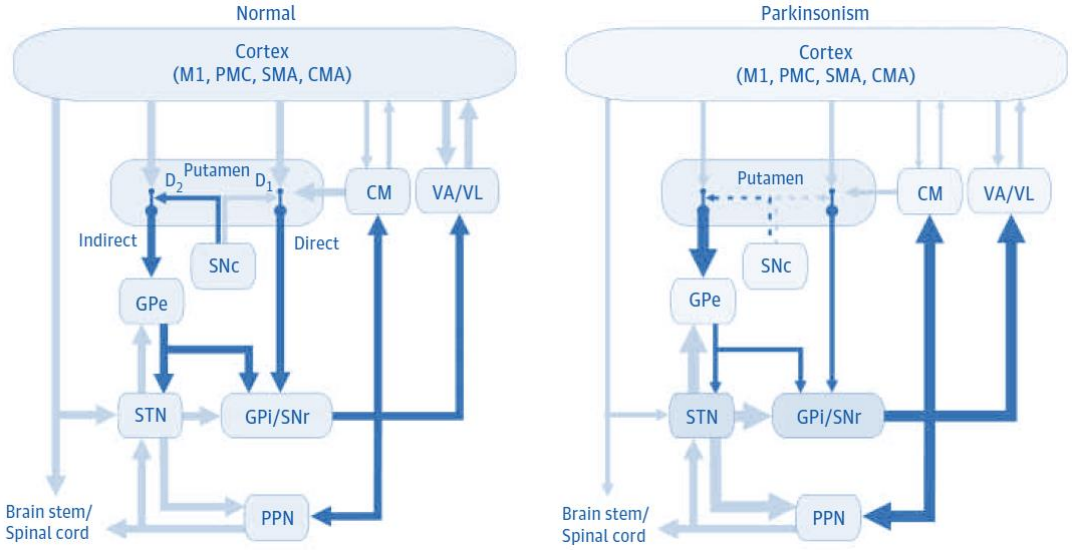

Figure 10 - Parkinsonism-related changes in overall activity ('rate model') in the basal motor circuit (modified from Galvan et al [97]).
Blue arrows indicate inhibitory connections; gray arrows indicate excitatory connections. The thickness of the arrows corresponds to their presumed activity.

The pathological alterations postulated by this model imply corresponding anatomical and biochemical changes, particularly in GABAergic activities, which have been measured with microdialysis, glutamate decarboxylase (GAD) assays and molecular imaging tracers targeting GABA receptors in both patients and animal models of PD (reviewed in [97]).
Microdialysis studies in animal models of PD showed that the level of GABA is increased in GPe, thus supporting an over-activation D2R-MSNs of the *indirect* pathway that inhibit the GPe[97]. Accordingly, measurements in the STN, which only contains GPe GABAergic terminals, also showed a decreased level of the GABA activity[97]. Instead, the GABA activity of the SNr was not found to be consistently increased, with studies reporting unchanged values[97]. Post mortem tissue studies in PD patients also questioned the pathologically relevant increase in GABAergic activity, showing unchanged values in different BG nuclei[97].
Protein and mRNA measurements of the GABA-synthesizing enzyme GAD also gave conflicting results, which showed increased GAD levels in D2R-MSNs of the *indirect* pathway as well as in GPi and SNr neurons of dopamine-depleted animals, but unchanged values in GPe and STN[97].
Molecular imaging findings showed instead, an expected reduction of GABA receptor density in GPe and an increased (compensatory) expression in GPi and SNr, thus supporting the hypothesis of the 'rate model'[97].
To further confirm this hypothesis, glutamatergic transmission has been assessed, but no consensus on the changes in the binding and expression of striatal ionotropic glutamate receptors (NMDA- and AMPA-type) has been found[97]. These receptors appear to be down-regulated in the GPi and SNr of patients and animal models of PD, thus possibly expressing a compensatory response to the increased STN glutamatergic drive, but confirmatory evidence is lacking[97].

Altogether these findings suggest that the 'rate model' over simplifies the alterations occurring in PD and does not provide an exhaustive explanation to muscular rigidity and parkinsonian tremor[106]. Moreover, lesion or alteration of the GPi, GPe or thalamic VA/VL nuclei failed to produce parkinsonism, thus suggesting that the 'rate model' is inadequate and that other alterations contribute to PD symptomatology[106]. These may include altered (i.e. oscillatory and/or synchronized) firing patterns[70,97,107].

Burst discharges
Bursts describe brief episodes of high frequency firing imposed on slower background activity.
They represent a distinct firing mode and are supported by the occasional activation of specific ion channels, thus likely encoding distinct information, qualitatively different from that conveyed by single spikes[108]. Burst also induce a greater synaptic and post-synaptic effect than spikes alone, thus domineering neural information flow[108].

The 'firing pattern model' suggests that exaggerated burst firings may disable an individual neuron's ability to process and relay information, thus limiting the computational function of the BG and impairing appropriate motor control (Figure 11)[70,97,107,109].

Normally, GPi, GPe and STN neurons do not fire in bursts: GPi fires continuously at high frequency, GPe fires at high frequency with pauses, and STN fires continuously at a middle frequency range. To the contrary, striatal dopamine depletion promotes burst firing[70,104].

Several studies in the primate MPTP and rodent 6-OHDA models of PD reported distinct changes in GPe, GPi and STN neurons burst firing [70,97,100,107]. Precisely, dopamine depletion was associated with an increase in burst firing (i.e. the proportions of time that the neurons spend in bursting activities), the proportions of spikes within bursts as well as the length of individual bursts[110]. Recordings in PD patients undergoing neurosurgical interventions also confirmed these alterations[103] and showed a specific relationship where dopaminergic denervation was not increased in subjects with essential tremor[104].

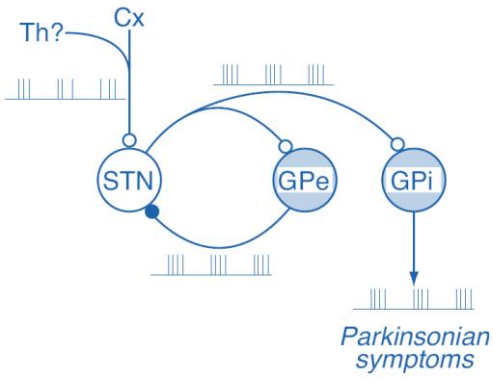

Figure 11 – the firing pattern model (modified form [105]).
Cx, cerebral cortex; GPe and GPi, external and internal segments of the globus pallidus; STN, subthalamic nucleus; Str, striatum; Th, thalamus

Excessive bursts may originate in GPe-STN microcircuit as suggested by dopamine-depleted cell-cultures of GPe and STN neurons that showed bursts if GPe-STN interconnections were present[76]. In particular, the reduction of STN direct dopaminergic innervation may enhance the effects of GPe GABAergic inputs, thus triggering STN rebound burst firing[97]. Accordingly, the activation of STN neurons D2 receptors normalized STN bursting in cell slices[97]. However, the relation between dopaminergic treatment and burst suppression is still unclear, as some studies reported a burst increase following dopamine replacement therapy in different BG nuclei in both patients and animal models of PD[70,97,107]

Overall, it is likely that excessive burst firing limits information processing in the cortico-striato-thalamic circuit, but it remains unclear whether they encode a pathological role *per se*.
Recent studies assessed this hypothesis by investigating the number, amplitude and duration of STN bursting activity in PD patients with and without medication[111–113]. Tinkhauser and colleagues found that STN bursting activity may be distinguished into short and long burst, which were small and high in amplitude, respectively[111–113]. The number of longer (high) bursts correlated with clinical impairment, while the opposite was true for short (small) bursts[111–113]. Moreover, motor improvement correlated with the decrease in burst duration *per se*[111–113]. Indeed, levodopa treatment lead to a relative increase of shorter, lower amplitude bursts[111–113]. Of note, the bursting activity described by Tinkhauser and colleagues does not directly refer to actual bursts (i.e. block of organized spikes) but rather an indirect representation of this pattern as assessed by means of an artificial and arbitrary analysis of local field potentials (LFPs), which reflect coordinated transmembrane and synaptic potential fluctuations of a large neural ensemble[114]. In this context, bursts are assumed to occur periodically and to contribute to the development of predominant neural oscillations[107,111–113].

Oscillations
If burst activity occurs periodically, it becomes oscillatory activity (although they remain independent properties of neural activity[115]).
Oscillatory activity is actually a nonspecific term that indicates periodical repetition of some neural activity, which can be single unit spikes, bursts or local field potentials (LFPs)[114].
Oscillations are thought to be crucial for efficient communication within and across brain circuits, representing a means for brain coordination[116,117]. They are conventionally divided into primary frequency bands in the delta (δ, 1–4 Hz), theta (θ, 4–8 Hz), alpha (α, 8–13 Hz), beta (β, 13–30 Hz) and gamma (γ, >30 Hz) ranges, as well as in sub-bands[118]. Different frequency bands provide distinct temporal and spatial resolution for processing, with low-frequency bands relying on large spatial regions and a long duration and high-frequency bands on small neural clusters and a short duration[119].

Single unit recordings in PD patients undergoing functional neurosurgery and MPTP-treated monkeys showed prominent oscillations in the θ-, α- and β-frequency bands in GPe, GPi, and STN neurons[97]. In particular, recordings in the GPi and STN of tremulous MPTP-treated monkeys showed a bimodal distributions (with spectral peak at 5 and 10 Hz) of the exaggerated oscillation[70,97], which, however, correlated poorly with rest tremor severity and persisted after tremor suppression[70,97].
Instead, single unit neuronal oscillations in MPTP-treated monkeys without rest tremor showed to correlate with bradykinesia and rigidity[70,97] and were suppressed by dopaminergic and surgical treatments, mirroring the improvement of spontaneous movements[70,97].

Brain oscillatory activity has been also widely studied by means of LFPs recordings, which by reflecting membrane, synaptic and post-synaptic potentials also encompass the highly regimented patterns of repetitive single unit firing[114]. Accordingly, several studies reported a correlation between LFPs oscillations in the β-band and single neurons activity, especially in the STN of PD patients[70,120]. In this nucleus, single unit firings with spectral peaks in the β-band have been shown to phase-couple with STN LFPs in 20–33% of instances across patients and non-human primates models of PD[121,122].
Although the same consistency is not confirmed in cases of tremor[121] and it is unclear to what extent this spikes-LFPs relationship holds true when moving to macroscales[123,124], it is undoubtedly the case that there are exaggerated α-[125] and β-oscillations[126,127] in the LFPs of STN and GPi neurons of untreated PD patients.

In PD, up to 95% of STN-LFPs show increased spectral power in the β-band[128,129], especially if compared to recordings after medication intake[127], which were instead associated with an increase in θ- and γ-oscillations[126]. Dopamine replacement therapy and surgical treatments consistently showed to suppress STN β-activity and this improvement correlates with clinical scores[130–133]. Just recently, also the β-power *per se* was shown to have some degree of correlation with PD clinical symptoms[134].
Of note, the β-band can be further subdivided into two, not mutually exclusive, sub-bands: lower (13–20 Hz) and upper (20–35 Hz) β-bands[129,135]. The lower β-band usually dominates over power in the upper band and seems more reactive to dopamine[136].
Dopamine replacement therapies were also shown to influence high frequency oscillations (HFO), determining a switch from ≈250 Hz to ≈250–350 Hz in STN-LFPs[137].

All these exaggerated oscillations tend to localize in distinctive functional territories of the BG, with β-oscillations mainly spotting 'motor' areas[88].
Recordings in animal models of PD reported an enhanced β-activity in the 'motor' functional territories of cortex, striatum, GP, STN, and SNr[70]. In line, STN β-oscillations were shown to vary with movements, being suppressed during movement preparation and execution, while showing a post-movement rebound[70]. This activity-pattern is likely physiological as it has been reported in healthy monkeys[138] and non-PD patients[139] and it closely mirrors movement-related cortical β-modulation[140,141].
The movement-related β-suppression is conserved also in PD, albeit longer latencies that mark a reduction of β-reactivity [142–144]. Of note, movement-related β-suppressions are not equal for every movement[135,145] and show a bilateral response, with greatest reductions for movements contralateral to the most dopamine depleted hemisphere[144,146]. While several studies assessing multi-joint upper arms goal-directed movements showed a clear β-suppression, this was not the case for more complex and distributed movements, such as gait[145,147–151].

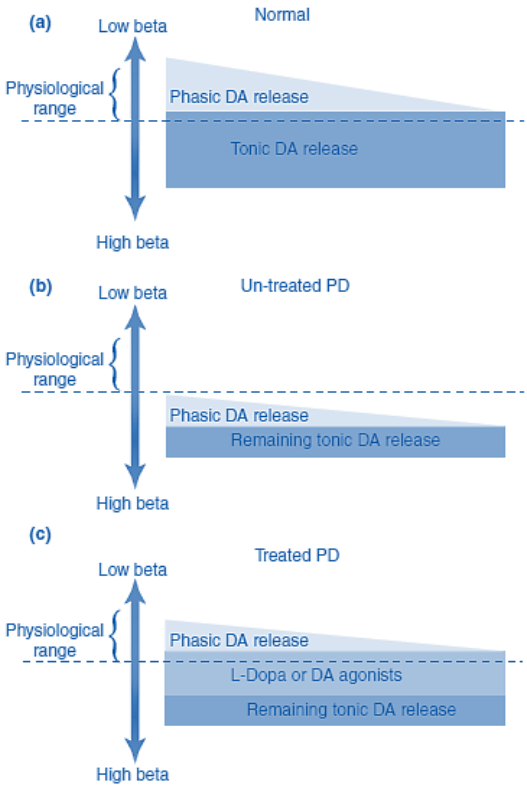

Figure 12 – Model of dopamine and β-activity relationship in health and in PD(modified form Jenkinson and Brown [153]).
(a) In the normal state, tonic and phasic dopamine release combine to give high levels of dopamine and beta oscillations are driven down into the physiological range. (b) In PD, loss of nigral dopaminergic (DA) neurons means that there is less presynaptic dopamine for release in the striatum and STN. Without treatment, net dopamine (i.e. the sum of tonic and phasic release modes), is at a low level. Beta oscillations are consequently elevated and vary with levels outside of the physiological range. (c) Treatment of PD patients with levodopa or dopamine agonists changes the set-point of the system. Boosting tonic dopamine activity brings variations in net dopamine owing to phasic release back within the physiological range. Consequently, beta oscillations are driven back to normal levels.

The suppression of β-activity is not limited to movement, but was also showed to follow recognition of salient cues that herald forthcoming action demands. Predictive cues could suppress STN β-oscillations even when unrelated to motor processing, but always in a dopamine-dependent fashion, thus being deficient in subjects with PD (Figure 12)[152].
Altogether these findings add to the formulation of a pathophysiological hypothesis centred on β-oscillations[153–155].
It has been proposed that β-oscillations signal immutability and promote the current (ongoing) state over a novel action selection, namely index the *status quo* (i.e. tonic muscular activity)[154]. The theory also posits that β-activity is a direct consequence of BG net dopamine levels, with low dopaminergic levels prompting exaggerated β-oscillations development and endurance (Figure 12).
Indeed, it has been suggested that, in the normal state, tonic and phasic dopamine release maintain high levels of net dopamine and ensure low and reactive β-oscillations, respectively. The dopamine loss occurring in PD reduces overall net dopamine levels, thus supporting an elevated and stable β-activity (Figure 12)[153–155]. Dopaminergic treatment can reverse this condition by boosting tonic

dopamine levels (Figure 12)[153–155]. Thus, the dopamine-related putative promotion of tonic motor activity by high and stable β-oscillations occurring in PD would explain bradykinesia, akinesia and rigidity (Figure 12)[153–155]. Indeed, excessive and persistent β-oscillations would not just lead to the maintenance of existing posture and muscular tone, but rather induce an unnecessary (pathological) reinforcement of muscle tone through the suppression of salient motor cues signalling novel motor actions[152]. Therefore, the mechanism by which exaggerated β-oscillations could cause PD symptoms is through an impairment of the information flow in the cortico-striato-thalamic motor loop[153–155].
Excessive β-oscillations would limit the computational capacity of the BG[153–155], causing it to be stuck in the β-rhythm whilst not being able to properly and timely convey β-motor commands to thalamic and brainstem targets, thus impairing motor processing and proper movements preparation and execution.

Although interesting, this theory met a number of criticisms. Studies in animal models of PD found that enhanced β-oscillations emerge days after the motor deficits and when dopamine depletion is extremely severe (≈90%), thus arguing against a causal role for these oscillations in the genesis of bradykinesia and rigidity[156,157]. The relation to dopaminergic depletion was also questioned by studies in patients with focal dystonia, a disease that does not show any dopaminergic alteration nor bradykinesia, in whom there was found an enhancement of β-oscillations similar to PD[158]. Moreover, β-oscillations are poor in patients and animal models of PD showing tremor, although bradykinesia is (and must be) also present[70,97,159]. Inconsistencies were also reported in the response to dopamine-independent treatment (i.e. surgical high frequency stimulation, HFS), which showed a marginal reduction of β-oscillations under therapeutic stimulation in MPTP-treated non-human primates [122].
The mechanisms by which excessive oscillations develop and why β-frequency is predominant are also unclear[153–155]. Striatal MSNs were initially suspected to generate this rhythm, but their very low and generally non-rhythmic activity makes it unlikely[160]. Still, some studies recorded β-oscillations in the striatum[119], especially in the FSIs after striatal dopamine depletion[161].
Alternatively to the striatum, the GPe[162] and the STN[100,122,133] have been proposed as possible sources of oscillations, as synchronous oscillatory activities have been observed in both nuclei, but the evidence is not conclusive[70,97,157]. Clearly, also the interplay between these two nuclei might play a role in oscillation development[76]. As proposed for burst firing, STN neurons may generate oscillatory β-bursts in response to GPe periodic inhibitory inputs, thus entraining GPe activity and creating a self-sustaining oscillatory loop[76]. Once generated, this β-oscillation can be transmitted by the STN to the GPi and propagated to the entire cortico-striato-thalamic circuit[153–155]. However, besides the lack of *in vivo* evidence for such a STN-GPe pacemaker, it is unlikely that this mechanism can develop β-oscillations as STN/GPe cultures only generate very low-frequency oscillations (< 1 Hz), even in dopamine depleted state[76]. The cortex has been therefore proposed as the primer of the oscillatory activity by modulation of the spontaneous striatal and STN firing[70,107]. Indeed, an altered susceptibility to cortical or thalamic inputs induced by the dopamine loss may provide a means for BG exaggerated synchronization to a predominant rhythm[163].

Abnormal synchrony
Oscillations reflect synchronous neural entrainment, thus meaning that neurons fire in a related fashion. This does not mean that neurons fire simultaneously, nor at the same frequency, as they can entrain out of phase and drive each other rhythms to harmonic frequencies (Figure 13)[164].

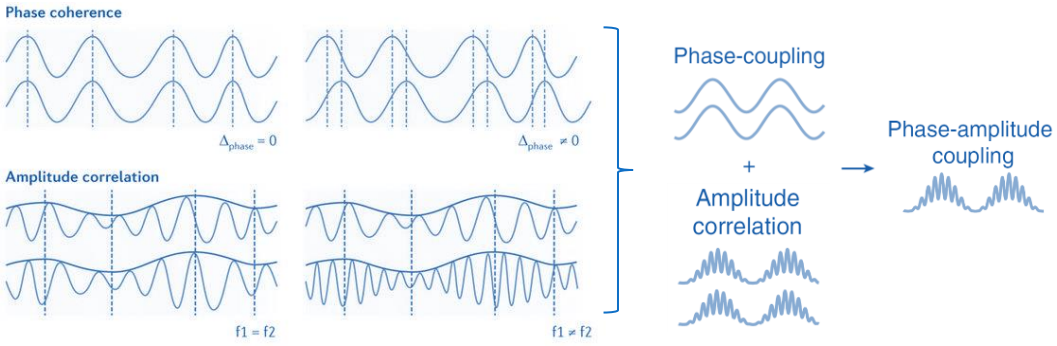

Figure 13 – Phase, Amplitude and phase-amplitude synchronization processes (modified from Siegel et al. [117] and from von Nicolai et al. [319]).
On the left, phase coherence is represented on top. It quantifies the consistency of the relative phase between two simultaneous signals that have the same frequency. The panels show an example of two oscillatory signals that are phase coherent with zero phase lag or with non-zero phase lag (i.e. phase-shifted). Amplitude correlation is showed in the bottom panel. It is a measure of the correlation of the envelopes of two simultaneous oscillatory signals (aka 'power-to-power correlation' or 'amplitude–amplitude coupling'). Amplitude correlation can be measured between oscillatory signals of the same or different underlying carrier frequencies. Furthermore, amplitudes can be positively correlated or negatively correlated (that is, anti-correlated). The panels show examples of a positive amplitude correlation between two oscillatory signals of the same or different underlying carrier frequencies. Importantly, phase coherence and amplitude correlation are independent of one another as showed by the interaction depicted at different frequency (f). The right panel shows phase-amplitude coupling processes, a detail description is reported in the text.

Unlike other brain areas, where synchronous neural oscillations give rise to networked activity patterns[118,165], in the BG synchronized network oscillations are supposed to be pathological[163,166–168].
Under normal conditions, BG neurons fire in an uncorrelated fashion, whereas dopamine depletion increases the synchrony between neighbouring BG neurons as well as between BG activity and cortical and thalamic rhythms[70,97,100,164].
After dopamine depletion, single unit activities within GPi, GPe, and STN showed synchronized firing[70], thus losing spatial discrimination of movement[169]. In particular, MPTP-treated monkeys showed aberrant proportion of neurons responding to movement compared to controls, with increased neurons encoding for similar movements[70,169].
Like single unit activities, LFPs also synchronize after dopamine depletion, thus generating the oscillatory activity discussed above. Indeed, LFPs themselves represent the transmembrane potential fluctuations or a neural ensemble, thus encompassing also single neuron activities. It follows that an increased β-power in LFPs recordings indicates that 'most' neurons are oscillating within this rhythm[114]. For this same reason, the amplitude of STN bursts discussed above reflects the degree of local neural synchronization, progressively increasing with burst duration[170]. It must be stressed, however, that spectral power (e.g. β-power) and neural synchrony are independent features of neural activity, expressing the amplitude and the phase of the oscillation, respectively. Accordingly, it has been shown that these two metrics can differ and carry distinctive information. The clinical improvement following dopamine-independent treatment (i.e. surgical HFS) in MPTP-treated non-human primates, for example, was associated with marginal reduction in β-oscillations but a major decrease in β-coupling[120]. Similarly, movement-related β-modulations do not necessarily mirror changes in neural-coherence [144,149].
Of relevance, besides local synchronization, LFPs signals were also shown to present coherent oscillations throughout the entire cortico-striato-thalamic circuit in dopamine depleted conditions[171]. In particular, the exaggerated β-oscillations were shown to be highly coherent to cortical β-rhythms[172].

Cortical neural activity is also altered in patients and animal models of PD, showing reduced and aberrant activation patterns (especially in M1 and the SMA) [97], and increased cortical neural synchrony over several areas[173] in both lower-frequency (1–7 Hz)[174] and β-bands[175]. The cortical coupling in the β-frequency band was found to correlate with the severity of PD symptoms, while its reduction after treatment correlated with clinical improvement[173]. Moreover, cortical-β power is modulated by movement preparation and execution [176].

These findings advanced the hypothesis that the exaggerated neural synchronization seen in animal models and patients with PD may be related to the dopaminergic striatal loss[177], but of cortical origin (Figure 14)[163,164,167].

Starting from these ideas, de Hemptinne and colleagues investigated the clinical relevance of intra-cortical and M1-STN synchronization by assessing the phase-amplitude coupling (PAC) of M1 and STN LFPs in PD and dystonic patients[163].
PAC (or nested oscillations) is a form of cross-frequency coupling in which the phase of the lower-frequency oscillation (nesting) drives the amplitude (i.e. spectral power) of the coupled higher-frequency oscillation (nested), thus resulting in synchronized faster-oscillation amplitude peaks to slow-oscillation phase.
In PD patients without medication, an exaggerated coupling has been found between the phase of the β-rhythm and the amplitude of broadband-γ activity (50-200 Hz) in M1[163,167], thus indicating that pyramidal neurons of PD patients fire (i.e. γ-frequency modulation) with unnatural periodicity (β-rhythm). Moreover, amplitude peaks in M1 γ-rhythms were also coupled with STN β-phase and M1-STN coherence did not correlate with the magnitude of the STN–M1 PAC, thus implying a causal connection[163]. Accordingly, therapeutic stimulation of the STN reversibly reduced PAC over a similar time course as that of the reduction of motor signs[167]. PAC computation across STN LFPs also showed to correlate with the clinical symptomatology and lead to the speculation that β-coupling might impede pro-kinetic HFO[178].
Most importantly, cortical γ-modulations preceded the STN β-trough, thus suggesting a role for cortical oscillations in entraining BG neurons into exaggerated synchronous firing[163,167].
However, a recent re-analysis from the same group of non-linear properties of M1 β-oscillations found a high degree of correlation with cortical γ-β PAC[179], thus suggesting that the latter might actually be an artefactual result of the spectral analysis of a non-sinusoidal signal[180]. Moreover, this abnormal PAC is not specific of PD, being present also in subjects with dystonia[158] and ET[181].

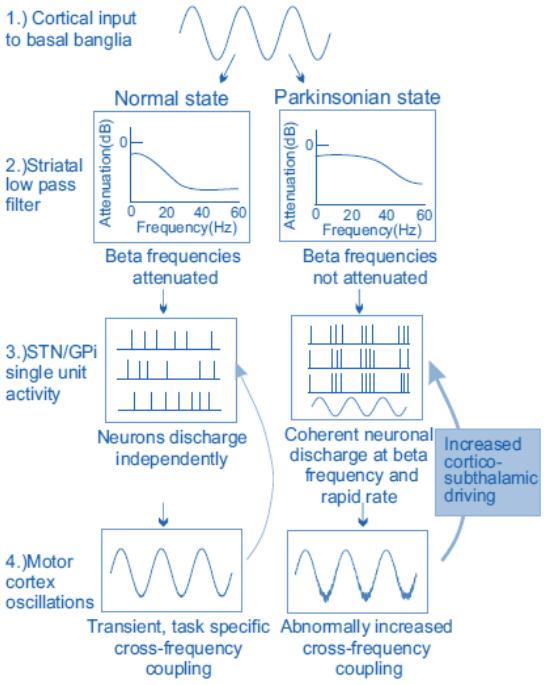

Figure 14 – **Model of cortex–basal ganglia interactions in the normal state and in PD (modified from de Hemptinne et al. [163])**
In general, cortical input to the striatum has a strong β-oscillatory component regardless of disease state. The normal corticostriatal circuitry acts as a low-pass filter with significant β-band attenuation, but the dopamine-denervated striatum produces less β-attenuation. In the Parkinsonian state, STN and internal globus pallidus (GPi) neurons have excessively synchronized activity in the β-band because of the change in the striatal filter. In the Parkinsonian state, excessively coherent basal ganglia β-band neuronal discharge drives M1 to have abnormally increased coupling between β-phase and broadband-γ amplitude. This aberrant coupling, in turn, further strengthens STN β-synchrony and excessive STN firing rate through the hyperdirect corticosubthalamic pathway.

2.5 Purpose of this work: parkinsonism as a network phenomenon

None of the current theory can properly explain the entire clinical spectrum of PD symptoms.

Although it is clear that parkinsonism is associated with increased firing, bursts, oscillations and neural synchrony, it is quite unlikely that specific electrophysiological abnormalities in the cortico-striato-thalamic circuit actually cause parkinsonism.
In fact, if the 'rate model' likely oversimplifies the dysfunction occurring in PD and fails to account for a series of observations (summarized in Figure 15)[182], the current leading hypothesis of an exaggerated neural synchrony leading to pathological oscillations is also inadequate[106,183].

Panel: Major clinical paradoxes and observations in Parkinson's disease that are not explained by the classic model

Origin of key features
Increased neuronal activity in the STN/GPi-SNr and the associated inhibition of the thalamocortical projection does not provide an explanation for the origin of tremor and rigidity in Parkinson's disease.

Pallidotomy abolishes dyskinesias
Levodopa-induced dyskinesias in patients with Parkinson's disease are eliminated by GPi lesions, which is incompatible with a model that associates excessive inhibition with the GPi/SNr and reduced basal ganglia efferent activity with dyskinesias.

No deficits after interrupting basal ganglia-cortical connections
Disruption of the motor circuit by surgical lesions (ie, thalamotomy or pallidotomy) does not aggravate bradykinesia in Parkinson's disease nor induces any new motor deficit.

GPi=globus pallidus pars interna. SNr=substantia nigra pars reticulata. STN=subthalamic nucleus.

Figure 15 – Inconsistencies in the rate model understanding of PD pathophysiology (modified from Rodriguez-Oroz et al [182]).

