Electrophysiological dissection of the neurophysiological and neuromuscular correlates of freezing phenomena in Parkinson's disease

Dissertation

zur Erlangung des Grades eines
Doktors der Naturwissenschaften

der Mathematisch-Naturwissenschaftlichen Fakultät
und
der Medizinischen Fakultät
der Eberhard-Karls-Universität Tübingen

vorgelegt
von

Marlieke Ardine Scholten
aus Groesbeek, Niederlande

Januar 2017

Tag der mündlichen Prüfung: 28-7-2017

Dekan der Math.-Nat. Fakultät: Prof. Dr. W. Rosenstiel

Dekan der Medizinischen Fakultät: Prof. Dr. I. B. Autenrieth

1. Berichterstatter: Prof. Rejko Krüger
2. Berichterstatter: Prof. Christoph Braun

Prüfungskommission: Prof. Rejko Krüger

Prof. Christoph Braun

Prof. Andreas Nieder

PD Dr. Daniel Weiss

Erklärung / Declaration:

Ich erkläre, dass ich die zur Promotion eingereichte Arbeit mit dem Titel:

„Electrophysiological dissection of the neurophysiological and neuromuscular correlates of freezing phenomena in Parkinson's disease"

selbstständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet habe. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

I hereby declare that I have produced the work entitled "Electrophysiological dissection of the neurophysiological and neuromuscular correlates of freezing phenomena in Parkinson's disease", submitted for the award of a doctorate, on my own (without external help), have used only the sources and aids indicated and have marked passages included from other works, whether verbatim or in content, as such. I swear upon oath that these statements are true and that I have not concealed anything. I am aware that making a false declaration under oath is punishable by a term of imprisonment of up to three years or by a fine.

Tübingen,……..... .....

Unterschrift / Signature

Contents

List of abbreviations

DBS	deep brain stimulation
EEG	electroencephalography
fMRI	functional magnetic-resonance imaging
FOG	freezing of gait
PD	Parkinson's disease
MLR	mesencephalic locomotor region
PPN	pedunculopontine nucleus
SNr	substantia nigra pars reticulata
STN	subthalamic nucleus
ULF	upper limb freezing

1. Summary

1.1 Abstract

Freezing phenomena including freezing of gait (FOG) highly disturb the quality of life in patients with Parkinson's disease (PD). Conventional therapy, like L-DOPA and deep brain stimulation of the subthalamic nucleus (STN-DBS) cannot satisfactorily relieve these symptoms, although it ameliorates most motor symptoms like rigidity and bradykinesia. These motor symptoms are primarily caused by degeneration of dopaminergic cells in the substantia nigra, which induces pathologically increased inhibitory output from the basal ganglia. STN-DBS may reduce the excessive basal ganglia output, however the working mechanism of STN-DBS and the influence on the neuromuscular network effects need further elucidation. In this context, we found that STN-DBS could lower pathologically increased low-frequency intermuscular synchronization during continuous finger tapping. Furthermore, STN-DBS strengthened the corticospinal connection during continuous finger tapping, displayed by the increased corticomuscular coherence in the tapping frequency.

With respect to freezing phenomena, it is necessary to further elucidate the pathophysiological mechanism. We therefore introduced a dual task to trigger upper limb freezing (ULF). Offline we defined criteria to detect freezing episodes from the biomechanical recording. We observed that during ULF cortical activity was increased in the alpha band compared to continuous finger tapping. During ULF, this increased alpha cortical activity started over the contralateral sensorimotor cortex and spread to the contralateral frontal cortex and the ipsilateral parietal cortex during the freezing episode. Furthermore, we observed that a higher number of ULF episodes was associated with an increased cortico-cortical beta synchronization. These findings – increased alpha activity and increased beta synchronization – could probably function as biomarker to predict freezing phenomena. This will enable us to decipher pathophysiological mechanisms of freezing phenomena further. Consequently, using this knowledge may improve therapy to meet the therapeutic need.

1.2 Zusammenfassung

‘Freezing’-Phänomene einschließlich Gang ‘Freezing’ (FOG) stören die Lebensqualität erheblich bei Patienten mit der Parkinson-Krankheit (PD). Mit konventionellen Therapien wie L-DOPA und die Tiefe Hirnstimulation des Nucleus Subthalamicus (STN-DBS) sind ‘Freezing’-Phänomene nur eingeschränkt behandelbar, obwohl sie die meisten Motorsymptome wie Rigor und Bradykinesie zufriedenstellend verbessern. Diese motorischen Symptome werden vor allem durch Degeneration der dopaminergen Zellen in der Substantia nigra verursacht, die eine pathologisch erhöhte Hemmung der Basalganglien induziert. STN-DBS kann den exzessiven Basalganglienausstoß verringern, jedoch sind der genaue Wirkmechanismus von STN-DBS und der Einfluss auf die neuromuskulären Netzwerkeffekte unvollständig charakterisiert. In diesem Zusammenhang stellten wir fest, dass STN-DBS die pathologisch verstärkte niederfrequente intermuskuläre Synchronisation während kontinuierlichem Finger-Tapping verringert. Darüber hinaus konnte STN-DBS die kortikospinale Synchronisation während kontinuierlichem Finger-Tapping stärken. Dies war anhand einer erhöhten kortikomuskulären Kohärenz in der Tappingfrequenz ersichtlich.

Hinsichtlich der ‘Freezing’-Phänomene ist es notwendig, den pathophysiologischen Mechanismus aufzuklären. Deswegen führten wir eine sogenannte Dual-Tasking-Aufgabe ein, um die Anzahl der ‘Freezing’-Phänomene bei Bewegungen der obere Extremität (ULF) zu steigern. Offline haben wir Kriterien definiert, um die ULF aus der biomechanischen Aufzeichnung zu detektieren. Wir beobachteten, dass während der ULF die kortikale Aktivität im Alpha-Band gegenüber dem kontinuierlichen Finger-Tapping erhöht war. Diese erhöhte alpha-kortikale Aktivität nahm über dem kontralateralen sensomotorischen Kortex ihren Ausgang und breitete sich während der ULF zum kontralateralen frontalen Kortex und ipsilateralen parietalen Kortex aus. Außerdem beobachteten wir, dass eine höhere Anzahl von ULF-Episoden mit einer erhöhten kortiko-kortikalen Beta-Synchronisation einherging. Diese spektralen Marker – erhöhte Alpha-Aktivität und erhöhte kortiko-kortikale Beta-Synchronisation – könnten möglicherweise als Biomarker fungieren, um ULF vorherzusagen. Dies ermöglicht es uns, pathophysiologische Mechanismen der ‘Freezing’-

Phänomene weiter zu entschlüsseln. Folglich können wir mit diesem Wissen die Therapie verbessern, um den therapeutischen Bedarf zu decken.

2. Synopsis

2.1. Parkinson's disease

2.1.1 Overview

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (de Lau and Breteler, 2006). It affects about 1% of the people above age 65 worldwide. In Europe, the approximate incidence rate is 5/100,000 per year (Von Campenhausen *et al.*, 2005). However, PD becomes increasingly prevalent with age. In this respect, the prevalence rises from 113/100,000 with age 50-59 years to 2953/100,000 with age 80+ years with a slightly higher prevalence of PD in men than in woman (Pringsheim *et al.*, 2014; Taylor *et al.*, 2007). Research to improve both the pathophysiological understanding of PD and therapy is important, because the aging world population increases the social and economic burden.

The etiology of PD remains largely unknown, however the genetic background is partly established. Approximately 5-10 % of the cases can be explained by monogenic autosomal dominant or autosomal recessive inheritance (Kalinderi *et al.*, 2016; Sharma *et al.*, 2012; Schiesling *et al.*, 2008). Monogenic disease is often characterized by an early onset (between 20 and 50 years) and a milder progression, especially in autosomal recessive inheritance. Nevertheless, the majority of the PD patients are sporadic, probably reflecting complex interaction of multiple genetic and environmental factors. These sporadic PD patients yield high clinical heterogeneity represented by the multiple genetic mutations discovered that may increase the susceptibility to PD (Schulte and Gasser, 2011). The most robust and consistently replicated associations have been found for *α-synuclein*, *MAPT*, *LRRK2,* and *GBA* (Kalinderi *et al.*, 2016).

PD is characterized by four cardinal motor symptoms: tremor at rest (frequency 4-6 Hz), rigidity, bradykinesia (or akinesia), and postural instability (Jankovic, 2008). Rest tremor typically disappears during movement or sleep. Rigidity is characterized as resistance and stiffness against movement of a joint. Bradykinesia is often the most prominent symptom and refers to slowness of movements. The

fourth cardinal symptom, postural instability, typically occurs in a later stage of the disease and causes impaired balance which may lead to falls. Other classic features of PD are a flexed posture and freezing of gait (FOG). Non-motor symptoms are more diverse among PD patients, although the most common are autonomic dysfunction, neuropsychiatric disorders, and sensory and sleep abnormalities (Chaudhuri and Schapira, 2009).

2.1.2 Pathophysiology and therapy

PD relates to degeneration of dopamine neurons in the substantia nigra. The substantia nigra is part of the basal ganglia, a diverse set of nuclei that amongst others regulate upper motor neuron circuits to initiate or suppress movements. Within the basal ganglia, a dopaminergic nigrostriatal pathway projects from the substantia nigra pars compacta to the striatum (consisting of the caudate nucleus and putamen), that controls a motor loop of neurons feeding forward to the motor cortex (Fitzgerald *et al.*, 2007a). The basal ganglia initiate movements and suppress unwanted movements via the direct and indirect pathway within the basal ganglia, which are in net effect excitatory (bearing dopamine-1 receptors) or inhibitory (bearing dopamine-2 receptors), respectively. In PD, the loss of dopamine may lead to an imbalance of the direct and indirect pathway interplay, with a preponderance of indirect pathway activation (Obeso *et al.*, 2008). The tonically active substantia nigra pars compacta loses activity and this attenuates the tonic facilitation of the D1 receptors and reduces the tonic inhibition of the D2 receptors. Thereby, the direct pathway is disengaged, the indirect pathway is activated by default (Figure 1). This automatic engagement of the indirect pathway disinhibits neurons of the subthalamic nucleus (STN) which increases the inhibition from the globus pallidus interna to the thalamocortical pathway, decreasing the thalamocortical motor output.

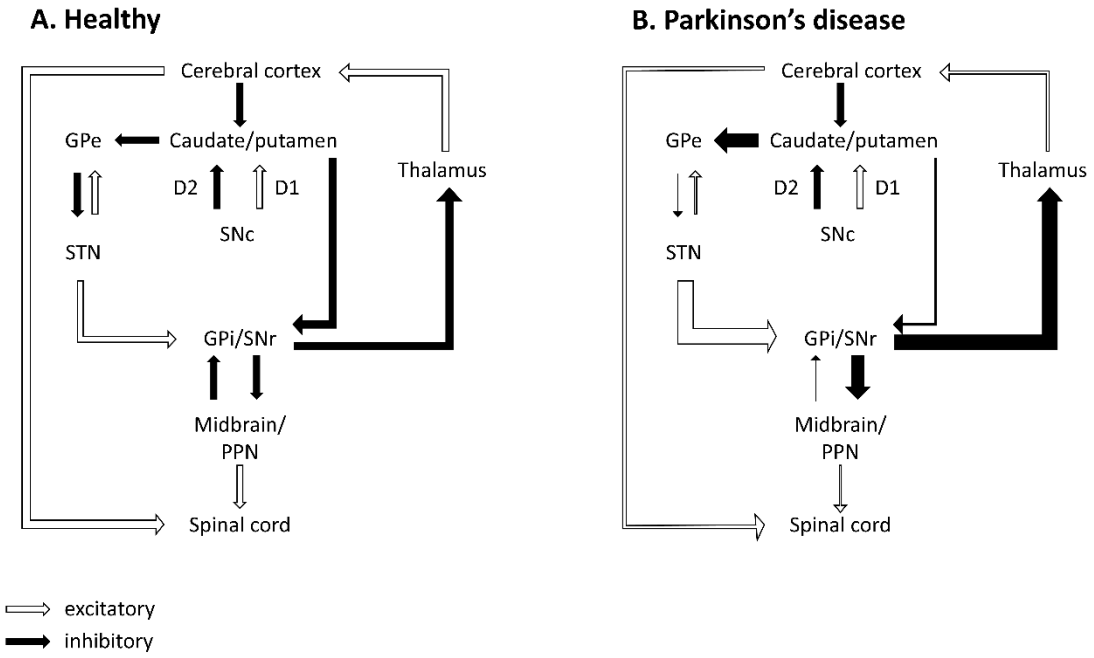

Figure 1 Model of the basal ganglia motor network in healthy subjects (A) and in PD (B) by degeneration of the dopamine neurons in the SNc.

Abbreviations: GPe = Globus pallidus, external segment; STN = Subthalamic nucleus; GPi = Globus pallidus, internal segment; SNr = Substantia nigra pars reticulata; SNc = Substantia nigra pars compacta; PPN = pedunculopontine nucleus (Krack *et al.*, 2010; Purves *et al.*, 2008).

It is thought that PD originates from a progressive failure of dopaminergic transmission. It is estimated that already 60% percent of the neurons in the substantia nigra pars compacta are lost when the first motor symptoms appear (Dauer and Przedborski, 2003). Although the motor symptoms of PD suggest a sole brain disease, there is evidence that pathological changes start in the olfactory bulb and enteric nerve plexus and then transfers via the dorsal motor nucleus of the vagus nerve to the lower brain stem and finally the midbrain (Hawkes *et al.*, 2007). This process has been divided in six stages and the starting olfactory or enteric origin would elucidate the pre-symptomatic non-motor symptoms, such as olfactory dysfunction and gastro-intestinal disorders (Braak *et al.*, 2003; Rey *et al.*, 2016). Moreover, emerging evidence point to additional neurodegeneration of various non-dopaminergic neurotransmitter systems (Stayte and Vissel, 2014).

Hitherto, the therapy of PD consists of symptomatic treatment strategies and modification of the disease course remains unachieved. Although progress has been made in symptomatic treatment, L-DOPA still controls the symptoms most effectively (Jankovic and Aguilar, 2008). L-DOPA is a precursor of dopamine and can pass the blood-brain barrier, unlike dopamine itself. In addition, dopamine agonist, catechol-o-methyl-transferase inhibitors and non-dopaminergic agents can relieve the symptoms. At young age, dopamine agonists are often preferred, because a long-term use of L-DOPA tends to increase the incidence of motor fluctuations including dyskinesia (Jankovic and Stacy, 2007; Marsden, 1994). L-DOPA particularly treats bradykinesia. In the more advanced disease stages, the therapeutic window becomes smaller, as is apparent by motor fluctuations including dyskinesia (Jankovic, 2005).

Therefore, in intermediate disease stages dominated by motor fluctuations, high frequency stimulation of the STN can stabilize motor symptom control and quality of life (Deuschl *et al.*, 2006; Schuepbach *et al.*, 2013). Deep brain stimulation (DBS) of the STN ameliorates bradykinesia, tremor, and rigidity as well as motor fluctuations (Kleiner-Fisman *et al.*, 2006) and, thus, enormously improves the quality of life (Deuschl *et al.*, 2006).

2.1.3 Mechanisms of subthalamic deep brain stimulation

DBS was first introduced in 1987 by implanting an electrode in the thalamic nucleus ventralis intermedius to suppress tremor (Benabid *et al.*, 1987). In 1993, a PD patient was implanted in the STN for the first time (Pollak *et al.*, 1993). There is an ongoing debate on the presumably multifold working mechanisms of DBS. The former invasive effective treatment therapy for PD was functional ablation. Since DBS mimics the effects of functional ablation, the hypothesis put forward that DBS inhibits the local neurons around the active contact(s) of the electrode (Agnesi *et al.*, 2013). However, some neurons show higher firing rates in direct surrounding of the electrode and the output of the stimulated nucleus is not decreased (Okun, 2012). Indeed, high frequency stimulation

of the STN activates the axonal output of the nucleus, such that the globus pallidus externus and internus exhibit increased firing rates time-locked to the stimulation pulse train (Johnson *et al.*, 2008). Furthermore, DBS has not only a local effect, but it affects the whole basal ganglia-thalamo-cortical network. The inhibition of the STN overactivity may strengthen the cortical control and thereby improve motor function (Kühn *et al.*, 2008; Salenius *et al.*, 2002; Weiss *et al.*, 2012).

Overactivity of the STN involves motor impairment caused by inhibited motor cortical activation (Devos *et al.*, 2004). The local field potential of the STN exhibit pathophysiological increased beta activity during overactivity, which can be reduced by movement, L-DOPA, or STN-DBS (Brown *et al.*, 2001; Giannicola *et al.*, 2010; Kuhn *et al.*, 2004). Furthermore, this reduction is associated with clinical improvement (Kühn *et al.*, 2008).

Also, in the cortex we observed a decreased beta cortico-cortical synchronization with therapy and this was associated with motor improvement (Findings can be found in chapter 5.2: Weiss et al., 2015: Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease). Cortico-cortical synchronization can be computed from a non-invasive multichannel electroencephalography (EEG). EEG is characterized by its sinusoidal waveforms, which can be divided in different frequency bands. These oscillations are measured in microvolt and is the additive effect of interactive cortical pyramidal neurons arranged in neighboring cortical cell columns. These interactions cause current flowing and EEG measures the current of the extracellular space (Fitzgerald *et al.*, 2007b).

Cortico-cortical synchronization is a measure of neuronal synchronization and is caused by communication of different brain areas. These local neuron ensembles integrate complex information that proved useful for neuronal processing and integration.

Using EEG and electromyographic recordings we observed pathophysiological neuromuscular correlates modulated by STN-DBS in PD patients. Therefore, we measured PD patients with the STN-DBS turned ‘On’ and ‘Off’, as well as age- and gender matched healthy controls during the

performance of finger tapping of the right index finger (Findings can be found in chapter 5.3: Scholten et al., 2016: Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease). We observed pathologically increased low frequency intermuscular synchronization between the agonist and antagonist muscle in PD patients 'Off' stimulation and this was lowered by STN-DBS. A high intermuscular synchronization might reflect common supraspinal input to the muscles. However, corticomuscular coherence was absent in this low frequency range excluding the cortex as direct possible supraspinal generator. Alternatively, spinal motor neurons might receive subcortical inputs conveying pyramidal motor output, e.g. through the nigro-reticulo-spinal pathway. This nigro-reticulo-spinal pathway can be influenced by dopaminergic medication (Delwaide *et al.*, 1993) and STN-DBS (Pötter-Nerger *et al.*, 2008), which may explain the decrease of intermuscular coherence when the STN-DBS was turned on.

Another interesting finding was the depressed corticomuscular coherence around the individual tapping frequency in PD patients as compared to healthy controls. Here, STN-DBS could enhance the corticomuscular coherence towards the level observed in healthy controls. Increased corticomuscular coherence indicates increased corticospinal contribution to spinal motor output, i.e. STN-DBS strengthened motor integration upon the corticospinal pathway. This is plausible, because STN-DBS inhibits the overactivity of the STN, thereby enhancing the activity in the STN-thalamo-cortical pathway (Limousin *et al.*, 1997).

2.2. Freezing phenomena

Festination of gait, often a precursor of FOG, was described for the first time by James Parkinson in 1817 in his work 'shaking palsy': *'…a propensity to bend the trunk forwards, and to pass from a walking to a running pace…'* (Parkinson, 2002). In 1877, Charcot is the first to describe festination at gait initiation, although the description is very terse: *'…this irresistible tendency to adopt a running pace depend exclusively on the centre of gravity being displayed forward by the inclination of the head and body. These are not constant and necessary phenomena; they are even frequently enough absent…'* (Charcot, 1877). Already in 1954, it was recognized that freezing phenomena are not restricted to gait: Schwab described the occurrence of what he termed 'motor blocks' in the upper limb, especially when accompanied by a dual task (Schwab *et al.*, 1954). In 1973, the freezing phenomenon itself was described by Andrews, associating it with a form of dystonia including co-contraction of the agonist and antagonist (Andrews, 1973).

Since then not many reports referred to freezing. Only in the last 15 years a growing interest in freezing phenomena and its underlying pathophysiology emerged. Freezing phenomena are characterized by an episodic inability to produce effective movement during the initiation or repetition of a movement. In FOG, patients report about their feet like glued on the ground, making it impossible to move forward effectively. Such episodes are characterized by a combination of frequency increment and amplitude decrease during a repetitive movement, such as walking in the so-called 'trembling-in-place-like freezing'. They are not restricted to idiopathic PD, but can also be recognized in atypical Parkinsonism like progressive supranuclear palsy and multisystem atrophy, or in focal lesions of specific brain structures such as brainstem, basal ganglia, or the frontal lobe (Nutt *et al.*, 2011).

FOG is typically more preponderant during restriction of space, such as in turning or in approaching an object or a narrow doorway. In addition, FOG can be triggered by increased cognitive load like dual tasking. It can often be prevented by sensory triggers, such as white stripes on the floor or an acoustic signal. In general, divided attention triggers FOG and focused attention on gait can alleviate

FOG (Bloem *et al.*, 2004; Rubinstein *et al.*, 2002). Due to its emergence in many common daily life activities, FOG interferes massively with the patients' mobility and therefore deteriorates the patient's quality of life (Moore *et al.*, 2007).

Many PD patients experience freezing and about 38% suffer regularly from FOG (Perez-Lloret *et al.*, 2014). As PD progresses, FOG is experienced more often. This aligns to decreased quality of life in patients suffering from FOG (Perez-Lloret *et al.*, 2014). FOG is one of the main reasons for falls, and can be explained by the fact that the feet stuck while the center of gravity proceeds forwards at the same time. In addition, most of the patients suffering from FOG have concomitant balance problems as further mechanism behind falls (Bloem *et al.*, 2004).

In the literature, a clinical working definition was proposed to capture FOG: it refers to 'an episodic inability to generate effective stepping in the absence of any known cause other than Parkinsonism or higher-level gait disorders. It is most commonly experienced during turning and step initiation, but also when faced with spatial constraint, stress and distraction. Focused attention and external stimuli (cues) can overcome the episode' (Giladi and Nieuwboer, 2008; Nutt *et al.*, 2011). Freezing phenomena typically last for several seconds but rarely last longer than thirty seconds. Freezing is generally categorized into at least three common phenomenologies: *i*) complete motor block also called 'akinesia', *ii*) trembling movement of the leg on the place, and *iii*) trembling in place with minimal forward progression (Schaafsma *et al.*, 2003).

2.2.1 Physiology of gait

Most of the knowledge about the hierarchical networks involved in the physiological control of gait was obtained from animal studies, mainly in the cat. Gait is initiated by either volitional processing in the cerebral cortex or emotional stimuli in the limbic system. After initiation, automatic processes in the cerebellum, basal ganglia, and brainstem accompany the initiated locomotion by projecting via descending pathways to the spinal cord in order to control posture appropriately (Takakusaki *et al.*,

2008). Animal studies showed that neural networks in the spinal cord can generate rhythmic limb movements when externally stimulated (Mori, 1987). These central pattern generators consist of organized groups of interneurons (Hagglund *et al.*, 2010). Human beings exhibit also central pattern generators, as shown by complete paraplegic patients. They demonstrate a patterned, locomotor-like activity when stimulating the lumbosacral spinal cord (Dimitrijevic *et al.*, 1998). The mesencephalic locomotor region (MLR) located in the midbrain of the brainstem projects via the medial reticular formation to the spinal locomotor system (Shumway-Cook and Woollacott, 2007) and is considered as the main locomotor center. It receives excitatory input from the cerebral cortex (mainly supplemental motor area and premotor cortex), limbic system, and cerebellum, as well as inhibitory input from the basal ganglia (Takakusaki, 2013). Predictive modulation of the step cycle is controlled by the cerebellum, which receives proprioceptive information via the spinocerebellar tract from peripheral nerves, muscles, joints, and skin.

2.2.2 Gait in Parkinson's disease

In contrast to other cardinal symptoms of PD, gait problems and postural instability respond poorly to dopaminergic medication, particularly in advanced disease stages. In the early disease stage dopaminergic medication can improve gait by increasing the stride length and stride velocity (Blin *et al.*, 1991). However, as disease progresses, dopaminergic medication often fails to improve gait (Grabli *et al.*, 2012) and this strengthens the view that the pathophysiology of PD also involves non-dopaminergic networks. Candidates are the cholinergic and glutamatergic networks within the MLR, which are located in the brainstem. The increased output from the main basal ganglia output centers in PD suppresses the activity in the MLR and pedunculopontine nucleus (PPN) within the brainstem. This suppresses locomotion and increases the postural muscle tone (Takakusaki *et al.*, 2003, 2011). Furthermore, a cholinergic lesion in the PPN of healthy monkeys caused a reduction of the step length and step velocity during locomotion. In PD, PPN cholinergic neurons degenerate over time (Hirsch *et al.*, 1987; Zweig *et al.*, 1989). This emphasizes that PPN cholinergic depletion could

associate to pathological locomotor integration in PD. The PPN is influenced by the basal ganglia, however a direct glutamatergic pathway from the STN to the PPN was not found in humans (Pahapill and Lozano, 2000). The effect of STN-DBS on gait is controversial. Overall, it may have a positive effect on gait speed (Roper *et al.*, 2016). Nevertheless, FOG lacks therapeutic benefit from STN-DBS in a substantial proportion of patients (Collomb-Clerc and Welter, 2015; Vercruysse *et al.*, 2014).

Biomechanical measures can improve the methodology of a study by providing objective and sensitive measures to observe gait improvement. They show us that STN-DBS can substantially improve the spatial parameters, such as stride length and stride velocity, but often not the temporal parameters, such as stride time (Faist, 2001; Ferrarin *et al.*, 2005). Furthermore, STN-DBS decreases the gait variability and gait asymmetry (Johnsen *et al.*, 2009), which have been associated with the occurrence of FOG (Fasano *et al.*, 2011; Plotnik *et al.*, 2008; Plotnik and Hausdorff, 2008). On the other hand, STN-DBS surgery may worsen the dynamic postural control (St George *et al.*, 2014), which is an important function in order to keep balance during perturbed standing.

In our experiment, we compared gait between stimulation turned on and off in PD patients. Therefore, we used three small wearable inertial sensors attached to the ankles and the lumbar region. These sensors comprise a three axial accelerometer, gyroscope, and magnetometer, which enabled us to compute accurately different gait parameters as stride length, stride time, and stride velocity. We found that STN stimulation significantly improve the stride length and the variability of the stride length, as well as gait asymmetry (Findings can be found in chapter 5.5: Scholten et al., *in prep*: Effects of subthalamic and nigral stimulation on gait in Parkinson's disease).

Enlarging the understanding of the pathophysiological working mechanisms of locomotion is unfortunately growing slowly, also due to the ambulatory character of gait, which impedes imaging of the brain. Therefore, previous imaging studies used virtual reality paradigms in the functional magnetic resonance imaging (fMRI) scanner to disentangle the locomotor network in PD patients. It is well known that the execution and imagery of a task show substantially overlapping neuronal

networks (Stinear *et al.*, 2006). These fMRI studies revealed that besides decreased perfusion of the MLR, gait disturbances in PD are associated with decreased posterior parietal perfusion (Karachi *et al.*, 2012; Snijders *et al.*, 2011). The posterior parietal cortex enables motor adaptation by modifying the walking pattern based on the visual input (Crémers *et al.*, 2012; Drew *et al.*, 2008). However, more work is needed to disentangle the pathophysiological mechanisms of gait in PD in order to optimize current therapies.

2.2.3 Detecting freezing of upper limb

We defined criteria to detect freezing of upper limb in a tapping task. Therefore, we instructed the subjects to tap continuously as quickly as possible with the right index finger. The exerted pressure of tapping was recorded by a force transducer. We defined biomechanical constraints to detect freezing phenomena. Similar to FOG, freezing phenomena are labeled as an episodic, unpredictable, and variable presentation. The gold standard to detect FOG is clinical observation of video recordings by an experienced neurologist. A frequently occurring subtype of FOG, trembling in place, can be identified by an increase in frequency of the horizontal acceleration measured on the legs. Normal gait is characterized by a main frequency between 0-3 Hz. In FOG episodes, the frequency spectrum increases to 3-8 Hz. Although it is unclear whether FOG and upper limb freezing (ULF) share the same neuropathological correlates (Barbe *et al.*, 2014; Nieuwboer *et al.*, 2009), ULF and FOG share the same clinical characteristics, i.e. an increased frequency and decreased amplitude. Therefore, we defined in our work criteria to detect ULF based on these characteristics: *i)* the amplitude deflection of the finger tap decreased over 50%, *ii)* the frequency of motor output increased above 3 Hz, and *iii)* the duration exceeded one second (Figure 1 in chapter 5.3: Scholten et al., 2016: Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease).