The aforementioned studies have shown that in patients or animal models of PD, synchronous oscillatory activity is associated with bradykinesia and rigidity, but this does not prove causality. The relation between exaggerated oscillations and motor symptoms is still based solely on correlative evidence.
The basis for the assumption of a causal relation between exaggerated oscillations and motor impairment relies extensively on a set of studies that imposed exaggerated β-oscillations either in the STN or the cortex, thus mainly causing bradykinesia. Indeed, the induction of 10 Hz[184] and 20 Hz[185–187] oscillations in the STN of PD patients through electrical stimulation as well as the artificial increase of cortical 20 Hz oscillations in healthy subjects by means of transcranial alternating-current stimulation (tACS)[188] was shown to cause a significant but subtle worsening of motor performance. However, another study assessing the development of β-oscillation with respect to dopamine depletion and motor impairment in non-human primate model of PD treated with progressive injection of low doses of MPTP found no dependency, with β-oscillation appearing several days after the clinical symptoms[156]. While rat models of PD showed inconsistent results, doubts on the causal effect of β-oscillations were corroborated by a computational model suggesting that moderate dopamine depletion is sufficient to induce clinical motor symptoms but β-oscillation requires severe degeneration[189,190]. Also studies in PD patients showed that the percentage of beta band oscillating cells can be independent from the degree of symptom amelioration upon application of levodopa[133] or STN high-frequency stimulation[191]. Thus, there is still conflicting evidence regarding a role for β-oscillation in PD motor impairment and, if a relation with bradykinesia seems possible, this is less the case for tremor and freezing of gait.

Although it may seem logical that the oscillations may result in tremor[192], a specific relationship between tremor and neural oscillations in both BG and cortex is missing[97].
Single unit recordings in the GPi and STN of tremulous MPTP-treated monkeys showed exaggerated oscillations with spectral peaks at 5 and 10 Hz, which, however, correlated poorly with rest tremor severity and persisted after tremor suppression[70,97]. This might be due to the fact that multiple independent oscillators are simultaneously active[97], yet it cannot explain why strong neural oscillations occur without overt tremor[97].
In PD patients with tremor, β-oscillations are poorly represented, although bradykinesia is (and must be) also present[70,97,159]. Most of the studies in patients with tremor found β-suppression[193] or oscillations in low-frequency bands, without overlap. Sparse evidence for β-oscillations in PD patients with tremor symptoms exists[194] and the harmonics of the low-frequency oscillations might still fall into β-rhythms[189], but the co-analysis of tremor related oscillations and β-oscillations showed no correlation and very different temporal and spatial properties[97,121,195], with tremor oscillations being more intermittent over time[121]. Moreover, a recent study trying to impose

asynchronous oscillations in the STN or the thalamus of PD patients to control tremor failed in controlling tremor amplitude albeit neural entrainment[196]. This difference in frequency-amplitude dependency suggests that the neural networks that generate tremor in PD are distributed, weakly coupled and timely loose[196].
This observation fits with the poor effect of dopaminergic treatment on both tremor severity and its oscillatory activity.

Similar to tremor, gait freezing is also poorly correlated with excessive neural oscillations[151]. Some studies actually showed increased β-oscillations in patients with FOG[197], but were biased in their sole comparison of groups of patents with and without FOG, so neglecting the primary characteristic of FOG: its episodic nature[8,198]. This comparison limits the proper analysis of FOG mechanism by overlooking activity changes occurring during ongoing freezing episodes. Accordingly, a recent study in 14 PD patients recording STN neural oscillations during ongoing FOG did not find a modulation of β-oscillations but rather an increase neural entropy, thus suggesting less coordinated processing[151]. Suggestions for a distributed brain network dysfunction in FOG came from studies assessing the cortical activity in PD patients, who showed reduced cognitive performances[199,200] and alteration of cortical metabolic[201] and oscillatory activity[202]. Interestingly, the one study assessing cortical oscillations in PD patients during ongoing freezing episodes did not find a modulation of β-oscillations but rather changes in low-frequency bands[202].
In the case of FOG the relation to neural oscillation is further complicated by the technical difficulty of performing neural recordings during a locomotion task. In line, the assessment of neural oscillations during walking is rather recent and produced conflicting results[145,147–151]. Some studies in PD could detect a suppression of β-band oscillations during walking, with even more pronounced suppression in those patients suffering from FOG[135], while others did not find any clear modulation, especially of the β-oscillations[149].

In summary, abnormal oscillations associated with bradykinesia and rigidity differ from tremor and FOG in multiple ways. First, bradykinesia and rigidity are correlated with β-frequency oscillations[154], whereas tremor[121] and FOG[202] are associated with independent low-frequency oscillations. Second, the cell populations associated with these latter symptoms appear to have unique anatomic distributions within the basal ganglia as well as distinctive connectivity patterns[121,203]. Third, dopamine-related modulation of neural oscillations have been linked with the reduction of bradykinesia and rigidity[154], but not tremor[97,121,195] or FOG. Finally, β-oscillations are chronically present[154], thus not reflecting the sporadic manifestation or worsening of tremor and FOG.

These observations suggest that the motor symptoms of PD may have distinctive pathophysiological mechanisms, which are associated with a wide spectrum of functional changes. These do not refer to the co-occurrence of increased firing, bursts, oscillations and neural synchrony in the BG nuclei, but rather to the dysfunction of a distributed neural network that goes beyond the BG and dopamine deficiency.
Some PD symptoms may reflect the alteration of brain areas that are not directly affected by neurodegeneration, nor severely deprived in dopamine, but still affected as part of a functional network. These functional alterations can be compensatory or detrimental, building up on the BG dysfunction. Of relevance, compensatory changes may also, in time, become detrimental and result in specific symptoms. These symptoms would be related to BG dysfunction and, yet, remain largely independent.
As a consequence, parkinsonism would not simply reflect a dopaminergic deficit but rather a network phenomenon composed of primarily dopaminergic symptoms as well as non-dopaminergic and extra-BG motor impairments. Again, the latter may reflect both a primarily functional deficit due to diffuse neurodegeneration as well as purely functional alterations sprouting from abnormal network activity.

I hypothesized that this could be particularly the case for tremor and freezing of gait.
In the case of tremor, it may emerge from combined but distinct alterations of the cortico-striato-thalamic and the cerebello-thalamic-cortical circuits. The dopaminergic deficit occurring in PD may alter the activity in the cortico-striato-thalamic circuit, possibly inducing pathological oscillation through exaggerated synchrony, and triggering a reaction of the cerebello-thalamic-cortical circuit through the modulation of locus coeruleus activity (LC). This noradrenergic nucleus bridges the two circuits and was shown to react to dopaminergic denervation by increasing its firing, thus mainly modulating cerebellar activity. Of note, this mechanism would be possible only in those PD patients in which the LC is temporarily spared from neurodegeneration, thus accounting for tremor incomplete

presentation. Hence, PD tremor would reflect a complex monoaminergic dysfunction (involving both dopamine and noradrenaline) that might emerge as a compensatory mechanism but, in time, becoming detrimental.
To verify this hypothesis and document a causal role for noradrenergic dysfunction in PD tremor, I examined the severity and time course of tremor in a combined animal model of PD, showing isolated dopaminergic or dopaminergic and noradrenergic deficit. The detailed hypothesis, methods, results and interpretations are presented in the next section.
In the case of freezing of gait, I hypothesized that it may reflect a timely deficit in the communication between the nodes of the locomotor network. In particular, I pictured FOG as the motor consequence of the neural failure in updating the locomotor program with the necessary information to face the approaching environmental demands. The application of an unappropriated motor program would lead to motor block.
In this view, the deficient update is supported by a failure in the communication between cortices and the BG.
To address this hypothesis and describe the neural mechanisms of FOG in PD, I examined the neural communication between the cortex and the STN in patients with PD during normal (effective) walking and during ongoing FOG episodes. As, for the tremor, the detailed hypothesis, methods, results and interpretations are presented in the studies section.

III. Studies

3.1 Tremor

3.1.1 Background

Tremor is a cardinal symptom of PD, yet its pathophysiology remains largely unclear. This is mainly due to the many peculiar features of tremor that challenge its mechanistic explanation.

In PD, tremor is worsened by psychological stress[33], while ceases during sleep[7], and it can herald the development of bradykinesia[204,205] but also disappear along the disease course[31]. Nonetheless, it does not correlate with striatal dopaminergic loss[206–208] and poorly responds to dopaminergic treatments, with large differences in tremor responsiveness between patients[209]. Hence, there is a dopamine paradox in resting tremor, as dopamine loss seems required for tremor development, but it is unrelated to tremor severity[209].
A plausible explanation is that tremor relies on a network dysfunction and involves different neurotransmitters other than dopamine.

The neural network that correlates with tremor was proposed by metabolic brain imaging findings that detected an abnormal activity in the cortico-striato-thalamic and cerebello-thalamo-cortical circuits of PD patients with rest tremor[192,210].
Electrophysiological studies confirmed these findings and detailed the cortical areas involved in tremor by assessing cortico-muscular coherence[211]. The cortical tremor network covers the M1, the somatosensory cortex (P1), the posterior parietal cortex, the SMA and cingulate areas[192,210]. Among these cortical regions, the M1 was suggested to play a pivotal role by a study that showed how inhibition of M1 activity with repetitive transcranial magnetic stimulation (rTMS) inputs can supress tremor in patients with PD[212].
At subcortical level, the putamen, STN and GP in the BG, the cerebellum, and the Vim nucleus of the thalamus were suggested to play a role in PD tremor genesis[192,210]. In particular, recordings in the STN, GPi and Vim of PD patients with rest tremor found neural bursts in phase with tremor[121,213–215]. HFS of these nuclei were also showed to control tremor severity in PD[192,210]. Recent studies also showed a beneficial effect through selective lesioning of the STN[216] and the Vim[217] by means of focused ultrasound ablation (reviewed in[218]). Unlike ablation surgeries (e.g. thalamotomy) however, the benefit of this refined lesioning faded over the course of a few months[217], thus highlighting the resilience of PD tremor. The recurrence of PD tremor after precise lesioning of selective structures further confirms that the neural networks generating tremor in PD are distributed, weakly coupled and timely loose as originally suggested by frequency-amplitude dependency evaluations[196]. It must also be acknowledged that tremor might develop from none of the abovementioned structures, but rather from an altered communication across them, thus reflecting a circuit dysfunction. Accordingly, a recent stereotactic surgery report showed that HFS of the zona incerta stably reduced tremor by over 90%[219]; a clinical trial assessing fiber targeting for tremor control is currently ongoing (but limited to ET patients)[220,221]. So, if the network of tremor is becoming more and more clear, the nature of its dysfunction (e.g. the relevance of a central oscillator) remains unknown[192,209,210].

To understand the functional dysfunction leading to tremor in PD, it is necessary to identify the neurotransmitters underling these changes.
Recent evidence advanced this line of research by assessing dopaminergic and serotonergic transporter availability in PD patients with and without tremor[35]. Patients were classified as tremor dominant, PIGD or indeterminate. As expected, tremor dominant patients had significantly less rigidity and bradykinesia over 2-years follow-up[35]. Moreover, the severity of rest tremor correlated with the loss of serotonin transporter in the brainstem raphe nuclei more than with the loss of dopamine transporter in the striatum[35]. Specifically, tremulous patients were shown to have lower raphe serotonin transporter availability, but less severe striatal dopaminergic deficits, compared with non-tremor patients[35]. The “tremor index” (i.e. product of tremor constancy and amplitude) was negatively correlated with serotonin binding in the raphe[35]. This suggests that patients with a relatively dopamine-resistant

tremor have more severe serotonergic deficiency, given the degree of dopamine depletion in the putamen. The predominant serotonin deficiency in patients with prominent rest tremor may also explain why such tremors disappear during sleep and why they are often less responsive to levodopa.
Although interesting, the data on serotoninergic/dopaminergic imbalance seems to better reflect the pathophysiological mechanism of postural tremor, rather than resting tremor[222,223]. The analysis was indeed performed in a wide patient collective (i.e. the Parkinson's Progressive Markers Initiative, PPMI), where ratings were restricted to the amplitude of postural tremor (whether it was re-emergent or not), thus mixing postural and (re-emergent) resting tremor and limiting the interpretation of the results[222]. Moreover, the treatment with drugs inhibiting the selective re-uptake of serotonin (SSRI) does not ameliorate resting tremor in PD, but rather has been associated with the occurrence and worsening of parkinsonism [7].

Current hypothesis
At present, PD rest tremor is still thought to mainly reflect a dopaminergic dysfunction that fed an aberrant activity in the cerebello-thalamic-cortical circuit[210,214,224,225]. Data from functional neuroimaging studies[225–227] have suggested that dopaminergic depletion of the GP may prompt tremor onset through a reduction of thalamic activity, which may be reduced by the potentiation of the self-inhibition of the thalamic cerebellar nucleus (i.e. the ventral-intermediate nucleus, Vim)[228]. In particular, Dirkx and colleagues performed a fMRI with co-registered EMG in PD patients with resting tremor before and after the assumption of levodopa, thus assessing how dopaminergic medications influence both tremor severity and its neural circuitry. They showed that dopamine reduces tremor amplitude by increasing thalamic inhibition, with a specific effect for the Vim, the thalamic nucleus that receives cerebellar inputs[228]. This effect correlated with the clinical response, thus suggesting that GP dopaminergic degeneration induce tremor by altering the Vim activity.

However, two independent molecular imaging studies did not find any difference in dopaminergic GP innervation in two large cohorts of PD patients[208,229] and Isaias and colleagues advanced an alternative hypothesized in which PD rest tremor reflects an increased noradrenergic activity of the locus coeruleus (LC)[208].

3.1.2 Working Hypothesis

If tremor reflects the dysfunction of distributed brain networks (i.e. cortico-striato-thalamic and cerebello-thalamo-cortical circuits[192,210,214,224,225]), then activity changes in brain areas spared from neurodegeneration may also be causal to tremor. In particular, reactive activity changes in unaffected brain areas that functionally connect to PD degenerated regions may be necessary, although not sufficient, to tremor development. This process is defined dynamic diaschisis[10] and postulate that, in a functional interconnected system, such as a loop or a hierarchically organized network, the alteration of the activity of one node necessarily alters the overall activity of the system, even when the output remains unchanged. Remote activity changes might initially be compensatory and limit the effect of an alteration on the outcome, however, in time, they can also become maladaptive or exhausting thus actively modifying the system outcome[230]. A practical example of this concept is the development of peripheral oedema in case of heart failure[231]. Given a reduced cardiac propulsive force, the kidneys withhold more sodium and water to increase the blood volume and support the bloodstream, but the reabsorbed water ends up mostly expanding the extracellular fluid (due to abnormal Starling forces) and, in time, its accumulates in the body, thus increasing the body weight, impairing the venous return to the heart, altering other organs functions (e.g. congestive hepatopathy) and ultimately worsening the overall condition.
Following this reasoning the hypothesis of Isaias and colleagues[208], I have further suggested that while tremor onset may be triggered by an increased noradrenergic output of the LC that primes the cerebello-thalamic-cortical circuit, the tremor frequency would rely on oscillatory signals generated by the cortico-striato-thalamic circuit following dopaminergic depletion. Tremor amplitude might be instead be regulated by the motor cortex, which may also define its body location

Locus coeruleus (LC)

The locus coeruleus, or A6 group, is located in the upper dorsolateral pontine tegmentum and represents the main source of norepinephrine (NE) of the brain, targeting several cortical and subcortical structures (Figure 16)[82,232]. The principal cortical targets of the LC are the sensory motor cortex, the prefrontal cortex, the parietal cortex, the medial prefrontal and anterior cingulate cortex, the entorhinal cortex, the hippocampus, the subiculum and the amygdala[232]. Outside the cortex, the LC projects to the basal forebrain cholinergic groups, nucleus basalis of Meynert, the thalamus with numerous axons spreading onto several thalamic nuclei, the hypothalamus, the superior colliculus and cerebellum[232]. Descending LC-NE projections target also the brainstem and the spinal cord, mainly reaching the brainstem reticular formation and the dorsal horn of the spinal cord (including the marginal zone containing spinothalamic neurons)[232]. The LC-NE also innervates the dopaminergic neurons in the SNc and in VTA[232], which projects back to the LC, thus constituting a closed microcircuit that can influence striatal dopaminergic realise.
Beside the dopaminergic innervation, LC afferences originates from prefrontal and anterior cingulate cortices, amygdala, hypothalamus, bed nucleus of the stria terminalis, preoptic region, periaqueductal gray, midbrain pontine reticular formation, PPN and the

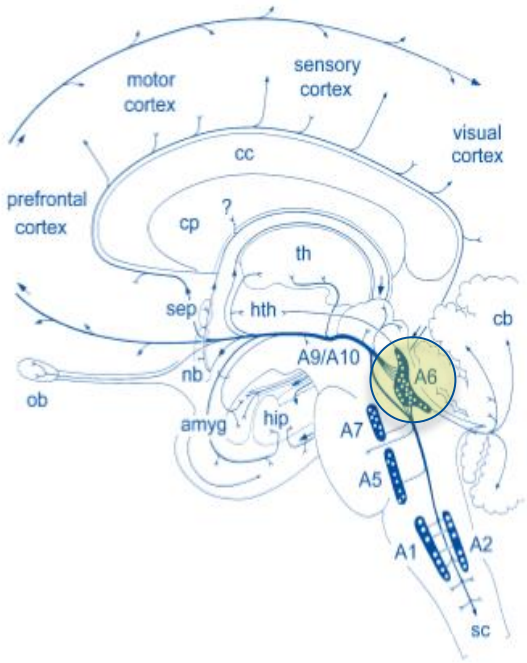

Figure 16 – Locus Coeruleus location and innervation on a sagittal plain (modified from Fuchs and Flügge [351]).
The noradrenergic neurons of the locus ceruleus (A6) project to the limbic (e.g. amyg) and cortical regions as well as to the thalamus (th), cerebellum (cb), and spinal cord (SC) They play an important role in the regulation of mood and attention. Other noradrenergic neural groups A1 , A2, A5, and A7 project to more restricted regions (not shown).

cerebellum[232]. The LC is also strongly connected with the dorsal raphe nucleus and the dorsal horn provides nociceptive inputs[232].

The LC contains about 45,000–50,000 neurons, which show stark tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) reactivity as reflection of the intense biosynthesis of NE. NE biosynthesis is performed by TH and DHB enzymes that transform tyrosine in dopamine and dopamine in NE, respectively[82]. NE and dopamine are then stored into the synaptic vesicle by the vesicular monoamine transporter 2 but can also be released with a non-synaptic mechanism, thus reaching glial cells and microvessels[82].
The synaptic effects of NE are determined by its uptake via the presynaptic norepinephrine transporter (NET) followed by its metabolism by mitochondrial monoamine oxidase (MAO) and cytosolic catechol-O-methyltransferase (COMT) [82]. The post-synaptic effects of NE are mediated by G-protein coupled receptors families, namely α1, α2 and β. The α1 and β receptors are mainly postsynaptic, whereas α2 are placed pre- and post-synaptically. The α1 and β receptors exert an excitatory effect mediated by the phospholipase C/inositol triphosphate/protein kinase C pathway and by the adenylyl cyclase (that increase the cyclic adenosine monophosphate, cAMP) pathway, respectively. The α2 receptors are, instead, negatively coupled with adenylyl cyclase and favour K+ currents while inhibiting presynaptic Ca2+ channels, thus reducing the overall neurotransmitter release. Through this mechanism the α2 receptors act as inhibitory autoreceptors. An exception to this inhibitory function is represented by the prefrontal cortical networks, where they exert an enhancing activity by inhibiting hyperpolarization-gated cAMP-regulated cation channels in pyramidal neurons[82].
Extra-synaptic realise of NE facilitates instead synaptic plasticity and activate via extracellular fluid dispersion the astrocytes and microvessels[82].
These complex transduction pathways sustain the functional effect of NE realise, which is the increase in the responsiveness of the neurons.
The functional effect of LC-NE is also determined by the discharge pattern of its neurons, which can present a tonic or a phasic firing[82].
Tonic firing is characterized by a sustained and highly regular discharge pattern (2–5 Hz) and it is associated with arousal and waking state, ceasing during REM sleep[82].
The baseline tonic firing is modified into a phasic firing during focused attention, which shows stimulus-locked train of activity. Such peculiar discharge sustains attention and task performances by filtering the irrelevant stimuli[82]. Hence, the LC-NE system plays a critical role in attention and stress response, but it is also relevant for the control of autonomic and sensory-motor functions[82]. Its interconnection with the BG and cerebellar circuits are particularly relevant for this latter function.

NE modulates dopamine release in the striatum via α2 receptors, which also promote STN burst firing[82]. In contrast, activation of α1 receptors exerts a different effect on the STN firing increasing its frequency but not the burst number[82].
LC-NE also exerts complex effects on the cerebellum, where it enhances the spontaneous GABAergic (inhibitory) activity of Purkinje cells[82].
These effects onto the BG and the cerebellum are particularly relevant in PD, where the LC can play a role in distinctive symptoms according to its activity [233].
The LC degenerates in PD and the loss of LC neurons and its activity can result in some specific PD symptoms. In particular, the loss of LC-NE innervation may have a major role in cognitive decline, with deficits in executive functions like focused attention and cognitive flexibility[82]. Moreover, the noradrenergic innervation loss in the motor thalamus (pallidonigral and cerebellar territories) may contribute to the abnormal thalamic neuron activity with impaired information transfer from the BG and cerebellum to the cortex. Accordingly, two recent molecular imaging studies have shown noradrenergic involvement in advanced PD patients with PD (average disease duration >6 years)[234,235]. Of relevance, even in these group of advanced PD patients, those with tremor showed a higher binding of NA reuptake transporters than tremor negative patients[235].
This hints on a possible role of the LC in an early phase of the disease and on a link between LC-activity and PD tremor. Precisely, in the initial phase of PD and only in a subgroup of patients, in which LC neurons do not precociously degenerates, the dopaminergic loss is followed by an activation of the LC[236]. This reactive increase in LC-NE release has been showed in animal models of PD[236] and suggests compensatory mechanism through which the LC attempts to restore normal BG firings[82]. Loss of noradrenergic LC neurons favour dopaminergic neurodegeneration in the 6-OHDA rat model of PD[237,238], whereas pharmacological or genetic blockade of NET

or administration of the α2 receptor agonist clonidine protects dopaminergic neurons[230]. These findings indicate that NE exerts a neuroprotective effects onto dopaminergic neurons by preventing of oxidative stress[82], thus possibly accounting for the benign disease progression of tremor-dominant PD patients.
The compensatory increases of LC-NE realise might, therefore, on one side protect residual SNc functioning, but on the other it may come at the expenses of tremor development[208].
In fact, besides being overactive following dopaminergic loss[237,238], the LC extensively projects to the cerebello-thalamic-cortical circuit, representing the sole source of noradrenaline (NA) in these structures[82,232,239]. The LC-NA directly modulates cerebellar activity[82], which is consistently reported to be enhanced in PD patients with tremor[240–244]. Furthermore, it has been shown that the flow of oscillatory activity in PD was primarily from the cerebellum to the cerebral cortex and to the muscles, thus arguing for a pivotal role of the cerebellum in sustaining PD tremor[245].
Although intriguing, evidence of a role for LC and its cerebellar projections in PD tremor remains elusive, mainly because of the difficulties in studying the LC activity in humans and classical models of PD[208].

Animal models
To avoid this limitations, the reserpine rat model of PD was investigated as it is one of the very few models manifesting resting tremor[246–248].

Current PD animal models often rely on genetically modified animals by altering the expression of SNCA, LRRK2, Parkin, PINK1, or DJ-, but none of these models actually express the key clinical and neuropathological features of PD[249].

To date, the most effective strategy to reproduce PD in animal is the toxin lesioning of dopaminergic neurons[249]. Given that environmental toxins (e.g. rotenone, paraquat or maneb) are not dopamine selective and have a high systemic toxicity, the most reliable toxins remain 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA) and reserpine[249].
MPTP and 6-OHDA enter the dopaminergic neuron through the dopamine transporter (DAT) and inhibit the complex I of the respiratory chain, thus inducing adenosine triphosphate (ATP) reduction, oxidative damage, protein aggregation, cell death, and dopamine loss[249].
While MPTP is mainly used in primate (due to natural rodent resilience to this drug), 6-OHDA is injected in rodents, where it destroys dopaminergic (DAT transporters), noradrenergic (NAT transporters) and serotoninergic (5-HT transporters) neurons[249]. Due to this poor selectivity, 6-OHDA destroys both LC and SNc neurons and it is unable to induce PD tremor.

An alternative to these toxins is reserpine. Reserpine is an alkaloid extracted from Rauwolfia serpentine; it inhibits the vesicular transporter of monoamines in the central nervous system (VMAT-2) and was actually one of the earliest models of PD. Although often criticized of being outdated, the reserpine model mimics the clinical, neurochemistry, and pharmacology features of PD[249].

The relationship between reserpine and PD was first reported by Carlsson et al, who showed that the reserpine motor effects were improved by L-DOPA in rodents[247]. Reserpine inhibits irreversibly VMAT-2 transporters and can induce akinesia, hypokinesia, limb rigidity and, most importantly, tremor[250]. The disruption of VMAT-2 cause intracellular monoamines storage depletions, leading to total monoamine depletion that includes dopamine, noradrenaline and serotonin, which reduces monoamines in nerve terminals, thus causing the development of the symptoms (Figure 17)[246,251–253]. The following accumulation of neurotoxic oxidation byproducts [254] also leads to neuronal degeneration over time[255,256].

To investigate a role of LC-NA for PD tremor onset the sole reserpine rat model was insufficient due to the temporary selectivity of reserpine, which (unlike 6-OHDA) does not alter LC or noradrenergic terminals for the first 72 hours[257]. Two groups of animal were therefore compared. One group was composed of simple reserpine rat models of PD and the second group by reserpinized rats pre-treated with N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4)[258].
The systemic injection of DSP-4 causes a depletion in NA levels, in the release capacity and in the activity of dopamine beta hydroxylase (DBH)[259]. In the first two weeks after treatment, the neurotoxin exclusively affects

and destroys NA terminal axons arising from the LC[259,260], presumably due to specific binding proprieties of the NA transporter in LC axon terminals that maximize DSP-4 affinity and uptake, leading to local alkylation of vital proteins[261]. Evaluations were focused on the cerebellum, given its established role in PD tremor[224,245,262].

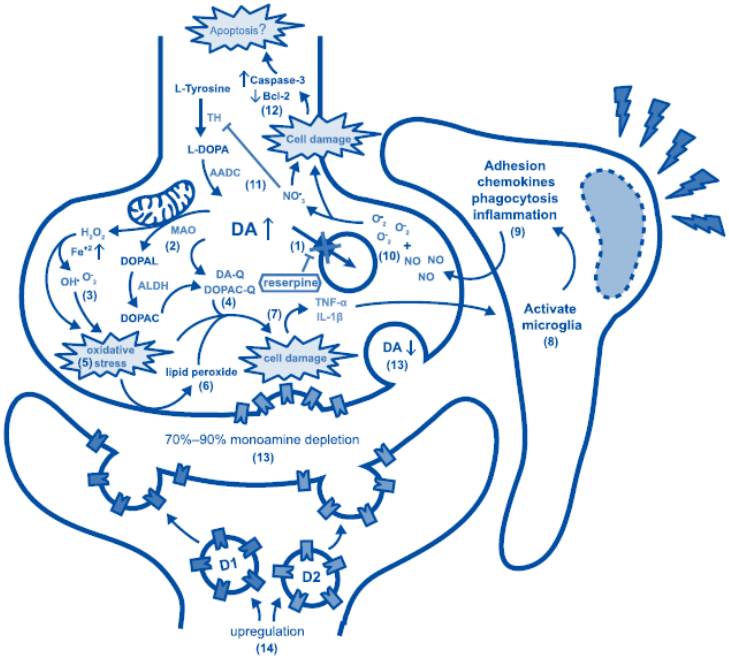

Figure 17 – Neurochemical and molecular events after reserpine treatment (modified from Leão et al [249]).
Reserpine administration determines (i) preclusion of dopamine (DA) storage; (ii) incensement of DA metabolites in the cytoplasm; (iii) generation of reactive oxygen species (ROS) and (iv) highly reactive quinones (DA-Q and DOPAC-Q); (v) increased oxidative stress and (vi) lipid peroxidation; (vii) accumulation of ROS and reactive quinones with pro-inflammatory signalling onset; (viii) activation of microglia by tumor necrosis factor (TNF)-α and interleukin (IL)-1β; (ix) amplification of pro-inflammatory signalling; (x) increment of nitric oxide (NO) and peroxynitrite (NO3-) formation with free superoxide (O2-); (xi) NO3- inhibition of tyrosine hydroxylase (TH) activity and (xii) reinforcement of cell damage by committing cell fate in pro-apoptotic signalling. In time, it also causes (xiii) monoamine depletion in synaptic cleft that results in (xiv) upregulation of D1 and D2 receptors on the pre- and postsynaptic membrane.

AADC, aromatic L-amino acid decarboxylase; ALDH, aldehyde dehydrogenases; MAO, monoamine oxidase.

3.1.3 Methods

To investigate a role for cerebellar LC-NA in PD tremor, I studied the reserpinized rat model of PD by comparing the motor symptoms of animals with and without selective lesioning of the cerebellar noradrenergic terminal axons by DSP-4 neurotoxin injection.

Animals and toxins injection procedures
I have investigated 15 male Sprague Dawley rats (Charles River Laboratories, Sulzfeld, Germany), which were constantly kept under standard conditions (21°C, 12-h light/dark cycle), with food and water available ad libitum. Animals were handled according to applicable international, national, and institutional guidelines for care and use of animals and all efforts were made to minimize animal suffering. The local institutional review board approved the experiments. At the time of experiment, animals weighed between 200-220 g.
Toxins injections were carried out by dr. Bolzoni Francesco and dr. Gabriele E.M. Biella as part of a collaborative project across the University Hospital and Julius-Maximilians-Universität Würzburg, the Università degli Studi di Milano and the Consiglio Nazionale delle Ricerche (CNR, Milan, Italy). In particular, the first group of seven rats (R-group) received only one intraperitoneal injection of 10 mg/kg of reserpine[263]. A second group of eight rats (DR-group) received one intraperitoneal injection of 50 mg/kg of DSP-4, to ensure sufficient degeneration of noradrenergic LC axon terminals[236], followed by a reserpine injection two weeks later (Figure 18).

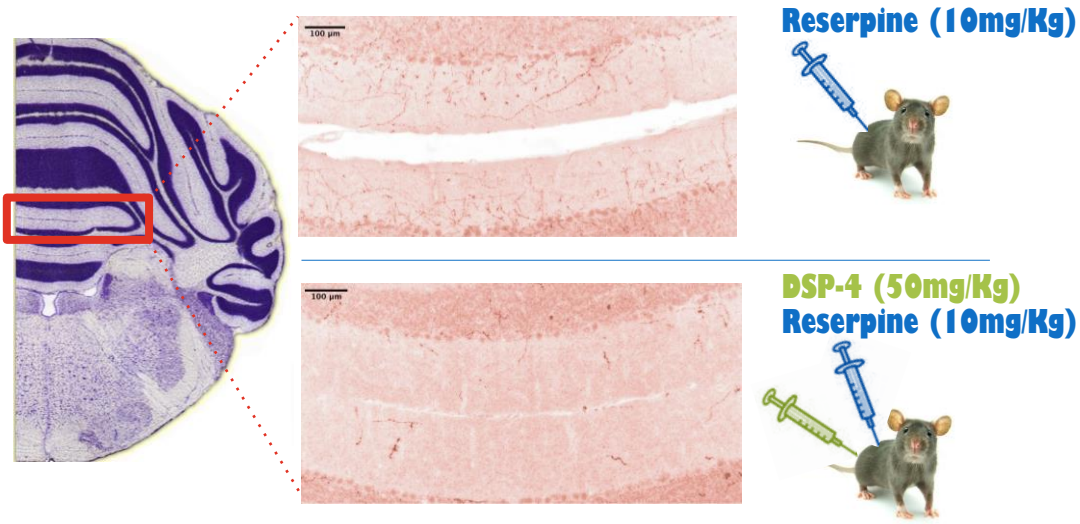

Figure 18- Schematic representation of the methods

Behavioural evaluations and kinematic analysis
Observations were carried out before the reserpine injection and for the following 3 h. In particular, all measurements took place in a dedicated room and at fixed timing (i.e. one week post-arrival, between 3pm and 5pm, with light on at 8am), following 30 min acclimatization. Animals were sacrificed just after the end of the experiments.
The severity of parkinsonian symptoms was scored with the visual 0-2 points scoring system originally proposed by Colpaert in his seminal paper of 1987[246]). Accordingly, score 2 was assigned when tremulous movements were visible immediately and clearly, score 1 when tremulous movements were intermittent and of modest amplitude, score 0 when no tremulous movements could be observed. The same scoring system was applied to akinetic rigid symptoms, namely: rigidity, hypokinesia, postural flexion of the back, and postural immobility. Scores were assigned by a single examiner, blinded for treatment conditions, and took place at 0 (injection time), 20, 40, 60, 80, 120 and 180 min after reserpine injection. DR group animals were also evaluated after DSP-4 injection only, at 14 days and one week previous reserpine injection. Measurements were repeated three times, and the average score was then used for statistical analysis.

To improve the evaluation of tremor and mirroring the experience with PD patients[264], tremor was evaluated also with accelerometers attached to the back limbs of the rat. Tremor was measured as the variation in the acceleration of the most tremulous limb. I computed the consistency of tremor (T%) as the percentage of the total time recorded (average of three sessions of ≈60 s at each time point). I have also measured the tremor frequency at each time point. Recordings from all the animals but one (r4, R-group) were available and were analyzed with Matlab-based (Mathworks) custom scripts. Kinematic data from r4 were excluded due to the presence of artifacts in the recordings.

Tissue preparation, immunolabeling and quantitative analyses

The animals were sacrificed just after the end of the recordings (i.e. 180 min following reserpine injection) by means of deep anesthesia with a combination of isoflurane and high dosage of pentobarbital. This procedure was followed by transcardial perfusion with cold heparinized saline solution. Tissues were fixed by a perfusion with 4% solution of paraformaldehyde in phosphate buffered saline (PBS). The brain was then removed and preserved in 4% paraformaldehyde until the histological analyses were performed.

Tissue preparation, sectioning, and immunofluorescence labelling were carried out by Frau Prof. Esther Asan at the Institute of Anatomy and Cell Biology, Julius-Maximilians-Universität Würzburg as by her previously described in Asan et al., 2003, 2005[265,266].

In brief, tissue blocks containing upper pons and cerebellum were washed in 0.01 M PBS (pH 7.4), successively infiltrated with 10 and 20% sucrose in PBS, frozen in liquid-nitrogen-cooled isopentane and stored at -80 °C. Serial 40 µm frontal vibratome sections were prepared after thawing of the tissue to room temperature (RT). Preincubation of free-floating sections in 5% normal goat serum (NGS; Sigma, Deisenhofen, Germany) and 1% Triton X-100 (TX100; Sigma) in PBS for 2 hours at RT was followed by incubation in the primary antibody solution for 48-72 hours at 4°C. Antibodies used were polyclonal rabbit-anti-tyrosine hydroxylase (TH; Millipore) or polyclonal rabbit-anti-dopamine-β-hydroxylase (Abcam) diluted 1:500 in 1% NGS, 0.5% TX100 and 0.05% NaN3 in PBS. After washing in PBS, sections were incubated in Cy3-labeled goat-anti-rabbit secondary antibody (Dianova, 1:600) in 0.5% TX100 in PBS for 2 hours at RT, washed in PBS, mounted on SuperfrostTM microscopic slides and covers lipped with Fluorogel (Electron Microscopy Sciences). Microphotographs for images were taken with a Keyence BZ 9000 microscope. Control sections subjected to the reaction sequence without primary antibodies did not show specific labelling.

Quantification of TH neuron and axon densities was done with a Zeiss Axiophot2 microscope using digital images acquired via CCD-camera and ImagePro 4.0 software. For axon density assessment, six images in the region showing the locus coeruleus were analysed on two sections per animal. Each structure within the molecular layer of the first lobule of the upper portion of the cerebellar vermis that demonstrated a continuous TH+ immunoreactive fiber profile was counted as one axon. For the neuron count, cell profiles displaying TH+ reactivity within the LC were quantified on two sections per animal. Axonal and neuronal densities per µm2 were then calculated after determination of the analysed cortical area and the area of the LC, respectively, with ImageJ.

Statistical analysis

I have performed the statistical analyses with the JMP statistical package (version 13, SAS Institute, Inc., Cary, NC, USA). In particular, the difference between groups was analysed using Wilcoxon rank-sum test, in each time point. Difference were considered statistically significant at $p<0.05$. Data are presented as mean ± standard deviation (SD);

3.1.4 Results

Verification of cerebellar noradrenergic denervation by means of DSP-4

The TH- (Figure 19A) and DBH-immunolabeling (Figure 19B) showed numerous noradrenergic terminal axons in the cerebellar cortex of R-group animals. In contrast, DR-group animals (Figures 19C, D) presented only few TH- and DBH-immunolabeled axonal in the cerebellar cortex. Quantitative analyses confirmed a ~90% reduction in the density of TH+ noradrenergic axon profiles in the DR-group (0.024±0.01 axons/µm^2) compared to the R group (0.270±0.04 axons/µm^2; p<0.001), thus proving that DSP-4 injections successfully caused a significant reduction of LC noradrenergic terminals.

However, the cell density of TH+ noradrenergic perikarya of the LC did not differ between the groups, with an average cell density of 1.37 neurons/µm^2 in DR animals and 1.33 neurons/µm^2 in R animals (p>0.05), thus confirming previous reports[259].

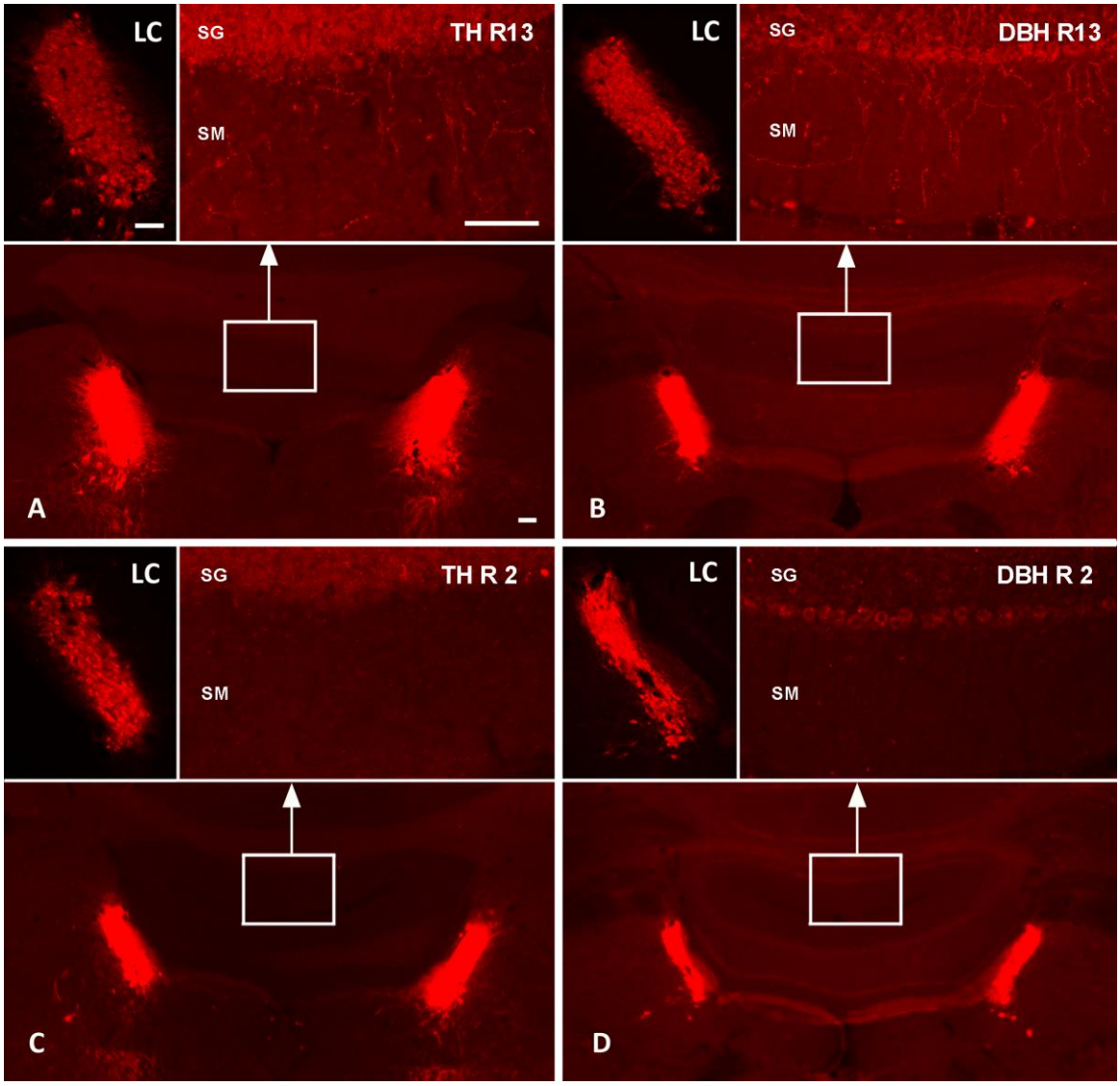

Figure 19 - Effects of DSP-4 on noradrenergic locus coeruleus neurons and their terminal axons in the cerebellum. TH- and DBH-fluorescence immunolabeling of the pontine brainstem and cerebellum of one reserpinized-only animal (R13; A, B) and one animal pre-treated with DSP-4 two weeks before reserpine (R2; C, D). The top left images in each panel show higher magnifications of the left LCs, the top right images show higher magnifications of the stratum granulosum (SG) and stratum moleculare (SM) of cerebellar vermal cortical areas indicated by white boxes in the overviews. In the reserpinized rat, TH- (A) and DBH-immunoreactions (B) label noradrenergic neurons in the LC and numerous noradrenergic terminal axons in the cerebellar cortex. In the DSP-4-treated animal, TH- (C) and DBH-immunoreactions (D) document a severe loss of cerebellar cortical noradrenergic terminal axons, while the LC neuronal cell bodies appear relatively spared. Bars: 100 µm. DBH, dopamine beta hydroxylase; LC, locus coeruleus; TH, tyrosine hydroxylase.

Symptoms severity at visual scoring

Pre-treatment with DSP-4 did not cause any motor symptom of the animals (DR-group) (Figure 20). After reserpine injection, DR-animals showed significantly less tremor as compared to R-animals (score at 40 min: 0.50±0.76 vs. 1.57±0.53, respectively, $p<0.01$; score at 60 min: 0.12 ± 0.35 vs. 1.14 ± 0.90, $p<0.01$) (Figure 20). Of relevance, tremor peaked at 40 min and decreased over time, vanishing after 120 min in all animals. On the contrary, reserpine induced marked rigidity, hypokinesia, postural flexion of the back, and postural immobility in both groups and for a sustained period (Figure 20). Accordingly, akinetic-rigid symptoms peaked later than tremor (i.e. 60-80 min) and never chase, thus not differing between the R- and DR-group at any time point (Figure 20).

Tremor consistency at accelerometer measurements

Kinematic measurement echoed the visual scoring, showing a significant difference in the consistency of tremor between R- and DR-group in all (relevant) time points (Figure 20). Tremor was only marginally present in DR-animals as compared to R-animals, with an average T% of 23.98±28.45 s vs. 45.46±35.66 s, respectively ($p<0.01$). On the contrary, the average frequency of tremor was similar between groups (DR-group: 25.86±13.34 Hz and R-group: 19.25±12.18 Hz; $p=0.06$) and never differed.

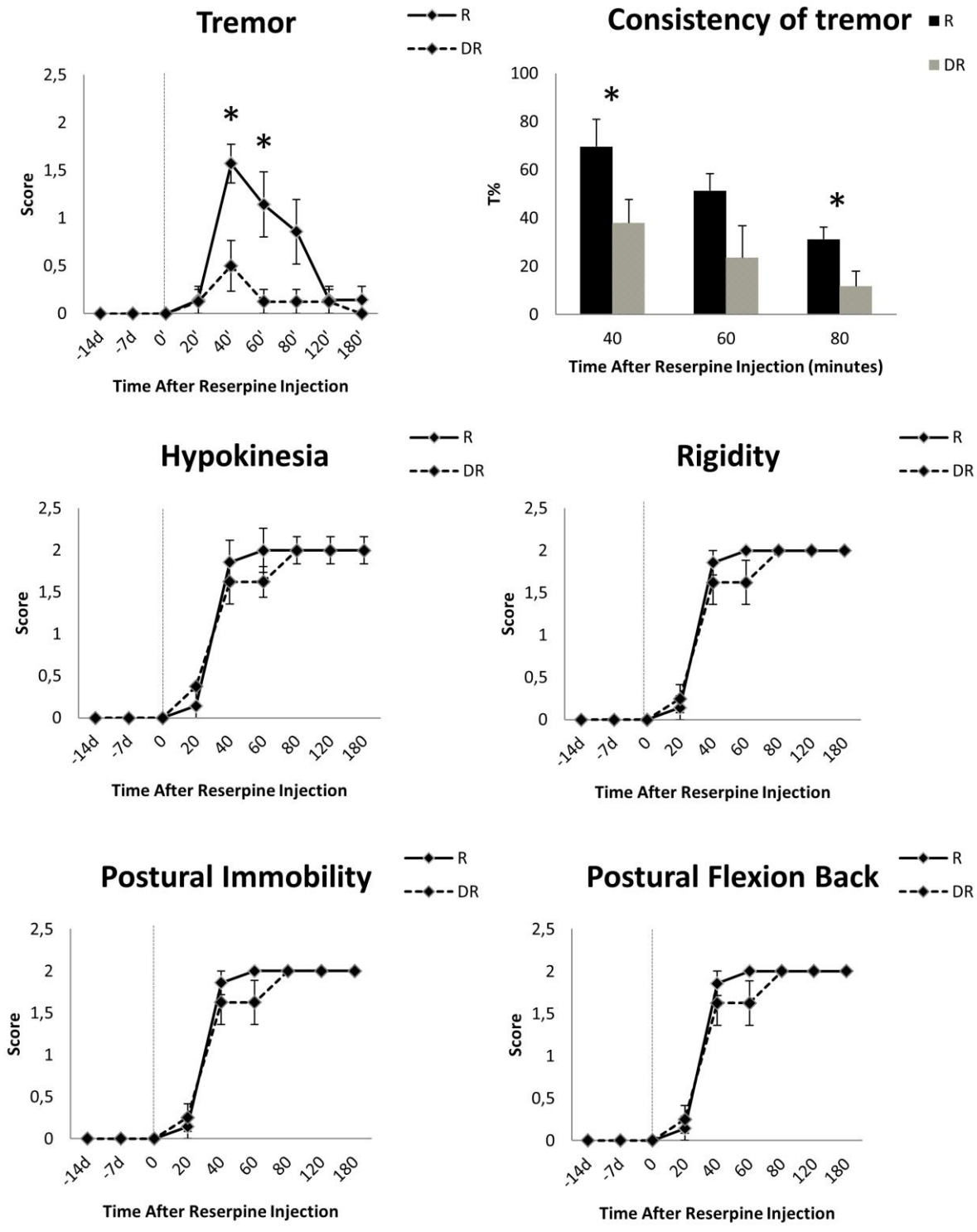

Figure 20 - Time course of the motor signs induced by 10 mg/kg of reserpine in rats.

Data are shown as mean (±standard error of mean, SEM) of seven reserpinized-only animals (R-group) and eight animals pre-treated with DSP-4 two weeks before reserpine (DR-group). For these animals only, the motor effects of DSP-4 are reported at 14 and 7 days before reserpine injection. Asterisks indicate two-tailed Wilcoxon rank-sum test. $p<0.05$. Tremor severity and consistency differed between groups, being more represented in the R-group. This difference was not mirrored by akinetic rigid symptoms, which were equally present and severe in the R- and DR-groups.

3.1.5 Interpretations and limitations

This study shows that cerebellar noradrenergic innervation impacts tremor onset, severity and consistency in the reserpinized model of PD. The lack of noradrenergic activity limited the development of tremor, while did not affect akinetic rigid symptoms that were equally present in both groups. When present, tremor amplitude but not frequency differed between groups. This mild tremor was expected, as DSP-4 injections induced a severe but incomplete (~90%) reduction of LC-NA terminals in the cerebellum. Of note, DSP-4 injections *per se* did not cause any parkinsonian symptoms.
Altogether these findings support a role for the LC in PD tremor and well extend to subjects with PD, suggesting that cerebellar noradrenergic innervation is actively involved in PD tremor development.

The LC is the main cerebellar source of NA, with LC-NA terminals primarily targeting the Purkinje cells[239]. The LC-NA has a direct inhibitory effect on Purkinje cells[267,268], thus facilitating the cerebellar output. This inhibitory effect follows phasic LC firing and is likely mediated by different mechanisms involving the hyperpolarization of the cell membrane, as well as activation of the adenylyl cyclase[269]. LC-NA also potentiates the GABAergic inhibitory effect of the stellate and basket interneuron projections to the Purkinje cell[267,270]. To a lesser extent, LC-NA can also enhance Purkinje cell activity by promoting climbing fibre and mossy fibre modulation[271,272]. Such bidirectional influence on Purkinje cells allows the noradrenergic neurons to selectively regulate cerebellar processes by promoting or suppressing distinctive signals encoded by the LC[267,273,274].
In PD, dopamine depletion is associated with a strong cerebellar compensatory response through the cerebello-thalamic-cortical circuit[262]. Indeed, functional neuroimaging studies have shown marked cerebellar activation in tremulous PD patients at rest and during the execution of motor tasks[240–244].
These compensatory cerebellar attempts may be primed by increased LC-NA activity. The downside of this augmented LC-NA activity is the development of PD tremor (Figure 21).
Molecular imaging findings showed that PD tremor suppression by HFS of the thalamic Vim was associated with a reduction in cerebellar regional cerebral blood flow[275]. Using a similar within-subject strategy, Mure and colleagues validated a tremor-related metabolic network in PD patients scanned while on- and off-stimulation of the Vim thalamic nucleus. This tremor circuit was mainly characterized by increased metabolic activity in the cerebellum, dorsal pons, and primary motor cortex, involving only marginally the putamen. Of relevance, the area that was originally identified as the dorsal pons encompass and can represent the LC area (Figure 19,[224]).

In line with a previous report[246], this experiment also showed that in reserpinized animals tremor present a distinctive dynamics, which differs from the one of akinetic rigid symptoms. Specifically, tremor showed an early peak and vanished over time, while akinetic rigid symptoms progressively increased (Figure 20). These almost opposite evolution patterns further support an independent mechanism for PD tremor and akinetic rigid symptoms. While the latter correlates with striatal dopaminergic depletion, tremor may rely on a temporary increase in LC-NA release[206–208,276]. This mechanism closely mirror reserpine effect that by blocking VMAT-2 progressively prevent the release of monoamines, above all NA, thus inducing a severe extracellular monoamine depletion[252,253]. Therefore, tremor dynamics closely follows extracellular monoamine fluctuations induced by reserpine, peaking at 40 min to fade over time (Figure 20).

Unlike tremor severity, the frequency of tremor never differs between the R- and DR-animals (Figure 20). Therefore, tremor frequency might rely on independent mechanisms, being unrelated to noradrenergic damage. A possible mechanism would be that dopaminergic and/or serotoninergic loss increase neuronal synchronicity and constrain the cerebello-thalamic-cortical circuit to oscillate at a definite frequency[192,210].

Of relevance, this idea of distinct mechanisms for tremor onset and frequency would reconcile many of the inconsistent findings in tremor research. In particular, this might clarify why single unit recordings in the GPi and STN of MPTP-treated monkeys showed exaggerated oscillations peaking at tremor frequency (i.e. 5 and 10 Hz) but did not correlate with tremor severity and were able to persist after tremor suppression[70,97]. Moreover, it also give a reasonable explanation to the observations that strong neural oscillations occur without overt tremor[97]. This interpretation is also valid in PD patients with tremor, in which oscillations in low-frequency

bands[121] and β-suppression[193] were reported. Indeed, low-frequency oscillations are independent from β-rhythms (i.e. are uncorrelated, with different temporal and spatial properties[97,121,195]) and can co-exist[121], becoming relevant only during tremor. The decoupling of tremor amplitude and tremor frequency also clarifies why imposing asynchronous oscillations in the STN or the thalamus of PD patients failed to control tremor amplitude, albeit neural entrainment[196]. Finally, this interpretation also fits with the poor effect of dopaminergic treatments, which suppress β-oscillations but marginally impact low-frequency oscillatory activity (i.e. under α-rhythms) and tremor severity. Accordingly, parkinsonian patients show a progressive worsening of akinetic rigid symptoms along with dopaminergic denervation, without changes in tremor frequency[276,277].

In summary, the most important observation of this study is that tremor relies on two distinct, necessary and not sufficient mechanisms.

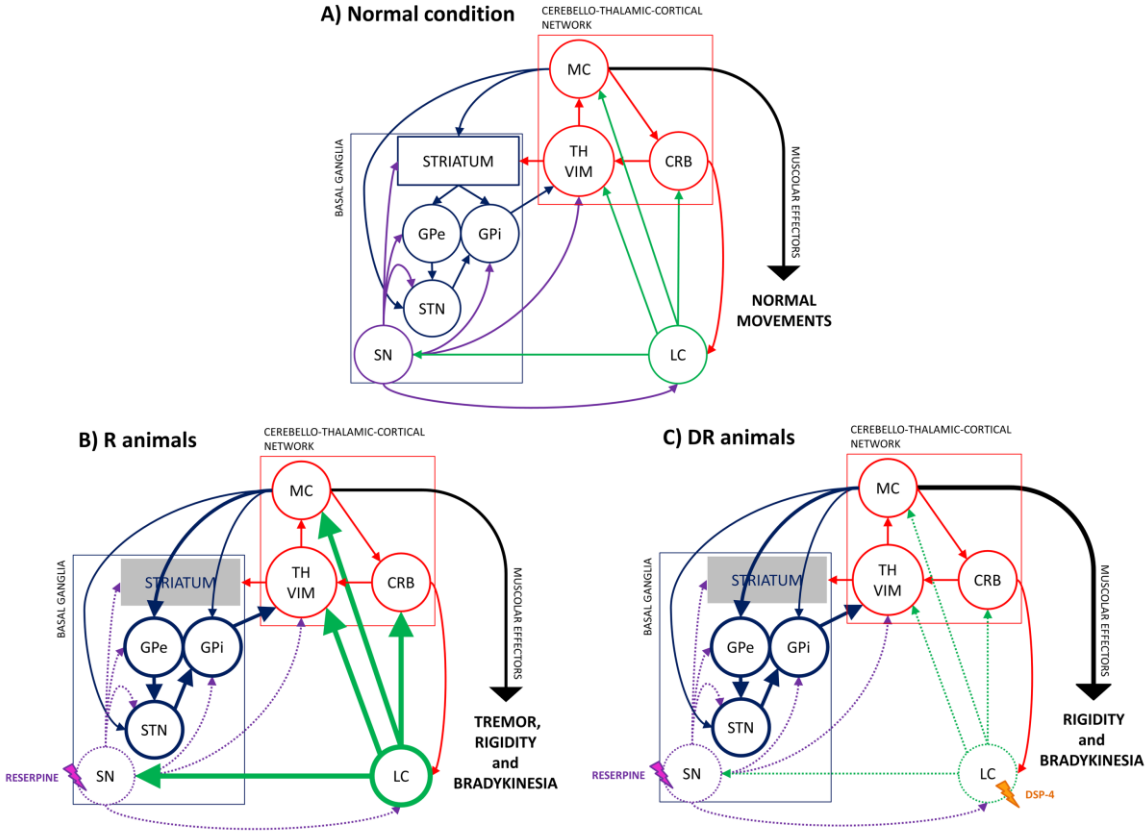

Figure 21 – Schematic representation of the influence of the locus coeruleus (LC) on the basal ganglia and cerebello-thalamic-cortical circuit.
(A) In the normal condition, the dopaminergic innervation arising from the substantia nigra (SN) and noradrenergic projections of the locus coeruleus (LC) are balanced and allow correct motor processing across the cortico-striatal-thalamic and the cerebello-thalamic-cortical circuits. In this condition, movements can be executed correctly. (B) The lack of dopamine determines the development of pathological oscillations in the basal ganglia circuitry and induces an exaggerated LC-noradrenergic (NA) release, which triggers tremor onset by gaiting the cerebello-thalamic-cortical network activity. Patients manifest tremor and the akinetic rigid symptoms of Parkinson's disease. (C) Degeneration of LC-NA cerebellar projections prevents the onset of tremor by reducing the receptivity of the cerebello-thalamic-cortical network. Parkinsonian patients would in this case manifest only akinetic rigid symptoms.

This study needs to account for several limitations. First, it lacks separated control groups treated with placebo and DSP-4 only. That's because, instead of a design with four parallel arms (i.e. placebo, D-group, R-group and DR-group), a delayed-start designed was performed. In this design, the first two weeks following DSP-4 injection (DR-group) are controlled against lack-of-treatments (R-group) and the active phase coincides with reserpine injection. This study design is commonly used in randomized clinical trial for rare disease, as it allows to minimize the population of study[278]. It requires that the treatment effects is not acute, but builds over time[278]. The current study met this criterion and allowed (i) assessing whether DSP-4 had any additive effects upon

behaviour (unrelated from those observed following reserpine) and (ii) measuring both the R and DR groups difference at baseline; moreover, it limited the number of animals scarified.
Second, the reserpine model of PD is still an inadequate approximation of the human condition and some have argued against its reliability[279]. In particular, limitations are: (i) reserpine does not induce neurodegeneration and protein aggregation (e.g. α-synuclein aggregates); (ii) it lacks of specificity, affecting all monoamines, and (iii) motor performance, monoamine content, and TH staining are partially restored after treatment interruption[249]. Nevertheless, the behavioral and neurochemical features of reserpine administration are highly reproducible, the reduction of many monoamines actually better resembles PD pathology as dopaminergic deficit alone and, for the specific purpose of this study, the reserpine rat model is one of the very few presenting tremor along with akinetic-rigid symptoms[249]. That said, the reserpinized rat still suffers from the limitations of an acute lesioning models, with diffuse neurotransmitters failure, and this does not closely mirror the early LC neuronal loss and Lewy body pathology, but rather approximates the functional results of these processes by decreasing NA storage and synthesis. This is obviously suboptimal and may have impacted some measurements, such as the frequency of tremor that was higher as the one presented by PD patients. Still early PD neurodegenerative processes remain unclear and might differ for some extent from patient to patients[19,20,25,280]. Reproduce the functional consequences of these neurodegenerative process by inducing a multi-neurotransmitter deficit is a reasonable approximation of the changes occurring in PD[249].
Third, the current research focuses on the cerebellum but reduction of noradrenergic activity may also affect other structures. One possibility is the PPN that, given the connections with the LC[79,82,281,282], may show reactive activity changes following noradrenergic modulation. However, the focus on the cerebellum was justified by the increasing evidence supporting a role of this structure in PD tremor[240–244,262]. Alterations of PPN might be more relevant for gait disorders[283], such as FOG, and will be discussed afterword.