2.2.4 Understanding freezing of upper limb

We detected episodes of ULF and regular tapping in the biomechanical signal and used them to observe the cortical signature from the EEG, which was synchronized to the biomechanical signal. During ULF, alpha activity (7-11 Hz) increased over the contralateral frontal, motor and parietal

cortex. Time-frequency analyses indicated that during ULF alpha activity increase started in the left motor cortex (contralateral to hand movement) and spread to left frontal and right parietal areas. Cortical alpha activity may reflect inhibition of unwanted movement, appearing mainly over the bilateral sensorimotor cortices during suppression of a self-paced finger movement (Sauseng *et al.*, 2013). Moreover, increased alpha activity over the sensorimotor cortex was associated with reduced corticospinal excitability, as was suggested from TMS studies (Sauseng *et al.*, 2009). In addition, alpha activity indicates increased focused attention on motor related processes (Cooper *et al.*, 2003; Klimesch *et al.*, 2007). This may be accomplished by active inhibition of cortical regions irrelevant for the execution of the task (Jensen and Mazaheri, 2010). This may explain why we observed an increased alpha activity during ULF as compared to regular tapping.

On the other hand, distraction as is present in dual tasking, triggers the occurrence of ULF episodes. We observed the cortical signatures during a dual task as compared to finger tapping only (single task) (Findings can be found in chapter 5.4: Scholten et al., 2016: Cortical correlates of susceptibility to upper limb freezing in Parkinson's disease). We focused on cortical activity and long-range cortico-cortical synchronization. We found a significant increase of beta range cortico-cortical synchronization over the left prefrontal cortex during dual tasking compared to single tasking. Furthermore, this increase was significantly correlated with the number of ULF episodes observed. This means that patients with a higher cortico-cortical synchronization in the beta band express a higher susceptibility for ULF episodes. Cortical beta synchronization may indicate an inhibition of the motor output, which can be caused by turning off the STN-DBS (Weiss *et al.*, 2015). In addition, increased beta synchronization was observed at the level of the STN and cortex and could be associated with impaired motor symptoms (Kühn *et al.*, 2008). The beta synchronization could be decreased with L-DOPA or STN-DBS. We observed the increased beta synchronization over the left prefrontal cortex and this may suggest a link to executive dysfunction, expressed by impaired movement automaticity (Taylor *et al.*, 1986). Furthermore, atrophy of the prefrontal cortex was linked to FOG (Kostic *et al.*, 2012).

2.2.5 Treating FOG

Although many studies were performed in relation to FOG, the treatment remains challenging. Medical treatment and DBS are not always effective, especially in the advanced disease stage and present an unmet therapeutic need.

However, different stimulation configuration options to improve axial disability and FOG have been reported. An elementary configuration is the 'better side reduction', which intends to decrease the amplitude voltage of the non-disease dominant side. As motor symptoms of PD are generally presented asymmetric, optimization of movement symmetry may improve gait. Therefore, it is favored to increase stimulation amplitude for the more severely affected leg and reduce the stimulation amplitude for the less severely affected leg (Fasano *et al.*, 2011). This strengthens the hypothesis that FOG is related with gait asymmetry, as higher gait asymmetry was found in PD patients with FOG compared to PD patients without FOG (Plotnik *et al.*, 2005).

Besides changing the voltage, other studies emphasized the effect of 'low frequency' 60 Hz stimulation. Although short-term effects are promising on FOG, the segmental symptoms worsened and thus will not be applicable for long term stimulation in a relevant proportion of patients (Moreau *et al.*, 2008). Moreover, tolerance of the effect on FOG was observed around six weeks from reprogramming (Ricchi *et al.*, 2012).

Further, the PPN within the dorsolateral mesencephalic tegmentum has been proposed as a relatively new target to treat gait disturbances including FOG (Breit *et al.*, 2006; Rauch *et al.*, 2010). In PD patients, a more severe loss of cholinergic neurons within the PPN have been be associated with gait disturbances and more frequent falling (Bohnen *et al.*, 2013; Bohnen and Albin, 2011; Karachi *et al.*, 2010). In animal models, electrical stimulation of the PPN induces a rhythmic stepping behavior (Karachi *et al.*, 2010). Low frequency stimulation of the PPN is thought to increase the neuronal activity and has been tested in PD patients with gait disturbances. However, the results are

controversial and PPN stimulation not consistently improve parkinsonian symptoms (Ferraye *et al.*, 2010; Moro *et al.*, 2010; Welter *et al.*, 2015), although a subjective improvement on FOG have been reported (Ferraye *et al.*, 2010; Thevathasan *et al.*, 2011; Welter *et al.*, 2015). Consequently, the patients eligible for this target as well as the clinical endpoints have to be refined.

Another entry point to access the brainstem locomotor area is the substantia nigra pars reticulata (SNr). In PD, the overactivity of the SNr may decrease the neuronal activity of the PPN (Karachi *et al.*, 2010; Pahapill and Lozano, 2000). Consequently, this might impede the control of the locomotor pattern modulated by the PPN. However, the SNr not only projects to the locomotor region and spinal cord, but also has ascending projections within the thalamo-cortical network. In our recent study, we observed the effect of concomitant STN and SNr stimulation. With this combined stimulation we co-stimulate segregate functional motor loops (Findings can be found in chapter 5.1: Weiss et al., 2013: Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial). With a frequency of 130 Hz, the SNr will be inhibited, and this may result in disinhibition of the PPN. This concomitant stimulation of the two nuclei may be advantageous since the primarily effective STN stimulation can be held constant, which implies a constant control of segmental symptoms. We observed that concomitant nigral stimulation did not improve overall axial disability as primary endpoint. However, it might benefit resistant FOG (Weiss *et al.*, 2013), as was suggested from the secondary endpoint analyses. A follow-up double-blind multi-center randomized controlled trial is under way to evaluate this preliminary finding (https://clinicaltrials.gov/show/NCT02588144, Combined Stimulation of STN and SNr for Resistant Freezing of Gait in Parkinson's Disease (STN+SNr)).

From animal experiments among mouse and monkeys we obtained that SNr is integrated in different network loops (Kitano *et al.*, 1998; Sherman *et al.*, 2015). In human, the network connections of SNr are hardly studied. One recent work in PD patients describes the differential effects of STN and SNr stimulation, where SNr improved the axial symptoms (Chastan *et al.*, 2009). Therefore, we aim to differentiate the effects of STN (stimulation of upper contact) respectively SNr stimulation

(stimulation of lower contact) on gait and the associated parameters. In this experiment, we used three small wearable inertial sensors attached to the ankles and the lumbar region. These sensors comprise a three axial accelerometer, gyroscope, and magnetometer, which enabled us to compute accurately different gait parameters as stride length, stride time, and stride velocity. Not only general spatio-temporal parameters can be determined, also gait asymmetry can be investigated. After analysis, we observed that STN stimulation significantly improved the stride length and the variability of the stride length (Findings can be found in chapter 5.5: Scholten et al., *in prep*: Effects of subthalamic and nigral stimulation on gait in Parkinson's disease). Both STN and SNr stimulation improved gait asymmetry. Said so, SNr stimulation may yield synergistic effect on STN stimulation, however, this will not exclude additional modulation of nigro-pontine locomotor contributions. This is subject to ongoing follow-up studies.

2.3. Outlook

We identified electrophysiological biomarkers of freezing phenomena on both muscular and cortical level. We found that ULF was accompanied by an increment in the alpha band on both the cortex and the antagonist muscle. On cortical level, this increase was observed over the contralateral sensorimotor, left frontal, and right parietal regions. This well-defined spatial increase of alpha activity could aid to detect freezing episodes during finger tapping and gait. However, increasing alpha activity is not specific to freezing phenomena, as it is also observed in event related desynchronization and synchronization processes as well as in attention-dependent cognitive processes. Directly before the occurrence of FOG an increase of theta activity (4-8 Hz) over the SMA region was observed (Shine *et al.*, 2014). Another possible biomarker we observed is an increase in cortico-cortical synchronization in the beta band during finger tapping, since this was associated with increased freezing susceptibility. The episodic nature of freezing requires a clinical application that ideally takes effect just before the freezing episode, to prevent it from occurring. A combination of biomarkers on the individual level, could promote diagnostic accuracy and might aid to detect transition states, i.e. time window along ongoing time series that are predicting an upcoming freezing event, while still giving time to intervene therapeutically in order to 'reset' the accumulating network imbalance finally ending up in a freezing phenomenon. Currently, machine learning algorithms can already detect FOG with sensitivity and specificity of more than 95 % using biomechanical data (Mazilu *et al.*, 2012). In order to develop an effective therapy, future work is required to investigate the mechanisms of the freezing pathophysiology.

In the future, freezing detection may enable personalized patterns of DBS delivery, i.e. adaptive stimulation pulses might be delivered upon upcoming locomotor network imbalance and increased susceptibility for freezing behavior. Exemplarily, if a transition from regular walking to FOG is predicted from electrophysiological or biomechanical surrogates, stimulation pulses might 'reset' the locomotor network imbalance in an ideal scenario. To date, the optimal feedback signal for closed loop DBS is unclear. However, signatures may differ across distinct PD motor symptoms, and

integration of multimodal features may be necessary to account for specificity and signal-to-noise-ratio issues on single trial detection. Exemplarily, beta oscillations on both the level of the STN and the cortex have been associated with worsening of rigidity. A recent study used the beta power of the local field potential to trigger STN stimulation. Stimulation was only administered when the beta power exceeded a certain threshold (Little *et al.*, 2013). This adaptive stimulation was 30% more effective than conventional open loop stimulation. These results are promising that once the optimal feedback has be found, STN-DBS can be personalized to meet the therapeutic need.

2.4. References

Agnesi F, Johnson MD, Vitek JL. Deep brain stimulation: how does it work? Handb. Clin. Neurol. 2013; 116: 39–54.

Andrews CJ. Influence of dystonia on the response to long-term L-dopa therapy in Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 1973; 36: 630–6.

Barbe MT, Amarell M, Snijders AH, Florin E, Quatuor EL, Sch??nau E, et al. Gait and upper limb variability in Parkinson's disease patients with and without freezing of gait. J. Neurol. 2014; 261: 330–342.

Benabid AL, Pollak P, Louveau A, Henry S, de Rougemont J. Combined (thalamotomy and stimulation) stereotactic surgery of the VIM thalamic nucleus for bilateral Parkinson disease. Appl. Neurophysiol. 1987; 50: 344–6.

Blin O, Ferrandez AM, Pailhous J, Serratrice G. Dopa-sensitive and Dopa-resistant gait parameters in Parkinson's disease. J. Neurol. Sci. 1991; 103: 51–54.

Bloem BR, Hausdorff JM, Visser JE, Giladi N. Falls and freezing of Gait in Parkinson's disease: A review of two interconnected, episodic phenomena. Mov. Disord. 2004; 19: 871–884.

Bohnen NI, Albin RL. The cholinergic system and Parkinson disease. Behav. Brain Res. 2011; 221: 564–73.

Bohnen NI, Frey KA, Studenski S, Kotagal V, Koeppe RA, Scott PJH, et al. Gait speed in Parkinson disease correlates with cholinergic degeneration. Neurology 2013; 81: 1611–1616.

Braak H, Tredici K Del, Rüb U, de Vos RA., Jansen Steur EN., Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 2003; 24: 197–211.

Breit S, Lessmann L, Unterbrink D, Popa RC, Gasser T, Schulz JB. Lesion of the pedunculopontine nucleus reverses hyperactivity of the subthalamic nucleus and substantia nigra pars reticulata in a 6-hydroxydopamine rat model. Eur. J. Neurosci. 2006; 24: 2275–2282.

Brown P, Oliviero A, Mazzone P, Insola A, Tonali P, Di Lazzaro V. Dopamine Dependency of Oscillations between Subthalamic Nucleus and Pallidum in Parkinson's Disease. J. Neurosci. 2001; 21

Von Campenhausen S, Bornschein B, Wick R, Bötzel K, Sampaio C, Poewe W, et al. Prevalence and incidence of Parkinson's disease in Europe. Eur. Neuropsychopharmacol. 2005; 15: 473–490.

Charcot JM. Clinical lectures on the diseases of the nervous system. Volume 3. London: London The New Sydenham society; 1877.

Chastan N, Westby GWM, Yelnik J, Bardinet E, Do MC, Agid Y, et al. Effects of nigral stimulation on locomotion and postural stability in patients with Parkinson's disease. Brain 2009; 132: 172–184.

Chaudhuri R, Schapira AH. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. Lancet Neurol. 2009; 8: 464–474.

Collomb-Clerc A, Welter M-L. Effects of deep brain stimulation on balance and gait in patients with Parkinson's disease: A systematic neurophysiological review. Neurophysiol. Clin. Neurophysiol. 2015; 45: 371–388.

Cooper NR, Croft RJ, Dominey SJ., Burgess AP, Gruzelier JH. Paradox lost? Exploring the role of alpha oscillations during externally vs. internally directed attention and the implications for idling and inhibition hypotheses. Int. J. Psychophysiol. 2003; 47: 65–74.

Crémers J, D'Ostilio K, Stamatakis J, Delvaux V, Garraux G. Brain activation pattern related to gait disturbances in Parkinson's disease. Mov. Disord. 2012; 27: 1498–1505.

Dauer W, Przedborski S. Parkinson's Disease: Mechanisms and Models. Neuron 2003; 39: 889–909.

Delwaide PJ, Pepin JL, Maertens de Noordhout A. The audiospinal reaction in parkinsonian patients reflects functional changes in reticular nuclei. Ann. Neurol. 1993; 33: 63–69.

Deuschl G, Schade-Brittinger C, Krack P, Volkmann J, Schäfer H, Bötzel K, et al. A randomized trial of

deep-brain stimulation for Parkinson's disease. N. Engl. J. Med. 2006; 355: 896–908.

Devos D, Labyt E, Derambure P, Bourriez JL, Cassim F, Reyns N, et al. Subthalamic nucleus stimulation modulates motor cortex oscillatory activity in Parkinson's disease. Brain 2004; 127: 408–19.

Dimitrijevic MR, Gerasimenko Y, Pinter MM. Evidence for a spinal central pattern generator in humans. In: Annals of the New York Academy of Sciences. Blackwell Publishing Ltd; 1998. p. 360–376.

Drew T, Andujar J-E, Lajoie K, Yakovenko S. Cortical mechanisms involved in visuomotor coordination during precision walking. Brain Res. Rev. 2008; 57: 199–211.

Faist M. Effect of bilateral subthalamic nucleus stimulation on gait in Parkinson's disease. Brain 2001; 124: 1590–1600.

Fasano A, Herzog J, Seifert E, Stolze H, Falk D, Reese R, et al. Modulation of gait coordination by subthalamic stimulation improves freezing of gait. Mov. Disord. 2011; 26: 844–851.

Ferrarin M, Rizzone M, Bergamasco B, Lanotte M, Recalcati M, Pedotti A, et al. Effects of bilateral subthalamic stimulation on gait kinematics and kinetics in Parkinson's disease. Exp. brain Res. 2005; 160: 517–27.

Ferraye MU, Debu B, Fraix V, Goetz L, Ardouin C, Yelnik J, et al. Effects of pedunculopontine nucleus area stimulation on gait disorders in Parkinson's disease. Brain 2010; 133: 205–214.

Fitzgerald MJT, Gruener G, Mtui E. Chapter 18: Basal Ganglia. In: Ozols I, Scott J, editor(s). Clinical Neuroanatomy and Neuroscience. Elsevier; 2007. a. p. 357–363.

Fitzgerald MJT, Gruener G, Mtui E. Chapter 30: Electroencephalography. In: Ozols I, Scott J, editor(s). Clinical neuroanatomy and Neuroscience. Elsevier Ltd; 2007. b. p. 329–339.

Giannicola G, Marceglia S, Rossi L, Mrakic-Sposta S, Rampini P, Tamma F, et al. The effects of levodopa and ongoing deep brain stimulation on subthalamic beta oscillations in Parkinson's

disease. Exp. Neurol. 2010; 226: 120–7.

Giladi N, Nieuwboer A. Understanding and treating freezing of gait in Parkinsonism, proposed working definition, and setting the stage. Mov. Disord. 2008; 23: S423–S425.

Grabli D, Karachi C, Welter M-L, Lau B, Hirsch EC, Vidailhet M, et al. Normal and pathological gait: what we learn from Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 2012; 83: 979–85.

Hagglund M, Borgius L, Dougherty KJ, Kiehn O. Activation of groups of excitatory neurons in the mammalian spinal cord or hindbrain evokes locomotion. Nat. Neurosci. 2010; 13: 246–252.

Hawkes CH, Tredici K Del, Braak H. Review: Parkinson's disease: a dual-hit hypothesis. Neuropathol. Appl. Neurobiol. 2007; 33: 599–614.

Hirsch EC, Graybiel AM, Duyckaerts C, Javoy-Agid F. Neuronal loss in the pedunculopontine tegmental nucleus in Parkinson disease and in progressive supranuclear palsy. Proc. Natl. Acad. Sci. U. S. A. 1987; 84: 5976–80.

Jankovic J. Motor fluctuations and dyskinesias in Parkinson's disease: Clinical manifestations. Mov. Disord. 2005; 20: S11–S16.

Jankovic J. Parkinson's disease: clinical features and diagnosis. J. Neurol. Neurosurg. Psychiatry 2008; 79: 368–76.

Jankovic J, Aguilar G. Current approaches to the treatment of Parkinson's disease. Neuropsychiatr. Dis. Treat. 2008; 4: 743–57.

Jankovic J, Stacy M. Medical management of levodopa-associated motor complications in patients with Parkinson's disease. CNS Drugs 2007; 21: 677–692.

Jensen O, Mazaheri A. Shaping functional architecture by oscillatory alpha activity: gating by inhibition. Front. Hum. Neurosci. 2010; 4: 186.

Johnsen EL, Mogensen PH, Sunde NA, Østergaard K. Improved asymmetry of gait in Parkinson's

disease with DBS: Gait and postural instability in Parkinson's disease treated with bilateral deep brain stimulation in the subthalamic nucleus. Mov. Disord. 2009; 24: 588–595.

Johnson MD, Miocinovic S, McIntyre CC, Vitek JL. Mechanisms and targets of deep brain stimulation in movement disorders. Neurotherapeutics 2008; 5: 294–308.

Kalinderi K, Bostantjopoulou S, Fidani L. The genetic background of Parkinson's disease: current progress and future prospects. Acta Neurol. Scand. 2016

Karachi C, Andre A, Bertasi E, Bardinet E, Lehericy S, Bernard FA. Functional Parcellation of the Lateral Mesencephalus. J. Neurosci. 2012; 32: 9396–9401.

Karachi C, Grabli D, Bernard FA, Tand?? D, Wattiez N, Belaid H, et al. Cholinergic mesencephalic neurons are involved in gait and postural disorders in Parkinson disease. J. Clin. Invest. 2010; 120: 2745–2754.

Kitano H, Tanibuchi I, Jinnai K. The distribution of neurons in the substantia nigra pars reticulata with input from the motor, premotor and prefrontal areas of the cerebral cortex in monkeys. Brain Res. 1998; 784: 228–38.

Kleiner-Fisman G, Herzog J, Fisman DN, Tamma F, Lyons KE, Pahwa R, et al. Subthalamic nucleus deep brain stimulation: Summary and meta-analysis of outcomes. Mov. Disord. 2006; 21

Klimesch W, Sauseng P, Hanslmayr S. EEG alpha oscillations: the inhibition-timing hypothesis. Brain Res. Rev. 2007; 53: 63–88.

Kostic VS, Agosta F, Pievani M, Kosti VS, Stefanova E, Ječmenica-Luki M, et al. Parkinson disease Pattern of brain tissue loss associated with freezing of gait in Pattern of brain tissue loss associated with freezing of gait in Parkinson disease. 2012

Krack P, Hariz MI, Baunez C, Guridi J, Obeso JA. Deep brain stimulation: from neurology to psychiatry? Trends Neurosci. 2010; 33: 474–484.

Kühn AA, Kempf F, Brücke C, Gaynor Doyle L, Martinez-Torres I, Pogosyan A, et al. High-frequency stimulation of the subthalamic nucleus suppresses oscillatory beta activity in patients with Parkinson's disease in parallel with improvement in motor performance. J. Neurosci. 2008; 28: 6165–73.

Kuhn AA, Williams D, Kupsch A, Limousin P, Hariz M, Schneider G-H, et al. Event-related beta desynchronization in human subthalamic nucleus correlates with motor performance. Brain 2004; 127: 735–746.

de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol. 2006; 5: 525–535.

Limousin P, Greene J, Pollak P, Rothwell J, Benabid AL, Frackowiak R. Changes in cerebral activity pattern due to subthalamic nucleus or internal pallidum stimulation in Parkinson's disease. Ann. Neurol. 1997; 42: 283–91.

Little S, Pogosyan A, Neal S, Zavala B, Zrinzo L, Hariz M, et al. Adaptive deep brain stimulation in advanced Parkinson disease. Ann. Neurol. 2013; 74: 449–57.

Marsden CD. Problems with long-term levodopa therapy for Parkinson's disease. Clin. Neuropharmacol. 1994; 17 Suppl 2: S32-44.

Mazilu S, Hardegger M, Zhu Z, Roggen D, Tröster G, Plotnik M, et al. Online Detection of Freezing of Gait with Smartphones and Machine Learning Techniques. In: Pervasive Computing Technologies for Healthcare (PervasiveHealth). IEEE; 2012. p. 123–130.

Moore O, Peretz C, Giladi N. Freezing of gait affects quality of life of peoples with Parkinson's disease beyond its relationships with mobility and gait. Mov. Disord. 2007; 22: 2192–2195.

Moreau C, Defebvre L, Destee A, Bleuse S, Clement F, Blatt JL, et al. STN-DBS frequency effects on freezing of gait in advanced Parkinson disease. Neurology 2008; 71: 80–84.

Mori S. Integration of posture and locomotion in acute decerebrate cats and in awake, freely moving cats. Prog. Neurobiol. 1987; 28: 161–195.

Moro E, Hamani C, Poon YY, Al-Khairallah T, Dostrovsky JO, Hutchison WD, et al. Unilateral pedunculopontine stimulation improves falls in Parkinson's disease. Brain 2010; 133: 215–224.

Nieuwboer A, Vercruysse S, Feys P, Levin O, Spildooren J, Swinnen S. Upper limb movement interruptions are correlated to freezing of gait in Parkinson's disease. Eur. J. Neurosci. 2009; 29: 1422–1430.

Nutt JG, Bloem BR, Giladi N, Hallett M, Horak FB, Nieuwboer A. Freezing of gait: Moving forward on a mysterious clinical phenomenon. Lancet Neurol. 2011; 10: 734–744.

Obeso JA, Rodríguez-Oroz MC, Benitez-Temino B, Blesa FJ, Guridi J, Marin C, et al. Functional organization of the basal ganglia: Therapeutic implications for Parkinson's disease. Mov. Disord. 2008; 23: 548–559.

Okun MS. Deep-Brain Stimulation for Parkinson's Disease The Cl inic a l Probl em. N Engl J Med 2012; 367: 1529–38.

Pahapill P, Lozano A. The pedunculopontine nucleus and Parkinson's disease. Brain 2000; 123 (Pt 9): 1767–83.

Parkinson J. An essay on the shaking palsy. 1817. J. Neuropsychiatry Clin. Neurosci. 2002; 14: 223–236; discussion 222.

Perez-Lloret S, Negre-Pages L, Damier P, Delval A, Derkinderen P, Destée A, et al. Prevalence, Determinants, and Effect on Quality of Life of Freezing of Gait in Parkinson Disease. JAMA Neurol. 2014; 71: 1–7.

Plotnik M, Giladi N, Balash Y, Peretz C, Hausdorff JM. Is Freezing of Gait in Parkinson's Disease Related to Asymmetric Motor Function? Ann Neurol 2005; 57: 656–663.

Plotnik M, Giladi N, Hausdorff JM. Bilateral coordination of walking and freezing of gait in Parkinson's disease. Eur. J. Neurosci. 2008; 27: 1999–2006.

Plotnik M, Hausdorff JM. The role of gait rhythmicity and bilateral coordination of stepping in the pathophysiology of freezing of gait in Parkinson's disease. Mov. Disord. 2008; 23

Pollak P, Benabid AL, Gross C, Gao DM, Laurent A, Benazzouz A, et al. [Effects of the stimulation of the subthalamic nucleus in Parkinson disease]. Rev. Neurol. (Paris). 1993; 149: 175–6.

Pötter-Nerger M, Ilic T V, Siebner HR, Deuschl G, Volkmann J. Subthalamic Nucleus Stimulation Restores Corticospinal Facilitation in Parkinson's Disease. Mov. Disord. 2008; 23: 2210–2215.

Pringsheim T, Jette N, Frolkis A, Steeves TDL. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov. Disord. 2014; 29: 1583–90.

Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia A-S, McNamara JO, et al. Chapter 18: Modulation of movement by the basal ganglia. In: Neuroscience. 2008. p. 453–474.

Rauch F, Schwabe K, Krauss JK. Effect of deep brain stimulation in the pedunculopontine nucleus on motor function in the rat 6-hydroxydopamine Parkinson model. Behav. Brain Res. 2010; 210: 46–53.

Rey NL, Steiner JA, Maroof N, Luk KC, Madaj Z, Trojanowski JQ, et al. Widespread transneuronal propagation of α-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease. J. Exp. Med. 2016; 213

Ricchi V, Zibetti M, Angrisano S, Merola A, Arduino N, Artusi CA, et al. Transient effects of 80 Hz stimulation on gait in STN DBS treated PD patients: A 15 months follow-up study. Brain Stimul. 2012; 5: 388–392.

Roper JA, Kang N, Ben J, Cauraugh JH, Okun MS, Hass CJ. Deep brain stimulation improves gait velocity in Parkinson's disease: a systematic review and meta-analysis. J. Neurol. 2016; 263: 1195–1203.

Rubinstein TC, Giladi N, Hausdorff JM. The power of cueing to circumvent dopamine deficits: a review of physical therapy treatment of gait disturbances in Parkinson's disease. Mov. Disord.

2002; 17: 1148–60.

Salenius S, Avikainen S, Kaakkola S, Hari R, Brown P. Defective cortical drive to muscle in Parkinson's disease and its improvement with levodopa. Brain 2002; 125: 491–500.

Sauseng P, Gerloff C, Hummel FC. Two brakes are better than one: The neural bases of inhibitory control of motor memory traces. Neuroimage 2013; 65: 52–58.

Sauseng P, Klimesch W, Gerloff C, Hummel FC. Spontaneous locally restricted EEG alpha activity determines cortical excitability in the motor cortex. 2009.

Schaafsma JD, Balash Y, Gurevich T, Bartels AL, Hausdorff JM, Giladi N. Characterization of freezing of gait subtypes and the response of each to levodopa in Parkinson's disease. Eur. J. Neurol. 2003; 10: 391–398.

Schiesling C, Kieper N, Seidel K, Krüger R. Review: Familial Parkinson's disease – genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. Neuropathol. Appl. Neurobiol. 2008; 34: 255–271.

Schuepbach WMM, Rau J, Knudsen K, Volkmann J, Krack P, Timmermann L, et al. Neurostimulation for Parkinson's Disease with Early Motor Complications. N. Engl. J. Med. 2013; 368: 610–622.

Schulte C, Gasser T. Genetic basis of Parkinson's disease: inheritance, penetrance, and expression. Appl. Clin. Genet. 2011; 4: 67–80.

Schwab RS, Chafetz ME, Walker S. Control of two simultaneous voluntary motor acts in normals and in parkinsonism. AMA. Arch. Neurol. Psychiatry 1954; 72: 591–8.

Sharma M, Ioannidis JPA, Aasly JO, Annesi G, Brice A, Van Broeckhoven C, et al. Large-scale replication and heterogeneity in Parkinson disease genetic loci. Neurology 2012; 79: 659–67.

Sherman D, Fuller PM, Marcus J, Yu J, Zhang P, Chamberlin NL, et al. Anatomical Location of the Mesencephalic Locomotor Region and Its Possible Role in Locomotion, Posture, Cataplexy, and

Parkinsonism. Front. Neurol. 2015; 6: 140.

Shine JM, Handojoseno AMA, Nguyen TN, Tran Y, Naismith SL, Nguyen H, et al. Abnormal patterns of theta frequency oscillations during the temporal evolution of freezing of gait in parkinson's disease. Clin. Neurophysiol. 2014; 125: 569–576.