In conclusion, this study advances the understanding of tremor pathophysiology, suggesting that tremor relies on two distinct, necessary and not sufficient mechanisms that involve different (related and yet largely independent) neurotransmitters, namely dopamine, serotonin and noradrenaline. Moreover, it supports the hypothesis that cerebellar noradrenergic LC projections specifically impact on tremor onset, severity and consistency. However, this evidence remains preliminary and further studies should be performed. In particular, the assessment of the dopaminergic/noradrenergic interplay at the onset of motor symptoms and along with disease progression in PD patients with and without tremor is particularly pressing. This possibility has recently become available thanks to the combination of novel molecular imaging compounds targeting the dopamine re-uptake transporter density (DaT) and NE re-uptake transporters (NET). Accordingly, this is the core of an ongoing project and will be discussed as future perspective.

3.2 Freezing of gait

3.2.1 Background

Freezing of gait (FOG) is a peculiar clinical phenomenon during which gait is suddenly impaired. FOG is defined as sudden and unpredictable failure of gait characterized by the inability to produce effective forward stepping [8,284]. The erratic nature of gait freezing episodes is particularly debilitating as it leads to falls because patients are caught always somehow unprepared. Indeed, it is quite peculiar that although patients are aware of the circumstances leading to FOG appearance, they cannot anticipate it. Especially for this reason FOG is a primary cause of falls that lead to injuries, hospitalization and increase mortality. It severely worsens patients quality of life, inducing fear of falling and prompting isolation and depression [48,285,286].
FOG is mostly observed at gait initiation and during turning, but it is also triggered by narrow passages (e.g. doorways), the execution of a secondary task (e.g. responding to a question while walking), or upon reaching a destination. FOG can also occur seemingly spontaneously while walking in open terrain, especially if under time constrains or spatial challenges (e.g. crowded places). Three main clinical presentations are recognised: (i) shuffling with small steps, (ii) trembling in place without forward movement and (iii) total akinesia [8,284].
FOG can be present in its different forms in cerebrovascular disorders, hydrocephalus and atypical parkinsonisms, such as primary progressive freezing gait (PPFG) or progressive supranuclear palsy (PSP). However, it is mostly seen in patients with PD [8,284].
In PD, it affects more than the half of the patients and up to 90% of those in Hoehn and Yahr stage IV [45]. It is more likely to occur when dopaminergic medications lose their effect (i.e. wearing-off or meds-off phases), but its relationship with these treatments is complex as levodopa can also induce or worsen FOG [43,50,287,288].
An effective treatment specific for FOG is currently missing and its management remains largely unsatisfactory. A main reason for this lack of treatment is that the pathophysiology of FOG is still unknown.
To understand the neural mechanisms of FOG and prior to present the present the current and working hypotheses of FOG pathophysiology, I will briefly discuss the current understanding of human locomotor control in physiological conditions.

Locomotor control in humans
Understanding the neural control of locomotion is challenging as it implies multisite brain in a standardized environment. Locomotion describes the ability of moving in the environment around us and it is the core of many daily activities, allowing standing, walking, jumping, swimming or running. Most of our knowledge on locomotor control derives form studies in animals, but it must be acknowledged that human locomotion represent a unique characteristic of our species[289].
Human walking is inherently unstable as we walk erected on two legs, striking the ground with the heel, when the leg is almost fully extended. This entails the simultaneous activation of flexors and extensors at heel-strike, which is not the case in any other animal (where these muscles activate in phase)[289]. Furthermore, unlike mammals[290], spinal cord injuries (e.g. transactions) are not followed by locomotion recovery when direct electrical stimulations are applied. Highly organized and patterned inputs seems indeed to be required to improve locomotion recovery [291,292].
This suggests a hierarchal organization of locomotor control, where spinal centres are modulated by supraspinal networks.
Accordingly, the spinal control of locomotion has been showed to mainly regulate stretch reflexes and sensory inputs processing (which modulate stance-to-swing transition [51]), while the spinal CPGs produces basic locomotor patters[293], but none of these structures initiate locomotion [51]. Of note, these features are similar across species. So, while spinal control of locomotion is shared across species and mainly regulates the execution of the movements, humans show a much more complex supraspinal locomotor control that accounts for the simultaneous processing of body orientation and motion in space, motion perception and spatial localization of objects in the extra-personal space[55].
Converging evidence support that such a complex task relies on the integration of different information through a distributed network, the supraspinal locomotor network. This network encompasses the cortex, the basal ganglia, the mesencephalic locomotor region (MLR) and the cerebellum [55,151,201,284,294].

Cortices are essential for execution of skilled locomotor tasks, visuomotor coordination, initiation and ongoing regulation of movements, and focused attention [55]. The execution of learned motor programs is performed by the sensory motor cortex (M1 and PA) that can finely tune muscles activation and limbs direction. The visuomotor coordination is performed by the posterior parietal cortex (PPA) that elaborates spatiotemporal relationships between environmental obstacles and body information, such as body schema. The initiation of the movements and their ongoing regulation rely on medial premotor cortices, and mainly on the supplementary motor area (SMA, F3) and the pre-supplementary motor area (pre-SMA, F6). In particular, while pre-SMA projections to the MLR are involved in movement initiation, the SMA regulates ongoing movements according to sensory, cognitive and motivational inputs in the manner of feed-forward motor commands or anticipatory postural adjustments (APAs)[295]. APAs are unconscious muscular activities counteracting the postural unbalance produced by novel, consciously-intended movement [296] and are essential to maintain balance control during walking, because posture must be optimized in advance to the purposeful action [55].
This function mainly relies on SMA reciprocal projections to the basal ganglia and the PPN, which innervates the pontine reticular formation and, in turn, the whole spinal segments through the reticulospinal tract (RST) [55]. RST neurons are able to supress the motor tone and work together with the vestibulospinal tract, which controls the overall postural tone level, to ensure the proper execution of programmed locomotor tasks [55].
Following APAs signals, MLR neurons (i.e. PPN and cuneiform nucleus) activate the medullary reticular formation, which via RST commands spinal CPGs that generates basic locomotor patterns [52,293]. MLR activity is modulated by two main structures, the cerebellum and the basal ganglia [53].
The cerebellum encodes internal postural models in space and elaborates sensory information that converge on the fastigial nuclei, which receives copies of spinal outputs and peripheral sensory, visual and vestibular information. This convergence of information may support an error-correction processes that amend the activities of MLR and cortical areas during locomotion [55].
The cerebellum may mainly refine muscular tone by in-parallel activation of fastigio-spinal, fastigio-reticular, and fastigio-vestibular pathways. Accordingly, cerebellar lesions with deficiency in these pathways are associated with hypotonia, which by reducing the accuracy of the sensory feedback can alter movement coordination and determine gait ataxia. Instead, the cerebellum is not involved in APAs achievement (as cerebellar lesions do not alter APAs), which may rely more on basal ganglia and SMA interactions [55].
Thanks to reciprocal connections to the cortex, the thalamus and the PPN, the basal ganglia refine cortical locomotor commands and timely shape APAs, which were found to be variable, hypometric and protracted in PD subjects.
It has been proposed that the GPi and SNr may provide a tonic inhibition onto the MLR and thalamus during resting, thus detaching cortical and spinal information flows, while cortical inputs during locomotion can engage the basal ganglia, which dynamically relieve (via the striatum) or increase (via the STN) MLR inhibition [297]. Therefore, the BG are particularly suited for adapting locomotion in response to contingent environmental changes and might not be constantly engaged during locomotion, but take action only when the ongoing locomotor pattern needs to be modified.
This led to the distinction of two locomotor networks: the “executive” and “planning” network of locomotion [294]. Execution of locomotion in a non-modulatory steady state goes from the cortical areas directly to the spinal central pattern generators (CPG), thereby bypassing the basal ganglia and the MLR. A sensory feedback loop runs from the spinal cord to the cerebellum and via the thalamus to the cortex, thus ensuring postural control [294]. For planning and modulation of locomotion, instead, the locomotor programs originate in the SMA and are transmitted through the BG and (via disinhibition) to the MLR, where they converge with sensory signals from the cerebellum. The MLR functionally represents an intersection for information form basal ganglia and cerebellar loops. Descending anatomical projections are directed to the medullary and pontine reticular formations (PMRF) and the spinal cord [294]. Cortical locomotor programs are further modulated via a cortico-striato-thalamic circuit.
When working effectively, these networks can provide a continuous locomotor control, able to face environmental challenges. However, failure of this system at multiple levels can impair the integration of the information needed for locomotion, thus inducing paroxysmal gait impairments (i.e. FOG). Central to the seamless functioning of this distributed network is a timely flow of information across its nodes that can yield to finely tune synchronization. This essential aspect of neural communication is supported by coordinated neural electrical activities, which due to their physical proprieties are referred and analysed as neural oscillations.

Neural oscillations and information flow across the brain: relevance to locomotion

How specific behavioural acts (e.g. locomotion, emotional processing or cognition) are encoded and ruled by functional activity of neuronal networks is among the most complex questions of neuroscience. In recent years, however, more and more studies suggested that the much of the motor and non-motor behaviours of which our brain is capable of can be achieved by precisely synchronizing distributed brain areas with distinct proprieties. This dynamic synchronization allow combining the functions of different brain areas to solve a given problem and it creates (with time and exercise) preferred anatomical connections that underlie acquired skills, thus being the basis of brain plasticity. An essential mean for dynamic brain coordination is synchronized neural oscillations that are sustained by coherent membrane potential fluctuations.

Neural oscillations represent periodic neural activities, thus encompassing single unit spikes, busts or local field potentials (LFPs) [114]. They are conventionally divided in frequency bands (e.g. δ [1–4 Hz], θ [4–8 Hz], α [8–13 Hz], β [13–30 Hz] and gamma γ [>30 Hz] [118] with distinct temporal and spatial proprieties that impact the related processing. Precisely, low-frequency bands rely on large spatial regions and long timing, while high-frequency bands originates from small neural cluster and in a short time[119], and allows long and stable or confined and fast synchronization, respectively. Therefore, just like anatomical segregation by which a neural inputs targets a specific brain spot by travelling in organized neuronal bundles, synchronization in distinct frequency bands may segregate specific neural processes. Of note, this latter communication mean enables to deliver different information through common effector pathways.[298] This is particularly relevant for cortical-basal ganglia processes as they run simultaneously along parallel functional loops that share common nuclei with a limited anatomical (i.e. spatial) segregation.

With respect to locomotion, oscillation dynamics were shown to be relevant in the spinal control of locomotion [51]. Several studies investigated the finely tune activation of the spinal networks that enable locomotion and describe many of the mechanisms [51]. Remarkably, the application of this knowledge to patients with chronic spinal lesions allowed the recovery of a voluntary control of locomotion [290–292]. Our understanding of the supraspinal neural network dynamics during gait and its derangements in FOG remains preliminary, calling for a deeper investigation.

Current hypothesis of FOG pathophysiology

At present, FOG is supposed to emerge from an overwhelming GABAergic inhibition of the MLR [297].

Following the theory that pathologic brain oscillations cause bradykinesia and considering freezing a bradykinetic sign, it was suggested that FOG may be a consequence of excessive synchronization of neuronal oscillations in the β-band [197].

Although conceivable, the interpretation of FOG as consequence of excessive β-synchronization suffers from a selection bias. The majority the evidence in favour of this view, indeed, comes from studies that compared patients who were chronically suffering from FOG with those who had never experienced this symptom, thus mainly addressing putative neuronal mechanisms preventing FOG rather than its actual pathophysiology.

Hence, it has been proposed that the GABAergic inhibition of the MLR causing FOG emerges from either paroxysmal bouts of decreased striatal activity (i.e. hypoactivation of the direct pathway in favour of the indirect pathway) or sudden increases in STN activity (i.e. activation of the hyperdirect pathway) [297]. Striatal activity drops might be triggered by deficient SMA signals (i.e. reduced excitatory glutamatergic inputs on the caudate nucleus), while STN activity enhancements may emerge as response to conflict. The processing of motor, cognitive, affective, or perceptual conflictual stimuli can, indeed, evoke a similar STN activation, which can be encoded in coherent θ-oscillations in the STN and medial pre-frontal cortices [299].

It was shown that during ongoing FOG episodes θ-oscillations are increased at cortical level (M1 and SMA), thus supporting an active role of the SMA in FOG development [202]. However, while cortical θ-oscillations are followed by coherent STN activity changes during conflict this is not the case in FOG.

Studies assessing STN-oscillations during ongoing FOG episodes did not show increases in θ- nor in β-oscillations, but reported a greater STN α-entropy (a measure of neural organization)[151] or longer β-burst duration (a temporal feature of β-synchronization) as compared to normal walking [300].

A causative role for the STN in FOG development is also questioned by the inconsistent results of STN stimulation. Indeed, while STN-HFS reduces its activation in response to conflict [93] and promotes impulsive behaviours, this effect does not controls FOG. A putative role for altered processing of conflict in FOG development is still possible through direct SMA-PPN connections, which are however excitatory [55].

The current model also only partially explains a typical cognitive issue associated with FOG, the impaired motor automaticity (i.e. dual task performances), which is interpreted as detrimental STN-modulation of the cerebellar cortex that leads to the inability to rely on previously learned habitual responses [297].
Therefore, despite validity in its circuitry and general conceptions, the current model fails to account for a series of clinical and neurophysiological evidence. For this reason, I have put forward a refined hypothesis of FOG pathophysiology.

3.2.2 Working Hypothesis

Unlike the current interpretation of FOG in PD as a sudden locomotor failure (i.e. lack of movement or "akinesia"), I envisioned that FOG would be an improper update of on-going motor programs to environmental needs and it could therefore be considered a "dyskinesia". This interpretation would fit all the conditions triggering FOG: from changes in the direction or timing of walking (e.g. turning, crossing obstacles, etc.), to gait initiation (i.e. the transition from upright standing to gait) or arrest.
In my hypothesis, gait freezing reflects a deficient update of the ongoing locomotor pattern with novel motor programs that are needed to face incoming environmental requests. This deficient update could be due to a reduced cortical (e.g. SMA) engagement in the production of *feedforward* motor programs, which are generated by the cortex and basal ganglia to adapt the locomotor pattern to the environmental contingencies [55,151,201,284,294].
In patients with PD, these motor updates are particularly relevant as the degeneration of dopaminergic nigrostriatal projections to the dorsal putamen affects motor automaticity [301], thus forcing parkinsonian patients to allocate attentional resources to effectively walk. The hyper-direct pathway, which directly connects the SMA with the STN, can support this active cortical control of locomotion, thus allowing a dynamic cortical-subcortical synchronization and the proper integration of the cortical *feedforward* motor programs with the ongoing locomotor plan. If this cortical-subcortical communication is altered (by a reduced compensatory activity or a second pathological hit), the locomotor update may be compromise and this may also cause FOG. As such, FOG would be due to a dysfunctional cortical planning, to a deficient integration (i.e. altered transmission) of the required motor information or both. In other terms, FOG would result from any cause able to determine a dysfunction in the locomotor networks dynamics that are essential to convey specific information supporting locomotion adaptation.
Central to my hypothesis is that the STN is engaged in locomotor control. As a fundamental preliminary step to studying FOG, I have first investigated the STN activity and connectivity during gait [149]. In particular, I have directly studied the role of STN in locomotor control by measuring its activity and connectivity in freely-moving PD patients. I have compared the STN β-power and inter-hemispheric coupling, amplitude cross-correlation and phase locking value across epochs of resting state, upright standing, and steady forward walking in eight subjects with PD. I reported a drop in the phase locking value in the β-frequency band during walking with respect to resting and standing, thus suggesting that not only the STN is engaged in locomotor control, but also that the information processing occurs for each body side separately [149]. This findings are in line with other works that reported a modulation of STN spectral power during locomotion [135,147,149,150,300].
Altogether these results suggest that the STN is involved in locomotion control as part of a distributed network and supports my hypothesis that alterations of cortical-STN communication might determine an "error cascade" that results in gait freezing (Figure 22).

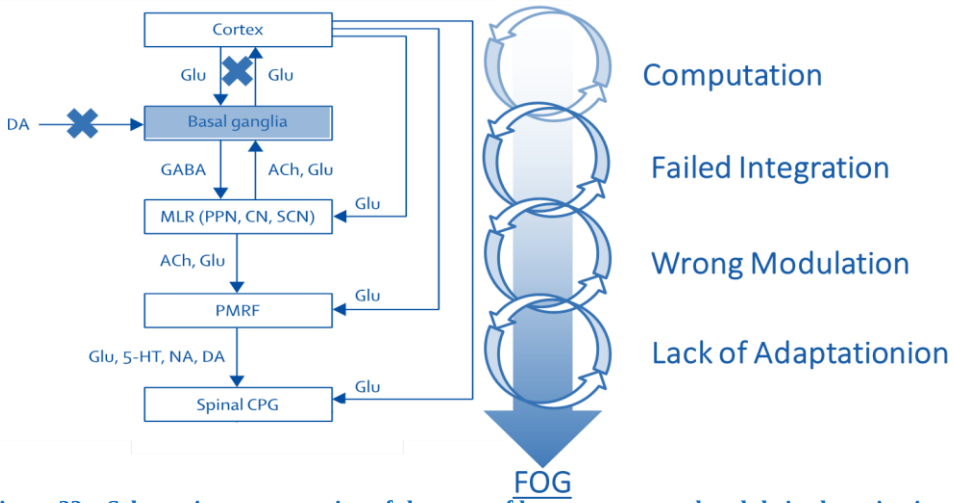

Figure 22 – Schematic representation of the steps of locomotor control and their alteration in freezing of gait.

To test this hypothesis, I have investigated cortical-subthalamic coupling and STN power spectral densities, β-bursts and inter-hemispheric coupling in seven freely moving subjects with PD. Multisite brain recordings during (effective) walking and ongoing episodes of gait freezing were combined with kinematic measurement and molecular brain imaging findings.

3.2.3 Methods

Subjects and surgery

I tested seven idiopathic PD patients who were diagnosed according to the UK Parkinson Disease Brain Bank criteria and treated with bilateral STN deep brain stimulation (DBS). DBS is a surgical treatment for selected PD patients and allows improving the motor and non-motor symptoms of PD as well as a sustained reduction of the medication intake by providing precise electrical inputs to target locations (e.g. STN or GPi) (see for review [302–304].

DBS implantations were performed in all patients but one (i.e. nwk01) at the University Hospital Wuerzburg (Wuerzburg, Germany) with the "Activa PC+S®" system (Medtronic, PLC). For subject nwk01, the electrodes were externalized and connected to a portable device ("AlphaDBS", Newronika S.r.l.) at battery replacement, which took place at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano.

These DBS systems are prototypes that, besides allowing (classical) therapeutic stimulation, allow on-demand recordings of STN LFP from the chronically-implanted electrodes. [305,306] All systems (i.e. "Activa PC+S®" and "AlphaDBS") and related hardware were provided under a request for application agreement by Medtronic, PLC or Newronika S.r.l., respectively. The companies had no impact on study design, patient selection, data analysis, or reporting of the results.

The surgical procedure has been previously detailed.[104,306] Briefly, the DBS electrode used was model 3389 model (Medtronic, PLC) that present four platinum–iridium cylindrical contacts of 1.5 mm each spaced by 0.5 mm. The contacts 0/8 were the lowermost, whereas contacts 3/11 were the uppermost; E0-3 refers to the right hemisphere and E8-11 to the left hemisphere. The STN coordinates (i.e. 12 mm lateral, 2 mm posterior, 4 mm ventral to the mid-commissural point) were individually adjusted according to patient's T2-weighted and susceptibility-weighted images (MAGNETOM Trio or Skyra, SIEMENS Healthcare, Erlangen, Germany). All electrode location were verified by intraoperative microelectrode recordings and stimulation as well as confirmed by means of intraoperative CT scan. The implantation procedures were performed by Dr. Frank Steigerwald, whereas the precise localization of the active (and recording) contacts was performed by Martin M. Reich by fusing pre- and postoperative scans (SureTuneTM, Medtronic, PLC).[307]

At the time of the experiment, I ensured that all patients were on stable dopaminergic treatment (for at least two months) and chronically stimulated (i.e. unchanged DBS parameters for at least two months). I tested the subjects about four years after surgery with the exception of patient nwk01 who was tested seven years after DBS due to battery replacement procedure.

To ensure the correct placement of the electrodes, I calculated the percentage improvement due to DBS as previously reported.[149] The demographic and clinical details of the sample are displayed in Table 1.

The local Institutional Review Board of the University Hospital Wuerzburg as well as the one of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano approved the study and all patients gave their written informed consent.

Table 1 – Demographic and clinical data.

Patients are grouped as suffering (i.e. FOG+) or not suffering (i.e. FOG-) from FOG. All patients were evaluated using the UPDRS-III within one month prior to implantation (pre-DBS) after overnight (>12 h) suspension of all dopaminergic drugs (meds-off) and upon receiving 1 to 1.5 times (range 200–300 mg) the levodopa-equivalent of the morning dose (meds-on). After surgery (post-DBS), patients were assessed in four conditions: (i) stimulation off for at least two hours (stim-off); (ii) bilateral STN stimulation (stim-on); (iii) meds-on (as pre-DBS); (iv) meds-on and stim-on.

DBS, deep brain stimulation; FOG, freezing of gait; LEDD, levodopa equivalent daily dose; UPDRS-III, Unified Parkinson's Disease Rating.

		Patient						
		FOG +					FOG -	
		wue03	**wue04**	**wue10**	**wue11**	**nwk01**	**wue06**	**wue07**
Sex		M	M	M	F	M	M	M
Age at onset (years)		43	47	46	42	41	36	51
Disease duration at experiment (years)		20	9	12	13	14	14	12
UPDRS-III pre-DBS (score)	Meds-off	40	26	69	55	40	46	43
	Meds-on	9	8	14	4	9	11	24
UPDRS-III post-DBS (score)	Meds-off/ stim-off	45	27	65	51	66	48	29
	Meds-on/ stim-off	23	9	20	13	18	11	8
	Meds-off/ stim-on	17	5	25	9	15	12	15
	Meds-on/ stim-on	14	8	5	14	9	6	9
LEDD pre-DBS (mg)		2725	658	1200	1300	960	1133	650
LEDD post-DBS (mg)		600	400	550	460	680	180	220

SPECT imaging

All patients performed FP-CIT and SPECT to measure the dopamine reuptake transporter (DAT) density as previously described.[149] This molecular imaging technique is a mainstay approach for diagnosis and differential diagnosis of PD [308] and can detect dopaminergic striatal tone, which was shown to play a modulatory role in locomotor synergies in PD [309]. The striatal DAT binding values of the patients were compared with the one of 15 healthy subjects (four males, 11 females; mean age ± standard deviation (SD) 62 ± 9 years, range 44–68 years). According to the DAT binding values of each patient, I identified the brain hemisphere with more (+) or less (-) dopaminergic innervation and the asymmetry index (AI). [149]

Protocol, set-up and biomechanical data processing

All patients were asked to walk barefoot over ground at a self-selected speed, over a 15 m path that included passing through one turning door (1 m wide) in the gait laboratory and two common doors (1.2 m and 1.6 m wide) outside the gait laboratory (Figure 23A). I specifically designed this pathway to mirror daily-life situations that require the adaptation of the gait pattern to different environmental conditions without the parallel engagement of cognitive tasks (i.e. dual task walking). To prevent dopaminergic medication bias, all patients were investigated in the morning and at least 12 h after the last intake of dopaminergic replacement therapy. Moreover, the DBS was switched off 2 h priori the experiment (i.e. meds-off/stim-off condition), thus ruling out the confounding effect due to stimulation. Despite these challenges, all patients were able to perform at least four trials (range 4–8).

Patient nwk01 was severely impaired by the symptoms and was only able to perform recordings in the gait laboratory.

Kinematic recordings of the lower limbs were carried for the entire walking path by means of two inertial recording units (IMU, Opal, APDM, Oregon, USA), with a sampling rate of 128 Hz. IMUs were placed on the outer anklebones, thus recording the kinematics of each leg separately. A representation of the complete set-up is depicted in (Figure 23A).

To identify the episodes of gait freezing I combined clinical evaluation and kinematic recordings. Precisely, walking trials were video-recorded with two synchronized cameras (VIXTA, BTS, Italy) and evaluated by two independent raters (NGP and IUI).[202] Gait freezing epochs were also defined by computing the wavelet spectrum of the angular velocity over the medial-lateral axis, which was characterized by a switch to higher frequency as compared to (effective) walking. This analysis as well as the computation of the biomechanical parameters was computed by Eng. Chiara Palmisano.

To specifically study the neural network dynamic changes related to gait freezing I selected five time frames, which anticipated, characterized and followed each freezing episode. In particular, I identified: (i) (effective) gait (Walking), 2 s-time epochs free of gait freezing; (ii) pre-freezing (F_{PRE}), 2 s-time epochs immediately preceding a freezing episode; (iii) freezing start (F_{START}), first 2 s of a freezing episode; (iv) freezing stop (F_{STOP}), last 2 s of the freezing episode; (v) post-freezing (F_{POST}), 2 s-time epochs after the resolution of a freezing episode (Figure 23B). The lengths of the epochs were defined according to the shortest freezing episode, which lasted 3 s. The total number of walking epochs was 296, for a total of 592 s of (effective) walking. Of relevance, the epochs of (effective) walking were recorded in the same environmental settings, thus controlling for its difficulties. In addition, gait freezing epochs were also directly compared with epochs of successful passing through doors (32 epochs of 2 s each), to disentangle ongoing gait freezing related changes from a more general gait stop signal (needed to open and pass through the door). The two subjects (i.e. wue06 and wue07) who did not suffer from FOG were considered separately.

In the gait laboratory, the kinematics of body segments during (effective) steady-state linear walking (reached before approaching the turning door) were computed with an optoelectronic system (SMART-DX400, BTS, Milano, Italy) that calculated the 3D coordinates of 29 spherical retro-reflective markers (15 mm diameter) fixed to anatomical landmarks.[149] The marker coordinates were low-pass filtered (cut-off frequency of 7 Hz) and interpolated. Eng. Chiara Palmisano extracted the kinematic parameters by means of custom scripts developed in Matlab® ambient (Matlab 2017b, The MathWorks, Inc., Natick, Massachusetts, USA) and provided the stride length, duration, and velocity (expressed as percentage of the subject's height) and the stance and double-support duration of each patient. For the calculation of the temporal parameters (i.e. stance and double-support) instead, recordings were time-normalized as a percentage of the stride duration. Averaged values are reported in Table 2, where the data from 11 healthy controls (nine males, three females; mean ± SD age 58 ± 5 years, range 50–66 years) matched for age and anthropometric measurements are also displayed (Table 2). Values for the patient and healthy control collectives were compared and tested for statistically significant differences (see below).

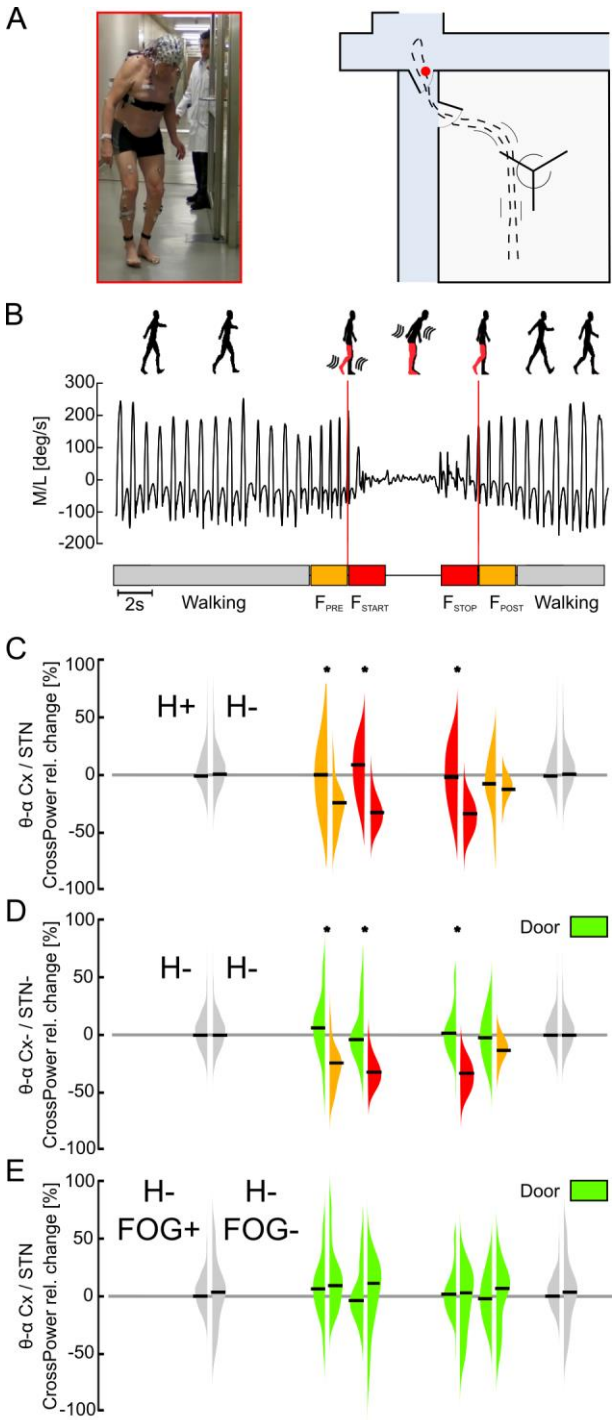

Figure 23 – Experimental set-up, gait freezing kinematic identification, and the corresponding neural correlate.
A. Experimental set-up. The walking path inside and outside the gait laboratory consisted of walking through a turning door (inside the gait laboratory) and two common doors outside, where a representative freezing episode took place (red dot and figure).
B. Kinematic representation of one freezing episode. Representative traces of the angular velocity over the medial-lateral axis during walking and gait freezing. We identified five time frames: (effective) walking is shown as light grey boxes (i.e. 2 s-time epochs free of gait freezing), FPRE and FSTOP are the orange boxes (i.e. 2 s-time epochs preceding and following a freezing episode, respectively), and FSTART and FSTOP are shown as red boxes (i.e. the first and the last 2 s of freezing, respectively).
C. Percentage relative change of cortical-subthalamic low-frequency coupling during gait freezing vs. (effective) walking. Effective walking (grey) was characterized by a sustained low-frequency (i.e. θ-α bands, 4-13 Hz) cortical-subthalamic coupling. During gait freezing (FSTART – FSTOP, red), the cortex and the STN decupled on the side with less striatal dopamine (i.e. H-). The decoupling was already evident before gait freezing (FPRE, orange) and vanished with the recovery of a normal locomotor pattern (FPOST, orange). The asterisk indicates statistical significance ($p<0.05$).
D. Percentage relative change of cortical-subthalamic low-frequency coupling during gait freezing vs. successful passing through a door. The cortex and the STN decoupled only during gait freezing, while no difference was detected during successful passing through a door. The asterisk indicates statistical significance ($p<0.05$).

E. Percentage relative change of cortical-subthalamic low-frequency coupling during passing through a door in subjects suffering vs. not suffering from FOG. Subjects suffering and not suffering from FOG showed the same cortical-subthalamic coupling during (effective) walking and successful passing through a door.

Cx, cortex (i.e. SMA, M1 and PA); FOG, freezing of gait; FPRE, pre-freezing; FSTART, freezing start; FSTOP, freezing stop; STN, subthalamic nucleus (STN+ and STN- refers to the side with more and less striatal dopaminergic innervation respectively); FOG+ and FOG- refers to the patients suffering or not suffering from FOG, respectively .

Electrophysiological signal recording and analysis

STN LFPs were combined with cortical recording obtained with a 64-channel portable EEG (MOVE, Brain Products, Germany); for signal synchronization please refer to [305]. I recorded the STN LFPs with a single bipolar contact configuration, thus limiting the recording volume. The recording contacts were chosen according to the chronic stimulation setting as a bipolar montage of the two contacts surrounding the stimulation cathode.[142,159] LFP signals were amplified by 1000, while the sampling frequency was 422 Hz. Recordings were re-sampled at 400 Hz for analysis. I acquired the EEG signals with the sampling frequency at 1000 Hz, which was then re-sampled at 400 Hz for analysis. Raw signal were preprocessed by Eng. Andrea Canessa as follows. First, a bandpass Kaiser windowed FIR filter (pass band [1–80] Hz, stop band [0.5–84] Hz, attenuation 60 dB) was applied to eliminate low and high frequency, thus focusing the analysis between 1 and 80 Hz. Second, the power line noise (50 Hz) was eliminated with bandstop 4th order Butterworth filters and EEG channels that were affected by a bad scalp-electrode connection were replaced with spherical spline interpolation. Stereotypical artefacts (e.g. blinks, heartbeat, and muscle tension) were instead removed by independent component analysis (ICA[310–312]) and a laplacian montage was applied to limit EMG artefacts.[313] LFPs and EEG signals were finally analyzed with MatLab-based custom script and the Fieldtrip Toolbox.[314]

Together Eng. Andrea Canessa and I decided the analysis and defined the electrophysiological metrics needed to assess the neural network dynamics changes during gait freezing. We focused on three measurements: (i) spectral power analysis (i.e. Power Spectral Density, PSD); (ii) β-burst duration and (iii) STN inter-hemispheric connectivity.

(i) We first computed the PSDs for all channels (64 EEG and two STN LFP channels) of each 2 s-epoch of the five conditions. To ensure an efficient spectral estimation of the relatively small number of trials a multitaper spectral analysis was preferred.[315] The time-frequency bandwidth was set to 1.5, resulting in two slepian tapers being used. We performed a spectral analysis between 4 Hz and 80 Hz, with a resolution of 1 Hz. For the sake of comparison between all five conditions, we normalized each frequency bin of the PSD with respect to the total power (integral of the PSD between 4 Hz and 80 Hz), separately for all channels:

$$PSD(f)_i = \frac{PSD(f)_i}{\int_4^{80} PSD(f)_i df}$$

As in Shine and coll.,[202] six regions of interest (ROIs) were defined (three for each hemisphere): SMAL {F1,F3,FC1,FC3}, SMAR {F2,F4,FC2,FC4}, M1L {C1,C3,CP1,CP3}, M1R {C2,C4,CP2,CP4}, PAL {P1,P3}, and PAR {P2,P4}, which overlay cortical areas involved in locomotor control.[201,284] Figure 24A shows the PSDs for the cortical and STN recordings. After selecting the ROIs, we identified for each patient the most prominent peak in the θ-α range (i.e. 4–13 Hz) of the averaged PSD across walking epochs. With respect to the STN recordings, we chose the individual peak in β-frequency (13–35 Hz) as the activity always peaked in this band. Then we compared the β-power of the two STN (STN+ and STN-, separately) between epochs of (effective) walking and timeframes of gait freezing. To this end, we estimated group level variance using the bootstrapping (20 repetitions; resampling with replacement) technique and estimated the confidence intervals between the 5th and 95th percentiles of the bootstrap distributions.

(ii) Since recent publication suggested a role for the temporal characteristics of the STN β-oscillations, we also investigated the (so-called) β-burst distribution between epochs of (effective) walking and gait freezing. Unlike the previous analysis, this calculation does not rely on the spectra distribution

of the waveform but on the changes of the envelope of the raw signal. Therefore, we first focused on the β-oscillations components of the raw signal by decomposing the raw LFPs with Wavelet transformation (morlet_transform script in Brainstorm – Morlet Wavelet, fc = 1, FWHM = 4) into frequency components between 13 and 35 Hz with the frequency resolution of 1 Hz. Then, we computed the wavelet amplitude envelope and selected the β-peak frequency (single frequency bin of 1 Hz) in the walking condition of each subject. To normalize data between subjects and conditions, we calculated the z-score of the wavelet amplitudes by subtracting and dividing them for the mean and the SD of the walking envelope, respectively. The β-burst was selected according to an amplitude threshold. As burst occurrence is correlated with total β-power in a trial [316,317], we selected the amplitude threshold by measuring for each patient the correlation coefficient between the mean β-amplitude and the number of peaks exceeding an amplitude threshold computed in all 2 s walking epochs. By repeating this procedure for different values of the threshold (range: 1-5), we obtained the relationship between the amplitude threshold and the correlation coefficient. We then averaged the curves and selected the maximum across all subjects, which was 1.35 and 1.45 for the STN in the less- and most-depleted hemisphere, respectively (Figure 24B). These values were used as thresholds to define the β-burst peak and, for each 2 s-epoch (all subjects and all conditions) we searched the β-bursts exceeding this threshold. We than measured the β-burst duration by sorting all the peaks according to their amplitude and identifying their Full Width Half Maximum. Of relevance, this method makes the identification of burst duration independent from the threshold used as cutoff. Small peaks that lay inside the duration of a higher one were excluded and considered as a single β-burst. We finally compared the β-burst duration between effective walking and gait freezing. For this analysis, we considered FSTART and FSTOP timeframes together and compared them with randomly-selected timeframes of (effective) walking of equal length, thus accounting for a possible confounding effect due to different recording durations.

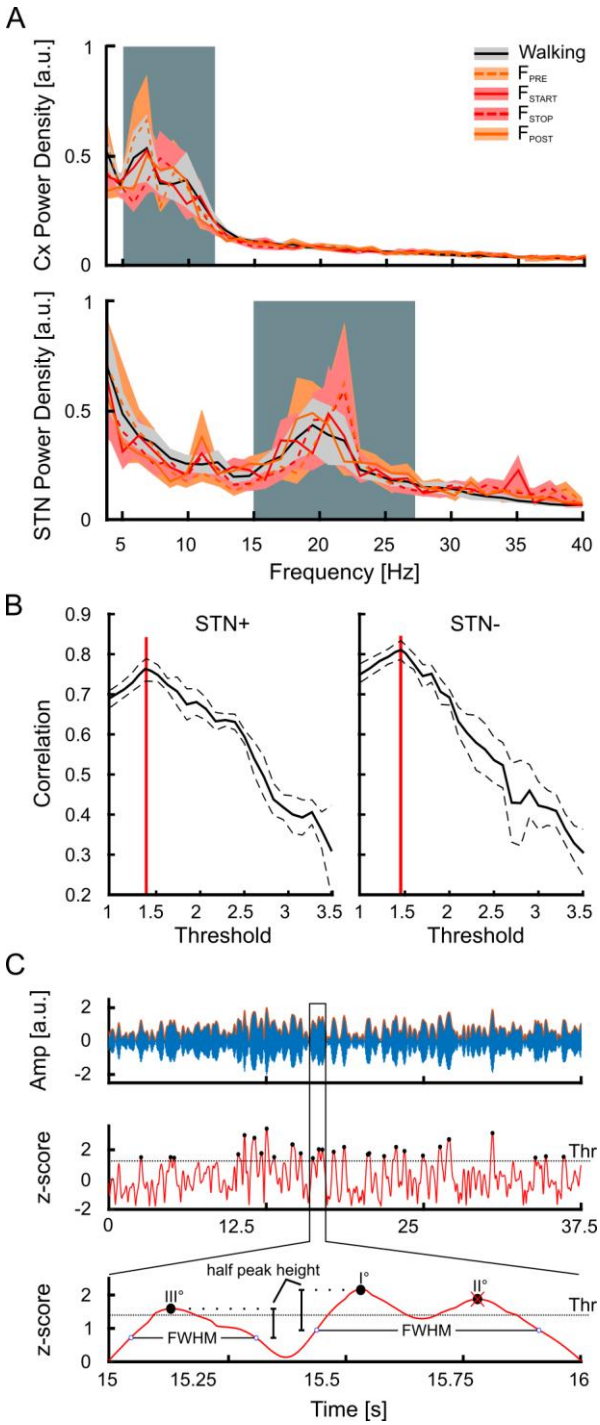

Figure 24 – Power spectral densities of cortical and subthalamic local field potentials and β-burst identification.
A. Cortical and STN power spectral densities. The cortical local field potentials in the selected ROIs displayed a bimodal distribution with two distinct activity peaks in the θ- and α-frequency bands. The STN power spectra also showed a bimodal distribution with a small peak at 11 Hz and a prominent peak in the β-frequency band. The background color indicates the frequency selected for further analysis.
B. β-burst identification. Pearson's correlation coefficient between average β-amplitude and number of β-peaks above the threshold computed in all 2-s walking epochs is reported for the two STN separately (STN+ and STN-). Black lines are the average of the curves for each subject. Dashed lines represent the standard error computed with the bootstrap technique. Red lines identify the values used as threshold for β-burst detection.
C. Top. A segment of the wavelet real part (blue line), superimposed with the wavelet amplitude (red line) derived from the wavelet transformed local field potentials in the beta peak frequency (20 Hz) for a single subject. Middle. The wavelet amplitude was z-scored subtracting and dividing, respectively, by the mean and the standard deviation of the wavelet amplitude across all epochs and conditions. When the z-score was above the cutoff threshold (black dotted line, computed as in B), we identified the location of the β-burst peak (black dots). We identified all peaks and sorted them according to their amplitude. Once sorted, starting from the higher peak we identified the burst duration with the Full Width Half Maximum (FWHM) method. Bottom. A close view of the identification of the burst duration. Starting from the higher peak (peak I°),

we found the closest points (blue circles) in which the z-scored wavelet amplitude went below the peak half amplitude. The time difference between these two points determined the burst duration. Since the location of peak II° lay inside the burst duration of peak I°, we eliminated it, considering these two peaks as belonging to a single burst. We replicated this procedure for all the smaller peaks, for example peak III°.
FPRE, pre-freezing; FSTART, freezing start; FSTOP, freezing stop; ROIs, regions of interest; STN, subthalamic nucleus (STN+ or STN- refers to the side with more and less striatal dopaminergic innervation respectively).

(iii) To study the (a) cortical-subthalamic and (b) inter-hemispheric subthalamic coupling, we computed the cross power spectral density (CPSD) between the LFP signals of the cortical areas and the STN, as well as between the two STN.[202,305] For the computation of the CPSD, we adopted the same normalization procedure used for the PSD estimation, separately for all pairs of channels:

$$\mathrm{CPSD(f)_{ij}} = \frac{\mathrm{CPSD(f)_{ij}}}{\sqrt{\int_4^{80} \mathrm{PSD(f)_i df} \int_4^{80} \mathrm{PSD(f)_j df}}}$$

(a) To assess for differences in the cortico-subthalamic coupling across the five conditions, we integrated the CPSD in the specific θ-α band, obtaining an index ($\theta\alpha CC^{k}_{CX/STN}$) to describe the cross coupling between each cortical ROI (CX = {SMA+/– , M1+/–, PA+/– }) and its own ipsilateral STN for all epochs k = {Walking, FPRE, FSTART, FSTOP, FPOST}.

(b) To assess the differences in the subcortical inter-hemispheric coupling in the five conditions instead, we integrated the CPSD in the specific β-band (13–35 Hz), obtaining an index ($\beta CC^{k}_{STN-/STN+}$,) to describe the coupling between the two STN for all epochs k = {Walking FPRE, FSTART, FSTOP, FPOST}.

We then normalized the θ-α and the β indexes for each patient, computing the percentage relative change with respect to the Walking epochs:

(a) $$\widehat{\theta\alpha CC^{k}_{CX/STN}} = \frac{\theta\alpha CC^{k}_{CX/STN} - \overline{\theta\alpha CC^{Walking}_{CX/STN}}}{\overline{\theta\alpha CC^{Walking}_{CX/STN}}} * 100$$

(b) $$\widehat{\beta CC^{k}_{STN-/STN+}} = \frac{\beta CC^{k}_{STN-/STN+} - \overline{\beta CC^{Walking}_{STN-/STN+}}}{\overline{\beta CC^{Walking}_{STN-/STN+}}} * 100$$

where $\overline{\theta\alpha CC^{Walking}_{CX/STN}}$ and $\overline{\beta CC^{Walking}_{STN-/STN+}}$ are the averages across the "Walking" epochs, respectively, for the θ-α and the β cross-coupling indexes of each subject.
Thanks to the normalization procedure, we could compare (effective) "Walking" epochs against all freezing gait epochs (i.e. FPRE, FSTART, FSTOP and FPOST), pooling the normalized indexes for all epochs across all patients. With the same procedure, we also were able to compare the epochs of FOG (i.e. FSTART and FSTOP) against epochs of successful passing through a door ("Door").

Statistical significance was measured by means of Wilcoxon rank sum test; all results were corrected for multiple comparisons by means of Bonferroni's correction. The significance level was set at $p<0.05$.

3.2.4 Results

Clinical, molecular imaging and biomechanical data

Table 1 shows the detailed demographic and clinical data. All subjects showed sustained improvement after DBS (average ± SD, 69.60 ± 9.93%) that mirrored the benchmark response from levodopa (73.85 ± 14.85%), thus supporting the correct placement of the electrodes.

With respect to molecular imaging findings, all patients showed a significant bilateral reduction of striatal DAT binding in comparison with healthy controls. For all patients there was an imbalanced DAT loss, with one hemisphere more dopamine-depleted than the opposite one (average ± SD: H- 67 ± 11%; H+ 58 ± 12%). This was evident in the average AI of the striatum, which was 27 ± 16. In all patients, the molecular imaging findings were in agreement with the clinical evaluations, always showing H- contralateral to the clinically most impaired body side. The individual values of striatal DAT binding have previously been reported for all patients[149] (apart from patient nwk01) (left caudate: 1.31; left putamen: 0.95; right caudate: 1.17; right putamen: 0.72).

Table 2 shows the biomechanical findings. In particular, PD patients showed reduced stride length and maximal velocity with respect to healthy controls. The combination of clinical and biomechanical observation defined 14 14 freezing episodes for a total time of 127 s.

Table 2 – Anthropometric and kinematic measurements

Stance and double-support duration are expressed as the percentage of the duration of the stride (i.e. the interval between two subsequent heel strikes of the same foot). The stride length and the stride velocity were calculated as a percentage of the body height of each subject (%BH). Data are shown as mean ± standard deviation; the asterisk indicates statistical significance ($p<0.05$).

ASIS, anterior-superior iliac spines; BH, body height; BMI, body mass index; PD, Parkinson's disease.

	PD patient	Healthy control
Body height (cm)	176.04 ± 7.90	174.24 ± 6.47
Inter ASIS distance (mm)	255.28 ± 33.18	290.07 ± 34.74
Foot length (mm)	254.07 ± 11.79	254.11 ± 15.22
Limb length (mm)	918.34 ±3 6.75	900.43 ± 29.62
Weight (kg)	85.80 ± 13.44	76.54 ± 10.74
BMI (Kg/m2)	27.74 ± 4.25	25.22 ± 3.58
Stride duration (s)	1.16 ± 0.05	1.13 ± 0.09
Stance duration (%stride)	64.60 ± 4.71	62.31 ± 1.62
Double support duration (%stride)	28.94 ± 9.54	24.58 ± 3.32
Stride length (%BH) *	56.81 ± 19.92	72.00 ± 6.41
Stride average velocity (%BH/s)	49.36 ± 18.10	64.17 ± 9.37
Stride max velocity (%BH/s) *	157.91 ± 44.63	199.63 ± 21.44

Subthalamic oscillatory activity and connectivity during gait freezing

The comparison of STN β-oscillations between (effective) walking and ongoing freezing episodes in term of β-power, duration of β-burst and subthalamic interhemispheric coupling did not yielded any significant difference (Figure 25A, B and C respectively).

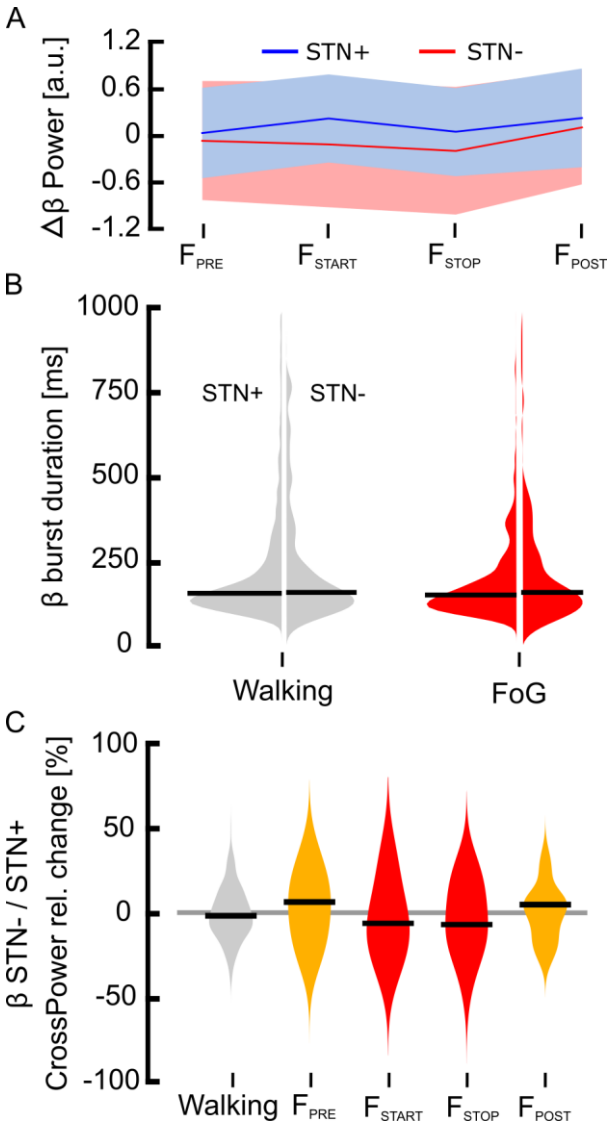

Figure 25 – Subthalamic oscillatory activity and connectivity during walking and gait freezing.

A. STN β-power. No differences were observed for the relative change of STN β power during gait freezing with respect to (effective) walking (i.e. zero) for the two STN and between them (STN+ vs. STN-).

B. β-burst duration. No difference was found in the distribution of the β-burst duration of (effective) walking vs. gait freezing for both STN and between them (STN+ vs. STN-).

C. Inter-hemispheric STN coupling. Despite a reduction in the coupling between the two STN during gait freezing vs. (effective) walking, this difference did not reach statistical significance.

FPRE, pre-freezing; FSTART, freezing start; FSTOP, freezing stop; FPOST, post-freezing; STN, subthalamic nucleus (STN+ and STN- refers to the side with more and less striatal dopaminergic innervation respectively).

Cortical-subthalamic network during gait freezing

To assess the cortical-subcortical network communication in gait freezing, I compared the cortical-subthalamic coupling between (effective) walking and ongoing freezing episodes. This comparison showed that in subjects with PD during (effective) walking, the cortex and the STN were stably coupled in a low-frequency band (i.e. θ-α rhythms). Gait freezing was instead characterized by a θ-α cortical-subthalamic decoupling in the hemisphere with less striatal dopaminergic innervation (H-, Figure 23C). Of relevance, this desynchronization started at the transition from (effective) walking into gait freezing (i.e. pre-freezing [FPRE]), lasted the entire freezing episode (from freezing start [FSTART] to freezing stop [FSTOP]), and resolved with recovery of the effective walking pattern (freezing end [FEND]) (Figure 23C). This modulation pattern was present in every patient.

To assess that θ-α cortical-subthalamic decoupling selectively signals gait freezing, I compared epochs of gait freezing with epochs of successful passing through a door and I found that the θ-α cortical-subthalamic decoupling

was specific for gait freezing as successful passing through a door was not associated with a cortical-subthalamic desynchronization (Figure 23D).

Finally, I assessed whether these neural network dynamics were specific for subjects with FOG. The θ-α cortical-subthalamic coupling was computed during walking (unperturbed and though a door) in two subjects not suffering from FOG (i.e. wue06 and wue07). These subjects mirrored the network dynamics (i.e. stable θ-α cortical-subthalamic coupling) of patients suffering from FOG during (effective) walking and successful passing through a door (Figure 23E).

3.2.5 Interpretations and limitations

The results of this study suggest that neural networks dynamics support locomotion control in subjects with PD and derangements of this synchronization is associated with FOG.

At present, supraspinal locomotor control in human requires the SMA and the basal ganglia for computation of *feedforward* locomotor commands that are integrated in the MLR with cerebellar *feedback* signals to descend to the medullary and pontine reticular formations and the spinal cord.[55] Dynamic synchronization across the cortex and the basal ganglia can play a relevant role for the adaptation of gait to environmental challenges as it can impact the computation ad convey of novel locomotor information to brainstem and spinal centers. [116,318]

Results from an animal study in rats support this hypothesis [319]. Von Nicolai and coll. investigated cortico-subcortical neural processing during gait (running) by performing synchronous multisite brain recording in the cortex and in the striatum of heathy animals. They reported that cortico-striatal θ-phase coupling correlates with running speed, thus suggesting a specific role for cortical-basal ganglia communication during gait.[319]
In line with this study, a distinct cortical-subcortical low-frequency (i.e. θ- and α-band) coupling during (effective) walking was found in both parkinsonian patients who suffer or do not suffer from FOG (Figure 23C and E). The disruption of this coupling anticipated and characterized the freezing episodes. Moreover, it was recovered when the effective walking pattern was regained (Figure 23C).
This cortical-subthalamic decoupling was limited to the hemisphere with greater dopaminergic denervation in a context of unbalanced dopaminergic loss between the two hemispheres, however no subthalamic activity changes were identified during gait freezing (Figure 24), thus suggesting that the dysfunctional dynamics responsible for FOG development may originate in the cortex.
A possible cause is a defective engagement of the SMA during gait. This dysfunction has been consistently described in PD patients [201,320,321], in which it also couples with basal ganglia pathology.[154,163,297] The dopaminergic loss affecting the putamen of PD patients has been associated to deficits in motor automaticity, which forces the subjects to allocate attentional resources to perform learned motor skills [301]. In this context, a poor engagement of the SMA during gait could impair the production of novel *feedforward* motor programs, such as APAs,[322] challenging a timely adaptation of gait to environmental requests (obstacles, distractions, etc.) and leading to gait freezing.[323]
In line with this interpretation, recent preliminary findings in subjects with PD and FOG investigated with functional magnetic resonance imaging and virtual reality showed that doorway-provoked gait freezing was associated with both selective hypoactivation of the (pre)SMA and cortical-subthalamic decoupling. [324]

It must be also acknowledged that in PD the deficient cortical-subcortical coupling likely combines with exaggerated STN β-oscillations that may support an excessive inhibition of the MLR via indirect activation of pallidal and substantia nigra GABAergic projections.[55,297] Indeed, despite a lack of subthalamic activity changes during gait freezing, the documented exaggerated STN β-oscillations can still reducing the dynamicity of motor processing.[300]

Few other studies have directly assessed the brain functional activity during ongoing freezing episodes, but limited the recordings to one brain region (i.e. cortex or STN).[151,202,300] In the seminal work by Shine and coll., cortical LFPs were recorded by means of a four-channel wireless EEG system in 24 subjects with PD and showed an increase in θ-activity over frontal midline electrodes during freezing episodes.[202] More recently, Anidi and coll. recorded STN LFPs in seven subjects with PD and STN-DBS and reported an increased duration of STN β-burst during gait freezing.[300] The discrepancies with the findings presented here likely relate to the different settings of the studies. In my study, FOG impaired forward walking, whereas in Shine and coll. it impaired turning[202] and Anidi and coll. studied a "freezing behavior" during repetitive stepping while seated.[300] Differences may also arise in response to the different environmental contingencies.[198] Furthermore, it is unclear whether different clinical presentations of gait freezing (e.g. trembling in place, shuffling forward, etc.) share a common electrophysiological substrate.[198]

This study has several limitations. First, the sample size was small, although similar to the one of other studies on this topic [300]. Due to the advanced stage of the disease, only six of nine patients who received the "Activa PC+S®" system could be included. Likewise only one of the six patients with the "AlphaDBS" system successfully completed the task. Notably, the "AlphaDBS" device could only be used at battery replacement, thus limiting the recruitment to severely impaired patients. Still, the replication of the findings in all patients and the consistency of the recordings with two different devices reinforce their validity. Second, the sporadic occurrence of FOG resulted in the recording of few episodes. The limited time prevented the computation of advanced neural metrics (e.g. effective connectivity measures). Hence, the analyses was limited to robust and well-established functional connectivity metrics that have been applied in other studies on FOG.[202,300] Moreover, the combined visual and kinematic detection of freezing episodes increased the temporal resolution. Third, the interpretation of the findings must be cautious. The lack of co-registration of EEG electrodes with individual MRIs prevented the identification of the source of the signals, which may not solely reflect the activity of specific cortical areas (e.g. the SMA).

In conclusion, this study provides direct evidence of locomotor network dynamics during effective gait and shows how derangements of this dynamic connectivity relate to gait freezing in PD. These findings support the interpretation of FOG as a "circuitopathy" that reflects dysfunctional dynamic diaschisis mechanisms. It follows that freezing of gait can be treated by different therapeutic strategies (e.g. neuromodulation techniques[325]) that target these network functional derangements rather than supply the neurodegenerated areas.

IV. Discussion and future directions

4.1 Clinical translation: retuning the networked dysfunction of parkinsonism with neuromodulation

My experiments suggest that some PD symptoms (i.e. resting tremor and gait freezing) directly reflect functional derangements of an integrated neural network. These results provide evidence for revising the pathophysiology of PD that can no more be reduced to neurodegeneration, but should encompass dysfunctional rearrangements of neuronal networks (distinctively for each sign of PD), thus recognizing PD as a circuitopathy.

Circuitopathy is an unspecific definition that does not focus on the biological alteration, but rather stresses the relevance of the dysfunctional consequences of biological damage. The etiologies of circuit disturbances can vary and may include damage to neural pathways, loss of neural elements and populations[326]. However, this also introduces the existence of disturbances in the functional activity of neural circuits, through disordered firing and pathological oscillatory activity in neural ensembles. Of note, unlike biological damage, these functional disturbances can be temporary, intermittent or paroxysmal. Moreover, they can affect multiple spatially and temporally distributed cerebral areas, thus producing a great variety of symptom combinations, which is exactly the phenomenology of PD.

Recognizing PD as a circuitopathy may seem disadvantageous at first as it implies increasing the complexity of PD pathophysiology, adding more questions to an already largely unclear neurological disorder. However, this also represents a resource to improve both our understanding of this disease and the care of our patients.
Recognizing the relevance of specific dysfunctional neural network dynamics can offer the remarkable opportunity to directly target these functional derangements for clinical benefit. Of note, this strategy is not dopamine-dependent, thus avoiding dopamine-related complications and potentially improving non-dopaminergic symptoms. Retuning neural dysfunction is unfortunately not straightforward, but still possible by means of neuromodulation techniques.

Neuromodulation allows modifying neural activity without harming the neurons by influencing the functional results of neurotransmission, namely the membrane and action potentials. The principles and the many techniques for a neuromodulatory therapeutic approach have been described elsewhere[327–329] and are beyond the scope of this thesis. These techniques can be divided into two main categories: non-invasive and invasive.