Shumway-Cook A, Woollacott MH. Chapter 12: Control of Normal Mobility. In: Sabatini P, Horowitz L, Seitz A, editor(s). Motor Control: translatinfg reasearch into clinical practice. Lippincott Williams & Wilkins; 2007. p. 299–329.

Snijders AH, Leunissen I, Bakker M, Overeem S, Helmich RC, Bloem BR, et al. Gait-related cerebral alterations in patients with Parkinson's disease with freezing of gait. Brain 2011; 134: 59–72.

Stayte S, Vissel B. Advances in non-dopaminergic treatments for Parkinson's disease. Front. Neurosci. 2014; 8: 113.

St George RJ, Carlson-Kuhta P, Nutt JG, Hogarth P, Burchiel KJ, Horak FB. The effect of deep brain stimulation randomized by site on balance in Parkinson's disease. Mov. Disord. 2014; 29: 949–953.

Stinear CM, Byblow WD, Steyvers M, Levin O, Swinnen SP. Kinesthetic, but not visual, motor imagery modulates corticomotor excitability. Exp. Brain Res. 2006; 168: 157–164.

Takakusaki K. Neurophysiology of gait: From the spinal cord to the frontal lobe. Mov. Disord. 2013; 28: 1483–1491.

Takakusaki K, Habaguchi T, Ohtinata-Sugimoto J, Saitoh K, Sakamoto T. Basal ganglia efferents to the brainstem centers controlling postural muscle tone and locomotion: a new concept for understanding motor disorders in basal ganglia dysfunction. Neuroscience 2003; 119: 293–308.

Takakusaki K, Obara K, Nozu T, Okumura T. Modulatory effects of the GABAergic basal ganglia neurons on the PPN and the muscle tone inhibitory system in cats. Arch. Ital. Biol. 2011; 149: 385–405.

Takakusaki K, Tomita N, Yano M. Substrates for normal gait and pathophysiology of gait disturbances with respect to the basal ganglia dysfunction. In: Journal of Neurology. D. Steinkopff-Verlag; 2008. p. 19–29.

Taylor AE, Saint-Cyr JA, Lang AE. Frontal lobe dysfunction in Parkinson's disease. The cortical focus of neostriatal outflow. Brain 1986: 845–83.

Taylor KSM, Cook JA, Counsell CE. Heterogeneity in male to female risk for Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 2007; 78: 905–906.

Thevathasan W, Coyne TJ, Hyam JA, Kerr G, Jenkinson N, Aziz TZ, et al. Pedunculopontine nucleus stimulation improves gait freezing in parkinson disease. Neurosurgery 2011; 69: 1248–1253.

Vercruysse S, Vandenberghe W, Münks L, Nuttin B, Devos H, Nieuwboer A, et al. Effects of deep brain stimulation of the subthalamic nucleus on freezing of gait in Parkinson's disease: a prospective controlled study. J Neurol Neurosurg Psychiatry 2014; 85: 871–7.

Weiss D, Breit S, Hoppe J, Hauser A-K, Freudenstein D, Krüger R, et al. Subthalamic nucleus stimulation restores the efferent cortical drive to muscle in parallel to functional motor improvement. Eur. J. Neurosci. 2012; 35: 896–908.

Weiss D, Klotz R, Govindan RB, Scholten M, Naros G, Ramos-Murguialday A, et al. Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease. Brain 2015; 138: 679–693.

Weiss D, Walach M, Meisner C, Fritz M, Scholten M, Breit S, et al. Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. Brain 2013; 136: 2098–2108.

Welter M-L, Demain A, Ewenczyk C, Czernecki V, Lau B, El Helou A, et al. PPNa-DBS for gait and balance disorders in Parkinson's disease: a double-blind, randomised study. J. Neurol. 2015; 262: 1515–25.

Zweig RM, Jankel WR, Hedreen JC, Mayeux R, Price DL. The pedunculopontine nucleus in Parkinson's disease. Ann. Neurol. 1989; 26: 41–46.

3. List of appended papers

1) Weiss D, Klotz R, Govindan RB, **Scholten M**, Naros G, Ramos-Murguialday A, et al. Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease. Brain. 2015;138(Pt 3):679–93.
2) Weiss D, Walach M, Meisner C, Fritz M, **Scholten M**, Breit S, et al. Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. Brain. 2013;136(7):2098–108.
3) **Scholten M**, Govindan RB, Braun C, Bloem BR, Plewnia C, Krüger R, et al. Cortical correlates of susceptibility to upper limb freezing in Parkinson's disease. Clin Neurophysiol. 2016a;127(6):2386–93.
4) **Scholten M**, Klotz R, Plewnia C, Wächter T, Mielke C, Bloem BR, et al. Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease. Clin Neurophysiol. 2016b;127(1):610–20.
5) **Scholten M**, Klemt J, Bunjes F, Bloem B, Gharabaghi A, Krüger R, Weiss D. Effects of subthalamic and nigral stimulation on gait in Parkinson's disease. *In preparation*.

4. Statement of contributions

Paper 1

Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial.

Daniel Weiss, Margarete Walach, Christoph Meisner, Melanie Fritz, Marlieke Scholten, Sorin Breit, Christian Plewnia, Benjamin Bender, Alireza Gharabaghi, Tobias Wächter, Rejko Krüger

Published in: Brain; year: 2013; volume: 136; issue: 7; pages: 2098-2108

I assisted in acquiring the data and entered the data in the databank.

Task	**Contributing authors**
Conceived and designed experiment	Daniel Weiss, Alireza Gharabaghi, Rejko Krüger
Performed experiments	Daniel Weiss, Tobias Wächter, Margarete Walach, Melanie Fritz, Marlieke Scholten
Entering data in the databank	Marlieke Scholten, Melanie Fritz
Statistical analysis of the data	Christoph Meisner, Daniel Weiss, Margarete Walach
Wrote manuscript	Daniel Weiss, Rejko Krüger
Revised the manuscript and gave the final approval	All Authors

Paper 2

Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease

Daniel Weiss, Rosa Klotz, Rathinaswamy B. Govindan, Marlieke Scholten, Georgios Naros, Ander Ramos-Murguialday, Friedemann Bunjes, Christoph Meisner, Christian Plewnia, Rejko Krüger, Alireza Gharabaghi

Published in: Brain; year: 2015; volume: 138; issue: Pt 3; pages: 679-693

This project was primarily designed and performed by Rosa Klotz and Daniel Weiss. I assisted Rosa Klotz in the data analysis.

Task	Contributing authors
Conceived and designed experiment	Daniel Weiss, Rosa Klotz, Georgios Naros, Alireza Gharabaghi, Rejko Krüger
Delivered hardware for the experiment	Friedemann Bunjes
Performed experiments	Rosa Klotz, Daniel Weiss
Analysis of the data (preprocessing, computing, statistical analyses)	Daniel Weiss, Rosa Klotz, Rathinaswamy B. Govindan, Marlieke Scholten, Christoph Braun, Christoph Meisner, Ander Ramos-Muruialday
Contributed analysis tools	Rathinaswamy B. Govindan
Wrote manuscript	Daniel Weiss, Rosa Klotz
Revised the manuscript and gave the final approval	All Authors

Paper 3

Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease

Marlieke Scholten, Rosa Klotz, Christian Plewnia, Tobias Wächter, Carina Mielke, Bastiaan R. Bloem, Christoph Braun, Ulf Ziemann, Rathinaswamy B. Govindan, Alireza Gharabaghi, Rejko Krüger, Daniel Weiss

Published in: Clin Neurophysiology; year:2016; volume: 127; issue: 1; pages: 610–20

For this project I performed the experiments and analyzed the data. I wrote the first draft of the manuscript.

Task	Contributing authors
Conceived and designed experiment	Marlieke Scholten, Daniel Weiss
Delivered hardware for the experiment	Christoph Braun
Performed experiments	Marlieke Scholten
Analysis of the data (consisting of preprocessing, computing, statistical analyses)	Marlieke Scholten, Rathinaswamy B. Govindan, Christoph Braun, Ulf Ziemann, Bas Bloem, Daniel Weiss,
Contributed analysis tools	Rathinaswamy B. Govindan
Wrote manuscript	Marlieke Scholten, Daniel Weiss
Revised the manuscript and gave the final approval	All Authors

Paper 4

Cortical correlates of susceptibility to upper limb freezing in Parkinson's disease

Marlieke Scholten, Rathinaswamy B. Govindan, Christoph Braun, Bastiaan R. Bloem, Christian Plewnia, Rejko Krüger, Alireza Gharabaghi, Daniel Weiss

Published in: Clin Neurophysiology; year:2016; volume: 127; issue: 6; pages: 2386-93

For his project, I designed and performed the experiments, I analyzed the data and wrote the first draft of the manuscript.

Task	**Contributing authors**
Conceived and designed experiment	Marlieke Scholten, Daniel Weiss
Delivered hardware for the experiment	Christoph Braun
Performed experiments	Marlieke Scholten
Analysis of the data (consisting of preprocessing, computing, statistical analyses)	Marlieke Scholten, Rathinaswamy B. Govindan, Christoph Braun, Bas Bloem, Daniel Weiss, Rathinaswamy B. Govindan
Contributed analysis tools	
Wrote manuscript	Marlieke Scholten, Daniel Weiss
Revised the manuscript and gave the final approval	All Authors

Manuscript

Effects of subthalamic and nigral stimulation on gait in Parkinson's disease

Marlieke Scholten, Johannes Klemt, Friedemann Bunjes, Bas Bloem, Rejko Krüger, Alireza Gharabaghi, Daniel Weiss

Will be submitted to Gait and Posture in January 2017

I designed the experiment together with Daniel Weiss and performed the measurements together with Johannes Klemt. I analyzed the data with the help of Friedemann Bunjes and Daniel Weiss. I wrote the first draft of the manuscript.

Task	**Contributing authors**
Conceived and designed experiment	Marlieke Scholten, Daniel Weiss
Performed experiments	Marlieke Scholten, Johannes Klemt
Analysis of the data (preprocessing, computing, statistical analyses)	Marlieke Scholten, Friedemann Bunjes, Daniel Weiss,
Wrote manuscript	Marlieke Scholten, Daniel Weiss
Revised the manuscript and gave the final approval	All Authors

5. Appended papers and manuscript

5.1 Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial

Published as:

Weiss D, Walach M, Meisner C, Fritz M, Scholten M, Breit S, et al. Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. Brain. 2013;136(7):2098–108.

doi:10.1093/brain/awt122 Brain 2013: 136; 2098–2108 | 2098

BRAIN
A JOURNAL OF NEUROLOGY

Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial

Daniel Weiss,[1,2,3] Margarete Walach,[1,2,3] Christoph Meisner,[4] Melanie Fritz,[1,2,3] Marlieke Scholten,[1,2,3] Sorin Breit,[1,2,3] Christian Plewnia,[2,5] Benjamin Bender,[6] Alireza Gharabaghi,[2,7] Tobias Wächter[1,2,3] and Rejko Krüger[1,2,3]

1 German Centre for Neurodegenerative Diseases (DZNE), Tübingen, Germany
2 Werner-Reichardt Centre for Integrative Neuroscience, University of Tübingen, Germany
3 Department for Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research, University of Tübingen, Germany
4 Clinical Epidemiology and Applied Biometry, University of Tübingen, Germany
5 Department of Psychiatry and Psychotherapy, University of Tübingen, Germany
6 Department of Diagnostic and Interventional Neuroradiology, University of Tübingen, Germany
7 Department of Neurosurgery, University of Tübingen, Germany

Correspondence to: Daniel Weiss,
Centre of Neurology and
Hertie Institute for Clinical Brain Research and
German Centre for Neurodegenerative Diseases,
University of Tübingen, Hoppe-Seyler-Str. 3,
D-72076 Tübingen,
Germany
E-mail: daniel.weiss@uni-tuebingen.de

Correspondence may also be addressed to: Rejko Krüger
E-mail: rejko.krueger@uni-tuebingen.de

Gait and balance disturbances typically emerge in advanced Parkinson's disease with generally limited response to dopaminergic medication and subthalamic nucleus deep brain stimulation. Therefore, advanced programming with interleaved pulses was put forward to introduce concomittant nigral stimulation on caudal contacts of a subthalamic lead. Here, we hypothesized that the combined stimulation of subthalamic nucleus and substantia nigra pars reticulata improves axial symptoms compared with standard subthalamic nucleus stimulation. Twelve patients were enrolled in this 2 × 2 cross-over double-blind randomized controlled clinical trial and both the safety and efficacy of combined subthalamic nucleus and substantia nigra pars reticulata stimulation were evaluated compared with standard subthalamic nucleus stimulation. The primary outcome measure was the change of a broad-scaled cumulative axial Unified Parkinson's Disease Rating Scale score (Scale II items 13–15, Scale III items 27–31) at '3-week follow-up'. Secondary outcome measures specifically addressed freezing of gait, balance, quality of life, non-motor symptoms and neuropsychiatric symptoms. For the primary outcome measure no statistically significant improvement was observed for combined subthalamic nucleus and substantia nigra pars reticulata stimulation at the '3-week follow-up'. The secondary endpoints, however, revealed that the combined stimulation of subthalamic nucleus and substantia nigra pars reticulata might specifically improve freezing of gait, whereas balance impairment remained unchanged. The combined stimulation of subthalamic nucleus and substantia nigra pars reticulata was safe, and of note, no clinically relevant neuropsychiatric adverse effect was observed. Patients treated with subthalamic nucleus and substantia nigra pars reticulata stimulation revealed no 'global' effect on axial motor domains. However, this study opens the perspective that concomittant stimulation of the

Received December 22, 2012. Revised March 27, 2013. Accepted March 28, 2013. Advance Access publication June 11, 2013

substantia nigra pars reticulata possibly improves otherwise resistant freezing of gait and, therefore, highly warrants a subsequent phase III randomized controlled trial.

Keywords: Parkinson's disease; DBS; gait; freezing; subthalamic nucleus

Abbreviations: STN–DBS = subthalamic nucleus deep brain stimulation; SNr = substantia nigra pars reticulata; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

Deep brain stimulation of the subthalamic nucleus (STN–DBS) in Parkinson's disease is an established treatment for segmental motor symptoms and motor fluctuations (Deuschl *et al.*, 2006; Kleiner-Fisman *et al.*, 2006; Weaver *et al.*, 2009) including early disease stages with beginning motor fluctuations (Schuepbach *et al.*, 2013). However, debilitating axial motor symptoms are frequently observed during disease progression (Nutt *et al.*, 2011) and contribute to a disproportional decline of the therapeutic response to standard dopaminergic treatment and to STN–DBS (Krack *et al.*, 2003; St George *et al.*, 2010; Castrioto *et al.*, 2011). We postulate that these different therapeutic outcomes of segmental and axial motor domains may mirror differential functional sub-loops of pathological motor network processing. Whereas standard STN–DBS may primarily facilitate the thalamo-cortico-spinal motor control improving segmental symptoms (Salenius *et al.*, 2002; Potter-Nerger *et al.*, 2008; Kuriakose *et al.*, 2010; Weiss *et al.*, 2012*a*), gait disturbances in advanced disease stages may be associated with defective motor processing of mesencephalic locomotor pathways (Ferraye *et al.*, 2010; Moro *et al.*, 2010) including descending nigropontine projections to spinal motor neurons (Potter *et al.*, 2008; Chastan *et al.*, 2009; Tsang *et al.*, 2010; Thevathasan *et al.*, 2011*b*; Weiss *et al.*, 2012*a*). An attractive approach to modulate nigropontine locomotor integration is to introduce co-stimulation of the substantia nigra pars reticulata (SNr) on a caudal electrode contact of a lead with rostral contacts located in the STN (Weiss *et al.*, 2011*a*). Advanced programming with so-called 'interleaved pulses' allows independent stimulation of contacts with different amplitudes and pulse widths at a common frequency (Weiss *et al.*, 2011*a*; Wojtecki *et al.*, 2011; Kovacs *et al.*, 2012) and therefore enables us to co-stimulate segregate functional motor loops at the level of the STN and SNr (Weiss *et al.*, 2011*a*).

Materials and methods

This investigator-initiated phase II double-blind randomized controlled trial was registered at ClinicalTrials.gov (NCT01355835) and a detailed study protocol was published elsewhere (Weiss *et al.*, 2011*b*). The trial was approved by the local Ethics committee in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Patients

Patients with advanced Parkinson's disease and gait and balance impairment resistant to optimized dopaminergic and STN–DBS treatment (Weiss *et al.*, 2011*b*) were enrolled if they met the following inclusion criteria: age 18–80 years, disease duration >5 years, idiopathic Parkinson's disease including genetic forms of typical Parkinson's disease, therapy with STN–DBS and Activa® impulse generator (Medtronic), axial UPDRS ⩾12 [sum score of Unified Parkinson's disease Rating Scale (UPDRS) II, items 13–15 and UPDRS III, items 27–31], one of the two rostral electrode contacts located in the STN area and the lowermost electrode contacts located in the caudal STN–SNr border zone, dopaminergic medication unchanged for 4 weeks before study enrolment, and implantation of STN–DBS electrodes for at least 6 months. Exclusion criteria were cognitive impairment (Mini-Mental State Examination <25 points), participation in other clinical trials during the past 3 months or during study enrolment, acute suicidal tendency or psychosis, other chronic pathological conditions interfering with the study protocol or interpretability of the data, and pregnancy. Comprehensive data on patient screening and patient enrolment are given in the CONSORT flow diagram (Fig. 1). Patients were screened for mutations in the most frequent Parkinson's disease associated genes. One patient (Patient PD11) was identified with a Parkin gene mutation (Supplementary material).

Study design

This study is a randomized double-blinded 2 × 2 cross-over single centre clinical trial. After trial commencement, there were no changes to methods or outcome assessments. We tested the hypothesis that combined STN + SNr stimulation is superior to improve axial motor symptoms compared with active subthalamic standard therapy after 3-week active treatment.

Patients underwent a detailed 'baseline' assessment after overnight withdrawal of dopaminergic medication (OFF medication, OFF stimulation). In the same session, 'immediate testings' of standard STN stimulation versus combined STN + SNr stimulation treatment were performed. These three treatment conditions [i.e. (Baseline) in OFF medication OFF stimulation; standard STN stimulation in OFF medication; and combined STN + SNr stimulation in OFF medication] were introduced 30 min before the clinical ratings in randomized order. This session performed in an OFF medication state was considered to assess short-term efficacy and to ensure that parameters on subthalamic contacts were optimally adjusted. At the end of the immediate testing patients entered the '3-week follow-up' stage with both standard STN stimulation and combined STN + SNr stimulation active treatment in randomized order, prepared by the Institute for Clinical Epidemiology and Applied Biometry, Tübingen, Germany using a computer generated randomization. Endpoint assessments were obtained at the end of the '3-week follow-up' period and included both clinical and anamnestic measures as detailed below. In this cross-over trial we did not consider a second baseline assessment after the first '3-week follow-up' period (before entering the second '3-week' period). Based on current literature it is highly improbable, that carry-over effects from either combined STN + SNr

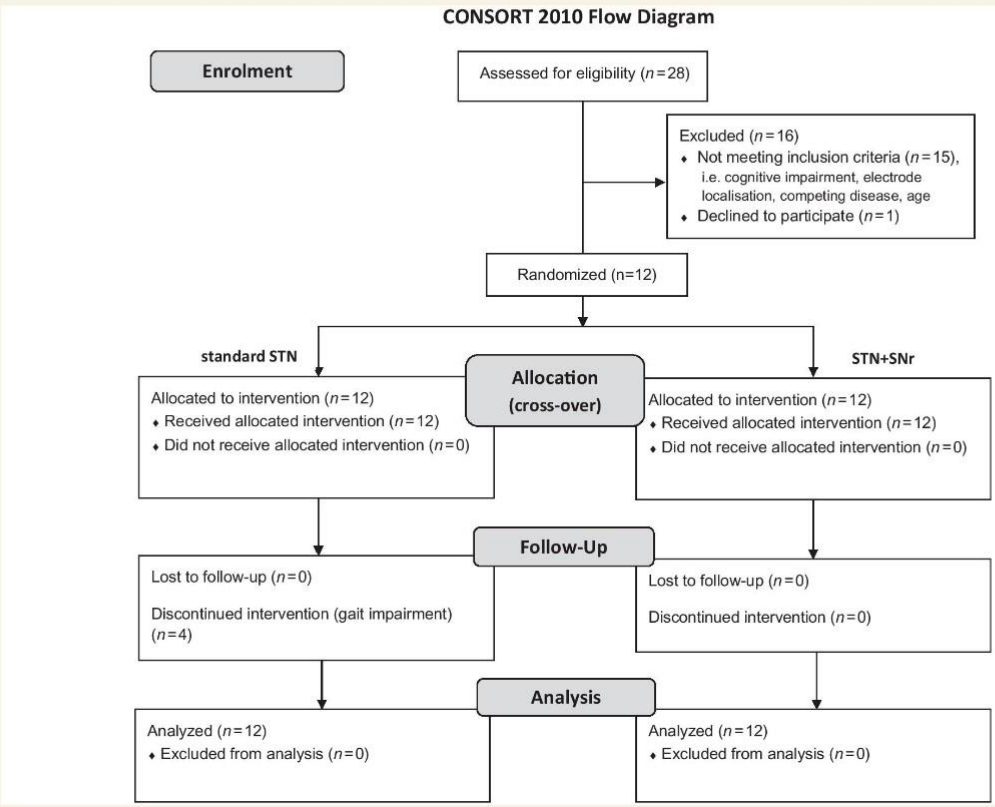

Figure 1 CONSORT chart. [STN + SNr] = combined STN + SNr stimulation.

stimulation or standard STN stimulation treatment might outlast a '3-week follow-up' given: (i) the immediate recurrence of motor symptoms when the stimulator is switched OFF; and (ii) clearly discriminable motor effects of subthalamic and nigral stimulation were demonstrated within short time intervals (Chastan *et al.*, 2009; Weiss *et al.*, 2011*a*). This was recently confirmed by an independent study that described fast clinical wash-out after turning off the DBS that was most pronounced in advanced disease stages (Cooper *et al.*, 2013). Therefore, and for ethical reasons, we did not implement a second baseline assessment that would have necessitated another L-DOPA and stimulation withdrawal after the first '3-week follow-up'.

Because both standard STN stimulation and combined STN + SNr stimulation similarly controlled for segmental symptoms and did not induce acute adverse events or other sensations using the study parameters applied, there was no indication that the patients were able to distinguish between the two stimulation programs. However, patients and the endpoint assessor may have noticed when stimulators were switched OFF in the baseline condition due to the recurrence of segmental Parkinson's disease symptoms. For ethical reasons patients were informed that the two programs of standard STN and combined STN + SNr stimulation were designed to study the differential therapeutic efficacy on gait measures.

The initial titration of subthalamic and nigral stimulation parameters was performed by the principal investigator (D.W.) who also stored the allocation code and held it closed until all endpoint assessments and final statistical analyses were performed. In order to keep both patients and endpoint assessors blinded to the treatment condition, parameters were changed several times between standard STN and combined STN + SNr stimulation before maintaining the intended program. Double-blind clinical endpoint assessments were performed by a specialized expert neurologist trained in Parkinson's disease and DBS treatment (T.W.). Patients were able to discontinue study treatment in accordance with the accepted ethical standard.

Electrode localization

Localization of the active STN and SNr contacts were determined by coregistration analyses of preoperative 3D T_1-weighted MPRAGE and postoperative 3D T_1-weighted FLASH sequences. Coregistration analyses were performed with Matlab 7.0 and the open-source toolbox SPM5 and indicated electrode localization of active contacts in the dorsolateral portions of STN and SNr, respectively (Fig. 2).

Therapy and stimulation parameters

At the time of study enrolment, all patients were implanted with Activa® pulse generators (enabling advanced interleaved programming). Some individual patients with longer follow-up periods of DBS therapy (up to 79 months) initially received Kinetra® pulse

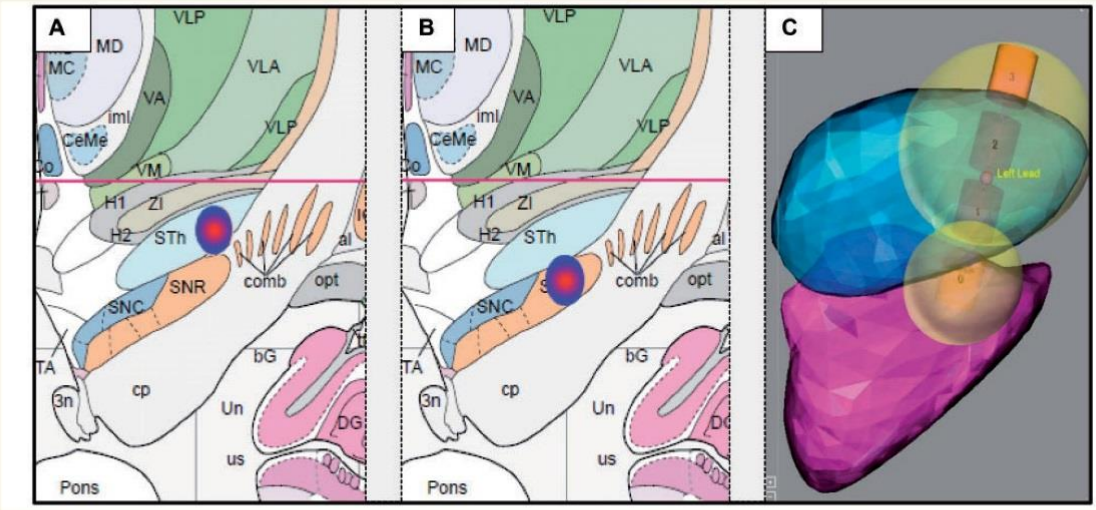

Figure 2 Localization of active electrode contacts of (**A**) dorsolateral STN and (**B**) dorsolateral SNr. Coordinates relative to the mid-commisural point (MCP) were: left STN -11.4 ± 0.8, -0.9 ± 2.0, -3.0 ± 1.7; right STN 13.5 ± 1.1, -0.5 ± 1.7, -2.2 ± 1.5; left SNr -10.0 ± 0.9, -3.4 ± 2.1, -6.4 ± 1.8; right SNr 12.1 ± 1.3, -3.3 ± 1.7, -5.8 ± 1.5 (x, y, z; x = medio-lateral, y = anterio-posterior, z = rostro-caudal). Electrode coordinates (mean ± standard deviation in x- and y-direction) are visualized in coronal view on the Atlas of the Human Brain with permission (Mai *et al*., 2007). (**C**) An additional illustrative image of electrode localization including a simulation on volume of tissue activated was kindly provided by Medtronic based on work by Yelnik *et al*. (2007) (atlas) and D'Haese *et al*. (2012) (atlas and algorithms).

generators; however these were changed to Activa® pulse generators after battery depletion during regular clinical follow-up. Activa® pulse generators were available at our study site from 2009.

The stimulation parameters applied during the study phase were established according to our stringent predefined study protocol (Weiss *et al.*, 2011*b*) to achieve the best individual parameters for active subthalamic contacts in patients with emerging gait disturbances. This protocol provides a standardized procedure including the concept of 'better side reduction' (Fasano *et al.*, 2011) in order to ensure best individual STN stimulation parameters before entering the study. Here, in general the rostral contacts 2 (second upper left STN of the quadripolar electrode) and 10 (second upper right STN) were chosen, that also prevented current spreading from the subthalamic active contacts to SNr. This programming was performed before patients entered the study.

Importantly, when entering the study protocol, we again ascertained that segmental motor symptoms were optimally controlled from standard STN stimulation. Therefore, the medication OFF session was considered to verify optimal stimulation parameters as gold standard (i.e. for tremor, bradykinesia and rigidity) before entering the 'immediate testing'. Nigral stimulation was standardized on a common pulse width of 60 µs and all subthalamic and nigral contacts were stimulated at a common frequency (125 Hz). Detailed information on the stimulation parameters is provided (Supplementary Tables 1 and 2).

Throughout the study the stimulation parameters of the active subthalamic contacts as well as medication were held constant, including 'immediate testing' and both '3-week follow-up' assessments. Of note, owing to the delayed onset of dyskinesia after introduction of combined STN + SNr stimulation, medication in one patient and nigral stimulation parameters in two patients had to be adjusted during the '3-week follow-up'' according to the intention-to-treat principle (detailed below).

Outcome measures

In this phase II study we primarily aimed to investigate a broad spectrum of axial motor symptoms. Therefore, a broad-scaled primary endpoint was defined as 'axial score' built from eight items of the anamnestic UPDRS II (items 13–15: falling unrelated to freezing, freezing when walking, walking) and the clinical UPRDS III (items 27–31: arising from chair, posture, gait, postural stability, body bradykinesia and hypokinesia), all 5-point rated. For the statistical evaluation the five rating points are represented by the numbers 0 to 4, which represent increasing levels of impairment on different axial motor domains including freezing of gait, independence of gait, balance and posture. This 'axial score' was summed of the ratings across the eight items (range 0–32). Secondary clinical endpoint assessments tested axial motor function (UPDRS III, items 27–31), balance (Berg Balance Scale; Berg *et al*., 1992), gait [timed walking test from Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease (CAPSIT-PD)], and freezing of gait (Freezing of Gait Assessment Course) (Ziegler *et al.*, 2010). The Freezing of Gait Assessment Course reliably detects freezing of gait given its episodic nature and dependence on environmental factors and includes elements like 'walking through a narrow door', 'turning in tight space' and 'dual tasking' that are well-known to provoke freezing of gait. These clinical ratings were obtained at baseline, upon 'immediate testing' and at '3-week follow-up' in all treatment conditions. Further anamnestic measures were assessed at baseline and at '3-week follow-up' on: (i) gait impairment related to freezing (Giladi Freezing of Gait

Questionnaire) (Giladi *et al.*, 2009); (ii) quality of life (PDQ-39); (iii) neuropsychiatric symptoms (Beck's Depression Index, Barrett Impulsiveness Scale); and (iv) non-motor symptoms (Non-motor Symptoms Scale) (Storch *et al.*, 2010). Note that the anamnestic scores and the primary endpoint (including anamnestic items) were not considered for 'immediate testing' as treatment conditions were separated by only 30 min.

Statistical analysis

The primary endpoint for the confirmatory statistical analysis was the difference in the 'axial score' between standard STN and combined STN + SNr stimulation at '3-week follow-up'. A sample size of 10 patients was estimated to be sufficient to detect a difference of 4 points on the primary outcome measure with 80% power, assuming a standard deviation of 4.0 (effect size: 1.0; NQuery Advisor 7.0). Assuming normal distribution a two-sided paired *t*-test with $\alpha = 0.05$ on the null hypothesis of equality of the two therapies was applied. The normality assumption on the primary endpoint was confirmed using the Shapiro-Wilk test. To adjust for a maximum of two dropouts $n = 12$ patients were enrolled.

Assuming normal distributions, the statistical analysis of the primary and all secondary outcomes includes a control for period effects. Therefore, unpaired *t*-tests were used to compare the sum of the scores in the two periods, i.e. the group of six patients who were randomized to standard STN stimulation followed by combined STN + SNr stimulation versus the group of six patients who were randomized to combined STN + SNr stimulation followed by standard STN stimulation (Wellek and Blettner, 2012). In the second step we analyzed the difference between the scores for combined STN + SNr stimulation and for standard STN stimulation. First we confirmed the normal distribution and if no evidence against the normality assumption was found ($P > 0.05$ on the Shapiro-Wilk tests) we compared the differences with paired *t*-tests. In case of evidence against the normality assumption we used sign tests.

The primary endpoint was statistically analyzed with the paired *t*-test. The outcome on the primary endpoint was decided on a two-sided significance level of 0.05. All secondary endpoints were analyzed with an exploratory intention and no confirmatory interpretation was drawn. As in this situation the 'use of multiple test procedures will not solve the problem of making valid statistical inference for hypotheses that were generated by the data' (Bender and Lange, 2001) findings from the exploratory analyses are subject to testing in confirmatory follow-up trials and accordingly not corrected for multiple comparisons here.

Moreover, given some clinical heterogeneity of our cohort concerning disease duration and time from DBS implantation (as typically observed along the variable endophenotypic spectrum of idiopathic Parkinson's disease) we additionally performed non-parametric testing on the primary endpoint and further on secondary endpoints without normal distribution (Non-motor Symptoms Scale, CAPSIT-PD, Berg Balance Scale) using a sign test. All measurements are presented with mean ± standard deviation for parametric tests and median (range) for non-parametric tests. The results presented were two-sided *P*-values without adjustments.

Results

Of 28 patients assessed for eligibility in our study centre 12 patients with advanced Parkinson's disease (nine male, age 65.0 ± 8.9 years) were enrolled between January 2011 and June 2012 at the Department for Neurodegenerative Diseases (Table 1). Reasons that precluded study participation were cognitive impairment (Mini-Mental State Examination < 25; $n = 5$), caudal electrode contact located outside STN–SNr border zone ($n = 4$), other disease that interfered with gait ($n = 5$), age > 80 years ($n = 1$), retracted consent ($n = 1$) (Fig. 1). The study cohort had age at Parkinson's disease onset of 47.0 ± 8.3 years, disease duration 17.6 ± 5.2 years, and time since DBS implantation 31.3 ± 24.4 (range: 6–79) months. The mean Mini-Mental State Examination score was 28.7 ± 1.3 (no patient <25). Four patients wished to discontinue standard STN stimulation treatment prematurely (Patients PD3, PD7, PD10 and PD11) owing to more pronounced gait impairment (Patients PD10 and PD11), immobility (Patients PD3 and PD7) or falls (Patient PD7). Three of these patients had been treated with combined STN + SNr stimulation first. A detailed overview on immediate (Table 2) and '3-week follow-up' results (Table 3) is given.

Table 1 Patient characteristics

ID	Age, years	Gender	Age at onset, years	Disease duration, years	Time with DBS, months	LED, mg	Axial score at enrolment
PD1	63	F	42	21	18	490	20
PD2	72	M	58	14	20	890	20
PD3	74	F	48	26	61	275	15
PD4	68	M	51	16	8	934	14
PD5	61	M	44	16	53	150	14
PD6	71	F	53	17	30	575	17
PD7	71	M	57	13	6	807	23
PD8	61	M	37	23	51	785	18
PD9	61	M	47	14	7	1098	12
PD10	67	M	41	26	79	440	14
PD11	41	M	31	10	10	350	14
PD12	70	M	55	15	33	1000	12

F = female, M = male; LED = L-DOPA equivalent dosage.

Table 2 Results from 'immediate testing'

	Baseline	'Immediate Testing'		
	OFF medication OFF stimulation	Standard STN stimulation	Combined STN + SNr stimulation	*P*-value
Secondary endpoints				
Axial UPDRS III (items 27–31)	11.17 ± 3.56	9.25 ± 4.67	8.17 ± 4.09	0.041[a]
Segmental UPDRS III (items 20–26)	38.0 ± 5.10	29.17 ± 6.62	27.58 ± 7.96	0.1347[a]
FOG-AC	22.17 ± 11.74	16.25 ± 12.78	8.67 ± 10.92	0.0056[a]
CAPSIT [steps]	18.5 (13–82)§	14.5 (8–51.5)§	14.5 (8.5–36)§	0.5488[b]
CAPSIT [time]	12 (6.5–105)§	7.5 (5.5–67.5)§	8.5 (5–28)§	0.7539[b]
CAPSIT [freezing]	0.5 (0–3)§	0.5 (0–3)§	0 (0–0.5)§	>0.99[b]
Berg Balance Scale	41.5 (11–56)§	47 (15–56)§	50 (9–56)§	0.7266[b]

FOG-AC = Freezing of Gait Assessment Course.
[a]*t*-Test.
[b]Sign Test.
§Median (Min–Max).

Table 3 Results from the '3-week follow-up'

	Baseline	'3-week follow-up'		
	OFF medication OFF stimulation	Standard STN stimulation	Combined STN + SNr stimulation	*P*-value
Primary endpoint (axial UPDRS II + III)	17.25 ± 4.31	14.25 ± 5.75	13.42 ± 6.47	0.470[a], 0.5078[b]
Secondary endpoints				
Segmental UPDRS III (items 20–26)	38.0 ± 5.10	28.75 ± 6.03	29.75 ± 5.53	0.5180[a]
Axial UPDRS III (items 27–31)	11.17 ± 3.56	8.08 ± 4.01	8.08 ± 4.38	>0.99[a]
FOG-AC	22.17 ± 11.74	14.42 ± 13.19	8.33 ± 10.91	0.0468[a]
CAPSIT [steps]	18.5 (13–82)§	14.25 (8–115)§	13 (8.5–28.5)§	0.2266[b]
CAPSIT [time]	12 (6.5–105)§	7.5 (4.5–71)§	7 (5–22.5)§	0.3438[b]
CAPSIT [freezing]	0.5 (0–3)§	0.25 (0–3.5)§	0 (0–0.5)§	0.0625[b]
Berg Balance Scale	41.5 (11–56)§	51.5 (19–56)§	51.5 (17–56)§	>0.99[b]
FOG-Q	14.67 ± 4.70	16.17 ± 3.83	14.50 ± 4.89	0.1013[a]
PDQ-39				
Mobility	53.96 ± 23.78	54.32 ± 27.23	49.38 ± 25.30	0.2925[a]
Activities of daily living	42.01 ± 20.45	45.08 ± 23.04	45.14 ± 22.46	0.4825[a]
Emotional well-being	26.74 ± 15.02	25.38 ± 21.45	23.96 ± 17.87	0.5697[a]
Stigma	21.88 ± 27.24	22.73 ± 25.35	20.31 ± 21.01	0.4592[a]
Social support	18.06 ± 23.26	18.94 ± 23.89	11.81 ± 10.93	0.2767[a]
Cognition	31.25 ± 24.28	23.30 ± 22.89	24.48 ± 21.89	0.4933[a]
Communication	40.97 ± 18.62	31.82 ± 21.99	36.81 ± 22.88	0.6250[a]
Bodily discomfort	35.42 ± 21.06	34.85 ± 22.61	36.81 ± 16.84	0.7623[a]
BDI	8.67 ± 3.37	7.91 ± 3.94	9.25 ± 5.55	0.3497[a]
NMSS				
Cardiovascular	1 (0–9)§	0 (0–6)§	0 (0–9)§	0.3750[b]
Sleep	9 (0–20)§	8 (0–24)§	11.5 (0–28)§	0.1797[b]
Mood	5.5 (2–18)§	8 (0–28)§	7 (0–49)§	0.7539[b]
Cognition	0 (0–12)§	0 (0–4)§	0 (0–13)§	>0.99[b]
Concentration	6 (0–27)§	4 (0–24)§	5 (0–32)§	0.2891[b]
Gastrointestinal	8 (0–25)§	8 (0–20)§	6.5 (0–20)§	0.7266[b]
Micturition	7 (0–30)§	8 (0–28)§	8.5 (0–18)§	>0.99[b]
Sexual function	4 (0–18)§	0 (0–12)§	1 (0–12)§	>0.99[b]
Sundries	7 (0–24)§	4 (0–26)§	9 (0–18)§	0.7266[b]
Barrett Impulsiveness Scale	62.6 ± 5.91	63.55 ± 4.3	61.67 ± 5.18	0.2894[a]
UPDRS IV	5.75 ± 1.96	6.27 ± 2.45	5.17 ± 3.04	0.2335[a]

FOG-AC = Freezing of Gait Assessment Course; FOG-Q = Freezing of Gait Questionnaire; CAPSIT = timed walking test from the Core Assessment Program; PDQ-39 = Parkinson's disease questionnaire (Quality of life, 39 items); BDI = Beck's Depression Scale Index; NMSS = Non-motor Symptoms Scale; UPDRS = Unified Parkinson's Disease Rating Scale. Two-sided *P*-values are given.
[a]*t*-Test.
[b]Sign Test, § Median (Min–Max).

Primary outcome parameter on axial motor impairment

At baseline (medication OFF, stimulation off) patients demonstrated severe impairment on the axial score as primary endpoint (17.25 ± 4.31). At '3-week follow-up', no statistically significant difference was found on the axial score between conditions [combined STN + SNr stimulation: 13.42 ± 6.47; standard STN stimulation: 14.25 ± 5.75; effect = 0.83 ± 3.86; 95% confidence interval (CI) − 1.62–3.82; $P = 0.470$; Fig. 3]. An additional non-parametric testing with the sign test revealed similar results. Four patients wished to discontinue standard STN stimulation treatment prematurely (Patient PD3: 3 h, Patient PD7: 19 days, Patient PD10: 2 days, Patient PD11: 9 days) but completed the entire combined STN + SNr stimulation follow-up. In these patients, the individual axial UPDRS scores improved in the combined STN + SNr stimulation condition compared with standard STN stimulation (Patient PD3: 16 versus 19; Patient PD7: 25 versus 27; Patient PD10: 9 versus 13; Patient PD11: 4 versus 9) with endpoint assessments performed according to the intention-to-treat principle. Three were randomized to combined STN + SNr stimulation first (Patients PD3, PD10 and PD11).

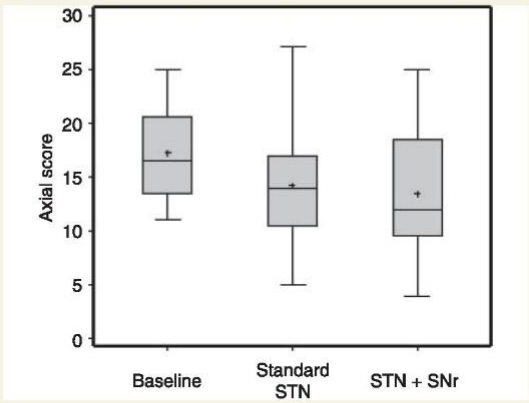

Figure 3 Primary endpoint at '3-week follow-up'. Results are given as box plots. *x*-axis: therapeutic condition; *y*-axis: axial score. [STN + SNr] = combined STN + SNr stimulation.

Secondary outcome measures: differentiation of distinct axial motor domains

For all secondary endpoints, no significant period effects were detected. In the 'immediate testing' and at '3-week follow-up'' the segmental UPDRS III (items 20–26) was improved with both standard STN stimulation and combined STN + SNr stimulation compared with medication OFF stimulation OFF (baseline), as expected (Tables 2 and 3). At baseline, patients presented with severe axial motor symptoms according to the axial UPDRSIII (items 27–31) (11.17 ± 3.56). Greater improvement was observed in the 'immediate testing' with combined STN + SNr stimulation compared with standard STN stimulation on only active subthalamic contacts (8.17 ± 4.09 versus 9.25 ± 4.67; $P = 0.041$), however, no difference was found at the '3-week follow-up' (8.08 ± 4.38 versus 8.08 ± 4.01; $P > 0.99$). Similarly, patients presented with severe freezing of gait at baseline according to the Freezing of Gait Assessment Course (22.17 ± 11.74). This improved more with combined STN + SNr stimulation compared with standard STN stimulation in the 'immediate testing' (8.67 ± 10.92 versus 16.25 ± 12.78; $P = 0.006$) and at the '3-week follow-up' (8.33 ± 10.91 versus 14.42 ± 13.19; $P = 0.047$). Of note, freezing of gait presented with similar severity both at 'immediate testing' and at '3-week follow-up' in both treatment conditions, although at '3-week follow-up' patients were ON their regular dopaminergic medication unlike 'immediate testing' (Fig. 4). In the CAPSIT-

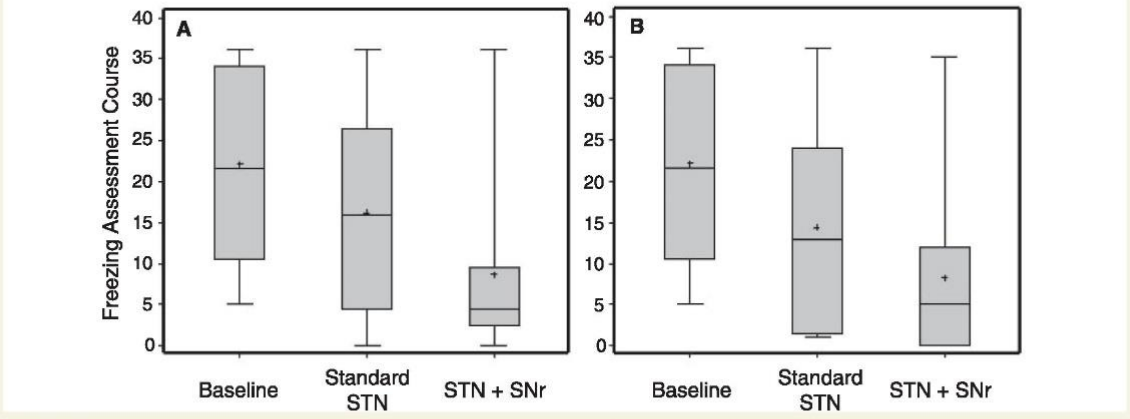

Figure 4 Secondary endpoint: results at (**A**) 'immediate testing' and at (**B**) '3-week follow-up' are given for the Freezing of Gait Assessment Course. Results are given as box plots. *x*-axis: therapeutic condition; *y*-axis: score of the Freezing of Gait Assessment Course. [STN + SNr] = combined STN + SNr stimulation.

PD timed walking test no relevant differences were observed between combined STN + SNr stimulation and standard STN stimulation in the number of steps and time. Freezing episodes occurred more frequently with standard STN stimulation compared with combined STN + SNr stimulation at '3-week follow-up' ($P = 0.063$; Supplementary material) but not at 'immediate testing'. In the Giladi Freezing of Gait Questionnaire, freezing of gait improved with combined STN + SNr stimulation compared with standard STN stimulation, although not significantly (14.50 ± 4.89 versus 16.17 ± 3.83; $P = 0.1$). No differences were observed in the Berg Balance Scale.

Ten of 12 patients wished to continue combined STN + SNr stimulation treatment at the end of the study.

Quality of life and non-motor issues

The PDQ-39 summary index was unchanged in both treatment arms. At baseline, patients presented with highest impairment of quality of life in the 'mobility' domain, as expected (53.96 ± 23.78). A slightly greater improvement was observed with combined STN + SNr stimulation compared with standard STN stimulation on 'mobility' (49.38 ± 25.30 versus 54.32 ± 27.23; not statistically significant) and 'social support' (11.81 ± 10.93 versus 18.94 ± 23.89; not statistically significant). No differences were identified in the distinct non-motor symptom domains.

Adverse events

In both treatment arms no serious adverse events were observed. Four patients wished to discontinue standard STN stimulation treatment prematurely. During combined STN + SNr stimulation active treatment, no acute side effects were observed, however, four adverse events were reported during the '3-week follow-up'. Two patients (Patients PD2 and PD9) reported delayed onset of dyskinesias within the first few days after introduction of combined STN + SNr stimulation, which completely resolved after therapy adjustment: in Patient PD2, stimulation amplitudes were lowered on the caudal contacts (−0.4V, both electrodes). Patient PD9 had already self-administered a reduction of the daily L-DOPA dosage by 125 mg when the patient informed the study site; this had already ameliorated the dyskinesias. The patient was rescheduled and as slight dyskinesias persisted, the SNr amplitudes were lowered by −0.1 V on both sides. After therapy adjustment both patients were followed for the complete '3-week follow-up' period according to the intention-to-treat principle. One patient (Patient PD8) reported at the '3-week follow-up' visit that a few intermittent episodes of double vision during combined STN + SNr stimulation treatment, each lasting for a few seconds, had occurred. Patient PD7 reported increased immobility and recurrent falls during the last week of follow-up under combined STN + SNr stimulation, whereas patient and caregiver consistently reported initial improvement of freezing of gait during the first 2 weeks.

Safety measures

No suicidality was reported. No change was found on the Beck's Depression Scale Index on group level; Patient PD1 presented with increased Beck's Depression Scale Index scores during combined STN + SNr stimulation compared to standard STN stimulation (18 versus 7). Patient PD7 reported visual 'benign hallucinations with insight retained' (UPDRS I, item 2) during combined STN + SNr stimulation consistent with a former personal history of hallucinations as documented in the preoperative records. UPDRS I, item 2 was unchanged at the group level between therapeutic conditions. No patient presented with psychosis. The comparison of standard STN stimulation and combined STN + SNr stimulation showed no differences between treatments on the Barrett Impulsivity Scale, on segmental motor symptoms (UPDRS III, items 20–26) and on motor fluctuations (UPDRS IV).

Discussion

In this randomized controlled phase II trial, intractable gait impairment as one of the major unmet needs in the treatment of advanced Parkinson's disease was treated with interleaved pulses of STN and SNr for the first time. This trial particularly addressed the therapeutic response of a broad spectrum of axial motor symptoms and secondarily disentangled the efficacy on distinct axial subdomains. The broad-scaled primary endpoint revealed no significant improvement of axial motor functioning with combined STN + SNr stimulation compared with stimulation on only active subthalamic contacts. Similarly, as a secondary endpoint analysis, there was only a slight improvement of the clinical axial motor items (UPDRS III, items '27-31') in the 'immediate testing' from combined STN + SNr stimulation compared with standard STN stimulation that did not present at the '3-week follow-up'. More specifically, we observed an improvement of freezing of gait on combined STN + SNr stimulation in the Freezing of Gait Assessment Course as secondary exploratory endpoint analysis, whereas postural control according to the Berg Balance Scale remained unchanged. This was in line with a (not statistically significant) five-point improvement in the mobility domain of the PDQ-39 with combined STN + SNr stimulation compared with standard STN stimulation. Although, no final conclusion can be drawn owing to the small sample size and exploratory nature of the secondary endpoints in this phase II trial, a difference of 3.2 points on the PDQ-39 'mobility' subdomain was identified as meaningful to improve the patients' subjective clinical impression in large Parkinson's disease cohorts (Peto *et al.*, 2001) and this may be verified in a larger follow-up trial. Of note, 4 of 12 patients discontinued standard STN stimulation treatment, three of them after switching from combined STN + SNr stimulation to the standard STN stimulation condition. Consistently, in all of these patients, the individual primary endpoint scores were superior with combined STN + SNr stimulation and 10 of 12 patients preferred to continue combined STN + SNr stimulation after completion of the study. Group-level data of the primary endpoint and anamnestic secondary outcome measures have to be interpreted

with caution given the premature drop-outs in only the standard stimulation on only active subthalamic contacts treatment arm.

Generally, it should be noted that the variability within the endophenotypic spectrum of idiopathic Parkinson's disease was also reflected by this study cohort and cannot be excluded. Even with genetic classifications, e.g. a spread in 'age at disease onset' and a variable disease progression was reported in *LRRK2* mutation carriers (Schiesling *et al.*, 2008). The clinical heterogeneity includes variable disease progression after STN–DBS with emerging axial symptoms resistant to standard therapy. However, regarding axial symptoms and cognitive decline, genetic biomarkers like the most common genetic susceptibility factor for Parkinson's disease, i.e. heterozygous mutations in the glucocerebrosidase (*GBA*) gene, might help to predict the individual profile of disease progression more accurately in the future (Weiss *et al.*, 2012*b*; Winder-Rhodes *et al.*, 2013). In this context, another strength of this study was to identify patients with Parkinson's disease with predominant freezing of gait as a future subgroup of interest for neuromodulation trials on the level of SNr. This is also important for related neurostimulation strategies on axial motor symptoms, as a heterogeneous spectrum of treatment response was observed in previous trials on pedunculopontine stimulation modulating balance or freezing of gait to a variable degree (Ferraye *et al.*, 2010; Hamani *et al.*, 2011). Therefore, the detailed phenotypic classification of patients with Parkinson's disease according to the clinical criteria identified here may help to reduce the heterogeneity of study cohorts in future trials on gait impairment.

Concomittant stimulation of the SNr was safe and well-tolerated. Mild side-effects were delayed by a few days and were resolved completely. The motor response of segmental symptoms remained unchanged and, similarly, motor fluctuations remained well controlled. Therefore, as a major advantage of the concomittant nigral stimulation, the best individual subthalamic stimulation parameters can be maintained for reprogramming. Most importantly, SNr stimulation was safe on non-motor issues and major neuropsychiatric domains including depressive symptoms, impulsivity, suicidality and psychotic symptoms. Previously, acute depressive (Bejjani *et al.*, 1999; Blomstedt *et al.*, 2008) or hypomanic clinical states (Ulla *et al.*, 2011) were described in few selected cases with high-frequency stimulation on SNr contacts, and similarly, mood changes were described to emerge basically on 'ventral subthalamic' contacts in the COMPARE trial (Okun *et al.*, 2009). However, the incidence of neuropsychiatric interference from SNr stimulation in unselected DBS cohorts remains undetermined. Recognizing these previous findings, we carefully monitored for neuropsychiatric symptoms and found that nigral stimulation may be applied safely. Nevertheless, patients with subthalamic and nigral stimulation should be followed with caution for neuropsychiatric symptoms. Larger cohorts and longer follow-up ranges are needed to draw a final conclusion.

To interpret the results of this and related studies on freezing of gait in advanced Parkinson's disease (Moreau *et al.*, 2008, 2012; Chastan *et al.*, 2009) and to strategize future directions of DBS for axial motor symptoms other aspects have to be considered: STN–DBS reprogramming (e.g. by modulating parameters to lower frequencies) was limited by recurrence of segmental symptoms (Moreau *et al.*, 2008; Ricchi *et al.*, 2012). This also applies when considering stimulation on a single SNr contact, which did not sufficiently control segmental motor symptoms (Chastan *et al.*, 2009) and, therefore, was not considered in this trial. Similarly, one might argue that the intermediate ventral subthalamic contact might have been more efficacious; however, several previous findings argue against this: the progressive amplitude increase on a dorsolateral subthalamic contact is likely to activate the ventral portion of the subthalamic nucleus, although, this was associated with a disproportional decline of gait impairment including freezing of gait (Moreau *et al.*, 2008). Consistently, no differential therapeutic response of gait or balance impairments was found with dorsal versus ventral subthalamic nucleus stimulation (McNeely *et al.*, 2011). The present study characterized a novel target of interest for future neuromodulation trials. The dorsolateral part of the SNr (Fig. 2) with mainly GABAergic and cholinergic projection neurons mediated our findings. Whereas, at the level of the pedunculopontine nucleus stimulation at lower frequencies below 35 Hz (Stefani *et al.*, 2007; Ferraye *et al.*, 2010; Moro *et al.*, 2010; Thevathasan *et al.*, 2011*a*) and at 70 Hz in unilateral stimulation (Moro *et al.*, 2010) was put forward for gait therapy, stimulation on high frequencies might be superior on the level of SNr in the light of previous converging experimental evidence: SNr demonstrated pathological overactivity in Parkinson's disease (Breit *et al.*, 2006) and high frequency stimulation may suppress SNr activity (Lafreniere-Roula *et al.*, 2010). Similarly, pharmacological inhibition of SNr activity presented with 'prokinetic' effects and elicited dyskinesias (Dybdal *et al.*, 2013) as similarly observed in two of our patients. Consistently, high-frequency SNr stimulation at 130 Hz (unlike 50 Hz) improved forelimb akinesia in a rat model of Parkinson's disease (Sutton *et al.*, 2013). GABAergic inhibitory output from the SNr to the pedunculopontine nucleus was demonstrated in animal research including experiments in rats (Childs and Gale, 1983; Grofova and Zhou, 1998), cat (Noda and Oka, 1986; Nakamura *et al*., 1989), and non-human primates (Carpenter *et al.*, 1981). Given the efferent monosynaptic GABAergic transmission from SNr to the pedunculopontine nucleus (Nandi *et al.*, 2008), high-frequency stimulation at the level of SNr might attenuate an overinhibitory drive.

A large body of clinical trials provides compelling evidence that axial impairment emerges along disease progression after primarily effective STN–DBS (Krack *et al.*, 2003; St George *et al.*, 2010; Castrioto *et al.*, 2011; Nutt *et al.*, 2011), however evidence-based data on how to treat these resistant symptoms is still limited. It has to be kept in mind that clinical trials generally select for cognitively competent Parkinson's disease patients given that axial and cognitive impairments may demonstrate with coincidence. Whether the potential benefit from nigral stimulation applies to a larger proportion of patients with advanced Parkinson's disease remains to be determined and this consideration may include patients with predominant preoperative freezing of gait that are often precluded from DBS treatments. Genetic predictors and endophenotypes might be defined to indicate optimal target selection (Weiss *et al.*, 2012*b*).

This phase II trial opens the perspective that concomittant SNr stimulation might improve intractable freezing of gait. Field steering applications like interleaved programming can be utilized as a reprogramming option if patients develop resistant freezing of gait

along disease progression. A larger randomized controlled phase III clinical trial to assess the efficacy of concomittant nigral stimulation on 'freezing of gait' and 'quality of life' is highly warranted.