Among non-invasive techniques the most commonly used are transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS). Although part of the same group, they present distinctive mechanisms of action that go from the tDCS-induced modulation of the ion currents and the neural membrane potential (through the application of very low current to the skull), to the TMS-generated electrical potentials that depolarize neurons and thereby trigger action potentials. These two neuromodulation techniques are of particular interest as they are non-invasive and allow direct targeting (with multiple and variegated stimulation settings) of the cortex, which is the final effector node of several circuitry derangements.

During my career, I have explored the possibility of using tDCS for the treatment of FOG (see 7.3 “Printouts”) [325]. The hypothesis was that anodal tDCS could improve FOG by increasing cortical excitability and sustaining cortico-subcortical interactions at gait planning. In a cross-over, double-blind, sham-controlled study, I investigated the effect of tDCS in 10 PD patients with medication refractory FOG [325]. Every patient underwent five consecutive days of anodal or sham tDCS, with an interval of one month between the two treatment (active or sham), during which the clinical efficacy of the treatment was evaluated [325]. Only the active tDCS treatment showed a significant improvement in the number and duration of FOG episodes [325]. A possible mechanism for this positive effect is represented by a plastic enhancement of cortical excitability, which may sustain the locomotor network activity and favour the cortical production and execution of anticipatory postural adjustments.

Similar studies have also been attempted with TMS, but the relatively poor control of the stimulation site (in the absence of a neuro-navigation system) combined with the localized nature of the stimulus have led to some inconsistencies [325]. I took advantage of the introduction of new brain navigation systems that integrate TMS with simultaneous EEG recordings, thus allowing direct monitoring of the functional effects of TMS pulses (see 7.3 "Printouts"). I had the opportunity to participate in the very first study applying this technique to study motor cortical areas other than M1 in PD. This first study aimed at characterizing cortical excitability changes related to levodopa (still the most potent pharmacological treatment available for PD) in patients with and without adverse events (i.e. dyskinesias). We studied 13 PD patients without dyskinesia and 10 with dyskinesia, before and after receiving fast-acting levodopa. The electrophysiological recordings of the SMA ipsilateral to the most dopamine-depleted striatum (measured with FP-CIT and SPECT) showed a positive correlation between cortical excitability changes and the clinical efficacy of levodopa. Dyskinetic patients showed an excessive and more widespread cortical excitability increase when levodopa-induced dyskinesias were present.

Despite the many encouraging results suggesting a possible role for non-invasive neuromodulation techniques in the treatment of PD and the optimal safety profile, it must be stressed that the clinical effect of tDCS and TMS remains variable and time-limited. The lack of standardized approaches and randomized clinical trials further challenges the interpretation of the results of the use of these techniques in routine clinical practice. These methods are therefore still limited to research alone.

This is not the case for deep brain stimulation (DBS) which (unlike other invasive neuromodulation techniques, such as extradural motor cortex stimulation[330]) do represent a therapeutically valuable option for PD treatment. DBS is an invasive neuromodulation technique that modulates neural activity by delivering controlled electrical inputs onto specific brain targets, which are implanted with an electrode carrying several (active) contacts (Figure 26)[331,332]. Data from a collective review of meta-analysis and multicentre controlled studies reported an improvement of 50% and 52% of the activities of daily living and motor symptoms after STN-DBS (meds-off vs. stim-on/meds-off state) [303,304]. In detail, the improvements were shown to be ≈75% for rigidity and tremor and ≈50% for bradykinesia and were associated to an amelioration of patients quality of life [303,304]. These positive effects are associated with an amelioration of motor fluctuations and allow the reduction of the dopaminergic medication by about ≈50%, which reduces the severity of levodopa-induced dyskinesia [303,304].

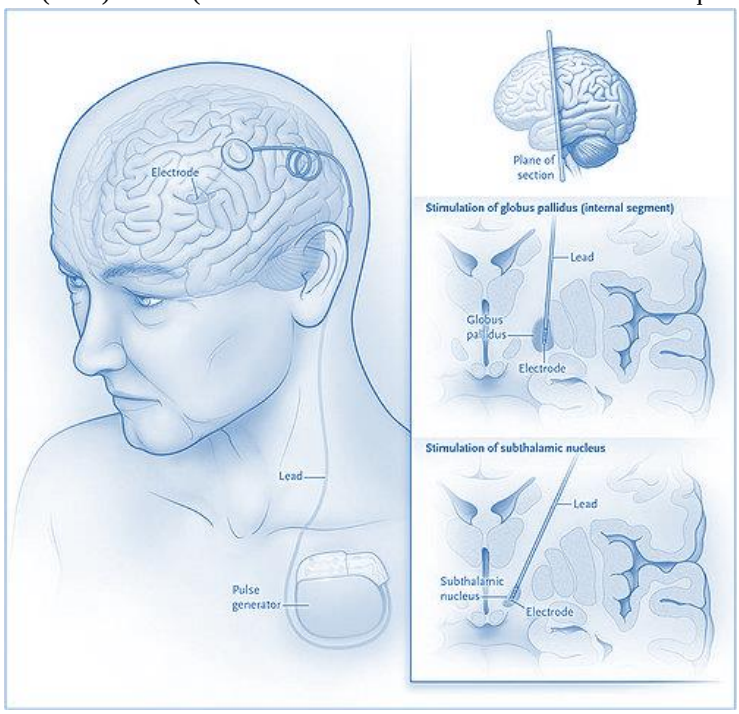

Figure 26 – Deep brain stimulation hardware depiction (modified from Okun et al. [332]).
The main components of DBS devices and the two most frequently implanted targets (i.e. STN and GPi) for PD are reported

These clinical benefits are strongly related to the delivery of the stimulation in precise brain locations which in the case of PD are: the Vim, the GPi and the STN [304]. The therapeutic target is actually smaller than the surgical one and consists of the neurons and axons that constitute the sensorimotor functional domain of the targeted nucleus. The stimulation outside of this domain can lead to the development of cognitive and behavioural side effects, which can compromise the motor improvement. As a preliminary investigation to the studies on STN activity and connectivity during gait as well as on the neural mechanism of gait freezing, I have explored the STN functional domains and described a distinctive neural signaling in the sensorimotor STN. These neurophysiological advancements, together with the evolution of the surgical procedure and of the neuroimaging techniques have

improved the targeting of the nuclei and allowed a better placement of the stimulation electrodes. Moreover, recent modifications of the power sources and of the stimulating electrodes themselves have allowed a better steering[333] and shaping[334–336] of the stimulation around the electrodes, thus improving the spatial-delivery of the stimulation onto the sensorimotor functional domain of the targeted nucleus.

It is now also becoming increasingly clear that the DBS effects go beyond the targeted nucleus, influencing the related neural networks by modifying neural plasticity [337]. Although still debated, several studies show that DBS disrupts pathological neuronal synchronization (i.e. β-oscillations and β-band phase amplitudes coupling in M1 [131,154,167,338,339 340,341]) that limits the communication within the cortico-striato-thalamic circuit by delivering chaotic desynchronizing inputs. In line with this, recent studies with MEG, fMRI or DTI showed both functional and structural connectivity changes after DBS [337], thus providing evidence that DBS is able to probe and regulate neural circuits. As such, DBS holds the possibility of targeting the pathophysiological networked alterations described in this thesis, thus possibly allowing a personalized treatment shaped on individual physio-markers.

Novel DBS algorithms that change the stimulation delivery over time according to a (pathological) signal would be useful for managing some of the troublesome side effects that still affect precisely placed and clinically effective DBS, above all the impairments in executive functioning [93] and in locomotion adaptation [342]. The origin of these troublesome DBS side effects is, indeed, likely related to the constant desynchronizing effect of DBS, which by disrupting the pathological PD over-synchronization also constrains physiological communication dynamics [340]. The impairment of dynamically synchronized hubs of a functional network (e.g. the SMA and the STN in the case of locomotor adaptation) can shatter the ability of adapting (motor) behaviours [343]. These side effects could be prevented by stimulation that adapts its current delivery to the presence of distinctive pathological activities, thus stimulating only when necessary. This technology is already on the way and it is known as adaptive DBS.

4.1.1 Adaptive deep brain stimulation

To optimize DBS benefit and prevent its interference with physiological synchronization processes that involve the network targeted with DBS, the stimulation delivery must be adjusted not only in space (i.e. precise target and stimulation shape) but also in the time domain.
In my study investigating neural mechanism of gait and gait freezing, I took advantage of novel DBS devices (Activa PC+s Medtronic, PLC and "AlphaDBS", Newronika srl) that allow the simultaneous recording and stimulation of the targeted brain area. Taking this technology a step forward, we could use it to directly regulate the delivery of the stimulation based on neural network derangements. Hence, new DBS devices will not be constantly active, but react to functional network derangements, thus possibly improving PD symptoms while sparing physiological processes.

Recent studies using different approaches have already proven the technical feasibility of feedback-controlled stimulation and reported promising clinical results. Two main algorithms have been tested for feedback-controlled stimulation: (i) close-loop DBS, which activates/stops the stimulation according to a sensed signal [344], and (ii) adaptive DBS, which modulates instant-per-instant one or more stimulation parameters (e.g. amplitude) according to the neural activity recorded [306,345].
The first application of feedback-controlled stimulation was performed by Rosin and colleagues in nonhuman primates, in which DBS was adjusted to neural spikes recorded in the ipsilateral M1 and was able to reduce the bradykinesia induced by MPTP pre-treatment[346]. Of relevance, Swann and colleagues recently put forward a similar approach in humans, adjusting DBS to cortical LFPs (γ-activity) as measured with EcoGA and replicating its feasibility and safety [347].
Little and colleagues, instead, proposed a 'closed-loop' algorithm by adjusting DBS to STN β- power in PD patients [344]. In particular, the individual STN β-oscillations were used to define a threshold and then filtered and tracked online for activating DBS. Despite the limited time and testing conditions, this approach topped conventional DBS benefit of 27% and with 56% less energy delivered [344]. Of note, this algorithm DBS followed β-power fluctuations and was reduced by dopaminergic treatments, thus potentially improving also levodopa-induced dyskinesia.
Similar results were reported by Rosa and coll. and Arlotti and coll. who tested 13 freely-moving PD patients with a portable adaptive DBS device that constantly modulated the amplitude of stimulation to STN β- power [306,345]. These studies also saw a reduction in dyskinesias through the use of adaptive DBS as compared to conventional DBS.

Taken together these findings suggest that closed-loop and adaptive DBS are technically possible, well tolerated and safe [333]. Still, to bring this technology to routine clinical use two steps still need to be made. First, we need to identify a reliable and consistent brain biomarker that can be used as a feedback signal. Secondly, we need new devices with better computational capacities at low battery costs.

Up to now, clinical studies have mostly relied on β-power changes to modulate the current delivery. Unfortunately, increased β-oscillations are not consistently found in all PD patients and their correlation to symptom severity is limited [134]. Moreover, as I have shown in this thesis, the neural derangements of some PD-related symptoms, such as FOG, are not detectable by only recording a single brain area (the STN) but at a network level (cortical-subthalamic decoupling). Last but not least, past studies were performed under highly controlled conditions, largely neglecting neural network changes during complex movements, such as freely moving patients walking in a everyday life environment. Future applications of feedback-controlled DBS should be based on more reliable signals.

With regards to the need for prolonged recordings, I took part in a very recent study that extended the brain recording time to a whole day (24 hours) and beyond. The device, a new "AlphaDBS" system, allowed STN LFPs recoding in the range 5-35 Hz continuously (see 7.3 "Printouts"). The most striking finding was that β-power changes could not distinguish wakefulness and sleep states that were characterized instead by a modulation of the inter-hemispheric coupling of the two STN. This further suggests that network signals can better represent brain activity changes and be a more accurate marker for feedback-controlled DBS.

In this context, the findings of my experiments on FOG are pivotal for new generation DBS devices. Indeed, I was able to describe a symptom (FOG)-specific biomarker (i.e. cortical-subthalamic decoupling) of a network derangement that could be used for network-signal controlled DBS.

New challenges await, in particular the recognition of multiple network signals, each of them specific for a distinctive motor (and non-motor) neurological sign of PD. This will require highly sensitive equipment with the ability to perform more complex and demanding computational analysis, but both these technical issues will likely be resolved thanks to the application of new and already available technologies, such as multi-segmented electrodes and more powerful processors.

The potential benefits of performing a network- instead of local-feedback DBS are many, the most important being the chance of topping conventional DBS. The goal of close-loop/adaptive DBS is to extend current clinical effects [333] and to avoid DBS-induced chronic side effects. The former applies for example to gait disturbances (including FOG), to speech problems and potentially non-motor symptoms. With regards to the possibility to avoid chronic side effects, we have recently described the appearance of DBS-induced gait derangements in patients with essential tremor months after implantation [336]. Also in PD, tremor control by high frequencies (usually >180 Hz) and DBS can be associated with discomfort and subtle impairment of gait control [348]. A preliminary attempt of a closed-loop DBS was performed adapting the stimulation delivery to EMG or peripheral limb acceleration signals [349]. This setup allowed a more effective suppression of tremor as compared with conventional DBS [349]. However, when targeting the phase relationship between the neuronal tremor-related oscillatory activity and the DBS pulses, only a minor effect on tremor amplitude could be reached (despite neural entrainment), thus suggesting a network origin for tremor [349] and the necessity of 'network' signals to control DBS.

In conclusion, adaptive DBS holds the promise of retuning specific dysfunctional neural network dynamics for PD. The studies performed to date have significant limitations and more solid evidence is needed to support the use of this technology in clinical practice. The experiments described in my thesis define what, in my opinion, is the framework for future studies to reliably bring this technology to the clinic. This will lead to great scientific discoveries on the functioning of the basal ganglia and on the true pathophysiological derangements of PD-related signs and more importantly to more efficacious and accessible treatments for our patients.

V. Bibliography

1. Marsden, C. D. The mysterious motor function of the basal ganglia: the Robert Wartenberg Lecture. *Neurology* **32,** 514–39 (1982).
2. Wolpert, D. M., Ghahramani, Z. & Jordan, M. I. An internal model for sensorimotor integration. *Science* **269,** 1880–2 (1995).
3. Gallivan, J. P., Chapman, C. S., Wolpert, D. M. & Flanagan, J. R. Decision-making in sensorimotor control. *Nat. Rev. Neurosci.* **19,** 519–534 (2018).
4. Grillner, S. Biological Pattern Generation: The Cellular and Computational Logic of Networks in Motion. *Neuron* **52,** 751–766 (2006).
5. Burn, D. *Oxford textbook of movement disorders*. *Oxford University Press* (2013).
6. Nonnekes, J. *et al*. Unmasking levodopa resistance in Parkinson's disease. *Mov. Disord.* (2016). doi:10.1002/mds.26712
7. Elias, W. J. & Shah, B. B. Tremor. *JAMA* **311,** 948 (2014).
8. Nutt, J. G. *et al.* Freezing of gait: Moving forward on a mysterious clinical phenomenon. *Lancet Neurol.* **10,** 734–744 (2011).
9. Isaias, I. U. A role for locus coeruleus in Parkinson tremor. (Univesrsità degli studi di Milano, 2012).
10. Price, C. J., Warburton, E. A., Moore, C. J., Frackowiak, R. S. & Friston, K. J. Dynamic diaschisis: anatomically remote and context-sensitive human brain lesions. *J. Cogn. Neurosci.* **13,** 419–29 (2001).
11. de Lau, L. M. & Breteler, M. M. Epidemiology of Parkinson's disease. *Lancet Neurol.* **5,** 525–535 (2006).
12. Ascherio, A. & Schwarzschild, M. A. The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* **15,** 1257–1272 (2016).
13. Quinn, N., Critchley, P. & Marsden, C. D. Young onset Parkinson's disease. *Mov. Disord.* **2,** 73–91 (1987).
14. Schrag, A., Ben-Shlomo, Y., Brown, R., David Marsden, C. & Quinn, N. Young-onset Parkinson's disease revisited—clinical features, natural history, and mortality. *Mov. Disord.* **13,** 885–894 (1998).
15. Jankovic, J. & Kapadia, A. S. Functional Decline in Parkinson Disease. *Arch. Neurol.* **58,** 1611 (2001).
16. Domingo, A. Genetics of Parkinson disease. *Handb. Clin. Neurol.* **147,** 211–227 (2018).
17. Abeliovich, A. & Gitler, A. D. Defects in trafficking bridge Parkinson's disease pathology and genetics. *Nature* **539,** 207–216 (2016).
18. Panzacchi, A. *et al.* A voxel-based PET study of dopamine transporters in Parkinson's disease: Relevance of age at onset. *Neurobiol. Dis.* **31,** 102–109 (2008).
19. Kalia, L. V. & Lang, A. E. Parkinson's disease. *Lancet* **386,** 896–912 (2015).
20. Lees, A. J., Hardy, J. & Revesz, T. Parkinson's disease. *Lancet* **373,** 2055–2066 (2009).
21. Klingelhoefer, L. & Reichmann, H. Pathogenesis of Parkinson disease—the gut–brain axis and environmental factors. *Nat. Rev. Neurol.* **11,** 625–636 (2015).
22. Holmqvist, S. *et al.* Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* **128,** 805–820 (2014).
23. Doppler, K. *et al.* Cutaneous neuropathy in Parkinson's disease: a window into brain pathology. *Acta Neuropathol.* **128,** 99–109 (2014).
24. Braak, H. *et al.* Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* **24,** 197–211
25. Jellinger, K. A. Is Braak staging valid for all types of Parkinson's disease? *J. Neural Transm.* 1–9 (2018). doi:10.1007/s00702-018-1898-9
26. Sauerbier, A., Jenner, P., Todorova, A. & Chaudhuri, K. R. Non motor subtypes and Parkinson's disease. *Parkinsonism Relat. Disord.* **22,** S41–S46 (2016).
27. Svensson, E. *et al*. Vagotomy and subsequent risk of Parkinson's disease. *Ann. Neurol.* **78,** 522–529 (2015).
28. Jankovic, J. Parkinson's disease: clinical features and diagnosis. *J. Neurol. Neurosurg. Psychiatry* **79,** 368–376 (2008).
29. Postuma, R. B. *et al.* MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* **30,** 1591–1601 (2015).
30. Fereshtehnejad, S.-M. & Postuma, R. B. Subtypes of Parkinson's Disease: What Do They Tell Us About Disease Progression? *Curr. Neurol. Neurosci. Rep.* **17,** 34 (2017).
31. Chen, W., Hopfner, F., Becktepe, J. S. & Deuschl, G. Rest tremor revisited: Parkinson's disease and other disorders. *Transl. Neurodegener.* **6,** (2017).
32. Bhatia, K. P. *et al*. Consensus Statement on the classification of tremors. from the task force on tremor of the International Parkinson and Movement Disorder Society. *Mov. Disord.* **33,** 75–87 (2018).
33. Deuschl, G., Bain, P. & Brin, M. Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. *Mov. Disord.* **13 Suppl 3,** 2–23 (1998).
34. Jankovic, J., Schwartz, K. S. & Ondo, W. Re-emergent tremor of Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **67,** 646–50 (1999).
35. Pasquini, J. *et al.* Progression of tremor in early stages of Parkinson's disease: a clinical and neuroimaging study. *Brain* **141,** 811–821 (2018).
36. Ceravolo, R. *et al.* Predictive value of nigrostriatal dysfunction in isolated tremor: a clinical and SPECT study. *Mov. Disord.* **23,** 2049–54 (2008).
37. Rahman, S., Griffin, H. J., Quinn, N. P. & Jahanshahi, M. Quality of life in Parkinson's disease: The relative importance of the symptoms. *Mov. Disord.* **23,** 1428–1434 (2008).
38. Maffoni, M., Giardini, A., Pierobon, A., Ferrazzoli, D. & Frazzitta, G. Stigma Experienced by Parkinson's Disease Patients: A Descriptive Review of Qualitative Studies. *Parkinsons. Dis.* **2017,** 1–7 (2017).
39. Fox, S. H. *et al.* International Parkinson and movement disorder society evidence-based medicine review: Update on treatments for the motor symptoms of Parkinson's disease. *Mov. Disord.* (2018). doi:10.1002/mds.27372
40. Aquino, C. C. & Fox, S. H. Clinical spectrum of levodopa-induced complications. *Mov. Disord.* **30,** 80–89 (2015).
41. Coelho, M. & Ferreira, J. J. Late-stage Parkinson disease. *Nat. Rev. Neurol.* **8,** 435–442 (2012).
42. Nonnekes, J. *et al*. Freezing of gait: a practical approach to management. *Lancet Neurol.* **14,** 768–778 (2015).
43. Espay, A. J. *et al.* "On" state freezing of gait in Parkinson disease: A paradoxical levodopa-induced complication. *Neurology* **78,** 454–457 (2012).
44. Contreras, A. & Grandas, F. Risk factors for freezing of gait in Parkinson's disease. *J. Neurol. Sci.* **320,** 66–71 (2012).
45. Perez-Lloret, S. *et al.* Prevalence, Determinants, and Effect on Quality of Life of Freezing of Gait in Parkinson Disease. *JAMA Neurol.* **71,** 884 (2014).
46. Schaafsma, J. D. *et al.* Characterization of freezing of gait subtypes and the response of each to levodopa in Parkinson's disease. *Eur. J. Neurol.* **10,** 391–398 (2003).
47. Okuma, Y. & Yanagisawa, N. The clinical spectrum of freezing of gait in Parkinson's disease. *Mov. Disord.* **23,** S426–S430 (2008).

48. Ehgoetz Martens, K. A. *et al.* The functional network signature of heterogeneity in freezing of gait. *Brain* **141,** 1145–1160 (2018).
49. Ehgoetz Martens, K. A., Ellard, C. G. & Almeida, Q. J. Does Anxiety Cause Freezing of Gait in Parkinson's Disease? *PLoS One* **9,** e106561 (2014).
50. Curtze, C., Nutt, J. G., Carlson-Kuhta, P., Mancini, M. & Horak, F. B. Levodopa Is a Double-Edged Sword for Balance and Gait in People With Parkinson's Disease. *Mov. Disord.* **30,** 1361–70 (2015).
51. Kiehn, O. Decoding the organization of spinal circuits that control locomotion. *Nat. Rev. Neurosci.* **17,** 224–238 (2016).
52. Marder, E. & Bucher, D. Central pattern generators and the control of rhythmic movements. *Curr. Biol.* **11,** R986–R996 (2001).
53. Bostan, A. C. & Strick, P. L. The basal ganglia and the cerebellum: nodes in an integrated network. *Nat. Rev. Neurosci.* **19,** 338–350 (2018).
54. Passingham, R. E. *The frontal lobes and voluntary action*. (Oxford University Press, 1993).
55. Takakusaki, K. Functional Neuroanatomy for Posture and Gait Control. *J. Mov. Disord.* **10,** 1–17 (2017).
56. Herrero, M. T., Barcia, C. & Navarro, J. M. Functional anatomy of thalamus and basal ganglia. *Child's Nerv. Syst.* **18,** 386–404 (2002).
57. Jones, E. G. Synchrony in the Interconnected Circuitry of the Thalamus and Cerebral Cortex. *Ann. N. Y. Acad. Sci.* **1157,** 10–23 (2009).
58. Rubin, J. E., McIntyre, C. C., Turner, R. S. & Wichmann, T. Basal ganglia activity patterns in parkinsonism and computational modeling of their downstream effects. *Eur. J. Neurosci.* **36,** 2213–2228 (2012).
59. Haber, S. & Mcfarland, N. R. The Place of the Thalamus in Frontal Cortical-Basal Ganglia Circuits. *Neurosci.* **7,** 315–324 (2001).
60. Haber, S. N. & Calzavara, R. The cortico-basal ganglia integrative network: the role of the thalamus. *Brain Res. Bull.* **78,** 69–74 (2009).
61. Caligiore, D. *et al.* Consensus Paper: Towards a Systems-Level View of Cerebellar Function: the Interplay Between Cerebellum, Basal Ganglia, and Cortex. *Cerebellum* **16,** 203–229 (2017).
62. Middleton, F. A. & Strick, P. L. Basal ganglia and cerebellar loops: motor and cognitive circuits. *Brain Res. Rev.* **31,** 236–250 (2000).
63. Bostan, A. C., Dum, R. P. & Strick, P. L. The basal ganglia communicate with the cerebellum. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 8452–6 (2010).
64. Hoshi, E., Tremblay, L., Féger, J., Carras, P. L. & Strick, P. L. The cerebellum communicates with the basal ganglia. *Nat. Neurosci.* **8,** 1491–1493 (2005).
65. Smith, Y., Raju, D. V, Pare, J.-F. & Sidibe, M. The thalamostriatal system: a highly specific network of the basal ganglia circuitry. *Trends Neurosci.* **27,** 520–7 (2004).
66. Lanciego, J. L., Luquin, N. & Obeso, J. A. Functional neuroanatomy of the basal ganglia. *Cold Spring Harb. Perspect. Med.* **2,** a009621 (2012).
67. Albin, R. L., Young, a B. & Penney, J. B. The functional anatomy of basal ganglia disorders. *Trends Neurosci.* **12,** 366–375 (1989).
68. Parent, A. & Hazrati, L. N. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res. Brain Res. Rev.* **20,** 91–127 (1995).
69. Parent, a. & Hazrati, L. N. Functional anatomy of the basal ganglia. II. The place of subthalamic nucleus and external pallidum in basal ganglia circuitry. *Brain Res. Rev.* **20,** 128–154 (1995).
70. Ellens, D. J. & Leventhal, D. K. Review: electrophysiology of basal ganglia and cortex in models of Parkinson disease. *J. Parkinsons. Dis.* **3,** 241–54 (2013).
71. Dahlström, A. & Fuxe, K. Localization of monoamines in the lower brain stem. *Experientia* **20,** 398–399 (1964).
72. Parker, J. G. *et al.* Diametric neural ensemble dynamics in parkinsonian and dyskinetic states. *Nature* **557,** 177–182 (2018).
73. Calabresi, P., Picconi, B., Tozzi, A., Ghiglieri, V. & Di Filippo, M. Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat. Neurosci.* **17,** 1022–1030 (2014).
74. Castle, M. *et al.* Thalamic innervation of the direct and indirect basal ganglia pathways in the rat: Ipsi- and contralateral projections. *J. Comp. Neurol.* **483,** 143–153 (2005).
75. Hikosaka, O., Takikawa, Y. & Kawagoe, R. *Role of the Basal Ganglia in the Control of Purposive Saccadic Eye Movements*. (2000). at <www.physrev.physiology.org>
76. Plenz, D. & Kital, S. T. A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. *Nature* **400,** 677–682 (1999).
77. Nambu, A., Tokuno, H. & Takada, M. Functional significance of the cortico-subthalamo-pallidal 'hyperdirect' pathway. *Neurosci. Res.* **43,** 111–117 (2002).
78. Martinez-Gonzalez, C., Bolam, J. P. & Mena-Segovia, J. Topographical organization of the pedunculopontine nucleus. *Front. Neuroanat.* **5,** 22 (2011).
79. Alam, M., Schwabe, K. & Krauss, J. K. The pedunculopontine nucleus area: Critical evaluation of interspecies differences relevant for its use as a target for deep brain stimulation. *Brain* **134,** 11–23 (2011).
80. Fu, Y., Paxinos, G., Watson, C. & Halliday, G. M. The substantia nigra and ventral tegmental dopaminergic neurons from development to degeneration. *J. Chem. Neuroanat.* **76,** 98–107 (2016).
81. Zaja-Milatovic, S. *et al.* Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. *Neurology* **64,** 545–7 (2005).
82. Benarroch, E. E. Locus coeruleus. *Cell Tissue Res.* (2017). doi:10.1007/s00441-017-2649-1
83. Hoover, J. & Strick, P. Multiple output channels in the basal ganglia. *Science (80-.).* **259,** (1993).
84. Alexander, G. E., DeLong, M. R. & Strick, P. L. Parallel Organization of Functionally Segregated Circuits Linking Basal Ganglia and Cortex. *Annu. Rev. Neurosci.* **9,** 357–381 (1986).
85. Alexander, G. E. & Crutcher, M. D. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci.* **13,** 266–271 (1990).
86. Pozzi, N. G. *et al.* Distinctive neuronal firing patterns in subterritories of the subthalamic nucleus. *Clin. Neurophysiol.* **127,** (2016).
87. Draganski, B. *et al.* Evidence for segregated and integrative connectivity patterns in the human Basal Ganglia. *J. Neurosci.* **28,** 7143–52 (2008).
88. Lambert, C. *et al.* Confirmation of functional zones within the human subthalamic nucleus: Patterns of connectivity and sub-parcellation using diffusion weighted imaging. *Neuroimage* **60,** 83–94 (2012).
89. Parent, A., Charara, A. & Pinault, D. Single striatofugal axons arborizing in both pallidal segments and in the substantia nigra in primates. *Brain Res.* **698,** 280–284 (1995).
90. Georgopoulos, A. P., DeLong, M. R. & Crutcher, M. D. Relations between parameters of step-tracking movements and single cell discharge in the globus pallidus and subthalamic nucleus of the behaving monkey. *J. Neurosci.* **3,** 1586–98 (1983).
91. Mink, J. W. The basal ganglia: Focused selection and inhibition of competing motor programs. *Prog. Neurobiol.* **50,** 381–425

(1996).
92. Mazzoni, P., Hristova, A. & Krakauer, J. W. Why don't we move faster? Parkinson's disease, movement vigor, and implicit motivation. *J. Neurosci.* **27,** 7105–16 (2007).
93. Frank, M. J., Samanta, J., Moustafa, A. a & Sherman, S. J. Hold your horses: impulsivity, deep brain stimulation, and medication in parkinsonism. *Science* **318,** 1309–1312 (2007).
94. Obeso, I. *et al.* The subthalamic nucleus and inhibitory control: Impact of subthalamotomy in Parkinson's disease. *Brain* **137,** 1470–1480 (2014).
95. Jahanshahi, M., Obeso, I., Rothwell, J. C. & Obeso, J. A. A fronto–striato–subthalamic–pallidal network for goal-directed and habitual inhibition. *Nat. Rev. Neurosci.* **16,** 719–732 (2015).
96. Hikosaka, O. & Isoda, M. Switching from automatic to controlled behavior: cortico-basal ganglia mechanisms. *Trends Cogn. Sci.* **14,** 154–161 (2010).
97. Galvan, A. & Wichmann, T. Pathophysiology of Parkinsonism. *Clin. Neurophysiol.* **119,** 1459–1474 (2008).
98. Schwartzman, R. J. & Alexander, G. M. Changes in the local cerebral metabolic rate for glucose in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate model of Parkinson's disease. *Brain Res.* **358,** 137–143 (1985).
99. Mallet, N., Ballion, B., Le Moine, C. & Gonon, F. Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. *J. Neurosci.* **26,** 3875–84 (2006).
100. Bergman, H., Wichmann, T., Karmon, B. & DeLong, M. R. The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. *J. Neurophysiol.* **72,** 507–20 (1994).
101. Hutchison, W. D. *et al.* Neurophysiological identification of the subthalamic nucleus in surgery for Parkinson's disease. *Ann. Neurol.* **44,** 622–628 (1998).
102. Gross, R. E., Krack, P., Rodriguez-Oroz, M. C., Rezai, A. R. & Benabid, A. L. Electrophysiological mapping for the implantation of deep brain stimulators for Parkinson's disease and tremor. *Mov. Disord.* **21,** (2006).
103. Hutchison, W. D. *et al.* Differential neuronal activity in segments of globus pallidus in Parkinson's disease patients. *Neuroreport* **5,** 1533–7 (1994).
104. Steigerwald, F. *et al.* Neuronal activity of the human subthalamic nucleus in the parkinsonian and nonparkinsonian state. *J. Neurophysiol.* **100,** 2515–2524 (2008).
105. DeLong, M. R. Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.* **13,** 281–285 (1990).
106. Montgomery, E. B. Basal ganglia physiology and pathophysiology: A reappraisal. *Park. Relat. Disord.* **13,** 455–465 (2007).
107. Nambu, A., Tachibana, Y. & Chiken, S. Cause of parkinsonian symptoms: Firing rate, firing pattern or dynamic activity changes? *Basal Ganglia* **5,** 1–6 (2015).
108. Krahe, R. & Gabbiani, F. Burst firing in sensory systems. *Nat. Rev. Neurosci.* **5,** 13–23 (2004).
109. Tachibana, Y., Iwamuro, H., Kita, H., Takada, M. & Nambu, A. Subthalamo-pallidal interactions underlying parkinsonian neuronal oscillations in the primate basal ganglia. *Eur. J. Neurosci.* **34,** 1470–1484 (2011).
110. Wichmann, T. & Soares, J. Neuronal firing before and after burst discharges in the monkey basal ganglia is predictably patterned in the normal state and altered in parkinsonism. *J. Neurophysiol.* **95,** 2120–33 (2006).
111. Tinkhauser, G. *et al.* The modulatory effect of adaptive deep brain stimulation on beta bursts in Parkinson's disease. *Brain* **140,** 1053–1067 (2017).
112. Tinkhauser, G. *et al.* Beta burst dynamics in Parkinson's disease OFF and ON dopaminergic medication. *Brain* **140,** 2968–2981 (2017).
113. Tinkhauser, G. *et al.* Beta burst coupling across the motor circuit in Parkinson's disease. *Neurobiol. Dis.* **117,** 217–225 (2018).
114. Buzsáki, G., Anastassiou, C. a. & Koch, C. The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes. *Nat. Rev. Neurosci.* **13,** 407–420 (2012).
115. Chan, V., Starr, P. A. & Turner, R. S. Bursts and oscillations as independent properties of neural activity in the parkinsonian globus pallidus internus. *Neurobiol. Dis.* **41,** 2–10 (2011).
116. Varela, F., Lachaux, J. P., Rodriguez, E. & Martinerie, J. The brainweb: phase synchronization and large-scale integration. *Nat. Rev. Neurosci.* (2001). doi:10.1038/35067550
117. Siegel, M., Donner, T. H. & Engel, A. K. Spectral fingerprints of large-scale neuronal interactions. *Nat. Rev. Neurosci.* **13,** 121–134 (2012).
118. Buzsáki, G. & Chrobak, J. J. Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. *Curr. Opin. Neurobiol.* **5,** 504–510 (1995).
119. Singh, A. Oscillatory activity in the cortico-basal ganglia-thalamic neural circuits in Parkinson's disease. *Eur. J. Neurosci.* (2018). doi:10.1111/ejn.13853
120. Kühn, A. a. *et al.* The relationship between local field potential and neuronal discharge in the subthalamic nucleus of patients with Parkinson's disease. *Exp. Neurol.* **194,** 212–220 (2005).
121. Moran, a., Bergman, H., Israel, Z. & Bar-Gad, I. Subthalamic nucleus functional organization revealed by parkinsonian neuronal oscillations and synchrony. *Brain* **131,** 3395–3409 (2008).
122. Moran, A., Stein, E., Tischler, H. & Bar-Gad, I. Decoupling neuronal oscillations during subthalamic nucleus stimulation in the parkinsonian primate. *Neurobiol. Dis.* **45,** 583–590 (2012).
123. Ray, S. & Maunsell, J. H. R. Network Rhythms Influence the Relationship between Spike-Triggered Local Field Potential and Functional Connectivity. *J. Neurosci.* **31,** 12674–12682 (2011).
124. Ray, S. & Maunsell, J. H. R. Differences in Gamma Frequencies across Visual Cortex Restrict Their Possible Use in Computation. *Neuron* **67,** 885–896 (2010).
125. Priori, A. *et al.* Rhythm-specific pharmacological modulation of subthalamic activity in Parkinson's disease. *Exp. Neurol.* **189,** 369–79 (2004).
126. Brown, P. *et al.* Dopamine dependency of oscillations between subthalamic nucleus and pallidum in Parkinson's disease. *J. Neurosci.* **21,** 1033–8 (2001).
127. Hammond, C., Bergman, H. & Brown, P. Pathological synchronization in Parkinson's disease: networks, models and treatments. *Trends Neurosci.* **30,** 357–364 (2007).
128. Little, S. & Brown, P. What brain signals are suitable for feedback control of deep brain stimulation in Parkinson's disease? *Ann. N. Y. Acad. Sci.* **1265,** 9–24 (2012).
129. de Solages, C., Hill, B. C., Koop, M. M., Henderson, J. M. & Bronte-Stewart, H. Bilateral symmetry and coherence of subthalamic nuclei beta band activity in Parkinson's disease. *Exp. Neurol.* **221,** 260–266 (2010).
130. Eusebio, A. *et al.* Deep brain stimulation can suppress pathological synchronisation in parkinsonian patients. *J. Neurol. Neurosurg. Psychiatry* **82,** 569–73 (2011).
131. Kühn, A. a., Kupsch, A., Schneider, G. H. & Brown, P. Reduction in subthalamic 8-35 Hz oscillatory activity correlates with clinical improvement in Parkinson's disease. *Eur. J. Neurosci.* **23,** 1956–1960 (2006).

132. Kühn, A. a. *et al.* Pathological synchronisation in the subthalamic nucleus of patients with Parkinson's disease relates to both bradykinesia and rigidity. *Exp. Neurol.* **215,** 380–387 (2009).
133. Weinberger, M. *et al.* Beta oscillatory activity in the subthalamic nucleus and its relation to dopaminergic response in Parkinson's disease. *J. Neurophysiol.* **96,** 3248–3256 (2006).
134. Neumann, W.-J. *et al.* Subthalamic synchronized oscillatory activity correlates with motor impairment in patients with Parkinson's disease. *Mov. Disord.* (2016). doi:10.1002/mds.26759
135. Storzer, L. *et al.* Bicycling suppresses abnormal beta synchrony in the Parkinsonian basal ganglia. *Ann. Neurol.* **82,** 592–601 (2017).
136. Litvak, V. *et al.* Resting oscillatory cortico-subthalamic connectivity in patients with Parkinson's disease. *Brain* **134,** 359–374 (2011).
137. Foffani, G. *et al.* 300-Hz subthalamic oscillations in Parkinson's disease. *Brain* **126,** 2153–2163 (2003).
138. Courtemanche, R., Fujii, N. & Graybiel, A. M. Synchronous, Focally Modulated β-Band Oscillations Characterize Local Field Potential Activity in the Striatum of Awake Behaving Monkeys. *J. Neurosci.* **23,** (2003).
139. Androulidakis, A. G. *et al.* Dopaminergic therapy promotes lateralized motor activity in the subthalamic area in Parkinson's disease. *Brain* **130,** 457–468 (2007).
140. Fogelson, N. *et al.* Different functional loops between cerebral cortex and the subthalmic area in parkinson's disease. *Cereb. Cortex* (2006). doi:10.1093/cercor/bhi084
141. Pfurtscheller, G. & Lopes da Silva, F. H. Event-related EEG/MEG synchronization and desynchronization: basic principles. *Clin. Neurophysiol.* **110,** 1842–1857 (1999).
142. Devos, D. *et al.* Predominance of the contralateral movement-related activity in the subthalamo-cortical loop. *Clin. Neurophysiol.* **117,** 2315–2327 (2006).
143. Little, S., Pogosyan, A., Kuhn, A. A. & Brown, P. Beta band stability over time correlates with Parkinsonian rigidity and bradykinesia. *Exp. Neurol.* **236,** 383–388 (2012).
144. Canessa, A. *et al.* Striatal Dopaminergic Innervation Regulates Subthalamic Beta-Oscillations and Cortical-Subcortical Coupling during Movements: Preliminary Evidence in Subjects with Parkinson's Disease. *Front. Hum. Neurosci.* **10,** 611 (2016).
145. Storzer, L. *et al.* Bicycling and Walking are Associated with Different Cortical Oscillatory Dynamics. *Front. Hum. Neurosci.* **10,** 61 (2016).
146. Alegre, M. *et al.* Movement-related changes in oscillatory activity in the human subthalamic nucleus: Ipsilateral vs. contralateral movements. *Eur. J. Neurosci.* **22,** 2315–2324 (2005).
147. Hell, F., Plate, A., Mehrkens, J. H. & Bötzel, K. Subthalamic oscillatory activity and connectivity during gait in Parkinson's disease. *NeuroImage Clin.* **19,** 396–405 (2018).
148. Quinn, E. J. *et al.* Beta Oscillations in Freely Moving Parkinson's Subjects Are Attenuated During Deep Brain Stimulation. doi:10.1002/mds.26376
149. Arnulfo, G. *et al.* Phase matters: A role for the subthalamic network during gait. *PLoS One* **13,** e0198691 (2018).
150. Fischer, P. *et al.* Alternating Modulation of Subthalamic Nucleus Beta Oscillations during Stepping. *J. Neurosci.* **38,** 5111–5121 (2018).
151. Syrkin-Nikolau, J. *et al.* Subthalamic neural entropy is a feature of freezing of gait in freely moving people with Parkinson's disease. *Neurobiol. Dis.* **108,** 288–297 (2017).
152. Oswal, a., Litvak, V., Sauleau, P. & Brown, P. Beta Reactivity, Prospective Facilitation of Executive Processing, and Its Dependence on Dopaminergic Therapy in Parkinson's Disease. *J. Neurosci.* **32,** 9909–9916 (2012).
153. Jenkinson, N. & Brown, P. New insights into the relationship between dopamine, beta oscillations and motor function. *Trends Neurosci.* **34,** 611–618 (2011).
154. Brittain, J. S. & Brown, P. Oscillations and the basal ganglia: Motor control and beyond. *Neuroimage* **85,** 637–647 (2014).
155. Little, S. & Brown, P. The functional role of beta oscillations in Parkinson's disease. *Parkinsonism Relat. Disord.* **20,** S44–S48 (2014).
156. Leblois, A. *et al.* Late emergence of synchronized oscillatory activity in the pallidum during progressive Parkinsonism. *Eur. J. Neurosci.* **26,** 1701–13 (2007).
157. Quiroga-Varela, A., Walters, J. R., Brazhnik, E., Marin, C. & Obeso, J. A. What basal ganglia changes underlie the parkinsonian state? The significance of neuronal oscillatory activity. *Neurobiol. Dis.* **58,** 242–248 (2013).
158. Wang, D. D. *et al.* Subthalamic local field potentials in Parkinson's disease and isolated dystonia: An evaluation of potential biomarkers. *Neurobiol. Dis.* **89,** 213–222 (2016).
159. Quinn, E. J. *et al.* Beta oscillations in freely moving Parkinson's subjects are attenuated during deep brain stimulation. *Mov. Disord.* **30,** 1750–1758 (2015).
160. Singh, A. *et al.* Human striatal recordings reveal abnormal discharge of projection neurons in Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* **113,** 9629–34 (2016).
161. Corbit, V. L. *et al.* Pallidostriatal Projections Promote β Oscillations in a Dopamine-Depleted Biophysical Network Model. *J. Neurosci.* **36,** 5556–71 (2016).
162. Guiyeom Kang & Lowery, M. M. A model of pathological oscillations in the basal ganglia and deep brain stimulation in parkinson's disease. in *2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society* 3909–3912 (IEEE, 2009). doi:10.1109/IEMBS.2009.5333557
163. de Hemptinne, C. *et al.* Exaggerated phase-amplitude coupling in the primary motor cortex in Parkinson disease. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 4780–5 (2013).
164. Wilson, C. J. Oscillators and Oscillations in the Basal Ganglia. *Neuroscientist* (2014). doi:10.1177/1073858414560826
165. Fries, P. Rhythms for Cognition: Communication through Coherence. *Neuron* **88,** 220–35 (2015).
166. Wilson, C. J. Oscillators and Oscillations in the Basal Ganglia. *Neurosci.* (2014). doi:10.1177/1073858414560826
167. de Hemptinne, C. *et al.* Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease. *Nat. Neurosci.* **18,** 779–786 (2015).
168. Swann, N. C. *et al.* Gamma Oscillations in the Hyperkinetic State Detected with Chronic Human Brain Recordings in Parkinson's Disease. *J. Neurosci.* **36,** 6445–6458 (2016).
169. Bronfeld, M. & Bar-Gad, I. Loss of Specificity in Basal Ganglia Related Movement Disorders. *Front. Syst. Neurosci.* **5,** 38 (2011).
170. Tinkhauser, G. *et al.* Beta burst dynamics in Parkinson's disease OFF and ON dopaminergic medication. *Brain* **140,** 2968–2981 (2017).
171. Dejean, C. *et al.* Evolution of the dynamic properties of the cortex-basal ganglia network after dopaminergic depletion in rats. *Neurobiol. Dis.* **46,** 402–13 (2012).
172. Mallet, N. *et al.* Disrupted dopamine transmission and the emergence of exaggerated beta oscillations in subthalamic nucleus and cerebral cortex. *J. Neurosci.* **28,** 4795–806 (2008).

173. Silberstein, P. *et al.* Cortico-cortical coupling in Parkinson's disease and its modulation by therapy. *Brain* **128,** 1277–1291 (2005).
174. Soikkeli, R., Partanen, J., Soininen, H., Pääkkönen, A. & Riekkinen, P. Slowing of EEG in Parkinson's disease. *Electroencephalogr. Clin. Neurophysiol.* **79,** 159–65 (1991).
175. Li, S., Arbuthnott, G. W., Jutras, M. J., Goldberg, J. a & Jaeger, D. Resonant antidromic cortical circuit activation as a consequence of high-frequency subthalamic deep-brain stimulation. *J. Neurophysiol.* **98,** 3525–3537 (2007).
176. Meziane, H. B. *et al.* Movement Preparation and Bilateral Modulation of Beta Activity in Aging and Parkinson's Disease. *PLoS One* **10,** e0114817 (2015).
177. Heimer, G., Bar-Gad, I., Goldberg, J. A. & Bergman, H. Dopamine replacement therapy reverses abnormal synchronization of pallidal neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primate model of parkinsonism. *J. Neurosci.* **22,** 7850–5 (2002).
178. van Wijk, B. C. M. *et al.* Localization of beta and high-frequency oscillations within the subthalamic nucleus region. *NeuroImage Clin.* **16,** 175–183 (2017).
179. Cole, S. R. *et al.* Nonsinusoidal beta oscillations reflect cortical pathophysiology in Parkinson's disease. *J. Neurosci.* (2017). doi:10.1523/JNEUROSCI.2208-16.2017
180. van Wijk, B. C. M. Is Broadband Gamma Activity Pathologically Synchronized to the Beta Rhythm in Parkinson's Disease? *J. Neurosci.* **37,** 9347–9349 (2017).
181. Lipski, W. J. *et al.* Dynamics of human subthalamic neuron phase-locking to motor and sensory cortical oscillations during movement. *J. Neurophysiol.* **118,** 1472–1487 (2017).
182. Rodriguez-Oroz, M. C. *et al. Review Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms*. *The Lancet Neurology* **8,** (2009).
183. Wichmann, T. & DeLong, M. R. Deep Brain Stimulation for Movement Disorders of Basal Ganglia Origin: Restoring Function or Functionality? *Neurotherapeutics* **13,** 264–283 (2016).
184. Timmermann, L. *et al.* Ten-Hertz stimulation of subthalamic nucleus deteriorates motor symptoms in Parkinson's disease. *Mov. Disord.* **19,** 1328–1333 (2004).
185. Chen, C. C. *et al.* Excessive synchronization of basal ganglia neurons at 20 Hz slows movement in Parkinson's disease. *Exp. Neurol.* **205,** 214–221 (2007).
186. Chen, C. C. *et al.* Stimulation of the subthalamic region at 20 Hz slows the development of grip force in Parkinson's disease. *Exp. Neurol.* **231,** 91–96 (2011).
187. Eusebio, A. *et al.* Effects of low-frequency stimulation of the subthalamic nucleus on movement in Parkinson's disease. *Exp. Neurol.* **209,** 125–130 (2008).
188. Pogosyan, A., Gaynor, L. D., Eusebio, A. & Brown, P. Boosting Cortical Activity at Beta-Band Frequencies Slows Movement in Humans. *Curr. Biol.* **19,** 1637–1641 (2009).
189. Stein, E. & Bar-Gad, I. Beta oscillations in the cortico-basal ganglia loop during parkinsonism. *Exp. Neurol.* **245,** 52–59 (2013).
190. Beck, M. H. *et al.* Short- and long-term dopamine depletion causes enhanced beta oscillations in the cortico-basal ganglia loop of parkinsonian rats. *Exp. Neurol.* **286,** 124–136 (2016).
191. Foffani, G. *et al.* Subthalamic oscillatory activities at beta or higher frequency do not change after high-frequency DBS in Parkinson's disease. *Brain Res. Bull.* **69,** 123–130 (2006).
192. Hallett, M. Tremor: Pathophysiology. *Parkinsonism Relat. Disord.* **20,** S118–S122 (2014).
193. Qasim, S. E. *et al.* Electrocorticography reveals beta desynchronization in the basal ganglia-cortical loop during rest tremor in Parkinson's disease. *Neurobiol. Dis.* **86,** 177–186 (2016).
194. Levy, R., Hutchison, W. D., Lozano, A. M. & Dostrovsky, J. O. High-frequency synchronization of neuronal activity in the subthalamic nucleus of parkinsonian patients with limb tremor. *J. Neurosci.* **20,** 7766–75 (2000).
195. Levy, R. *et al.* Effects of apomorphine on subthalamic nucleus and globus pallidus internus neurons in patients with Parkinson's disease. *J. Neurophysiol.* **86,** 249–260 (2001).
196. Cagnan, H. *et al.* Stimulating at the right time: phase-specific deep brain stimulation. *Brain* **140,** 132–145 (2017).
197. Toledo, J. B. *et al.* High beta activity in the subthalamic nucleus and freezing of gait in Parkinson's disease. *Neurobiol. Dis.* **64,** 60–65 (2014).
198. Nieuwboer, A. & Giladi, N. Characterizing freezing of gait in Parkinson's disease: Models of an episodic phenomenon. *Mov. Disord.* **28,** 1509–1519 (2013).
199. Amboni, M., Barone, P. & Hausdorff, J. M. Cognitive contributions to gait and falls: evidence and implications. *Mov. Disord.* **28,** 1520–33 (2013).
200. Vercruysse, S. *et al.* Freezing beyond gait in Parkinson's disease: A review of current neurobehavioral evidence. *Neurosci. Biobehav. Rev.* **43,** 213–227 (2014).
201. Tard, C. *et al.* Brain Metabolic Abnormalities During Gait With Freezing in Parkinson'S Disease. *Neuroscience* **307,** 281–301 (2015).
202. Shine, J. M. *et al.* Abnormal patterns of theta frequency oscillations during the temporal evolution of freezing of gait in parkinson's disease. *Clin. Neurophysiol.* **125,** 569–576 (2014).
203. Shine, J. M. *et al.* Freezing of gait in Parkinson's disease is associated with functional decoupling between the cognitive control network and the basal ganglia. *Brain* **136,** 3671–3681 (2013).
204. Brooks, D. J. *et al.* Isolated tremor and disruption of the nigrostriatal dopaminergic system: an 18F-dopa PET study. *Neurology* **42,** 1554–60 (1992).
205. Ghaemi, M. *et al.* Monosymptomatic resting tremor and Parkinson's disease: A multitracer positron emission tomographic study. *Mov. Disord.* **17,** 782–788 (2002).
206. Isaias, I. U. *et al.* [123I]FP-CIT striatal binding in early Parkinson??s disease patients with tremor vs. akinetic-rigid onset. *Neuroreport* **18,** 1499–1502 (2007).
207. Benamer, T. S. *et al.* Accurate differentiation of parkinsonism and essential tremor using visual assessment of [123I]-FP-CIT SPECT imaging: the [123I]-FP-CIT study group. *Mov. Disord.* **15,** 503–10 (2000).
208. Isaias, I. U. *et al.* A role for locus coeruleus in Parkinson tremor. *Front. Hum. Neurosci.* **5,** 179 (2011).
209. Nieuwhof, F., Panyakaew, P., van de Warrenburg, B. P., Gallea, C. & Helmich, R. C. The patchy tremor landscape. *Curr. Opin. Neurol.* 1 (2018). doi:10.1097/WCO.0000000000000582
210. Hallett, M. Parkinson's disease tremor: pathophysiology. *Parkinsonism Relat. Disord.* **18,** S85–S86 (2012).
211. Timmermann, L. *et al.* The cerebral oscillatory network of parkinsonian resting tremor. *Brain* **126,** 199–212 (2003).
212. Ni, Z. & Chen, R. Transcranial magnetic stimulation to understand pathophysiology and as potential treatment for neurodegenerative diseases. *Transl. Neurodegener.* **4,** 22 (2015).
213. Magnin, M., Morel, A. & Jeanmonod, D. Single-unit analysis of the pallidum, thalamus and subthalamic nucleus in parkinsonian patients. *Neuroscience* **96,** 549–64 (2000).

214. Cagnan, H. *et al.* The nature of tremor circuits in parkinsonian and essential tremor. *Brain* **137,** 3223–3234 (2014).
215. Zirh, T. A., Lenz, F. A., Reich, S. G. & Dougherty, P. M. Patterns of bursting occurring in thalamic cells during parkinsonian tremor. *Neuroscience* **83,** 107–21 (1998).
216. Martínez-Fernández, R. *et al.* Focused ultrasound subthalamotomy in patients with asymmetric Parkinson's disease: a pilot study. *Lancet Neurol.* **17,** 54–63 (2018).
217. Fasano, A. *et al.* Magnetic Resonance Imaging-Guided Focused Ultrasound Thalamotomy in Parkinson Tremor: Reoperation After Benefit Decay. *Mov. Disord.* **33,** 848–849 (2018).
218. Krishna, V., Sammartino, F. & Rezai, A. A Review of the Current Therapies, Challenges, and Future Directions of Transcranial Focused Ultrasound Technology. *JAMA Neurol.* **75,** 246 (2018).
219. Blomstedt, P. *et al.* Deep brain stimulation in the caudal zona incerta versus best medical treatment in patients with Parkinson's disease: a randomised blinded evaluation. *J. Neurol. Neurosurg. Psychiatry* **89,** 710–716 (2018).
220. Coenen, V. A. Deep braIn Stimulation for Tremor TractographIC Versus Traditional (DISTINCT). (2015). at <https://clinicaltrials.gov/ct2/show/NCT02491554?cond=Tremor&cntry=DE&rank=1>
221. Sajonz, B. E. A. *et al.* Deep Brain Stimulation for Tremor Tractographic Versus Traditional (DISTINCT): Study Protocol of a Randomized Controlled Feasibility Trial. *JMIR Res. Protoc.* **5,** e244 (2016).
222. Jankovic, J. Parkinson's disease tremors and serotonin. *Brain* **141,** 624–626 (2018).
223. Hassan, A. No handshake agreement on postural tremor subtypes in Parkinson disease? *Neurology* **90,** 581–582 (2018).
224. Mure, H. *et al.* Parkinson's disease tremor-related metabolic network: Characterization, progression, and treatment effects. *Neuroimage* **54,** 1244–1253 (2011).
225. Helmich, R. C., Hallett, M., Deuschl, G., Toni, I. & Bloem, B. R. Cerebral causes and consequences of parkinsonian resting tremor: a tale of two circuits? *Brain* **135,** 3206–3226 (2012).
226. Helmich, R. C., Janssen, M. J. R., Oyen, W. J. G., Bloem, B. R. & Toni, I. Pallidal dysfunction drives a cerebellothalamic circuit into Parkinson tremor. *Ann. Neurol.* **69,** 269–281 (2011).
227. Dirkx, M. F., Zach, H., Bloem, B. R., Hallett, M. & Helmich, R. C. The nature of postural tremor in Parkinson disease. *Neurology* **90,** e1095–e1103 (2018).
228. Dirkx, M. F. *et al.* Dopamine controls Parkinson's tremor by inhibiting the cerebellar thalamus. *Brain* **140,** aww331 (2017).
229. Lee, J.-Y. *et al.* Pallidal dopaminergic denervation and rest tremor in early Parkinson's disease: PPMI cohort analysis. *Parkinsonism Relat. Disord.* (2018). doi:10.1016/j.parkreldis.2018.02.039
230. Rommelfanger, K. S. & Weinshenker, D. Norepinephrine: The redheaded stepchild of Parkinson's disease. *Biochem. Pharmacol.* **74,** 177–190 (2007).
231. Navas, J. P. & Martinez-Maldonado, M. Pathophysiology of edema in congestive heart failure. *Heart Dis. Stroke* **2,** 325–9
232. Samuels, E. & Szabadi, E. Functional Neuroanatomy of the Noradrenergic Locus Coeruleus: Its Roles in the Regulation of Arousal and Autonomic Function Part II: Physiological and Pharmacological Manipulations and Pathological Alterations of Locus Coeruleus Activity in Humans. *Curr. Neuropharmacol.* **6,** 254–285 (2008).
233. Isaias, I. U. *et al.* Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease. *BMC Neurol.* **11,** 88 (2011).
234. Sommerauer, M. *et al.* Evaluation of the noradrenergic system in Parkinson's disease: an 11C-MeNER PET and neuromelanin MRI study. *Brain* **141,** 496–504 (2018).
235. Nahimi, A. *et al.* Noradrenergic deficits in Parkinson's disease imaged with ^{11}C-MeNER. *J. Nucl. Med.* jnumed.117.190975 (2017). doi:10.2967/jnumed.117.190975
236. Delaville, C., Navailles, S. & Benazzouz, A. Effects of noradrenaline and serotonin depletions on the neuronal activity of globus pallidus and substantia nigra pars reticulata in experimental parkinsonism. *Neuroscience* **202,** 424–33 (2012).
237. Srinivasan, J. & Schmidt, W. J. Potentiation of parkinsonian symptoms by depletion of locus coeruleus noradrenaline in 6-hydroxydopamine-induced partial degeneration of substantia nigra in rats. *Eur. J. Neurosci.* **17,** 2586–92 (2003).
238. Wang, T., Zhang, Q.-J., Liu, J., Wu, Z.-H. & Wang, S. Firing activity of locus coeruleus noradrenergic neurons increases in a rodent model of Parkinsonism. *Neurosci. Bull.* **25,** 15–20 (2009).
239. Aston-Jones, G. & Cohen, J. D. An integrative theory of locus coeruleus-norepinephrine function: Adaptive Gain and Optimal Performance. *Annu. Rev. Neurosci.* **28,** 403–450 (2005).
240. Ferrucci, R., Bocci, T., Cortese, F., Ruggiero, F. & Priori, A. Cerebellar transcranial direct current stimulation in neurological disease. *Cerebellum & Ataxias* **3,** 16 (2016).
241. Catalan, M. J., Ishii, K., Honda, M., Samii, A. & Hallett, M. A PET study of sequential finger movements of varying length in patients with Parkinson's disease. *Brain* **122 (Pt 3),** 483–95 (1999).
242. Wu, T. & Hallett, M. A functional MRI study of automatic movements in patients with Parkinson's disease. *Brain* **128,** 2250–2259 (2005).
243. Wu, T., Wang, L., Hallett, M., Li, K. & Chan, P. Neural correlates of bimanual anti-phase and in-phase movements in Parkinson's disease. *Brain* **133,** 2394–409 (2010).
244. Mentis, M. J. *et al.* Enhancement of brain activation during trial-and-error sequence learning in early PD. *Neurology* **60,** 612–9 (2003).
245. Muthuraman, M. *et al.* Cerebello-cortical network fingerprints differ between essential, Parkinson's and mimicked tremors. *Brain* **141,** 1770–1781 (2018).
246. Colpaert, F. C. Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rat. *Neuropharmacology* **26,** 1431–40 (1987).
247. CARLSSON, A., LINDQVIST, M. & MAGNUSSON, T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* **180,** 1200 (1957).
248. CARLSSON, A. The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacol. Rev.* **11,** 490–3 (1959).
249. Leão, A. H. F. F., Sarmento-Silva, A. J., Santos, J. R., Ribeiro, A. M. & Silva, R. H. Molecular, Neurochemical, and Behavioral Hallmarks of Reserpine as a Model for Parkinson's Disease: New Perspectives to a Long-Standing Model. *Brain Pathol.* **25,** 377–390 (2015).
250. FLACH, F. F. Clinical effectiveness of reserpine. *Ann. N. Y. Acad. Sci.* **61,** 161–6 (1955).
251. Fernandes, V. S. *et al.* Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's disease. *Behav. Brain Res.* **231,** 154–163 (2012).
252. Gerlach, M. & Riederer, P. Animal models of Parkinson's disease: an empirical comparison with the phenomenology of the disease in man. *J. Neural Transm.* **103,** 987–1041 (1996).
253. Tieu, K. A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harb. Perspect. Med.* **1,** a009316 (2011).
254. Caudle, W. M., Colebrooke, R. E., Emson, P. C. & Miller, G. W. Altered vesicular dopamine storage in Parkinson's disease: a