Acknowledgements

We are grateful to Ruth Bösel and Dagmar Henke (Department of Biometry) for support in data management and statistical analyses. We wish to thank John Hammargren (Medtronic Inc., Minnesota, USA) for reconstruction of an exemplary image of volume of tissue activated by *interleaved* STN + SNr stimulation. We greatly acknowledge Dr. Robert Chen (MBBChir, MSc, FRCPC) for proof reading and the valuable comments on the manuscript (Toronto Western Hospital, Edmond J. Safra Program in Parkinson's Disease, University Health Network, and the Division of Neurology, University of Toronto, Canada).

Funding

The study was supported by a Research Grant (AKF 259-0-0) of the Eberhard Karls University Tübingen and by Medtronic Europe Sarl. Daniel Weiss is supported by a research grant of the German Research Council (DFG) WE5375/1-1 and was supported by a Research Grant of the Medical Faculty of the University of Tübingen (AKF 259-0-0). Daniel Weiss received speaker's honoraria and a travel grant from Medtronic, Abott Pharmaceutical, UCB, and the Movement Disorder Society. Margarete Walach received a travel grant from GlaxoSmithKline. Christoph Meisner received reimbursement from the Department of Neurodegenerative Diseases as provided by Medtronic study support. Melanie Fritz received a travel grant from Ipsen Pharmaceuticals.

Christian Plewnia received resarch grants from the German Research Council (DFG; PL 525/1–1), the University of Tübingen (AKF #238-0-0) and the Werner Reichardt Centre for Integrative Neuroscience (CIN, PP2011_11). He received speaker's honoraria by Inomed Medizintechnik GmbH. Sorin Breit was supported by a Research Grant of the Medical Faculty of the University of Tübingen (AKF 246-0-1). Benjamin Bender received a travel grant by Bayer Vital and was supported by research grants of the German Research Council (DFG) BE4609/1-1 and the Wilhelm-Schuler-Stiftung. Alireza Gharabaghi is supported by grants from the German Research Council [DFG GH 94/2-1, DFG EC 307], Federal Ministry for Education and Research [BFNT 01GQ0761, BMBF 16SV3783, BMBF 03160064B, BMBF V4UKF014, and European Union [ERC 2276329]. Alireza Gharabaghi received speaker's honoraria and travel grants from Medtronic. Tobias Wächter: received speaker's honoraria and travel reimbursement for scientific meetings from Medtronic, Solvay, Abbott Pharma, Cephalon, Merz Pharmaceuticals, Ipsen Pharma and Schwarz Pharma. He has also received financial support for research from and conducted commissioned research for Medtronic, Abbott Pharma, Merz Pharmaceuticals, Ipsen Pharma and Pharm-Allergan and worked on advisory boards for Ipsen Pharma and Merz Pharmaceuticals. Rejko Krüger serves as Editor of European Journal of Clinical Investigation, Journal of Neural Transmission and Associate Editor of BMC Neurology; has received research grants of the German Research Council (DFG; KR2219/2-3 and KR2119/8-1), the Michael J Fox Foundation, the Fritz Thyssen foundation (10.11.2.153) and the Federal Ministry for Education and Research [BMBF, NGFNplus; 01GS08134], as well as speaker's honoraria and/or travel grants from UCB Pharma, Cephalon, Abott Pharmaceutical, Takeda Pharmaceuticals and Medtronic.

Supplementary material

Supplementary material is available at *Brain* online.

References

Bejjani BP, Damier P, Arnulf I, Thivard L, Bonnet AM, Dormont D, et al. Transient acute depression induced by high-frequency deep-brain stimulation. N Engl J Med 1999; 340: 1476–80.

Bender R, Lange S. Adjusting for multiple testing–when and how? J Clin Epidemiol 2001; 54: 343–9.

Berg KO, Wood-Dauphinee SL, Williams JI, Maki B. Measuring balance in the elderly: validation of an instrument. Can J Public Health 1992; 83: S7–11.

Blomstedt P, Hariz MI, Lees A, Silberstein P, Limousin P, Yelnik J, et al. Acute severe depression induced by intraoperative stimulation of the substantia nigra: a case report. Parkinsonism Relat Disord 2008; 14: 253–6.

Breit S, Lessmann L, Unterbrink D, Popa RC, Gasser T, Schulz JB. Lesion of the pedunculopontine nucleus reverses hyperactivity of the subthalamic nucleus and substantia nigra pars reticulata in a 6-hydroxydopamine rat model. Eur J Neurosci 2006; 24: 2275–82.

Carpenter M, Carleton SC, Keller JT, Conte P. Connection of the subthalamic nucleus in the monkey. Brain Res 1981; 224: 1–29.

Castrioto A, Lozano AM, Poon YY, Lang AE, Fallis M, Moro E. Ten-year outcome of subthalamic stimulation in Parkinson disease: a blinded evaluation. Arch Neurol 2011; 68: 1550–6.

Chastan N, Westby GW, Yelnik J, Bardinet E, Do MC, Agid Y, et al. Effects of nigral stimulation on locomotion and postural stability in patients with Parkinson's disease. Brain 2009; 132: 172–84.

Childs JA, Gale K. Neurochemical evidence for nigrotegmental GABAergic projection. Brain Res 1983; 258: 109–114.

Cooper SE, McIntyre CC, Fernandez HH, Vitek JL. Association of deep brain stimulation washout effects with Parkinson disease duration. JAMA Neurol 2013; 70: 95–9.

Deuschl G, Schade-Brittinger C, Krack P, Volkmann J, Schafer H, Botzel K, et al. A randomized trial of deep-brain stimulation for Parkinson's disease. N Engl J Med 2006; 355: 896–908.

Dybdal D, Forcelli PA, Dubach M, Oppedisano M, Holmes A, Malkova L, et al. Topography of dyskinesias and torticollis evoked by inhibition of substantia nigra pars reticulata. Mov Disord 2013; 28: 460–8.

Fasano A, Herzog J, Seifert E, Stolze H, Falk D, Reese R, et al. Modulation of gait coordination by subthalamic stimulation improves freezing of gait. Mov Disord 2011; 26: 844–51.

Ferraye MU, Debu B, Fraix V, Goetz L, Ardouin C, Yelnik J, et al. Effects of pedunculopontine nucleus area stimulation on gait disorders in Parkinson's disease. Brain 2010; 133: 205–14.

Giladi N, Tal J, Azulay T, Rascol O, Brooks DJ, Melamed E, et al. Validation of the freezing of gait questionnaire in patients with Parkinson's disease. Mov Disord 2009; 24: 655–61.

Grofova I, Zhou M. Nigral innervation of cholinergic and glutamatergic cells in the rat mesopontine tegmentum: light and electron microscopic anterograde tracing and immunohistochemical studies. J Comp Neurol 1998; 395: 359–79.

Hamani C, Moro E, Lozano AM. The pedunculopontine nucleus as a target for deep brain stimulation. J Neural Transm 2011; 118: 1461–8.
Kleiner-Fisman G, Herzog J, Fisman DN, Tamma F, Lyons KE, Pahwa R, et al. Subthalamic nucleus deep brain stimulation: summary and meta-analysis of outcomes. Mov Disord 2006; 21 (Suppl 14): S290–304.
Kovacs N, Janszky J, Nagy F, Balas I. Changing to interleaving stimulation might improve dystonia in cases not responding to pallidal stimulation. Mov Disord 2012; 27: 163–5.
Krack P, Batir A, Van Blercom N, Chabardes S, Fraix V, Ardouin C, et al. Five-year follow-up of bilateral stimulation of the subthalamic nucleus in advanced Parkinson's disease. N Engl J Med 2003; 349: 1925–34.
Kuriakose R, Saha U, Castillo G, Udupa K, Ni Z, Gunraj C, et al. The nature and time course of cortical activation following subthalamic stimulation in Parkinson's disease. Cereb Cortex 2010; 20: 1926–36.
Lafreniere-Roula M, Kim E, Hutchison WD, Lozano AM, Hodaie M, Dostrovsky JO. High-frequency microstimulation in human globus pallidus and substantia nigra. Exp Brain Res 2010; 205: 251–61.
McNeely ME, Hershey T, Campbell MC, Tabbal SD, Karimi M, Hartlein JM, et al. Effects of deep brain stimulation of dorsal versus ventral subthalamic nucleus regions on gait and balance in Parkinson's disease. J Neurol Neurosurg Psychiatry 2011; 82: 1250–5.
Moreau C, Defebvre L, Destee A, Bleuse S, Clement F, Blatt JL, et al. STN-DBS frequency effects on freezing of gait in advanced Parkinson disease. Neurology 2008; 71: 80–4.
Moreau C, Delval A, Defebvre L, Dujardin K, Duhamel A, Petyt G, et al. Methylphenidate for gait hypokinesia and freezing in patients with Parkinson's disease undergoing subthalamic stimulation: a multicentre, parallel, randomised, placebo-controlled trial. Lancet Neurol 2012; 11: 589–96.
Moro E, Hamani C, Poon YY, Al-Khairallah T, Dostrovsky JO, Hutchison WD, et al. Unilateral pedunculopontine stimulation improves falls in Parkinson's disease. Brain 2010; 133: 215–24.
Nakamura Y, Tokuno H, Moriizumi T, Kitao Y, Kudo M. Monosynaptic nigral inputs to the pedunculopontine tegmental nucleus neurons which send their axons to the medial reticular formation in the medulla oblongata. An electron microscopic study in the cat. Neurosci Lett 1989; 103: 145–50.
Nandi D, Jenkinson N, Stein J, Aziz T. The pedunculopontine nucleus in Parkinson's disease: primate studies. Br J Neurosurg 2008; 22 (Suppl 1): S4–8.
Noda T, Oka H. Distribution and morphology of tegmental neurons receiving nigral inhibitory inputs in the cat: an intracellular HRP study. J Comp Neurol 1986; 244: 254–66.
Nutt JG, Bloem BR, Giladi N, Hallett M, Horak FB, Nieuwboer A. Freezing of gait: moving forward on a mysterious clinical phenomenon. Lancet Neurol 2011; 10: 734–44.
Okun MS, Fernandez HH, Wu SS, Kirsch-Darrow L, Bowers D, Bova F, et al. Cognition and mood in Parkinson's disease in subthalamic nucleus versus globus pallidus interna deep brain stimulation: the COMPARE trial. Ann Neurol 2009; 65: 586–95.
Peto V, Jenkinson C, Fitzpatrick R. Determining minimally important differences for the PDQ-39 Parkinson's disease questionnaire. Age Ageing 2001; 30: 299–302.
Potter M, Herzog J, Siebner HR, Kopper F, Steigerwald F, Deuschl G, et al. Subthalamic nucleus stimulation modulates audiospinal reactions in Parkinson disease. Neurology 2008; 70: 1445–51.
Potter-Nerger M, Ilic TV, Siebner HR, Deuschl G, Volkmann J. Subthalamic nucleus stimulation restores corticospinal facilitation in Parkinson's disease. Mov Disord 2008; 23: 2210–5.
Ricchi V, Zibetti M, Angrisano S, Merola A, Arduino N, Artusi CA, et al. Transient effects of 80 Hz stimulation on gait in STN DBS treated PD patients: a 15 months follow-up study. Brain Stimul 2012; 5: 388–92.
Salenius S, Avikainen S, Kaakkola S, Hari R, Brown P. Defective cortical drive to muscle in Parkinson's disease and its improvement with levodopa. Brain 2002; 125: 491–500.
Schiesling C, Kieper N, Seidel K, Kruger R. Review: Familial Parkinson's disease—genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. Neuropathol Appl Neurobiol 2008; 34: 255–71.
Schuepbach WM, Rau J, Knudsen K, Volkmann J, Krack P, Timmermann L, et al. Neurostimulation for Parkinson's disease with early motor complications. N Engl J Med 2013; 368: 610–22.
St George RJ, Nutt JG, Burchiel KJ, Horak FB. A meta-regression of the long-term effects of deep brain stimulation on balance and gait in PD. Neurology 2010; 75: 1292–9.
Stefani A, Lozano AM, Peppe A, Stanzione P, Galati S, Tropepi D, et al. Bilateral deep brain stimulation of the pedunculopontine and subthalamic nuclei in severe Parkinson's disease. Brain 2007; 130: 1596–607.
Storch A, Odin P, Trender-Gerhard I, Fuchs G, Reifschneider G, Ray Chaudhuri K, et al. Non-motor Symptoms Questionnaire and Scale for Parkinson's disease. Cross-cultural adaptation into the German language [in German]. Nervenarzt 2010; 81: 980–5.
Sutton AC, Yu W, Calos ME, Smith AB, Ramirez-Zamora A, Molho ES, et al. Deep brain stimulation of the substantia nigra pars reticulata improves forelimb akinesia in the hemiparkinsonian rat. J Neurophysiol 2013; 109: 363–74.
Thevathasan W, Coyne TJ, Hyam JA, Kerr G, Jenkinson N, Aziz TZ, et al. Pedunculopontine nucleus stimulation improves gait freezing in Parkinson disease. Neurosurgery 2011a; 69: 1248–53; discussion 1254.
Thevathasan W, Pogosyan A, Hyam JA, Jenkinson N, Bogdanovic M, Coyne TJ, et al. A block to pre-prepared movement in gait freezing, relieved by pedunculopontine nucleus stimulation. Brain 2011b; 134: 2085–95.
Tsang EW, Hamani C, Moro E, Mazzella F, Poon YY, Lozano AM, et al. Involvement of the human pedunculopontine nucleus region in voluntary movements. Neurology 2010; 75: 950–9.
Ulla M, Thobois S, Llorca PM, Derost P, Lemaire JJ, Chereau-Boudet I, et al. Contact dependent reproducible hypomania induced by deep brain stimulation in Parkinson's disease: clinical, anatomical and functional imaging study. J Neurol Neurosurg Psychiatry 2011; 82: 607–14.
Weaver FM, Follett K, Stern M, Hur K, Harris C, Marks WJ Jr, et al. Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. JAMA 2009; 301: 63–73.
Weiss D, Breit S, Hoppe J, Hauser AK, Freudenstein D, Kruger R, et al. Subthalamic nucleus stimulation restores the efferent cortical drive to muscle in parallel to functional motor improvement. Eur J Neurosci 2012a; 35: 896–908.
Weiss D, Breit S, Wachter T, Plewnia C, Gharabaghi A, Kruger R. Combined stimulation of the substantia nigra pars reticulata and the subthalamic nucleus is effective in hypokinetic gait disturbance in Parkinson's disease. J Neurol 2011a; 258: 1183–5.
Weiss D, Brockmann K, Srulijes K, Meisner C, Klotz R, Reinbold S, et al. Long-term follow-up of subthalamic nucleus stimulation in glucocerebrosidase-associated Parkinson's disease. J Neurol 2012b; 259: 1970–2.
Weiss D, Wachter T, Meisner C, Fritz M, Gharabaghi A, Plewnia C, et al. Combined STN/SNr-DBS for the treatment of refractory gait disturbances in Parkinson's disease: study protocol for a randomized controlled trial. Trials 2011b; 12: 222.
Wellek S, Blettner M. On the proper use of the crossover design in clinical trials: part 18 of a series on evaluation of scientific publications. Dtsch Arztebl Int 2012; 109: 276–81.
Winder-Rhodes SE, Evans JR, Ban M, Mason SL, Williams-Gray CH, Foltynie T, et al. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. Brain 2013; 136: 392–9.
Wojtecki L, Vesper J, Schnitzler A. Interleaving programming of subthalamic deep brain stimulation to reduce side effects with good motor outcome in a patient with Parkinson's disease. Parkinsonism Relat Disord 2011; 17: 293–4.
Ziegler K, Schroeteler F, Ceballos-Baumann AO, Fietzek UM. A new rating instrument to assess festination and freezing gait in Parkinsonian patients. Mov Disord 2010; 25: 1012–8.

5.2 Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease

Published as:

Weiss D, Klotz R, Govindan RB, Scholten M, Naros G, Ramos-Murguialday A, et al. Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease. Brain. 2015;138(Pt 3):679–93.

Brain Advance Access published January 2, 2015

doi:10.1093/brain/awu380 BRAIN 2015: Page 1 of 15 | 1

BRAIN
A JOURNAL OF NEUROLOGY

Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease

Daniel Weiss,[1,2,3] Rosa Klotz,[1,2,3] Rathinaswamy B. Govindan,[4] Marlieke Scholten,[1,2,3] Georgios Naros,[3,5] Ander Ramos-Murguialday,[6,7] Friedemann Bunjes,[1,2] Christoph Meisner,[8] Christian Plewnia,[3,9] Rejko Krüger[1,2,3,10] and Alireza Gharabaghi[3,5]

Dynamic modulations of large-scale network activity and synchronization are inherent to a broad spectrum of cognitive processes and are disturbed in neuropsychiatric conditions including Parkinson's disease. Here, we set out to address the motor network activity and synchronization in Parkinson's disease and its modulation with subthalamic stimulation. To this end, 20 patients with idiopathic Parkinson's disease with subthalamic nucleus stimulation were analysed on externally cued right hand finger movements with 1.5-s interstimulus interval. Simultaneous recordings were obtained from electromyography on antagonistic muscles (right flexor digitorum and extensor digitorum) together with 64-channel electroencephalography. Time-frequency event-related spectral perturbations were assessed to determine cortical and muscular activity. Next, cross-spectra in the time-frequency domain were analysed to explore the cortico-cortical synchronization. The time-frequency modulations enabled us to select a time-frequency range relevant for motor processing. On these time-frequency windows, we developed an extension of the phase synchronization index to quantify the global cortico-cortical synchronization and to obtain topographic differentiations of distinct electrode sites with respect to their contributions to the global phase synchronization index. The spectral measures were used to predict clinical and reaction time outcome using regression analysis. We found that movement-related desynchronization of cortical activity in the upper alpha and beta range was significantly facilitated with 'stimulation on' compared to 'stimulation off' on electrodes over the bilateral parietal, sensorimotor, premotor, supplementary-motor, and prefrontal areas, including the bilateral inferior prefrontal areas. These spectral modulations enabled us to predict both clinical and reaction time improvement from subthalamic stimulation. With 'stimulation on', interhemispheric cortico-cortical coherence in the beta band was significantly attenuated over the bilateral sensorimotor areas. Similarly, the global cortico-cortical phase synchronization was attenuated, and the topographic differentiation revealed stronger desynchronization over the (ipsilateral) right-hemispheric prefrontal, premotor and sensorimotor areas compared to 'stimulation off'. We further demonstrated that the cortico-cortical phase synchronization was largely dominated by genuine neuronal coupling. The clinical improvement with 'stimulation on' compared to 'stimulation off' could be predicted from this cortical decoupling with multiple regressions, and the reduction of synchronization over the right prefrontal area showed a linear univariate correlation with clinical improvement. Our study demonstrates wide-spread activity and synchronization modulations of the cortical motor network, and highlights subthalamic stimulation as a network-modulating therapy. Accordingly, subthalamic stimulation may release bilateral cortical computational resources by facilitating movement-related desynchronization. Moreover, the subthalamic nucleus is critical to balance inhibitory and facilitatory cortical players within the motor program.

1 German Centre of Neurodegenerative Diseases (DZNE), 72076 Tübingen, Germany
2 Department for Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research, University of Tübingen, 72076 Tübingen, Germany
3 Werner Reichardt Centre for Integrative Neuroscience, 72076 Tübingen, Germany
4 Foetal Medicine Institute, Division of Foetal and Transitional Medicine, Children's National Health System, M3118C Washington, DC, USA

Received July 28, 2014. Revised October 17, 2014. Accepted November 6, 2014.

5 Division of Functional and Restorative Neurosurgery, Department of Neurosurgery, University of Tübingen, 72076 Tübingen, Germany
6 Institute of Medical Psychology and Behavioural Neurobiology, University of Tübingen, 72076 Tübingen, Germany
7 TECNALIA, Health Technologies, 200003 San Sebastian, Spain
8 Clinical Epidemiology and Applied Biometry, University of Tübingen, 72076 Tübingen, Germany
9 Department of Psychiatry and Psychotherapy, Neurophysiology & Interventional Neuropsychiatry, 72076 Tübingen, Germany
10 Clinical and Experimental Neuroscience, Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg and Centre Hospitalier de Luxembourg (CHL), 1210 Luxembourg, Luxembourg

Correspondence to: Daniel Weiss, MD,
Department for Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research,
Department of Neurosurgery,
Centre for Integrative Neuroscience,
University of Tübingen,
Germany
E-mail: daniel.weiss@uni-tuebingen.de

Correspondence may also be addressed to: Alireza Gharabaghi, MD, Division of Functional and Restorative Neurosurgery, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany E-mail: alireza.gharabaghi@uni-tuebingen.de

Keywords: Parkinson's disease; subthalamic nucleus; deep brain stimulation; synchronization, cortex

Abbreviations: MRD = movement-related desynchronization; STN-DBS = subthalamic nucleus–deep brain stimulation; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

Dynamic modulations of large-scale network activity and synchronization are inherent to a broad spectrum of cognitive processes (Fell and Axmacher, 2011; Engel *et al.*, 2013). Dysregulation of the concerted interplay in such networks parallels several neuropsychiatric disease states (Uhlhaas and Singer, 2006), and this includes the pathological motor 'off state' in Parkinson's disease (Salenius *et al.*, 2002; Timmermann *et al.*, 2003; Kuhn *et al.*, 2006; Weiss *et al.*, 2012; Hirschmann *et al.*, 2013). Although a comprehensive understanding of neuronal activation and synchronization on the multiple levels of the motor network is still enigmatic (i.e. topography, time, and frequency domain interactions), 'intrinsic coupling modes' were expected to modulate in a context-dependent manner (Engel *et al.*, 2013). Generally speaking, such multilevel organization enables patterning and integration of several consecutive steps and features of processes such as motor integration, including inhibition, relay or execution, and feedback processing to optimize motor performance and behavioural success.

In particular, pathological motor states such as Parkinson's disease are paralleled by excessive synchronization of the basal ganglia—cortical motor network in the beta band, and this may critically interfere with efficient motor integration (Kuhn *et al.*, 2006; Eusebio *et al.*, 2011; Little *et al.*, 2013; Kahan *et al.*, 2014). Consistent with this notion dopaminergic neurodegeneration impacts significant maladaptive activity and connectivity at widely-distributed levels including both subthalamic and motor cortical activity, as well as corticospinal synchronization (Salenius *et al.*, 2002; Kumru *et al.*, 2004; Potter-Nerger *et al.*, 2008; Weiss *et al.*, 2012; Herz *et al.*, 2013). Given that daily life motor function needs rapid and ongoing adjustments of the motor program, it is plausible and supported by experimental evidence that cortical activity and synchronization require short-latency dynamic adjustments. Similarly, a disturbance of these dynamic processes may be of critical relevance for Parkinson's disease motor symptoms as was shown in cortical event-related desynchronization of internally generated movement in Parkinson's disease (Brown and Marsden, 1999; Magnani *et al.*, 2002; Devos *et al.*, 2004).

Here, we set out to study the dynamic large-scale cortical activity and cortico-cortical synchronizations during externally-paced motor processing in patients with Parkinson's disease treated with subthalamic nucleus deep brain stimulation (STN-DBS). We hypothesize that subthalamic stimulation facilitates the movement-related desynchronization (MRD) of cortical activity. With this spectral measure we probe to predict the clinical outcome and reaction time performance from STN-DBS using multiple regression models. Moreover, we apply the time-frequency cross-coherence as a time- and frequency-sensitive measure to characterize the dynamic modulations of cortico-cortical coherence across motor execution. To comprehensively characterize the complex multilevel organization of cortico-cortical synchronization, we use the global synchronization index which quantifies the overall cortico-cortical synchronization. Applying this novel approach we differentiate the complex cortico-cortical synchronization map into 2D topographic representations and demonstrate that our findings were explained by genuine neuronal coupling. Furthermore, we characterize the modulations of cortical motor network synchronization induced by subthalamic stimulation.

Table 1 Clinical characteristics

Patient	Age	Gender	Duration of disease (years)	Duration of DBS (years)	Hoehn and Yahr	LED (mg)
PD1	51	M	11	2	2.5	400
PD2	64	F	16	1	2.5	1210
PD3	52	M	15	3	2	520
PD4	58	M	11	4	2	350
PD5	60	M	15	4	2	150
PD6	55	M	10	1	2	300
PD7	59	M	17	2	3	630
PD8	77	M	7	2	1.5	300
PD9	71	F	26	1	4	200
PD10	53	M	13	4	2	700
PD11	62	F	18	2	4	300
PD12	52	M	16	4	2	300
PD13	52	M	11	1	3	870
PD14	58	M	20	6	4	530
PD15	71	F	22	4	4	750
PD16	51	M	14	5	2	500
PD17	40	M	7	2	2	450
PD18	50	F	21	3	4	680
PD19	75	F	22	6	2	150
PD20	60	M	22	2	4	910

M = male; F = female; LED = L-DOPA equivalent dosage in mg.

Materials and methods

Patients

Twenty-four patients with idiopathic Parkinson's disease and STN-DBS were recorded, and 20 patients were referred for final data analysis (15 male, age 58.6 ± 9.4 years, disease duration 15.7 ± 5.3 years, time with STN-DBS 3.0 ± 1.6 years, all right-handed according to the Edinburgh Handedness Inventory; Table 1). Four patients were excluded from final data analyses: two of them because of reduced EMG quality; one patient did not sufficiently adhere to the experimental paradigm, and one patient did not tolerate the DBS reprogramming and discontinuation of stimulation. All patients were implanted with a quadripolar electrode with iridium contacts (type 3389, Medtronic). Patients were excluded if the Mini-Mental State Examination scored <25 points or if there were other neurological, medical or psychiatric conditions interfering with interpretability of the data. All patients participated with written informed consent and permission of the local ethics committee of the University of Tübingen.

Study experiments were performed after overnight withdrawal of dopaminergic medication. Patients were tested with both 'stimulation off' (StimOff) and bilateral 'stimulation on' (StimOn) in randomized order. Generally, a reliable washout of the clinical DBS effect can be achieved within 30 min after 'switching off DBS' in advanced disease stages as in our cohort (Cooper *et al.*, 2013; Weiss *et al.*, 2013). Motor Unified Parkinson's Disease Rating Scale (UPDRS) III assessments were obtained in each therapeutic condition, and a 'segmental UPDRS III subscore' (items 20–26) was additionally built. Before testing, stimulation parameters were reprogrammed to bipolar settings in case of chronic monopolar stimulation to reduce the DBS artefact in EEG recordings. Therefore, the negative active contact was held constant and polarized against a more dorsal contact. To obtain equivalent clinical efficacy of stimulation, stimulation amplitudes (constant voltage) were increased by 30% after bipolarization as suggested elsewhere (Silberstein *et al.*, 2005; Weiss *et al.*, 2011). Clinical outcome and individual stimulation parameters are given (Table 2).

Paradigm

Patients performed externally paced finger movements of fingers II–V of the right hand that were visually cued in random order with a fixed 1.5-s interstimulus interval. Four red circles were arranged horizontally, and illumination of one of the circles indicated the 'Go' signal for the corresponding finger. Patients were instructed to respond as fast and accurate as possible by button press. Patients were carefully instructed to keep their fingers in permanent contact with the buttons before, during and after a motor response. Adherence to this requirement was monitored online by the investigator. A small-amplitude finger press of ~2 mm was sufficient to elicit the motor response at full depression of the button, and this time-point was registered and referred to as time point '0' for data segmentation. This paradigm was chosen to stabilize the movement characteristics in StimOff and StimOn conditions. A trial was timed-out after 1 s if there was no response. Each patient performed four blocks of 84 random stimuli. In this study, we were interested to study motor integration on externally paced movements with relatively brief interstimulus intervals to be more sensitive for the cortical activation patterns that relate to the direct motor execution process. Similar studies were conducted with interstimulus intervals as short as 500 ms (Gerloff *et al.*, 1998; Herz *et al.*, 2014), and included spectral time-frequency analyses (Hege *et al.*, 2014) using wavelets to avoid standard Fourier transform window length limitation to study low frequencies. We determined reaction time and per cent of correct responses as behavioural motor performance measures.

Electrophysiological recordings

Sixty-four-channel surface EEG was recorded using linked earlobe references and a frontal ground (BrainAmp, Brainproducts). EMG of the right flexor digitorum superficialis and extensor digitorum communis muscles was recorded simultaneously using bipolar, pre-gelled Ag/AgCl surface electrodes (Norotrode, Myotronics-Noromed Inc.). Triggers from 'Go' signals and the subsequent motor responses were registered synchronously to the electrophysiological recording and used for offline data segmentation. EEG and EMG were sampled at 1000 Hz.

Data processing

Before the spectral analyses EEG data were band-pass filtered from 0.5 to 200 Hz and EMG data from 10 to 300 Hz. Afterwards, EMG was full-wave rectified. Both EEG and EMG data were visually inspected and corrected for episodic muscle and movement artefacts. EEG data principal

Table 2 Clinical outcome and individual stimulation parameters

Patient	Segmental UPDRS III (items 20–26)		Left STN	Right STN
	StimOff	StimOn		
PD1	26	6	3–2+, 4.5 V, 60 µs, 130 Hz	7–6+, 4.0 V, 60 µs, 130 Hz
PD2	14	11	2–3+, 3.5 V, 60 µs, 130 Hz	6–7+, 4.2 V, 60 µs, 130 Hz
PD3	25	8	1–2+, 4.3 V, 90 µs, 130 Hz	6–7+, 3.2 V, 90 µs, 130 Hz
PD4	11	2	3–2+, 4.8 V, 60 µs, 130 Hz	7–6+, 4.2 V, 60 µs, 130 Hz
PD5	24	10	2–3+, 5.0 V, 90 µs, 130 Hz	5–6+, 3.5 V, 60 µs, 130 Hz
PD6	12	0	2–1+, 3.5 V, 60 µs, 130 Hz	6–5+, 4.5 V, 90 µs, 130 Hz
PD7	17	7	1–2+, 4.6 V, 60 µs, 120 Hz	5–6+, 4.6 V, 60 µs, 120 Hz
PD8	15	9	2–1+, 6.0 V, 60 µs, 180 Hz	6–5+, 3.0 V, 60 µs, 180 Hz
PD9	29	8	2–3+, 6.5 V, 90 µs, 130 Hz	4–5+, 4.5 V, 60 µs, 130 Hz
PD10	18	2	1–3+, 3.5 V, 60 µs, 130 Hz	6–7+, 2.5 V, 60 µs, 130 Hz
PD11	22	7	2–3+, 5.0 V, 90 µs, 120 Hz	6–7+, 4.5 V, 90 µs, 120 Hz
PD12	17	8	2–3–1+, 4.6 V, 120 µs, 180 Hz	6–5+, 4.0 V, 90 µs, 180 Hz
PD13	7	5	2–3+, 4.2 V, 120 µs, 130 Hz	6–7+, 3.9 V, 120 µs, 130 Hz
PD14	24	8	2–3+, 2.5 V, 60 µs, 130 Hz	6–5+7+, 2.6 V, 120 µs, 130 Hz
PD15	17	9	3–2+, 2.0 V, 120 µs, 125 Hz	7–6+, 2.8 V, 90 µs, 125 Hz
PD16	32	18	1–3+, 4.9 V, 90 µs, 130 Hz	6–7+, 5.2 V, 90 µs, 130 Hz
PD17	24	5	0–3+, 5.6 V, 60 µs, 130 Hz	5–7+, 3.5 V, 120 µs, 130 Hz
PD18	25	16	2–3–1+, 2.7 V, 60 µs,130 Hz	6–7+, 3.9 V, 90 µs, 130 Hz
PD19	18	7	2–3+, 3.3 V, 120 µs, 125 Hz	6–7+, 3.3 V, 120 µs, 125 Hz
PD20	29	16	3–2+, 4.2 V, 60 µs, 130 Hz	6–7+, 3.5 V, 60 µs, 130 Hz

components were extracted using EEGlab and components corresponding to eye blinks, eye movements, frontal or temporal muscle artefacts, or cardiovascular artefacts were removed. Then, the components were transformed back to the channel-by-time series. Finally, reference-free EEG data were obtained using a short Laplacian spatial filter. This step was considered to reduce cortical volume conduction and to improve the spatial resolution of the cortical representations. In this sense, we report our findings by indicating electrode positions that overly neuroanatomic areas of interest or maxima/minima of the spectral topographic distributions. We refer to these representations by indicating cortical areas underlying the electrodes. Data were segmented from −800 ms to +400 ms relative to the registration of the button press at time '0'.

Spectral analyses

Time-frequency measures of cortical activity

To determine the movement-related cortical and muscular activity on the segmented data we computed the event-related spectral perturbation as implemented in EEGlab newtimef function. To obtain better frequency resolution, we used a Hanning-tapered sinusoidal wavelet transform beginning with a three-cycle wavelet that continued to expand slowly and reached half of the cycles on the highest frequency as suggested elsewhere (Delorme and Makeig, 2004). Our approach resulted in a frequency resolution of 2 Hz. We considered a frequency range from 10 to 100 Hz for the muscular spectra (time range: −632 to +232 ms relative to the registration of the finger tap at time '0'), and from 8–100 Hz for the cortical spectra (time range: −591 to +192 ms). Time-frequency samples were normalized on the mean spectral power of the entire epoch at each specific frequency. Statistical procedures on the time-frequency representations are described below.

Time-frequency analysis of cortico-cortical coherence

The event-related cortico-cortical cross-coherence spectra were computed from 10–30 Hz (time range: −632 to +232 ms). The magnitude of cross-coherence varies between 0 and 1 with a value of 0 indicating complete absence of correlation and 1 indicating perfect correlation. We calculated the cortico-cortical cross-coherence between cortical regions of interest, i.e. on electrodes over the bilateral sensorimotor areas ('C3', 'C4'), supplementary motor area ('FCz') and bilateral dorsolateral prefrontal region ('F3', 'F4'). Here, we selected regions of interest to analyse the interhemispheric time-frequency cross-coherence ('C3C4' and 'F3F4'), as well as the cross-coherence over the bilateral sensorimotor areas and both supplementary-motor area ('C3FCz', 'C4FCz') and dorsolateral prefrontal areas ('C3F3', 'C4F4').

Global phase synchronization index and its topographic differentiation

To study the multidimensional cortico-cortical synchronization processes in a more comprehensive way, we introduced the global synchronization index measure. We calculated the pairwise phase synchronization index (ψ) for each pair of electrodes and represented this in a matrix form as an 'association matrix'. We eigenvalue decomposed the association matrix and defined the global synchronization index (γ) as the ratio of the sum of the eigenvalues greater than a

defined tolerance value with respect to the sum of all the eigenvalues. With this definition, γ would take on a value of one in case of perfect synchrony between all possible channels and a value of zero for complete asynchrony between the channels. We obtained the tolerance value using a bootstrap approach (detailed mathematical procedure in the online Supplementary material). To obtain the topographic distribution of the global cortico-cortical synchronization, we identified the electrode sites that contributed significantly to γ based on their eigenvectors (detailed mathematical procedure in the Supplementary material). This was obtained for both the StimOff and StimOn conditions separately. The global synchronization index and its topographic differentiation were applied on the EEG time series of interest after Laplace transform and band-pass filtering for the frequency range of interest. If the global synchronization index was non-zero for a subject on both conditions, we considered these data for further analysis.

We selected three movement-related time windows of interest driven by the results from the spectral time-frequency analyses. The selection of the respective time-series was derived from the muscular activity and cortico-cortical cross-coherence time-frequency analyses, and will be defined along with the result presentation. Together, the procedure allows us to monitor coupling and uncoupling of distinct areas within the global cortical movement-related processing stream on the basis of the phase synchronization index. The detailed mathematical-methodological algorithm is provided in the Supplementary material.

Additionally, we analysed whether phase synchronization in our study represented genuine neuronal synchronization as opposed to the spurious synchronization from non-interacting sources as present in volume conduction. This problem has been discussed as immanent to EEG research and highly non-trivial, and accordingly no general consensus, but several valuable approaches have been put forward in this sense. Of note, most approaches were conceptualized under the premise that volume conduction occurs with zero-phase delay (Nolte *et al.*, 2004; Hipp *et al.*, 2012; Haufe *et al.*, 2013). Therefore, to analyse if the global synchronization index and its topographic differentiation in our study reflected genuine neuronal synchronization, we analysed the phase differences of significant cortico-cortical synchronizations. Briefly, to test this, we assumed that there were three different categories of phase delays in our sequence x, namely (i) values close to zero (<0.1); (ii) values close to pi (pi – pi / 10 to pi + pi / 10); and (iii) all other values, where Category (iii) is compatible with genuine neuronal synchronization as opposed to Categories (i) and (ii) that were expected to reflect instantaneous coupling from non-interacting sources. A detailed mathematical background and statistical consideration on the comparison of the three categories is provided in the Supplementary material.

Statistical analyses

Muscular activity was in part affected by tonic background activity in some patients, i.e. from incomplete relaxation or rigidity, as can be expected in advanced Parkinson's disease stages. To this end, we improved the signal-to-noise ratio by masking the muscular time-frequency samples on the 95% bootstrap significance level with reference to the entire trial epoch, i.e. non-significant muscular activation samples were zeroed out on the individual subject level. This included a correction for multiple comparisons on the multiple time-frequency samples with the false discovery rate. Afterwards, the muscular activation onset was determined from the time-frequency event-related spectral perturbation. We defined the time point of muscular activation onset when activity first exceeded the bootstrap significance level. These onset times were used to compare muscular activation onset of right flexor digitorum superficialis and right extensor digitorum communis muscles between conditions (StimOff versus StimOn) with paired samples t-tests. Group level data of muscular activation were obtained as grand averages from these thresholded individual spectra. Clinical and performance measures were compared between conditions using paired samples t-tests.

Spectral time-frequency measures of cortical MRD and cortico-cortical cross-coherence were compared between StimOff and StimOn using a non-parametric framework. We determined the Monte-Carlo estimates from the permutation distribution. Permutation statistics were computed for each sample in the time-frequency space as implemented in the Fieldtrip open source toolbox (Oostenveld *et al.*, 2011). We used 1000 random permutations on a dependent samples t-test and an adjusted alpha level of $P < 0.025$ per tail. As these sample-wise statistics produce a massive number of comparisons [i.e. the product of the number of channels (64) by times (200) by frequencies (46)], the cluster-based correction method was demonstrated to treat the multiplicity problem effectively without losing sensitivity for spectral modulations (Maris and Oostenveld, 2007).

We used separate multiple regressions to explore whether (i) clinical improvement on the segmental UPDRS motor score; or (ii) reaction time improvement as dependent variables (StimOff minus StimOn) were associated with a change in cortical MRD (StimOff minus StimOn). Therefore, we took the difference in MRD averaged over the time-frequency window of interest (StimOff minus StimOn) as derived from the event-related spectral perturbation analyses. We optimized our model by inserting electrodes placed over the left prefrontal, premotor, supplemental motor, and sensorimotor areas. As MRD may yield similar patterns across neighbouring electrodes, we removed electrodes from the model if there was statistical evidence for collinearity.

The global synchronization index was compared between the StimOff and StimOn conditions using a paired t-test with the directed hypothesis that StimOff condition will show higher global phase synchronization than the StimOn condition. A one-tailed $P < 0.05$ was considered statistically significant. To compare the topographic differentiation between StimOff and StimOn, we performed one-tailed paired t-tests ($P < 0.05$) on the eigenvector of each cortical channel between the StimOff and StimOn conditions hypothesizing on higher phase synchronization in StimOff than StimOn. We controlled the false positives with the false discovery rate (Benjamini and Hochberg, 1995).

Results

Clinical outcome, performance and muscular activation

StimOn led to a significant improvement of motor symptoms compared to StimOff on the total UPDRS III [22.3 ± 9.7 versus 57.0 ± 13.6; $t(19) = 14.184$, $P < 0.001$]

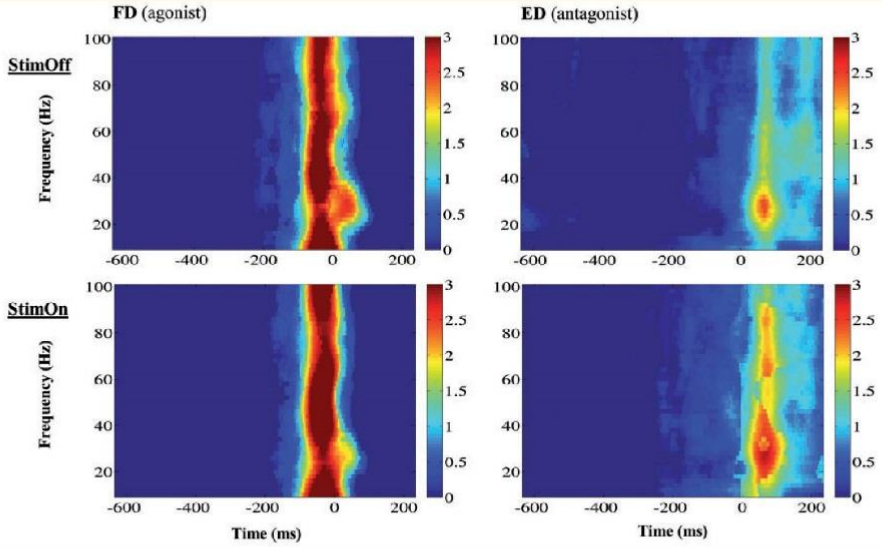

Figure 1 Grand averages of the time-frequency spectra of muscular movement-related spectral pertubations (individual spectra were bootstrap thresholded). No significant differences of M. flexor digitorum (agonist; FD) or M. extensor digitorum (antagonist; ED) activation patterns were found between StimOff and StimOn, i.e. no differences in activation onset, activation strength and frequency response were present, respectively. *x*-axis: Time (ms), where time '0' denotes registration of the finger tap; *y*-axis: Frequency (Hz); activity is coded in colour with warm colours indicating stronger activity (colour bar).

and on the segmental UPDRS III subscore [8.1 ± 4.6 versus 20.3 ± 6.7; $t(19) = 10.532$, $P < 0.001$], as expected. Each patient exhibited an improvement on the total UPDRS III motor score of at least 30% (individual data not shown), and individual improvement of the segmental UPDRS subscore (Table 2). Reaction time was significantly shorter with StimOn compared to StimOff (649.7 ± 137.9 ms versus 712.2 ± 117.0 ms; $P = 0.032$) and the per cent correct responses increased with StimOn compared with StimOff (85.4% ± 14.9 versus 74.8% ± 19.0; $P = 0.016$).

The time course of muscular activation was similar with both StimOff and StimOn (Fig. 1). Right flexor digitorum superficialis muscle activation onset did not differ between therapy conditions [StimOff: −123.5 ± 53 ms, StimOn: −134.7 ± 51 ms; $t(18) = 1.025$, $P = 0.319$]. Right extensor digitorum communis muscle activation onset was similar with −11.0 ± 72 ms with StimOff and −35.3 ± 92 ms with StimOn [$t(17) = 1.017$, $P = 0.324$]. Activation strength and frequency response according to the event-related spectral perturbation of both right flexor digitorum superficialis and right extensor digitorum communis muscles did not differ between StimOff and StimOn conditions both on the raw and the bootstrap-thresholded spectral perturbations.

Movement-related spectral perturbation of cortical activity

In the StimOff condition, alpha (8–12 Hz) and beta band (14–30 Hz) MRD was pronounced on the C3 and C4 electrodes overlying the bilateral sensorimotor areas. This also involved the central and (to a lesser degree) the bilateral frontal electrodes. In the C3 electrode alpha and beta MRD onset occurred at approximately −350 ms relative to fingertap registration at time '0' and outlasted the fingertap for approximately +40 ms. A slight movement-related increase of low and high gamma band activity was observed in the frequency range from 40–80 Hz and from −50 to +70 ms. In StimOn, MRD in the alpha and beta range was significantly stronger compared to StimOff most pronounced over left frontal FC3 and sensorimotor C3 electrodes. Moreover, MRD covered a wider cortical area including the electrodes overlying the bilateral both prefrontal areas (F1, F3, Fz, F2, F9, F10), premotor (FC3, FC4), supplementary-motor (FCz), sensorimotor (C3, C4), and parietal areas (P3, P4). At the left C3 electrode, MRD onset occurred at approximately −350 ms similar to StimOff, however it outlasted the fingertap for +160 ms, and was thereby significantly longer compared with StimOff. There was no difference in gamma activity between conditions. The comprehensive cortical movement-related spectral perturbations are given in Fig. 2.

We conducted several subanalyses to ensure that MRD was significantly modulated by StimOn. First, we ensured that there was no constant suppression of the cortical alpha and beta rhythms throughout the 1.5-s interval. We found that over the C3 electrode of interest, MRD showed significant activity decrease from the epoch mean in both StimOff and StimOn conditions (permutation statistics with $P < 0.05$ including correction for multiple

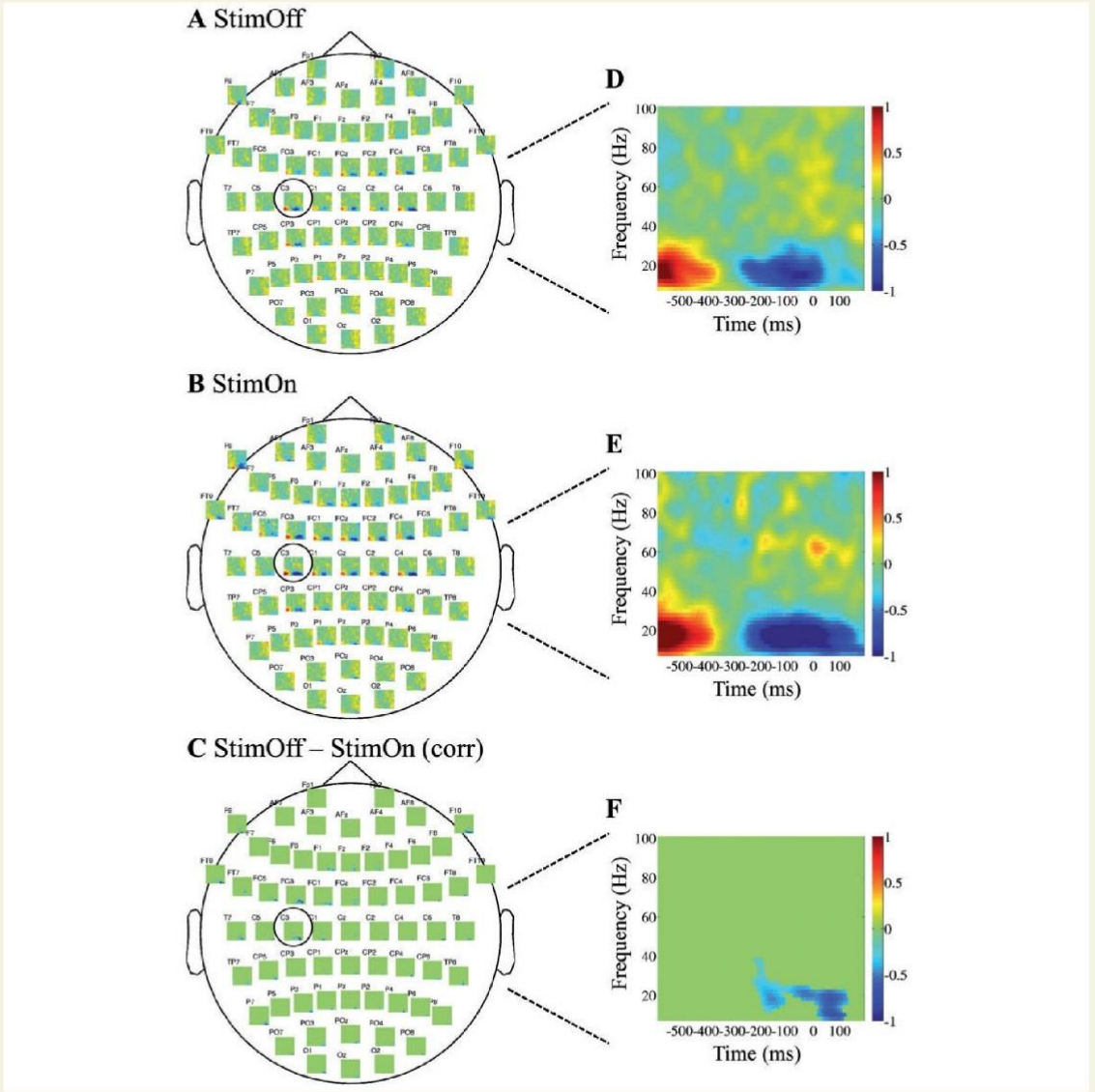

Figure 2 Time-frequency spectra of the cortical movement-related spectral pertubations (multiplots). At each of the 64 electrode positions, a time-frequency plot is given. Grand average spectra of the cortical electrodes are given in StimOff (**A**) and StimOn (**B**), and StimOn minus StimOff (**C**) including the statistical comparison and correction for multiple comparisons using a cluster-based correction method (Maris and Oostenveld, 2007). Non-significant time-frequency samples are 'zeroed out' and given in green. The time-frequency representations of the left sensorimotor region of interest ('C3') are given for StimOff (**D**), StimOn (**E**), and StimOn minus StimOff (**F**). *x*-axis: Time (ms), where time '0' denotes registration of the finger tap; *y*-axis: Frequency (Hz).

comparisons with the false discovery rate). This demonstrates that there was significant movement-related activity modulation and that the task rate did not lead to a continuous and general suppression of the alpha and beta rhythms (not shown). Next, one might argue that StimOn could have introduced a constant decrease of activity between 8–30 Hz. Therefore, we analysed the cortical power spectrum on the entire epoch from −800 to +400 ms and found no significant differences of cortical activity in any of the electrodes (Supplementary Fig. 1). A further important aspect we consider, that higher MRD deflections might arise from higher prestimulus amplitudes (Lemm *et al.*, 2009). Although our experimental paradigm differed from this previous work, we found that cortical activity from

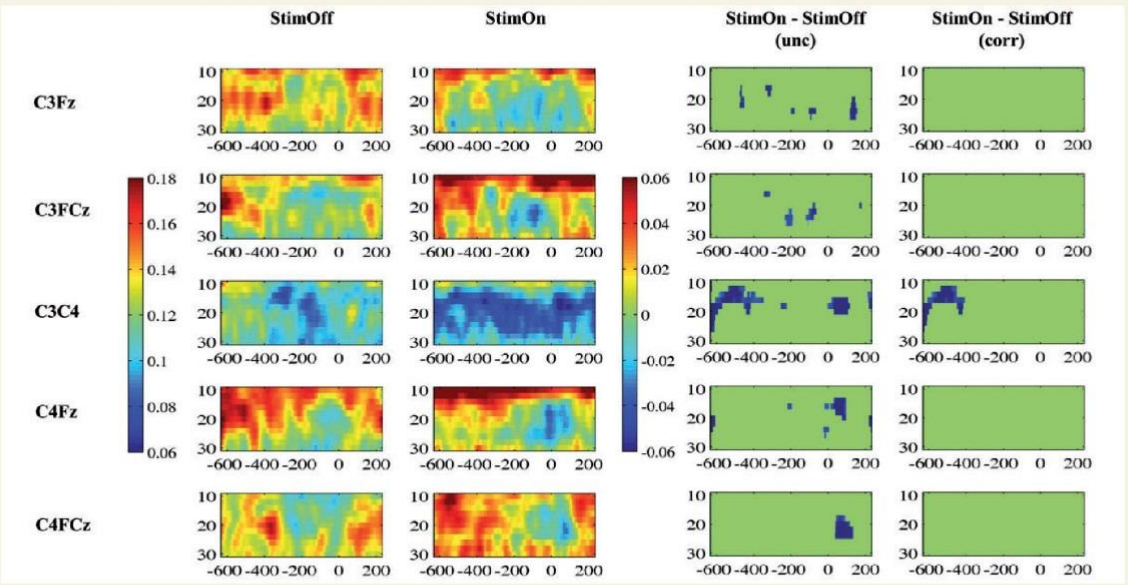

Figure 3 Time-frequency cross-coherence between cortical regions of interest. The first column indicates the grand average spectra in StimOff, the second column in StimOn. The coherence magnitude is indicated by the colour bar (scale from 0.06 to 0.16). As third column, the statistical comparison between StimOff and StimOn (uncorrected) is given, and the fourth column includes correction for multiple comparisons. The difference in coherence magnitude StimOn minus StimOff is indicated by the right colour bar, and non-significant time-frequency samples are 'zeroed out' and masked in green (scaled from 0.06 to −0.06). *x*-axis: Time (ms), where time '0' denotes registration of the finger tap; *y*-axis: Frequency (Hz).

−800 to −400 ms (before MRD onset) and between 8–30 Hz did not show significant differences between StimOff and StimOn in any of the channels (not shown).

We designed the multiple regression model from electrodes over the cortical areas of interest involved in motor integration. We modelled the clinical outcome and reaction time improvement from stimulation from MRD differences between StimOff and StimOn in these cortical areas. For the MRD difference between StimOff and StimOn, we took the mean power of each cortical electrode at the time-frequency window of interest with maximum MRD (frequency range: 14–24 Hz, time range of interest: −198 to −1 ms). We achieved the highest coefficient of determination in predicting UPDRS improvement when applying the five electrodes of interest 'F1', 'FC1', 'FCz', 'C1', 'C5' ($R^2 = 0.61$, $F = 4.293$, $P = 0.014$). Similarly, the reaction time improvement could be modelled with electrodes 'F1', 'FC3', 'Cz', 'C1', 'C5'; ($R^2 = 0.66$, $F = 5.306$, $P = 0.006$).

Cortico-cortical coherence and phase synchronization

The analysis of cortico-cortical time-frequency cross-coherence from 10–30 Hz was considered to explore the characteristics of movement-related coherence. We found movement-related coherence decrease mainly between 'C3FCz', 'C4FCz' and between 'C3F3' pair without significant differences between StimOff and StimOn. This pattern was different in the interhemispheric electrode pairs: both 'F3F4' and 'C3C4' showed a more constant coherence decrease over the whole time range. This was most pronounced in StimOn and reached statistical significance compared to StimOff in the 'C3C4' pair in the premovement phase from −632 to approximately −400 ms (Fig. 3).

For the phase synchronization analysis we selected the frequency range of 14–24 Hz as derived from the significant coherence decrease in 'C3C4'. We selected three time epochs of interest, i.e. the 'premovement period' from −600 to −401 ms which was before cortical MRD and EMG onset; the 'movement period' from −200 to −1 ms, which covered the maximum of cortical MRD, cortico-cortical coherence decrease, and agonist EMG activation; and the early 'postmovement period' from 0 ms to +199 ms. Phase synchronization analysis was considered (i) to analyse neuronal synchronization; and (ii) to analyse the global cortical synchronization as well as distinct cortical contributions to the global cortical synchronization.

The global synchronization index was larger in StimOff compared to StimOn ('premovement period': significant in 12 patients, StimOff > StimOn; $P = 0.0096$; 'movement period': significant in 10 patients, StimOff > StimOn,

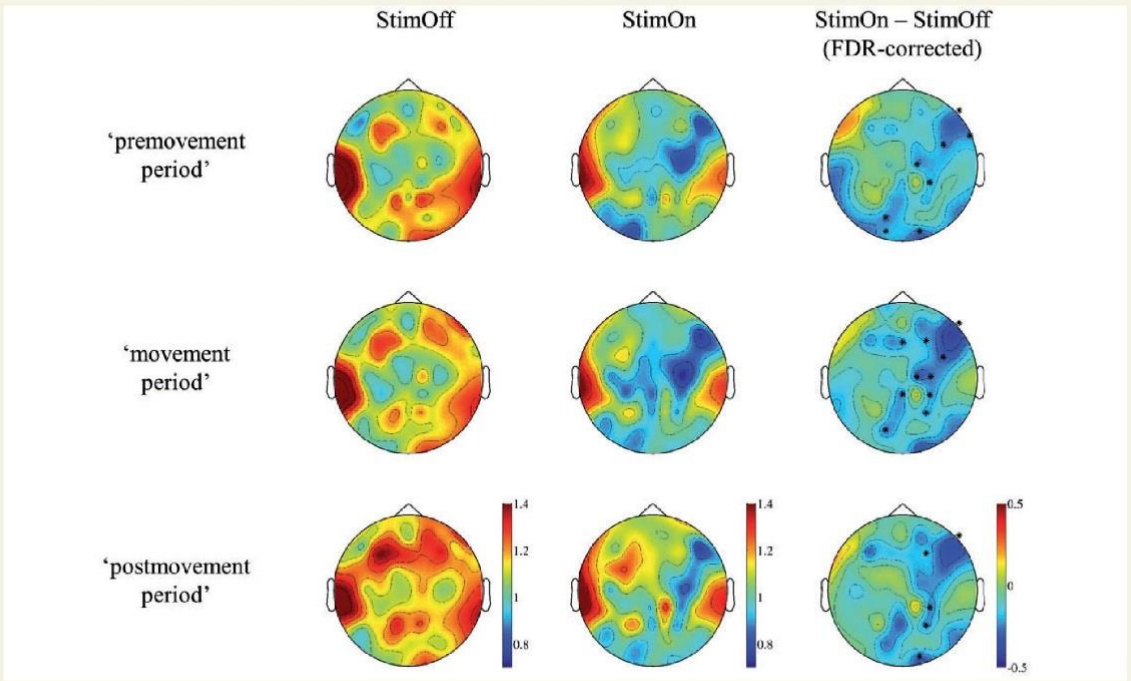

Figure 4 Topographic differentiation of the cortico-cortical phase synchronization in StimOff and StimOn, and difference of StimOn minus StimOff. In the latter column significant desynchronizations between conditions are indicated by asterisks. Colour bars indicate the magnitude of cortico-cortical phase-synchronization with warmer colors indicating stronger phase synchronization.