premature demise. *Trends Neurosci.* **31,** 303–308 (2008).
255. Dröge, W. Free Radicals in the Physiological Control of Cell Function. *Physiol. Rev.* **82,** 47–95 (2002).
256. Tsang, A. H. K. & Chung, K. K. K. Oxidative and nitrosative stress in Parkinson's disease. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1792,** 643–650 (2009).
257. Reis, D. J., Joh, T. H. & Ross, R. A. Effects of reserpine on activities and amounts of tyrosine hydroxylase and dopamine-beta-hydroxylase in catecholamine neuronal systems in rat brain. *J. Pharmacol. Exp. Ther.* **193,** (1975).
258. Jonsson, G., Hallman, H., Ponzio, F. & Ross, S. DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine)--a useful denervation tool for central and peripheral noradrenaline neurons. *Eur. J. Pharmacol.* **72,** 173–88 (1981).
259. Fritschy, J. M. & Grzanna, R. Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals. *Neuroscience* **30,** 181–97 (1989).
260. Grzanna, R., Berger, U., Fritschy, J. M. & Geffard, M. Acute action of DSP-4 on central norepinephrine axons: biochemical and immunohistochemical evidence for differential effects. *J. Histochem. Cytochem.* **37,** 1435–1442 (1989).
261. Zaczek, R., Fritschy, J. M., Culp, S., De Souza, E. B. & Grzanna, R. Differential effects of DSP-4 on noradrenaline axons in cerebral cortex and hypothalamus may reflect heterogeneity of noradrenaline uptake sites. *Brain Res.* **522,** 308–14 (1990).
262. Wu, T. & Hallett, M. The cerebellum in Parkinson's disease. *Brain* **136,** 696–709 (2013).
263. Alachkar, A., Brotchie, J. M. & Jones, O. T. Locomotor response to l-DOPA in reserpine-treated rats following central inhibition of aromatic l-amino acid decarboxylase: Further evidence for non-dopaminergic actions of l-DOPA and its metabolites. *Neurosci. Res.* **68,** 44–50 (2010).
264. Lieber, B., Taylor, B. E. S., Appelboom, G., McKhann, G. & Connolly, E. S. Motion Sensors to Assess and Monitor Medical and Surgical Management of Parkinson Disease. *World Neurosurg.* **84,** 561–566 (2015).
265. Asan, E. *et al.* Ciliary neurotrophic factor in the olfactory bulb of rats and mice. *Neuroscience* **120,** 99–112 (2003).
266. Asan, E. *et al.* The corticotropin-releasing factor (CRF)-system and monoaminergic afferents in the central amygdala: investigations in different mouse strains and comparison with the rat. *Neuroscience* **131,** 953–67 (2005).
267. Woodward, D. J., Moises, H. C., Waterhouse, B. D., Yeh, H. H. & Cheun, J. E. The cerebellar norepinephrine system: inhibition, modulation, and gating. *Prog. Brain Res.* **88,** 331–41 (1991).
268. Hoffer, B. J., Siggins, G. R., Woodward, D. J. & Bloom, F. E. Spontaneous discharge of Purkinje neurons after destruction of catecholamine-containing afferents by 6-hydroxydopamine. *Brain Res.* **30,** 425–30 (1971).
269. Siggins, G. R., Hoffer, B. J. & Bloom, F. E. Cyclic adenosine monophosphate: possible mediator for norepinephrine effects on cerebellar Purkinje cells. *Science* **165,** 1018–20 (1969).
270. Moises, H. C., Waterhouse, B. D. & Woodward, D. J. Locus coeruleus stimulation potentiates local inhibitory processes in rat cerebellum. *Brain Res. Bull.* **10,** 795–804 (1983).
271. Lippiello, P. *et al.* Noradrenergic modulation of the parallel fiber-Purkinje cell synapse in mouse cerebellum. *Neuropharmacology* **89,** 33–42 (2015).
272. Guo, C. *et al.* Purkinje Cells Directly Inhibit Granule Cells in Specialized Regions of the Cerebellar Cortex. *Neuron* **91,** 1330–1341 (2016).
273. Moises, H. C. & Woodward, D. J. Potentiation of GABA inhibitory action in cerebrllum by locus coeruleus stimulation. *Brain Res.* **182,** 327–44 (1980).
274. Moises, H. C., Burne, R. A. & Woodward, D. J. Modification of the visual response properties of cerebellar neurons by norepinephrine. *Brain Res.* **514,** 259–75 (1990).
275. Deiber, M.-P. *et al.* Thalamic stimulation and suppression of parkinsonian tremor. *Brain* **116,** 267–279 (1993).
276. Spiegel, J. *et al.* Striatal FP-CIT uptake differs in the subtypes of early Parkinson's disease. *J. Neural Transm.* **114,** 331–335 (2007).
277. Zaidel, A., Arkadir, D., Israel, Z. & Bergman, H. Akineto-rigid vs. tremor syndromes in Parkinsonism. *Curr. Opin. Neurol.* **22,** 387–93 (2009).
278. Spineli, L. M., Jenz, E., Großhennig, A. & Koch, A. Critical appraisal of arguments for the delayed-start design proposed as alternative to the parallel-group randomized clinical trial design in the field of rare disease. *Orphanet J. Rare Dis.* **12,** 140 (2017).
279. Duty, S. & Jenner, P. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br. J. Pharmacol.* **164,** 1357–1391 (2011).
280. Giguère, N., Burke Nanni, S. & Trudeau, L.-E. On Cell Loss and Selective Vulnerability of Neuronal Populations in Parkinson's Disease. *Front. Neurol.* **9,** 455 (2018).
281. Samuels, E. & Szabadi, E. Functional Neuroanatomy of the Noradrenergic Locus Coeruleus: Its Roles in the Regulation of Arousal and Autonomic Function Part I: Principles of Functional Organisation. *Curr. Neuropharmacol.* **6,** 235–253 (2008).
282. Mena-Segovia, J. & Bolam, J. P. Rethinking the Pedunculopontine Nucleus: From Cellular Organization to Function. *Neuron* **94,** 7–18 (2017).
283. Burn, D. J. *et al.* Motor subtype and cognitive decline in Parkinson's disease, Parkinson's disease with dementia, and dementia with Lewy bodies. *J. Neurol. Neurosurg. Psychiatry* **77,** 585–589 (2006).
284. Snijders, A. H. *et al.* Physiology of freezing of gait. *Ann. Neurol.* **80,** 644–659 (2016).
285. Bloem, B. R., Hausdorff, J. M., Visser, J. E. & Giladi, N. Falls and freezing of gait in Parkinson's disease: A review of two interconnected, episodic phenomena. *Mov. Disord.* **19,** 871–884 (2004).
286. Okuma, Y., Silva de Lima, A. L., Fukae, J., Bloem, B. R. & Snijders, A. H. A prospective study of falls in relation to freezing of gait and response fluctuations in Parkinson's disease. *Parkinsonism Relat. Disord.* **46,** 30–35 (2018).
287. Fasano, A. & Lang, A. E. Unfreezing of gait in patients with Parkinson's disease. *Lancet Neurol.* **14,** 675–677 (2015).
288. Nonnekes, J. *et al.* Freezing of gait: a practical approach to management. *Lancet Neurol.* **14,** 768–778 (2015).
289. Capaday, C. The special nature of human walking and its neural control. *Trends Neurosci.* **25,** 370–376 (2002).
290. Capogrosso, M. *et al.* A brain–spine interface alleviating gait deficits after spinal cord injury in primates. *Nature* **539,** 284–288 (2016).
291. Wagner, F. B. *et al.* Targeted neurotechnology restores walking in humans with spinal cord injury. *Nature* **563,** 65–71 (2018).
292. Formento, E. *et al.* Electrical spinal cord stimulation must preserve proprioception to enable locomotion in humans with spinal cord injury. *Nat. Neurosci.* 1 (2018). doi:10.1038/s41593-018-0262-6
293. Minassian, K., Hofstoetter, U. S., Dzeladini, F., Guertin, P. A. & Ijspeert, A. The Human Central Pattern Generator for Locomotion: Does It Exist and Contribute to Walking? *Neurosci.* **23,** 649–663 (2017).
294. la Fougère, C. *et al.* Real versus imagined locomotion: A [18F]-FDG PET-fMRI comparison. *Neuroimage* (2010). doi:10.1016/j.neuroimage.2009.12.060
295. Nachev, P., Kennard, C. & Husain, M. Functional role of the supplementary and pre-supplementary motor areas. *Nat. Rev. Neurosci.* **9,** 856–69 (2008).
296. Baldissera, F. G. & Tesio, L. APAs Constraints to Voluntary Movements: The Case for Limb Movements Coupling. *Front. Hum.*

Neurosci. **11,** 152 (2017).
297. Lewis, S. J. G. & Shine, J. M. The Next Step: A Common Neural Mechanism for Freezing of Gait. *Neuroscientist* **22,** 72–82 (2016).
298. Fogelson, N. *et al.* Reciprocal interactions between oscillatory activities of different frequencies in the subthalamic region of patients with Parkinson's disease. *Eur. J. Neurosci.* **22,** 257–266 (2005).
299. Zavala, B., Zaghloul, K. & Brown, P. The subthalamic nucleus, oscillations, and conflict. *Mov. Disord.* **30,** 328–338 (2015).
300. Anidi, C. M. *et al.* Neuromodulation targets pathological not physiological beta bursts during gait in Parkinson's disease. *Neurobiol. Dis.* (2018). doi:10.1016/J.NBD.2018.09.004
301. Wu, T., Hallett, M. & Chan, P. Motor automaticity in Parkinson's disease. *Neurobiol. Dis.* **82,** 226–234 (2015).
302. Benabid, A. L., Chabardes, S., Mitrofanis, J. & Pollak, P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet. Neurol.* **8,** 67–81 (2009).
303. Kleiner-Fisman, G. *et al.* Subthalamic nucleus deep brain stimulation: Summary and meta-analysis of outcomes. *Mov. Disord.* **21,** S290–S304 (2006).
304. Deuschl, G. *et al.* A Randomized Trial of Deep-Brain Stimulation for Parkinson's Disease. *N. Engl. J. Med.* **355,** 896–908 (2006).
305. Canessa, A. *et al.* Striatal dopaminergic innervation regulates subthalamic beta-oscillations and cortical-subcortical coupling during movements: Preliminary evidence in subjects with Parkinson's disease. *Front. Hum. Neurosci.* **10,** (2016).
306. Arlotti, M. *et al.* Eight-hours adaptive deep brain stimulation in patients with Parkinson disease. *Neurology* 10.1212/WNL.0000000000005121 (2018). doi:10.1212/WNL.0000000000005121
307. Reich, M. M. *et al.* Progressive gait ataxia following deep brain stimulation for essential tremor: adverse effect or lack of efficacy? *Brain* **139,** 2948–2956 (2016).
308. Isaias, I. U. & Antonini, A. Single-Photon Emission Computed Tomography in Diagnosis and Differential Diagnosis of Parkinson's Disease. *Neurodegener. Dis.* **7,** 319–329 (2010).
309. Isaias, I. U. *et al.* The Influence of Dopaminergic Striatal Innervation on Upper Limb Locomotor Synergies. *PLoS One* **7,** e51464 (2012).
310. Jung, T.-P. *et al.* Removal of eye activity artifacts from visual event-related potentials in normal and clinical subjects. *Clin. Neurophysiol.* **111,** 1745–1758 (2000).
311. Onton, J. & Makeig, S. *Event-Related Dynamics of Brain Oscillations*. *Progress in Brain Research* **159,** (Elsevier, 2006).
312. Onton, J., Westerfield, M., Townsend, J. & Makeig, S. Imaging human EEG dynamics using independent component analysis. *Neurosci. Biobehav. Rev.* **30,** 808–22 (2006).
313. Fitzgibbon, S. P. *et al.* Surface Laplacian of Central Scalp Electrical Signals is Insensitive to Muscle Contamination. *IEEE Trans. Biomed. Eng.* **60,** 4–9 (2013).
314. Oostenveld, R., Fries, P., Maris, E. & Schoffelen, J.-M. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput. Intell. Neurosci.* **2011,** 156869 (2011).
315. Thomson, D. J. Spectrum estimation and harmonic analysis. *Proc. IEEE* **70,** 1055–1096 (1982).
316. Shin, H., Law, R., Tsutsui, S., Moore, C. I. & Jones, S. R. The rate of transient beta frequency events predicts behavior across tasks and species. *Elife* **6,** (2017).
317. Little, S., Bonaiuto, J., Barnes, G. & Bestmann, S. Motor cortical beta transients delay movement initiation and track errors. *bioRxiv* 384370 (2018). doi:10.1101/384370
318. Benda, J., Longtin, A. & Maler, L. A Synchronization-Desynchronization Code for Natural Communication Signals. *Neuron* **52,** 347–358 (2006).
319. von Nicolai, C. *et al.* Corticostriatal Coordination through Coherent Phase-Amplitude Coupling. *J. Neurosci.* **34,** 5938–48 (2014).
320. Hanakawa, T. *et al.* Mechanisms underlying gait disturbance in Parkinson's disease: a single photon emission computed tomography study. *Brain* **122 (Pt 7),** 1271–82 (1999).
321. Iseki, K. *et al.* Gait disturbance associated with white matter changes: A gait analysis and blood flow study. *Neuroimage* **49,** 1659–1666 (2010).
322. Jacobs, J. V., Nutt, J. G., Carlson-Kuhta, P., Stephens, M. & Horak, F. B. Knee trembling during freezing of gait represents multiple anticipatory postural adjustments. *Exp. Neurol.* **215,** 334–341 (2009).
323. Schlenstedt, C. *et al.* Are Hypometric Anticipatory Postural Adjustments Contributing to Freezing of Gait in Parkinson's Disease? *Front. Aging Neurosci.* **10,** 36 (2018).
324. Matar, E. *et al.* 025 The neural correlates of doorway freezing in parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **89,** A10.3-A11 (2018).
325. Valentino, F. *et al.* Transcranial direct current stimulation for treatment of freezing of gait: A cross-over study. *Mov. Disord.* **29,** 1064–1069 (2014).
326. Lozano, A. M. & Lipsman, N. Probing and Regulating Dysfunctional Circuits Using Deep Brain Stimulation. *Neuron* **77,** 406–424 (2013).
327. Ferrucci, R. & Priori, A. in *Handbook of clinical neurology* **155,** 393–405 (2018).
328. Guleyupoglu, B., Schestatsky, P., Edwards, D., Fregni, F. & Bikson, M. Classification of methods in transcranial Electrical Stimulation (tES) and evolving strategy from historical approaches to contemporary innovations. *J. Neurosci. Methods* **219,** 297–311 (2013).
329. Hallett, M. Transcranial Magnetic Stimulation: A Primer. *Neuron* **55,** 187–199 (2007).
330. Cilia, R. *et al.* Cerebral activity modulation by extradural motor cortex stimulation in Parkinson's disease: a perfusion SPECT study. *Eur. J. Neurol.* **15,** 22–8 (2008).
331. Kringelbach, M. L., Jenkinson, N., Owen, S. L. F. & Aziz, T. Z. Translational principles of deep brain stimulation. *Nat. Rev. Neurosci.* **8,** 623–635 (2007).
332. Okun, M. S. Deep-Brain Stimulation for Parkinson's Disease. *N. Engl. J. Med.* **367,** 1529–1538 (2012).
333. Kühn, A. A. & Volkmann, J. Innovations in deep brain stimulation methodology. *Mov. Disord.* **32,** 11–19 (2017).
334. Reich, M. M. *et al.* Short pulse width widens the therapeutic window of subthalamic neurostimulation. *Ann. Clin. Transl. Neurol.* **2,** 427–432 (2015).
335. Reich, M. M. *et al.* Reply: Clinical approach to delayed-onset cerebellar impairment following deep brain stimulation for tremor. *Brain* **140,** (2017).
336. Reich, M. M. *et al.* Progressive gait ataxia following deep brain stimulation for essential tremor: adverse effect or lack of efficacy? *Brain* **139,** (2016).
337. van Hartevelt, T. J., Fernandes, H. M., Stevner, A. B. A., Deco, G. & Kringelbach, M. L. Neural Plasticity in Human Brain Connectivity: The Effects of Deep Brain Stimulation. *The Rewiring Brain* 527–546 (2017). doi:10.1016/B978-0-12-803784-3.00025-1
338. Eusebio, A. & Brown, P. Synchronisation in the beta frequency-band--the bad boy of parkinsonism or an innocent bystander? *Exp.*

Neurol. **217,** 1–3 (2009).
339. Shreve, L. A. *et al.* Subthalamic oscillations and phase amplitude coupling are greater in the more affected hemisphere in Parkinson's disease. *Clin. Neurophysiol.* **128,** 128–137 (2017).
340. Wilson, C. J., Beverlin, B. & Netoff, T. Chaotic desynchronization as the therapeutic mechanism of deep brain stimulation. *Front. Syst. Neurosci.* **5,** 50 (2011).
341. Montgomery, E. B. *Deep Brain Stimulation Programming*.
342. Pötter-Nerger, M. & Volkmann, J. Deep brain stimulation for gait and postural symptoms in Parkinson's disease. *Mov. Disord.* **28,** 1609–1615 (2013).
343. Varela, F., Lachaux, J.-P., Rodriguez, E. & Martinerie, J. The brainweb: Phase synchronization and large-scale integration. *Nat. Rev. Neurosci.* **2,** 229–239 (2001).
344. Little, S. *et al.* Adaptive deep brain stimulation in advanced Parkinson disease. *Ann. Neurol.* 449–457 (2013). doi:10.1002/ana.23951
345. Rosa, M. *et al.* Adaptive deep brain stimulation controls levodopa-induced side effects in Parkinsonian patients. *Mov. Disord.* **32,** 628–629 (2017).
346. Rosin, B. *et al.* Closed-Loop Deep Brain Stimulation Is Superior in Ameliorating Parkinsonism. *Neuron* **72,** 370–384 (2011).
347. Swann, N. C. *et al.* Adaptive deep brain stimulation for Parkinson's disease using motor cortex sensing. *J. Neural Eng.* **15,** 046006 (2018).
348. Fasano, A., Aquino, C. C., Krauss, J. K., Honey, C. R. & Bloem, B. R. Axial disability and deep brain stimulation in patients with Parkinson disease. *Nat. Rev. Neurol.* **11,** 98–110 (2015).
349. Meidahl, A. C. *et al.* Adaptive Deep Brain Stimulation for Movement Disorders: The Long Road to Clinical Therapy. *Mov. Disord.* **32,** 810–819 (2017).
350. Braak, H. *et al.* Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* **24,** 197–211 (2003).
351. Fuchs E & Flügge G. *Monoaminergic neurons innervate almost all brain areas.* | *Open-i*. (2004).

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VII. Appendix

7.1 List of figures and tables

7.2 List of publications

First, second or last author:

1. **Pozzi, N.G.**, Arnulfo, G., Canessa, A., Steigerwald, F., Nickl, R., Homola, G. A., [...] & Isaias, I. U. (2016). Distinctive neuronal firing patterns in subterritories of the subthalamic nucleus. Clinical Neurophysiology, 127(11), 3387-3393.
2. *Arnulfo, G. and ***Pozzi, N.G.**, Palmisano, C., Leporini, A., Canessa, A., Brumberg, J., [...] & Isaias, I. U. (2018). Phase matters: A role for the subthalamic network during gait. PloS one, 13(6), e0198691.Palmisano, C; Brandt, G; Pozzi, NG; Brumberg, J; Leporini, A; Marotta, G; Cavallari, P; Frigo, CA; Pezzoli, G; Isaias, IU; ,A selective role of striatal dopamine in the timing of anticipatory postural adjustments at gait initiation in patients with Parkinson's disease,Parkinsonism & Related Disorders,46,,e76-e77,2018,Elsevier
3. Picascia, M., **Pozzi, N.G.**., Todisco, M., Minafra, B., Sinforiani, E., Zangaglia, R., [...] & Pacchetti, C. (2018). Cognitive disorders in normal pressure hydrocephalus with initial parkinsonism in comparison with "de novo" Parkinson's disease. European journal of neurology. *In press.*
4. Canessa, A., **Pozzi, N.G.**., Arnulfo, G., Brumberg, J., Reich, M. M., Pezzoli, G., [...] & Isaias, I. U. (2016). Striatal dopaminergic innervation regulates subthalamic beta-oscillations and cortical-subcortical coupling during movements: preliminary evidence in subjects with parkinson's disease. Frontiers in human neuroscience, 10, 611.
5. Reich, M.M.*, Brumberg, J*; **Pozzi, N.G.**; Marotta G; Roothans, J; Åström M; [...] & Isaias, I. U. (2016) Progressive gait ataxia following deep brain stimulation for essential tremor: adverse effect or lack of efficacy? Brain, 139, 2948-2956.
6. Reich, M. M., **Pozzi, N.G.**., Brumberg, J., Åström, M., Volkmann, J., & Isaias, I. U. (2017). Reply: Clinical approach to delayed-onset cerebellar impairment following deep brain stimulation for tremor. Brain, 140(5), e28-e28.
7. **Pozzi, NG**; Pacchetti, C., Back to the future: 30th anniversary of deep brain stimulation for Parkinson's disease. Functional neurology, 32,1,5,2017, CIC Edizioni Internazionali

Co-author:

8. Turco, F., Canessa, A., Olivieri, C., **Pozzi, N.G.**., Palmisano, C., Arnulfo, G., [...] & Isaias, I. U. (2018). Cortical response to levodopa in Parkinson's disease patients with dyskinesias. European Journal of Neuroscience.
9. Sinforiani, E., Pacchetti, C., Picascia, M., **Pozzi, N.G.**., Todisco, M., & Vitali, P. (2018). Clinical and Cognitive Features of Idiopathic Normal Pressure Hydrocephalus. In Hydrocephalus-Water on the Brain. IntechOpen.
10. Cilia, R., Laguna, J., Cassani, E., Cereda, E., **Pozzi, N.G.**., Isaias, I. U., [...] & Pezzoli, G. (2017). Mucuna pruriens in Parkinson disease: a double-blind, randomized, controlled, crossover study. Neurology, 89(5), 432-438.

Studies published before entering the PhD program:

11. Alfonsi, E., Terzaghi, M., Cosentino, G., Tassorelli, C., Manni, R., Pozzi, N., [...] & Fresia, M. (2016). Specific patterns of laryngeal electromyography during wakefulness are associated to sleep disordered breathing and nocturnal stridor in multiple system atrophy. Parkinsonism & related disorders, 31, 104-109.
12. Picascia, M., Minafra, B., Zangaglia, R., Gracardi, L., **Pozzi, N.G.**., Sinforiani, E., & Pacchetti, C. (2016). Spectrum of cognitive disorders in idiopathic normal pressure hydrocephalus. Functional neurology, 31(3), 143.
13. **Pozzi, N.G.**., De Marzi, R., Zangaglia, R., Minafra, B., & Pacchetti, C. (2015). Paroxysmal Dystonia with Axonal Neuropathy Resulting from Benignant Insulinoma: Case Report. Movement Disorders Clinical Practice, 2(1), 69-7
14. Ricca, I., Cereda, C., Bianchi, M., Zangaglia, R., **Pozzi, N.G.**., Picascia, M., [...]& Pacchetti, C. (2014, January). Neuropsychological assessment and clinical description of a patient carrying two GBA gene in cis mutations affected by atypical early-onset parkinsonism and dementia. In JOURNAL OF ALZHEIMERS DISEASE (Vol. 41, pp. S44-S45). NIEUWE HEMWEG 6B, 1013 BG AMSTERDAM, NETHERLANDS: IOS PRESS.

15. T Tinazzi, M., Fasano, A., Geroin, C., Morgante, F., Ceravolo, R., Rossi, S., [...]& Cossu, G. (2015). Pisa syndrome in Parkinson disease An observational multicenter Italian study. Neurology, 85(20), 1769-1779.
16. Minafra, B., Fasano, A., **Pozzi, N.G.**., Zangaglia, R., Servello, D., & Pacchetti, C. (2014). Eight-years failure of subthalamic stimulation rescued by globus pallidus implant. Brain stimulation, 7(2), 179-181.
17. **Pozzi, N.G.**., Minafra, B., Zangaglia, R., De Marzi, R., Sandrini, G., Priori, A., & Pacchetti, C. (2014). Transcranial direct current stimulation (tDCS) of the cortical motor areas in three cases of cerebellar ataxia. The Cerebellum, 13(1), 109-112.
18. Tassorelli, C., De Icco, R., Alfonsi, E., Bartolo, M., Serrao, M., Avenali, M., [...]& Nappi, G. (2014). Botulinum toxin type A potentiates the effect of neuromotor rehabilitation of Pisa syndrome in Parkinson disease: a placebo controlled study. Parkinsonism & related disorders, 20(11), 1140-1144.
19. Valentino, F., Cosentino, G., Brighina, F., **Pozzi, N.G.**., Sandrini, G., Fierro, B., ... & Pacchetti, C. (2014). Transcranial direct current stimulation for treatment of freezing of gait: A cross- over study. Movement Disorders, 29(8), 1064-1069.

7.3 Curriculum Vitae

7.4 Printouts of the articles published during the PhD-program

- **Pozzi, N.G.**, Arnulfo, G., Canessa, A., Steigerwald, F., Nickl, R., Homola, G. A., [...] & Isaias, I. U. (2016). Distinctive neuronal firing patterns in subterritories of the subthalamic nucleus. Clinical Neurophysiology, 127(11), 3387-3393.

- *Arnulfo, G. and ***Pozzi, N.G.**, Palmisano, C., Leporini, A., Canessa, A., Brumberg, J., [...] & Isaias, I. U. (2018). Phase matters: A role for the subthalamic network during gait. PloS one, 13(6), e0198691.Palmisano, C; Brandt, G; Pozzi, NG; Brumberg, J; Leporini, A; Marotta, G; Cavallari, P; Frigo, CA; Pezzoli, G; Isaias, IU; ,A selective role of striatal dopamine in the timing of anticipatory postural adjustments at gait initiation in patients with Parkinson's disease,Parkinsonism & Related Disorders,46,,e76-e77,2018,Elsevier

- Picascia, M., **Pozzi, N.G.**., Todisco, M., Minafra, B., Sinforiani, E., Zangaglia, R., [...] & Pacchetti, C. (2018). Cognitive disorders in normal pressure hydrocephalus with initial parkinsonism in comparison with "de novo" Parkinson's disease. European journal of neurology. *In press.*

- Canessa, A., **Pozzi, N.G.**., Arnulfo, G., Brumberg, J., Reich, M. M., Pezzoli, G., [...] & Isaias, I. U. (2016). Striatal dopaminergic innervation regulates subthalamic beta-oscillations and cortical-subcortical coupling during movements: preliminary evidence in subjects with parkinson's disease. Frontiers in human neuroscience, 10, 611.

- Reich, M.M.*, Brumberg, J*; **Pozzi, N.G.**; Marotta G; Roothans, J; Åström M; [...] & Isaias, I. U. (2016) Progressive gait ataxia following deep brain stimulation for essential tremor: adverse effect or lack of efficacy? Brain, 139, 2948-2956.

- Reich, M. M., **Pozzi, N.G.**., Brumberg, J., Åström, M., Volkmann, J., & Isaias, I. U. (2017). Reply: Clinical approach to delayed-onset cerebellar impairment following deep brain stimulation for tremor. Brain, 140(5), e28-e28.

- **Pozzi, NG**; Pacchetti, C., Back to the future: 30th anniversary of deep brain stimulation for Parkinson's disease. Functional neurology, 32,1,5,2017, CIC Edizioni Internazionali

Affidavit

I hereby confirm that my thesis entitled Parkinson's disease revisited: multiple circuitopathies is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Wuerzburg, 28.02.19
Place, Date Signature

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation Neuinterpretation des Morbus Parkinson als multiple Netzwerkerkrankungen eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Wuerzburg, 28.02.19
Ort, Datum Unterschrift