$P = 0.009$; early 'postmovement period': significant in 10 patients, StimOff > StimOn, $P = 0.021$). The topographic differentiation of cortico-cortical phase synchronization revealed desynchronization over the left fronto-central area in StimOff in both 'premovement period' and 'movement period' (Fig. 4). This was similar in StimOn. As significant difference, we observed a stronger desynchronization over the right prefrontal, premotor, and sensorimotor areas in all three time segments in StimOn compared to StimOff (Fig. 4).

Next, we analysed whether our findings reflected genuine neuronal synchronization. Using Chi-square test, we found that the three categories of phase differences [i.e. (i) values close to zero (<0.1); (ii) values close to pi (pi – pi / 10 to pi + pi / 10); and (iii) all other phase differences] occurred with significantly different proportions ($P < 0.0001$). McNemar's test was used to determine whether the frequencies of occurrences of the categories were equal for each pair of categories. This pairwise test showed that events in Category (iii) were significantly different from Categories (i) or (ii) across all the subjects after adjusting for multiple comparisons ($P < 0.001$). Phase differences in Category (iii) occurred in at least 98% in each single patient with significant phase synchronization. Therefore, the observed phase synchronizations dominantly reflected genuine neuronal synchronization.

The desynchronization over the right prefrontal, premotor, sensorimotor area (electrodes 'F10', 'FC6' and 'C2' predicted clinical improvement on the UPDRS III with a high coefficient of determination in the 'premovement period' ($R^2 = 0.84$, $F = 13.875$, $P = 0.002$), whereas no significant prediction was achieved during the 'movement period' ($R^2 = 0.55$, $F = 2.488$, $P = 0.158$). A linear correlation between UPDRS improvement and the decrease in phase synchronization of the 'F10' electrode was achieved in the 'premovement period' (Pearson's $r = 0.91$, $P < 0.001$; Fig. 5), indicating that clinical improvement correlated with reduced phase synchronization on the right inferior prefrontal area. No significant predictions on the reaction time outcome were obtained from the multiple regressions, however there was a significant correlation of reaction time decrease and synchronization decrease with StimOn compared to StimOff on the 'F10' electrode (Pearson's $r = 0.67$, $P = 0.016$).

Discussion

Here, we demonstrate that subthalamic stimulation modulates both large-scale cortical motor-network activity and synchronization in Parkinson's disease. Our study brought to light several cortical network mechanisms of Parkinson's

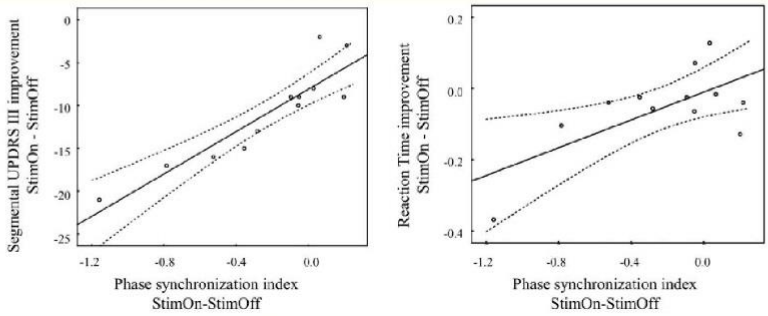

Figure 5 Linear correlation of the topographic differentiation of the global phase synchronization index. Indices are shown for the right inferior prefrontal cortex ('F10' electrode; difference StimOn minus StimOff) and clinical improvement (*left*) in the UPDRS III (StimOn minus StimOff) and reaction time difference (*right*; StimOn minus StimOff) in the 12 patients with significant global phase synchronization in both StimOff and StimOn conditions. The correlations were performed on data from the 'premovement period' in which 12 patients had significant phase synchronization.

disease motor impairment and therapeutic neuromodulation on the level of STN. First, StimOn strengthened the cortical processing by facilitating the MRD in the alpha and beta frequency ranges. With this MRD difference between StimOff and StimOn, we were able to predict both the clinical outcome on the segmental UPDRS III and reaction time improvement in StimOn. The most accurate predictions were achieved when using electrodes over the left sensorimotor, premotor, prefrontal, and midline areas. As further core finding, the cortico-cortical long-range synchronization was significantly decreased with StimOn. This included a reduction of interhemispheric cross-coherence between the bilateral sensorimotor areas, and decreased global phase synchronization in a frequency range between 14–24 Hz. The topographic differentiation pointed to similar desynchronization and resynchronization patterns of phase synchronization on the left (contralateral) hemisphere in both StimOff and StimOn. In contrast, phase synchronization was significantly reduced on electrodes over the right (ipsilateral) prefrontal, premotor, and sensorimotor areas in StimOn compared to StimOff.

Modulation of cortical activity

We found a wide-spread facilitation of cortical MRD with subthalamic nucleus stimulation over the bilateral prefrontal, premotor, supplementary motor, and sensorimotor areas, and this topography complied well with the known cortical connectivity of the STN (Albin *et al.*, 1989; Nambu *et al.*, 1997, 2002). Interestingly, this finding enabled us to predict both clinical and reaction time improvements. Intriguingly, the subthalamic nucleus may facilitate, relay, or inhibit motor cortical processing according to its remote cortical connectivity and, in Parkinson's disease, an over-inhibitory subthalamic tone in the beta frequency range was associated with abnormal motor cortical inhibition and defective corticospinal motor control (Salenius *et al.*, 2002; Kuriakose *et al.*, 2010; Weiss *et al.*, 2012). Our findings are in line with these previous observations. As local subthalamic beta band activity has been shown to be decreased with DBS (Kuhn *et al.*, 2008; Eusebio *et al.*, 2011), and beta band coupling of STN and cortex has been demonstrated particularly in the resting state and during akinesia (Sharott *et al.*, 2005; Weiss *et al.*, 2012), network effects on cortical beta band activity could be assumed during STN-DBS. Accordingly, beta band rhythm modulations may constitute a network-wide mechanism to balance motor execution and inhibition which is considered a core function of the subthalamo-cortical relay (Aron and Poldrack, 2006; Frank, 2006; Weiss *et al.*, 2014). Similarly, the prefrontal cortices are involved in executive motor functions and include response selection in attention-demanding motor skills particularly in motor programs that are not well rehearsed (Jahanshahi, 2013). Accordingly, increase of subthalamic beta band activity was found when conflict responses were inhibited successfully, and the frontal cortex was proposed as the core processor to mediate this motor inhibition presumably via hyperdirect cortico-subthalamic projections (Brittain *et al.*, 2012). Similar evidence for prefrontal contributions came from a PET study on motor timing in Parkinson's disease that found impaired striatal-prefrontal connectivity (Jahanshahi *et al.*, 2010) which improved with dopaminergic therapy (Jahanshahi *et al.*, 2010; Kwak *et al.*, 2010). This impairment to regulate the degree of prefrontal activation in Parkinson's disease (Postuma and Dagher, 2006) probably parallels several executive dysfunctions (Hallett, 2008). It has been postulated that this impairment relates rather to an overinhibitory basal ganglia control on prefrontal activity than to an intrinsic prefrontal dysfunction *per se* (Dirnberger *et al.*, 2005). In line with this consideration, pallidotomy increased prefrontal and supplementary motor activation (Samuel *et al.*, 1997). Our findings add to this view. Given that subthalamic stimulation promotes

movement-related activity decrease of the prefrontal area, this implies remote activity control of the prefrontal cortex and STN. This effect may rather reflect network modulation on remote subthalamic and cortical control than modulation of a single distinct basal ganglia pathway such as the hyperdirect pathway *per se*. The consideration of remote subthalamo-cortical interplay is corroborated by previous findings. It was put forward that STN-cortical modulation by subthalamic neurostimulation occurs on separate pathways in parallel, and that these distributed modulations result in a prokinetic net effect. The most important and reproduced explanations considered a desensitization of STN afferents (including those from the hyperdirect cortico-subthalamic pathway) (Gradinaru *et al.*, 2009; Kahan *et al.*, 2014), and as a paralleling mechanism remote antidromic motor cortex modulation from subthalamic stimulation was revealed (Li *et al.*, 2012; Gradinaru *et al.*, 2009). Additionally, cortical activity modulation may occur on the direct pathway, i.e. by facilitating cortical processing on the thalamo-cortical route. In line with our findings it was moreover noted that increases of MRD may reflect a facilitation of thalamo-cortical activation (Steriade and Llinas, 1988; Lemm *et al.*, 2009). Together, if an exaggerated inhibitory subthalamic tone (as present in the 'off state') is attenuated with DBS, motor output from the basal ganglia on thalamo-cortical pathways may be facilitated, and conversely, attenuation of cortical beta band activity during motor processing may result in less inhibitory cortical afferents to the STN (e.g. via the hyperdirect pathway with the well-known frontal and central-motor contributions).

Subthalamic stimulation decreases large-scale cortico-cortical coherence and phase synchronization

This study addressed the movement-related modulations of cortico-cortical cross-coherence in Parkinson's disease and its modulation with subthalamic stimulation. Both StimOff and StimOn showed similar movement-related coherence decrease from 14–24 Hz most pronounced on the left hemisphere. This involved the interhemispheric connections, as well as connectivity of the bilateral sensorimotor areas and supplementary motor area. As significant difference, StimOn yielded a stronger and permanent decrease of the interhemispheric coherence between the bilateral sensorimotor areas in the premovement phase. For the phase synchronization analysis we selected a frequency range from 14–24 Hz and three time periods of interest. As core finding, we identified a global reduction of cortico-cortical phase synchronization with StimOn compared to StimOff in all time segments. Next, we provided a comprehensive topographic distribution of cortico-cortical phase-synchronization of distinct cortical regions with respect to their contributions to the global cortical motor network synchronization. Major contributions to cortical desynchronization came from the right (ipsilateral) hemisphere, presumably from the prefrontal, premotor, and sensorimotor areas, and interestingly, this allowed us to model the therapeutic improvement from DBS.

In healthy subjects, interhemispheric (ordinary) coherence between the bilateral sensorimotor areas seems to be tightly associated with motor cortical excitability, as inhibitory 1 Hz repetitive transcranial magnetic stimulation applied to the left hand primary motor representation in healthy subjects resulted in increased interhemispheric coherence between the bilateral motor areas (Strens *et al.*, 2002). Similarly, active motor inhibition led to a phasic increase of cortical activity and was paralleled by increased interhemispheric coherence between the bilateral sensorimotor and bifrontal areas (Shibata *et al.*, 1998). Adding to these findings in healthy subjects, there is ample evidence on defective interhemispheric inhibition in Parkinson's disease. Cortico-cortical functional connectivity in the alpha and beta bands was pathologically enhanced in early stage Parkinson's disease compared to healthy controls (Olde Dubbelink *et al.*, 2013), whereas extensive beta band cortico-cortical coherence characterized the later disease stage (Stoffers *et al.*, 2008*a*, *b*). Probably the most intriguing evidence for impaired interhemispheric inhibition in Parkinson's disease comes from pathological mirror movement (Poisson *et al.*, 2013; Spagnolo *et al.*, 2013). It was discussed that reduced transcallosal inhibition or increased transcallosal facilitation may lead to a less lateralized brain activation, which, in turn could favour the occurrence of mirror movements (Li *et al.*, 2007; Poisson *et al.*, 2013). Consistent with this, smaller and shorter ipsilateral silent periods indicated impaired interhemispheric inhibition (Spagnolo *et al.*, 2013). In the later disease stages, interhemispheric beta band coherence is pathologically increased particularly in the resting state. Interestingly, higher interhemispheric coherence correlated with more advanced motor impairment (Silberstein *et al.*, 2005), and this was reduced with both L-DOPA and STN-DBS. Our study adds to this framework by demonstrating that interhemispheric coherence between the bilateral sensorimotor areas was more effectively decreased by STN-DBS in the premovement phase. Finally, this demonstrates the importance of the subthalamic nucleus in regulating the interhemispheric interplay during movement preparation.

Cortico-cortical phase synchronization was substantially desynchronized in StimOn compared to StimOff over the right (ipsilateral) prefrontal, premotor, and sensorimotor areas. Most importantly, this desynchronization predicted the motor improvement on the segmental UPDRS III items most accurately in the 'premovement period', and the reduction in phase synchronization over the right inferior prefrontal area in StimOn compared to StimOff was highly correlated with clinical motor improvement. This indicates that the right prefrontal area may play a critical role in Parkinson's disease motor impairment. Following our findings, an entrainment of the right inferior prefrontal area to motor inhibition can be assumed and was most

pronounced in the 'premovement period'. Further corroborative findings lend support to this view and help to delineate the functionality behind this finding.

Cortico-cortical phase synchronization is considered to reflect neuronal mechanisms to synchronize neurons to a common (cognitive) program, i.e. to 'bind' or to 'hold together' distinct features of a program. Thereby, neuronal synchronization may strengthen information exchange between distributed players during well-defined transient windows in the multidimensional time-frequency-topography space (Fell and Axmacher, 2011). Therefore, we propose that the observed right-hemispheric decrease of phase desynchronization reflects the decoupling of potentially inhibitory cortical processors from the motor program. Inhibitory contributions from these areas can be assumed, as a particular role of the right prefrontal cortex was demonstrated in executive motor control, particularly in 'motor inhibition' (Rubia *et al.*, 2001; Aron *et al.*, 2003; Aron and Poldrack, 2006; Zhang *et al.*, 2012; Hege *et al.*, 2014). Similarly, concordant activation of both STN and the right inferior frontal gyrus were demonstrated during motor response suppression and this pointed to the functional inhibitory synergism of both structures (Aron and Poldrack, 2006). More specific to Parkinson's disease, activity of the right prefrontal area was modulated in Parkinson's disease during repetitive bimanual finger movement in a functional MRI study, i.e. activity decreased in unimpaired movement but increased during involuntary motor blocks. Interestingly, these activity modulations further entrained the right primary motor cortex, dorsal premotor cortex, and the supplementary motor area (Vercruysse *et al.*, 2014), which closely relates to the distributions of the right-hemispheric decrease in phase synchronization in our study.

Methodological considerations

We primarily aimed to study the time period around movement execution instead of a longer premovement planning phase as would have been available during internally generated movement. Therefore, we decided to study externally-paced movement on relatively narrow interstimulus intervals of 1.5 s. This paradigm and the settings of our wavelet analysis led to the limitation that we were not able to study the (post-movement) event-related resynchronization. However, we were able to characterize MRD in narrow-interval externally-paced movement and its modulation with subthalamic stimulation. Studying externally-paced movement is similarly important, as this paradigm closely adheres to the everyday motor demands and requires rapid repetitive motor responses to external triggering. Cortical negativity and connectivity measures were studied on externally paced movements with even much shorter interstimulus intervals of 0.5 s (Gerloff *et al.*, 1998; Herz *et al.*, 2014). Nevertheless, we ensured that the alpha and beta band MRD modulations were significant from epoch mean, which stands against a general suppression of these rhythms in parallel to the relatively rapid task rate. Moreover, we confirmed that StimOn did not lead to a general suppression of the alpha and beta rhythms. Importantly, we also excluded differences in cortical activity before MRD onset as a confounder of our findings as discussed elsewhere (Lemm *et al.*, 2009). Together, these additional analyses substantiate that MRD was facilitated in StimOn.

Our pattern of cortical MRD is in good accordance with previous findings and therefore supports the validity of our analyses including those on cortico-cortical phase synchronization: internally-generated movement MRD occurs over the sensorimotor area contralateral to the intended limb movement during the premovement phase, but becomes largely bilateral and symmetric during the movement execution phase. In Parkinson's disease, internally-generated movements presented with a general impairment of the premovement desynchronization (Magnani *et al.*, 2002), and the mu-rhythm MRD was delayed in Parkinson's disease (Defebvre *et al.*, 1998; Magnani *et al.*, 2002). 'MRD impairment' may emerge and worsen along disease progression (Devos and Defebvre, 2006). Consistent with our findings, one previous study found that the prefrontal MRD mainly occurs in the movement phase and less in the premovement phase of internally-paced movements with both STN-DBS and L-DOPA, but could spread to the premovement phase 'off' therapy (Devos *et al.*, 2004). This corroborates our findings that the prefrontal MRD relates to initiation or delay of the motor command *per se*, i.e. inhibitory versus executive control.

Another important aspect of this study was to ensure that the observed modulations of cortico-cortical phase synchronization indeed reflected genuine neuronal synchronization as opposed to volume conduction from non-interacting sources (Nolte *et al.*, 2004; Hipp *et al.*, 2012; Haufe *et al.*, 2013). We demonstrated that the phase differences in our study were largely dominated by genuine synchronization but significantly different from volume conductions characteristics.

Stimulation artefacts were limited although not absent by using bipolar DBS configurations. We found that DBS caused a sharp artefact at the stimulation frequency itself and on harmonic frequencies. Importantly, however, we verified that the individual power spectra were not affected in the major frequency range of interest below 30 Hz. Moreover, the statistical comparisons of StimOff and StimOn confirmed that cortical activity was not affected by DBS (spectra in StimOff and StimOn presented as Supplementary Fig. 1).

Taken together, our study identifies important mechanisms of subthalamic stimulation on cortical network processes. Two major mechanisms were demonstrated, in that subthalamic stimulation facilitates MRD, and decouples potentially motor inhibitory cortical processors from the global motor network processing stream during motor preparation and motor execution. The present findings contribute to a more comprehensive understanding of the

large-scale motor network effects of subthalamic neurostimulation and raise attractive novel candidate regions and biomarkers for future neuromodulation strategies.

Acknowledgements

We would like to thank Dr R. B. Lenin for statistical guidance (Department of Mathematics, University of Central Arkansas, Conway, AR, USA).

Funding

Daniel Weiss is supported by a research grant of the German Research Council (DFG) WE5375/1-1 and was supported by a Research Grant of the Medical Faculty of the University of Tübingen (AKF 259-0-0). Daniel Weiss received speaker's honoraria and a travel grant from Medtronic, Abott Pharmaceutical, UCB, and the Movement Disorder Society. Christian Plewnia received research grants from the German Research Council (DFG; PL 525/1–1), the University of Tübingen (AKF #238-0-0) and the Werner Reichardt Centre for Integrative Neuroscience (CIN, PP2011_11). He received speaker's honoraria by Inomed Medizintechnik GmbH. Rejko Krüger serves as Editor of European Journal of Clinical Investigation, Journal of Neural Transmission and Associate Editor of BMC Neurology; has received research grants of the Fonds National de Recherche de Luxembourg (FNR; PEARL), German Research Council (DFG; KR2219/2-3 and KR2119/8-1), the Michael J Fox Foundation, the Fritz Thyssen foundation (10.11.2.153) and the Federal Ministry for Education and Research [BMBF, COURAGE-PD and Mito-PD], as well as speaker's honoraria and/or travel grants from UCB Pharma, Abbvie, St. Jude Medicals, and Medtronic.

Alireza Gharabaghi is supported by grants from the German Research Council [DFG EC 307], and the Federal Ministry for Education and Research [BFNT 01GQ0761, BMBF 16SV3783, BMBF 03160064B, BMBF V4UKF014. Alireza Gharabaghi received speaker's honoraria and travel grants from Medtronic.

Supplementary material

Supplementary material is available at *Brain* online.

References

Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989; 12: 366–75.

Aron AR, Fletcher PC, Bullmore ET, Sahakian BJ, Robbins TW. Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. Nat Neurosci 2003; 6: 115–6.

Aron AR, Poldrack RA. Cortical and subcortical contributions to Stop signal response inhibition: role of the subthalamic nucleus. J Neurosci 2006; 26: 2424–33.

Benjamini YH, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Stat Methodol 1995: 289–300.

Brittain JS, Watkins KE, Joundi RA, Ray NJ, Holland P, Green AL, et al. A role for the subthalamic nucleus in response inhibition during conflict. J Neurosci 2012; 32: 13396–401.

Brown P, Marsden CD. Bradykinesia and impairment of EEG desynchronization in Parkinson's disease. Mov Disord 1999; 14: 423–9.

Cooper SE, McIntyre CC, Fernandez HH, Vitek JL. Association of deep brain stimulation washout effects with Parkinson disease duration. JAMA Neurol 2013; 70: 95–9.

Defebvre L, Bourriez JL, Derambure P, Duhamel A, Guieu JD, Destee A. Influence of chronic administration of L-DOPA on event-related desynchronization of mu rhythm preceding voluntary movement in Parkinson's disease. Electroencephalogr Clin Neurophysiol 1998; 109: 161–7.

Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 2004; 134: 9–21.

Devos D, Defebvre L. Effect of deep brain stimulation and L-Dopa on electrocortical rhythms related to movement in Parkinson's disease. Prog Brain Res 2006; 159: 331–49.

Devos D, Labyt E, Derambure P, Bourriez JL, Cassim F, Reyns N, et al. Subthalamic nucleus stimulation modulates motor cortex oscillatory activity in Parkinson's disease. Brain 2004; 127(Pt 2): 408–19.

Dirnberger G, Frith CD, Jahanshahi M. Executive dysfunction in Parkinson's disease is associated with altered pallidal-frontal processing. Neuroimage 2005; 25: 588–99.

Engel AK, Gerloff C, Hilgetag CC, Nolte G. Intrinsic coupling modes: multiscale interactions in ongoing brain activity. Neuron 2013; 80: 867–86.

Eusebio A, Thevathasan W, Doyle Gaynor L, Pogosyan A, Bye E, Foltynie T, et al. Deep brain stimulation can suppress pathological synchronisation in parkinsonian patients. J Neurol Neurosurg Psychiatry 2011; 82: 569–73.

Fell J, Axmacher N. The role of phase synchronization in memory processes. Nat Rev Neurosci 2011; 12: 105–18.

Frank MJ. Hold your horses: a dynamic computational role for the subthalamic nucleus in decision making. Neural Netw 2006; 19: 1120–36.

Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical deconstruction of parkinsonian neural circuitry. Science 2009; 324: 354–9.

Gerloff C, Uenishi N, Nagamine T, Kunieda T, Hallett M, Shibasaki H. Cortical activation during fast repetitive finger movements in humans: steady-state movement-related magnetic fields and their cortical generators. Electroencephalogr Clin Neurophysiol 1998; 109: 444–53.

Hallett M. The intrinsic and extrinsic aspects of freezing of gait. Mov Disord 2008; 23(Suppl 2): S439–43.

Haufe S, Nikulin VV, Muller KR, Nolte G. A critical assessment of connectivity measures for EEG data: a simulation study. Neuroimage 2013; 64: 120–33.

Hege MA, Preissl H, Stingl KT. Magnetoencephalographic signatures of right prefrontal cortex involvement in response inhibition. Hum Brain Mapp 2014.

Herz DM, Siebner HR, Hulme OJ, Florin E, Christensen MS, Timmermann L. Levodopa reinstates connectivity from prefrontal to premotor cortex during externally paced movement in Parkinson's disease. Neuroimage 2013; 90C: 15–23.

Herz DM, Siebner HR, Hulme OJ, Florin E, Christensen MS, Timmermann L. Levodopa reinstates connectivity from prefrontal

to premotor cortex during externally paced movement in Parkinson's disease. Neuroimage 2014; 90: 15–23.
Hipp JF, Hawellek DJ, Corbetta M, Siegel M, Engel AK. Large-scale cortical correlation structure of spontaneous oscillatory activity. Nat Neurosci 2012; 15: 884–90.
Hirschmann J, Ozkurt TE, Butz M, Homburger M, Elben S, Hartmann CJ, et al. Differential modulation of STN-cortical and cortico-muscular coherence by movement and levodopa in Parkinson's disease. Neuroimage 2013; 68: 203–13.
Jahanshahi M. Effects of deep brain stimulation of the subthalamic nucleus on inhibitory and executive control over prepotent responses in Parkinson's disease. Front Syst Neurosci 2013; 7: 118.
Jahanshahi M, Jones CR, Zijlmans J, Katzenschlager R, Lee L, Quinn N, et al. Dopaminergic modulation of striato-frontal connectivity during motor timing in Parkinson's disease. Brain 2010; 133 (Pt 3): 727–45.
Kahan J, Urner M, Moran R, Flandin G, Marreiros A, Mancini L, et al. Resting state functional MRI in Parkinson's disease: the impact of deep brain stimulation on 'effective' connectivity. Brain 2014; 137 (Pt 4): 1130–44.
Kuhn AA, Kempf F, Brucke C, Gaynor Doyle L, Martinez-Torres I, Pogosyan A, et al. High-frequency stimulation of the subthalamic nucleus suppresses oscillatory beta activity in patients with Parkinson's disease in parallel with improvement in motor performance. J Neurosci 2008; 28: 6165–73.
Kuhn AA, Kupsch A, Schneider GH, Brown P. Reduction in subthalamic 8-35 Hz oscillatory activity correlates with clinical improvement in Parkinson's disease. Eur J Neurosci 2006; 23: 1956–60.
Kumru H, Summerfield C, Valldeoriola F, Valls-Sole J. Effects of subthalamic nucleus stimulation on characteristics of EMG activity underlying reaction time in Parkinson's disease. Mov Disord 2004; 19: 94–100.
Kuriakose R, Saha U, Castillo G, Udupa K, Ni Z, Gunraj C, et al. The nature and time course of cortical activation following subthalamic stimulation in Parkinson's disease. Cereb Cortex 2010; 20: 1926–36.
Kwak Y, Peltier S, Bohnen NI, Muller ML, Dayalu P, Seidler RD. Altered resting state cortico-striatal connectivity in mild to moderate stage Parkinson's disease. Front Syst Neurosci 2010; 4: 143.
Lemm S, Muller KR, Curio G. A generalized framework for quantifying the dynamics of EEG event-related desynchronization. PLoS Comput Biol 2009; 5: e1000453.
Li JY, Espay AJ, Gunraj CA, Pal PK, Cunic DI, Lang AE, et al. Interhemispheric and ipsilateral connections in Parkinson's disease: relation to mirror movements. Mov Disord 2007; 22: 813–21.
Li Q, Ke Y, Chan DC, Qian ZM, Yung KK, Ko H, et al. Therapeutic deep brain stimulation in Parkinsonian rats directly influences motor cortex. Neuron 2012; 76: 1030–41.
Little S, Pogosyan A, Neal S, Zavala B, Zrinzo L, Hariz M, et al. Adaptive deep brain stimulation in advanced Parkinson disease. Ann Neurol 2013; 74: 449–57.
Magnani G, Cursi M, Leocani L, Volonte MA, Comi G. Acute effects of L-dopa on event-related desynchronization in Parkinson's disease. Neurol Sci 2002; 23: 91–7.
Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. J Neurosci Methods 2007; 164: 177–90.
Nambu A, Tokuno H, Inase M, Takada M. Corticosubthalamic input zones from forelimb representations of the dorsal and ventral divisions of the premotor cortex in the macaque monkey: comparison with the input zones from the primary motor cortex and the supplementary motor area. Neurosci Lett 1997; 239: 13–6.
Nambu A, Tokuno H, Takada M. Functional significance of the cortico-subthalamo-pallidal 'hyperdirect' pathway. Neurosci Res 2002; 43: 111–7.
Nolte G, Bai O, Wheaton L, Mari Z, Vorbach S, Hallett M. Identifying true brain interaction from EEG data using the imaginary part of coherency. Clin Neurophysiol 2004; 115: 2292–307.
Olde Dubbelink KT, Stoffers D, Deijen JB, Twisk JW, Stam CJ, Hillebrand A, et al. Resting-state functional connectivity as a marker of disease progression in Parkinson's disease: A longitudinal MEG study. Neuroimage Clin 2013; 2: 612–9.
Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011; 2011: 156869.
Poisson A, Ballanger B, Metereau E, Redoute J, Ibarolla D, Comte JC, et al. A functional magnetic resonance imaging study of pathophysiological changes responsible for mirror movements in Parkinson's disease. PLoS One 2013; 8: e66910.
Postuma RB, Dagher A. Basal ganglia functional connectivity based on a meta-analysis of 126 positron emission tomography and functional magnetic resonance imaging publications. Cereb Cortex 2006; 16: 1508–21.
Potter-Nerger M, Ilic TV, Siebner HR, Deuschl G, Volkmann J. Subthalamic nucleus stimulation restores corticospinal facilitation in Parkinson's disease. Mov Disord 2008; 23: 2210–5.
Rubia K, Russell T, Overmeyer S, Brammer MJ, Bullmore ET, Sharma T, et al. Mapping motor inhibition: conjunctive brain activations across different versions of go/no-go and stop tasks. Neuroimage 2001; 13: 250–61.
Salenius S, Avikainen S, Kaakkola S, Hari R, Brown P. Defective cortical drive to muscle in Parkinson's disease and its improvement with levodopa. Brain 2002; 125(Pt 3): 491–500.
Samuel M, Ceballos-Baumann AO, Turjanski N, Boecker H, Gorospe A, Linazasoro G, et al. Pallidotomy in Parkinson's disease increases supplementary motor area and prefrontal activation during performance of volitional movements an H2(15)O PET study. Brain 1997; 120(Pt 8): 1301–13.
Sharott A, Magill PJ, Harnack D, Kupsch A, Meissner W, Brown P. Dopamine depletion increases the power and coherence of beta-oscillations in the cerebral cortex and subthalamic nucleus of the awake rat. Eur J Neurosci 2005; 21: 1413–22.
Shibata T, Shimoyama I, Ito T, Abla D, Iwasa H, Koseki K, et al. The synchronization between brain areas under motor inhibition process in humans estimated by event-related EEG coherence. Neurosci Res 1998; 31: 265–71.
Silberstein P, Pogosyan A, Kuhn AA, Hotton G, Tisch S, Kupsch A, et al. Cortico-cortical coupling in Parkinson's disease and its modulation by therapy. Brain 2005; 128(Pt 6): 1277–91.
Spagnolo F, Coppi E, Chieffo R, Straffi L, Fichera M, Nuara A, et al. Interhemispheric balance in Parkinson's disease: a transcranial magnetic stimulation study. Brain Stimul 2013; 6: 892–7.
Steriade M, Llinas RR. The functional states of the thalamus and the associated neuronal interplay. Physiol Rev 1988; 68: 649–742.
Stoffers D, Bosboom JL, Deijen JB, Wolters E, Stam CJ, Berendse HW. Increased cortico-cortical functional connectivity in early-stage Parkinson's disease: an MEG study. Neuroimage 2008a; 41: 212–22.
Stoffers D, Bosboom JL, Wolters E, Stam CJ, Berendse HW. Dopaminergic modulation of cortico-cortical functional connectivity in Parkinson's disease: an MEG study. Exp Neurol 2008b; 213: 191–5.
Strens LH, Oliviero A, Bloem BR, Gerschlager W, Rothwell JC, Brown P. The effects of subthreshold 1 Hz repetitive TMS on cortico-cortical and interhemispheric coherence. Clin Neurophysiol 2002; 113: 1279–85.
Timmermann L, Gross J, Dirks M, Volkmann J, Freund HJ, Schnitzler A. The cerebral oscillatory network of parkinsonian resting tremor. Brain 2003; 126(Pt 1): 199–212.
Uhlhaas PJ, Singer W. Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. Neuron 2006; 52: 155–68.
Vercruysse S, Spildooren J, Heremans E, Wenderoth N, Swinnen SP, Vandenberghe W, et al. The neural correlates of upper limb motor

blocks in Parkinson's disease and their relation to freezing of gait. Cereb Cortex 2014; 24: 3154–66.

Weiss D, Breit S, Hoppe J, Hauser AK, Freudenstein D, Kruger R, et al. Subthalamic nucleus stimulation restores the efferent cortical drive to muscle in parallel to functional motor improvement. Eur J Neurosci 2012; 35: 896–908.

Weiss D, Govindan RB, Rilk A, Wachter T, Breit S, Zizlsperger L, et al. Central oscillators in a patient with neuropathic tremor: evidence from intraoperative local field potential recordings. Mov Disord 2011; 26: 323–7.

Weiss D, Lam JM, Breit S, Gharabaghi A, Kruger R, Luft AR, et al. The subthalamic nucleus modulates the early phase of probabilistic classification learning. Exp Brain Res 2014; 232: 2255–62.

Weiss D, Walach M, Meisner C, Fritz M, Scholten M, Breit S, et al. Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. Brain 2013; 136(Pt 7): 2098–108.

Zhang J, Hughes LE, Rowe JB. Selection and inhibition mechanisms for human voluntary action decisions. Neuroimage 2012; 63: 392–402.

5.3 Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease

Published as:

Scholten M, Klotz R, Plewnia C, Wächter T, Mielke C, Bloem BR, et al. Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease. Clin Neurophysiol. 2016b;127(1):610–20.

Clinical Neurophysiology 127 (2016) 610–620

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease

Marlieke Scholten [a,b,c,d,*], Rosa Klotz [a,b,c], Christian Plewnia [c,f], Tobias Wächter [e], Carina Mielke [a,b,c], Bastiaan R. Bloem [g], Christoph Braun [c,h,i], Ulf Ziemann [c,j], Rathinaswamy B. Govindan [k], Alireza Gharabaghi [c,l], Rejko Krüger [a,b,c,m], Daniel Weiss [a,b,c,*]

[a] Department of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research (HIH), University of Tuebingen, Germany
[b] German Centre of Neurodegenerative Diseases (DZNE), University of Tuebingen, Tuebingen, Germany
[c] Centre for Integrative Neuroscience (CIN), University of Tuebingen, Tuebingen, Germany
[d] Graduate School of Neural & Behavioural Sciences, International Max Planck Research School, University of Tuebingen, Tuebingen, Germany
[e] Centre of Rehabilitation, Bad Gögging, Passauer Wolf, Germany
[f] Department of Psychiatry and Psychotherapy, University of Tuebingen, Tuebingen, Germany
[g] Radboud University Medical Centre, Donders Institute for Brain, Cognition and Behavior, Department of Neurology, Nijmegen, The Netherlands
[h] MEG Center, University of Tuebingen, Tuebingen, Germany
[i] CIMeC, Center of Mind/Brain Sciences, University of Trento, Italy
[j] Department of Neurology and Stroke, and Hertie Institute for Clinical Brain Research (HIH), University of Tuebingen, Tuebingen, Germany
[k] Division of Fetal and Transitional Medicine, Children's National Medical Center, Washington, DC, USA
[l] Division of Functional and Restorative Neurosurgery, Department of Neurosurgery, University of Tuebingen, Tuebingen, Germany
[m] Clinical and Experimental Neuroscience, Luxembourg Center for Systems Biomedicine (LCSB), University of Luxembourg and Centre Hospitalier de Luxembourg (CHL), Luxembourg

ARTICLE INFO

Article history:
Accepted 13 February 2015
Available online 28 February 2015

Keywords:
Parkinson's disease
EEG
Subthalamic nucleus
Deep brain stimulation
Upper limb freezing
Freezing of gait

HIGHLIGHTS

- Subthalamic stimulation reduces exaggerated low-frequency intermuscular coherence during continuous fingertapping in iPD.
- We implemented criteria to detect and segment freezing events of upper limb movement for electrophysiological data analyses.
- Freezing of upper limb movement presents with increased low-frequency muscular and cortical activation, likely reflecting increased cortical motor inhibition.

ABSTRACT

Objective: The pathophysiology of deep brain stimulation mechanisms and resistant freezing phenomena in idiopathic Parkinson's disease (iPD) remains incompletely understood. Further studies on the neuromuscular substrates are needed.
Methods: We analyzed 16 patients with advanced iPD and bilateral subthalamic nucleus stimulation, and 13 age- and gender-matched healthy controls. Patients were tested after overnight withdrawal of medication with 'stimulation off' (StimOff) and 'stimulation on' (StimOn). Subjects performed continuous tapping of the right index finger with simultaneous recordings of biomechanical registration, EMG of finger flexors and extensors, and EEG. First, we analyzed EEG and EMG spectral measures comparing StimOff with healthy controls and StimOff with StimOn (irrespective of freezing). Second, we contrasted 'regular (unimpaired) tapping' and 'freezing' resistant to subthalamic neurostimulation as obtained in StimOn.
Results: iPD showed increased intermuscular coherence around 8 Hz in StimOff that was reduced in StimOn. This 8 Hz muscular activity was not coherent to cortical activity. 'Freezing' episodes showed increased muscle activity of finger flexors and extensors at 6–9 Hz, and increased cortical activity at

Abbreviations: ED, extensor digitorum communis muscle; EEG, electroencephalography; EMG, electromyography; FD, flexor digitorum superficialis muscle; iPD, idiopathic Parkinson's disease; SM1, primary sensorimotor area; StimOff, deep brain stimulation turned off; StimOn, deep brain stimulation turned on; STN-DBS, deep brain stimulation of the subthalamic nucleus; ULF, upper limb freezing; UPDRS, Unified Parkinson's Disease Rating Scale.

* Corresponding authors at: Department for Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research and German Center for Neurodegenerative Diseases, University of Tuebingen, Hoppe-Seyler-Str. 3, 72076 Tuebingen, Germany. Tel.: +49 7071 29 85650; fax: +49 7071 29 8349.
E-mail addresses: marlieke.scholten@uni-tuebingen.de (M. Scholten), daniel.weiss@uni-tuebingen.de (D. Weiss).

http://dx.doi.org/10.1016/j.clinph.2015.02.012

7–11 Hz. During transition from regular tapping to 'freezing' the cortical activity first increased over the left sensorimotor area followed by a spread to the left frontal and right parietal areas.
Conclusions: We identified neuromuscular motor network features of subthalamic neurostimulation therapy and resistant upper limb freezing that point to increased low-frequency muscular and cortical activity.
Significance: Together, our findings demonstrate several motor network abnormalities associated with upper limb freezing that may translate into future research on freezing of gait in iPD.

1. Introduction

Symptomatic treatment of idiopathic Parkinson's disease (iPD) includes deep brain stimulation of the subthalamic nucleus (STN-DBS) to control segmental motor symptoms and motor fluctuations (Deuschl et al., 2006; Schuepbach et al., 2013). However, freezing shows a less favorable therapeutic response (Vercruysse et al., 2014; Weiss et al., 2013) and a closer pathophysiological understanding is highly warranted. Functional imaging pointed to both cortical and subcortical network abnormalities in freezers, and implicated the subthalamic nucleus, as well as the mesencephalic locomotor region and higher-order cortical areas in freezing pathophysiology (Herman et al., 2013; Shine et al., 2013b). Much less is known about the electrophysiological neuromuscular mechanisms behind freezing (Nutt et al., 2011).

Here, we studied the motor network mechanisms of two major clinical issues: first, we explored the effect of subthalamic neurostimulation on the motor network characteristics during fingertapping (irrespective of freezing). This is of interest, as several studies pointed to large-scale motor network effects of DBS. As such, subthalamic neurostimulation modulated cortical activity in terms of movement-related desynchronization (Devos et al., 2004; Weiss et al., 2015). Moreover, STN-DBS increased motor cortex excitability (Kuriakose et al., 2010). Similarly, there is evidence for both defective corticospinal interactions and muscle activation at abnormally low frequencies <10 Hz in the clinical 'off' state (Salenius et al., 2002; Weiss et al., 2012). As spinal antagonistic motor neurons have segregated motor neuron pools (Jessell et al., 2011), antagonistic motor control can be studied with surface EMG. A distinct impairment of antagonistic spinal reciprocal inhibition via inhibitory 1a-interneurons was demonstrated (Meunier et al., 2000). Moreover, both cortical (Bertolasi et al., 1998) and subcortical modulatory influences (Raoul et al., 2012) on antagonistic motor control were revealed.

Second, we captured freezing events of continuous fingertapping (referred to as 'upper-limb freezing', ULF) and compared freezing to regular movement. Freezing phenomena are not restricted to gait, but may entrain fingertapping as well. ULF and freezing of gait occur with high clinical co-incidence (Nieuwboer et al., 2009; Vercruysse et al., 2013), although there is an ongoing controversy whether both phenomena share common network mechanisms (Barbe et al., 2014). As common characteristic, both ULF and freezing of gait showed increased motor output in the frequency range from 4 to 8 Hz, whereas regular fingertapping occurred from 1 to 3 Hz (Vercruysse et al., 2012). Previous functional imaging research pointed to a specific role of the subthalamic nucleus in gait freezers (Fling et al., 2013; Shine et al., 2013a; Vercruysse et al., 2013). Similar evidence came from clinical studies that pointed to basal ganglia outputs in terms of STN (Vercruysse et al., 2014) and the substantia nigra pars reticulata (Weiss et al., 2013) as important modulators of gait freezing. Moreover, cortical processing as well as subcortico–cortical interactions seem to be relevant. In this sense, patients with gait freezing showed higher functional connectivity between the supplementary motor area and the mesencephalic locomotor region (Fling et al., 2013). Moreover, ULF was paralleled by increased cortical BOLD activity in the left dorsolateral prefrontal cortex, motor cortex, supplementary motor area, and dorsal premotor area (Vercruysse et al., 2013). A recent EEG study found increased cortical theta band activity during both transition from normal movement to freezing and freezing itself (Shine et al., 2014). Together, these findings substantiate the view that freezing phenomena are paralleled by maladaptive processing on both cortical and subcortical levels.

We recorded iPD patients with STN-DBS and healthy controls with biomechanical registration, EEG, and EMG of antagonistic muscles during continuous fingertapping. We studied the electrophysiological neuromuscular mechanisms, i.e., (i) activation patterns of cortex and antagonistic muscles, (ii) synchronization between antagonistic muscles (intermuscular coherence), and (iii) synchronization between cortex and muscles (corticomuscular coherence) on two major clinical issues. First, we studied iPD patients both in 'stimulation off' (StimOff) and 'stimulation on' (StimOn) conditions, and compared StimOff to age- and gender-matched healthy controls. We hypothesized that iPD show increased intermuscular coherence in StimOff, and we postulated that subthalamic stimulation would decrease it. Second, we introduced additional dual task interference in the StimOn condition in order to provoke freezing phenomena, i.e., ULF (Spildooren et al., 2010). We selected StimOn in order to capture freezing episodes resistant to STN-DBS therapy. We hypothesized that ULF shows increased cortical activity since iPD motor impairment demonstrated with impaired cortical activity desynchronization (Devos et al., 2004; Weiss et al., 2015), and since inhibition of preplanned movement was associated with transient cortical activity increase (Sauseng et al., 2012). Moreover, we expected increased muscular low-frequency activation and increased intermuscular coherence during freezing, the latter as a consequence of impaired antagonistic motor control.

2. Methods

2.1. Subject characteristics

Seventeen iPD patients treated with STN-DBS and fourteen age- and gender matched healthy controls (HC) were recruited from the Department for Neurodegenerative Diseases, University of Tuebingen, Germany. Two subjects were excluded from the analyses (one iPD patient did not tolerate the discontinuation of the DBS therapy, and one HC did not adhere to the task requirements). Therefore, data are presented from sixteen iPD patients (iPD1–iPD16, eleven male, age 61.2 ± 11.1 years) and thirteen age- and gender-matched HC (HC1–HC13, eight male, age 62.6 ± 10.6 years).

The disease duration of iPD patients was 13.6 ± 6.8 years. Inclusion criteria were: patients diagnosed with iPD and akinetic-rigid symptom dominance (determined by neurologist and patient history), treatment with STN-DBS for at least 3 months, and age >18 years. The akinetic-rigid symptom dominance was further supported as patients were examined in 'stimulation off' (StimOff) during the study (UPDRS III: item 20 'tremor' of head, upper- and lower limbs summed; median [IQR] 0.0 [0.0 1.5]; item 22 'rigidity' of head and upper limbs summed: 5.5 [5.0 8.0]; items 23–26 'diadochokinesia': 20.0 [14.8 23.3]). Exclusion criteria were: resting tremor, cognitive impairment (Mini Mental State Examination <25 (Folstein et al., 1975)) and other competing neurological or neuromuscular disease conditions. The study was approved by the local Ethics committee of the University of Tuebingen in accordance to the Declaration of Helsinki. All subjects provided written informed consent prior to study inclusion. All subjects were right-handed as documented with the Edinburgh Handedness Scale. All patients were identified as 'freezers' (score > 1) according to the Freezing of Gait Questionnaire (FOG-Q; Giladi et al., 2000), however gait 'freezing' was not treated as formal inclusion criterion. The iPD patient characteristics are displayed in Table 1.

2.2. Experimental set up and paradigm

Anamnestic scores (FOG-Q, MMST, and Edinburgh handedness score) were determined on the day before the recordings. All recordings took place in the morning after overnight withdrawal of dopaminergic medication. UPDRS III was determined in both StimOff and StimOn. Subjects were seated comfortably with the right arm supported by an arm rest. They were instructed to perform continuous tapping with the right index finger as quickly and precisely as possible, whilst the index finger was in permanent contact with the force transducer. The subjects were instructed to precisely push the force transducer with a predefined strength of 2 N, and were provided with ongoing visual real-time feedback of the exerted pressure on a computer-screen placed 1 m in front. Due to the weak contraction strength and the permanent contact of finger and force transducer, the performance of this motor task required only minimum spatial displacement of a few millimeters of the index finger when exerting the pressure. This experimental setup was chosen to stabilize the tapping and movement characteristics across patients and subjects. Since modulation and rescaling of the contraction strength are known to modulate the magnitude of corticomuscular coherence, we aimed to standardize the contraction strength by visual feedback (Kilner et al., 2000; Mima and Hallett, 1999). Importantly, no external cues or rhythms were presented that could have prevented freezing episodes (Giladi and Hausdorff, 2006; Rahman et al., 2008). All subjects and patients were instructed to tap continuously and – if present – to try immediately to overcome movement arrests. Subjects performed continuous fingertapping in ten blocks of 20 s. Between blocks a pause of ten seconds was given to prevent fatigue.

2.3. Electrophysiological recordings

During the fingertapping, bipolar surface EMG of the flexor digitorum superficialis (FD) and extensor digitorum communis (ED) muscles using Ag/AgCl electrodes (Myotronics, Kent, USA) and a 36-channel EEG were recorded simultaneously to the biomechanical signal measured by the force transducer. For the EEG-recordings, linked earlobes were used as reference and FPz was used as ground electrode. All signals were sampled at 1000 Hz and monitored online using a BrainVision Recorder (Brain Products version 1.20, MES Electronics, Gilching, Germany). Impedances were kept below 5 kOhm.

2.4. Differences between HC and iPD StimOff, and effect of subthalamic stimulation

First, HC and iPD patients were recorded with 'fingertapping only'. We aimed to compare iPD patients in the StimOff condition with HC. Next, we studied the influence of STN-DBS and compared the StimOff and StimOn conditions. Therefore, the iPD patients performed 'fingertapping only' in both StimOff and StimOn in randomized order. In order to limit the potential influence of clinical carry-over effects, each treatment condition was active for at least 20 min prior to the recordings. This is considered sufficient to limit potential carry-over effects in advanced disease stages (Cooper et al., 2013). Bipolar stimulation settings were used in StimOn to minimize DBS artifacts in the EEG recordings. If pre-existing, the best individual bipolar parameters were maintained. In case of

Table 1
Baseline characteristics of the iPD patients.

Number	Age (yrs)	Gender	Disease duration (yrs)	FOG-Q	UPDRS III ('18–26')		'Diadochokinesia'	
					StimOff	StimOn	StimOff	StimOn
1*	61	M	7	2	18	11	10	7
2*	53	M	10	5	56	39	30	20
3	58	M	15	2	22	14	7	2
4	74	M	8	7	32	12	17	9
5	67	M	25	10	25	18	16	11
6*	41	M	10	10	16	8	9	4
7	63	M	14	3	27	19	19	12
8	67	F	16	15	36	33	24	21
9*	65	F	24	15	29	22	15	12
10	68	F	15	13	24	15	14	10
11	64	F	12	15	31	27	22	20
12*	63	F	19	14	32	25	21	20
13*	32	M	2	6	42	30	28	20
14*	74	M	8	8	34	25	23	18
15*	66	M	8	4	35	23	22	19
16*	63	M	24	15	45	34	28	21

Abbreviations: FOG-Q = Freezing of Gait Questionnaire (max score = 24). UPDRS = Unified Parkinson Disease Rating Scale, item 18–26, item 22 included only review of head and arms (max score = 80), diadochokinesia subscore of UPDRS item 23–26 (max score = 32), M = male, F = female, StimOff = deep brain stimulation turned off, StimOn = deep brain stimulation turned on.

* Patients with freezing of upper limb.

chronic treatment with monopolar stimulation, a bipolar program similar to the effective monopolar setting was established: therefore, we held the negative active contact constant and polarized against the most dorsal neighboring contact. In this situation, amplitudes were increased by 30% in order to achieve similar clinical efficacy (Silberstein et al., 2005). All patients received high frequency stimulation (twelve patients at 130 Hz, three at 125 Hz, and one at 180 Hz). The mean stimulation amplitudes were 3.7 ± 1.0 V (left STN) and 3.4 ± 1.1 V (right STN), and the mean pulse widths were 73.1 ± 18.9 μs (left STN) and 67.5 ± 13.4 μs (right STN). The clinical motor state in StimOff and StimOn was documented (UPDRS III). Ratings were restricted to the UPDRS III items 18–26, and 'rigidity' item 22 was obtained from head and upper extremities only, as subjects were largely immobile in sitting positions owing to EEG and EMG installed. A 'diadochokinesia' subscore was summed from the UPDRS items 23–26.

2.5. Comparison of 'regular tapping' and 'upper limb freezing'

After the recordings with 'fingertapping only', iPD patients performed the fingertapping in StimOn during dual task interference (StimOn interference). We introduced dual task interference, in order to reliably obtain freezing episodes under research laboratory conditions (Camicioli et al., 1998; O'Shea et al., 2002; Spildooren et al., 2010). StimOn was considered as therapeutic condition, as we aimed to capture ULF resistant to subthalamic stimulation therapy as a widely recognized limitation of PD state-of-the-art therapy in advanced disease stages (Krack et al., 2003; Vercruysse et al., 2014; Weiss et al., 2013). We only analyzed the ULF episodes during StimOn interference in order to study freezing resistant to subthalamic stimulation. Although ULF episodes also occurred 'spontaneously' in StimOff (23 episodes in six patients) and to lesser extent in StimOn (five episodes in three patients), we did not concatenate ULF episodes across different clinical conditions. In the StimOn interference condition, we obtained clear-cut transitions from 'regular tapping' to 'freezing' (which is important for the time–frequency analysis as explained below) (Fig. 1). HC also performed the fingertapping during dual task interference. We chose a phonemic verbal fluency task in which the subjects generated as many words as possible from a given initial letter. All subjects received the same set of letters that consisted of the ten most-used German consonants (one consonant per block of 20 s). The order of the presented letters was randomized across subjects. The subjects were instructed to equally share their attention between the index fingertapping and the verbal fluency task (Bloem et al., 2006). The performance on the verbal fluency task (number of words generated) was audio-recorded and evaluated offline. All subjects could generate words at each letter. The total number of words generated was 53.2 ± 14.2 in HC and 52.9 ± 15.3 in iPD patients. A one-way ANOVA was conducted to examine the difference between iPD patients and HC on the total number of words generated treating the years of education as covariate. Hence, there was no difference between HC and iPD in number of words generated ($F_{1,24} = 0.445$; $P = 0.511$).

2.6. Analyses

Motor performance was analyzed as 'tapping frequency' and 'tapping regularity'. The tapping frequency was expressed as number of peaks over time (Hz). The regularity of tapping was defined as coefficient of the peak-to-peak interval variability and given as the standard deviation of the interpeak interval normalized by the mean interpeak interval. EEG, EMG, and force transducer signals were analyzed with Matlab (R2012b, The Mathworks, Nattick, MA, USA) based on the open-source toolboxes EEGlab (v13.1.1b) (Delorme and Makeig, 2004) and Fieldtrip (Oostenveld et al., 2011).

For offline preprocessing (prior to any spectral analyses), EMG signals were band-pass filtered from 10 to 300 Hz, notch filtered for the 50 Hz line artifact, and full-wave rectified. EMG can be considered as a broad-band oscillatory activity (carrier activity) whose amplitude is modulated by a low frequency signal (<25 Hz) (Farmer et al., 1993). In the spectrum, such modulation signals result in the broadening of the carrier band, yet, are not reflected by an individual peak in the low frequency range. Rectifying the band-pass filtered EMG signal is a simple but efficient approach to demodulate the EMG activity and to extract the low-frequency modulation signal (Ward et al., 2013). EEG signals were filtered from 1 to 200 Hz and notch filtered at 50 Hz. For both EMG and EEG signals, a finite impulse response filter was used. EEG signals were visually inspected and a block of 20 s was rejected if it was affected by muscle artifacts. Similarly, time series were rejected if affected by tremor. EEG Principal Component Analysis was performed. Components were rejected if corresponding to eye blinks or to transient muscle artifacts (from facial or temporal muscles). Then, the remaining components were transformed back to the time domain. Reference-free EEG data were obtained using the Hjorth transform as spatial filter (Hjorth, 1980). From each of the performance blocks, the first two seconds and the last second were removed in order to achieve blocks with stable motor performance.

2.6.1. Operationalized offline detection of upper limb freezing (ULF) episodes

ULF episodes were selected and segmented semi-automatically to ensure reproducibility of our offline data-analyses. For this purpose, we used an operationalized algorithm that was recently developed to evaluate the clinical and biomechanical characteristics of freezing in upper limb movement (Nieuwboer et al., 2009). A time-series in the biomechanical signal was categorized as ULF episode, if (i) the amplitude deflection of the finger tap decreased below 1 N (i.e., below 50% of the requested force modulation of 2 N), (ii) the duration exceeded one second, and (iii) the frequency of motor output increased above 3 Hz based on the biomechanical registration. (Fig. 1). We automatically selected all episodes satisfying criteria one and two, i.e., 50% amplitude decrease lasting more than one second. Then, we visually determined if there was motor output at frequencies > 3 Hz. The data length of 'regular tapping' was adjusted to the data length of ULF and balanced for the contribution of a single subject to the total data pool.

2.6.2. Frequency domain analyses of cortical and muscular activity

For 'fingertapping only', cortical and muscular activity and corticomuscular coherence were analyzed in the frequency domain using spectral analyses. EEG and EMG signals were divided into half-overlapping segments with a duration of two seconds. The choice of two seconds was made in order to achieve a frequency resolution of 0.5 Hz. For data in each window, power (the squared absolute of the Fourier transform) and cross-spectrum, which is the product of the Fourier transform of one signal and the complex conjugate of the Fourier transform of the other signal, were calculated. These spectral quantities were averaged over all windows.

Coherence of the signals x and y is defined as the ratio of the estimate of the cross-spectrum, P_{xy} at frequency f, to the product of the autospectra, P_{xx} and P_{yy} at frequency f:

$$Coh_{xy}(f) = \frac{P_{xy}(f)^2}{P_{xx}(f)P_{yy}(f)}$$

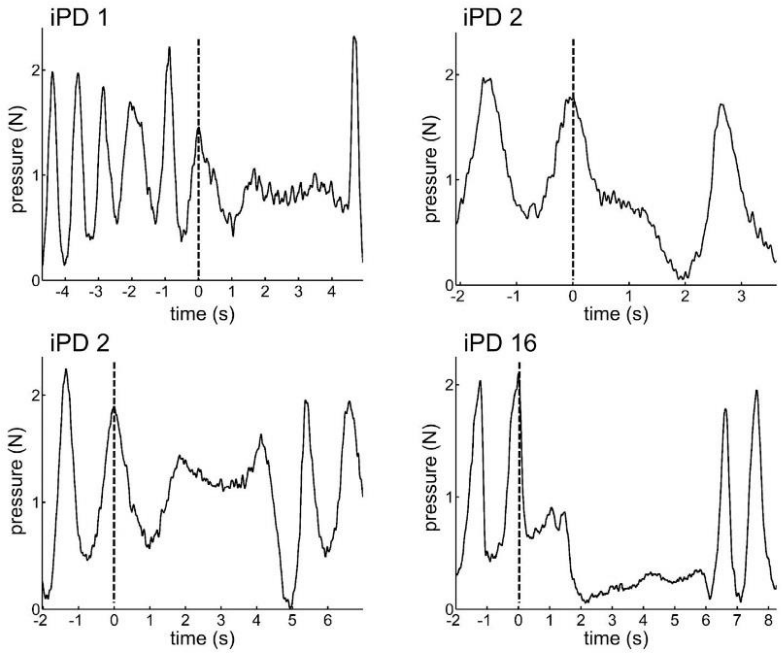

Fig. 1. Biomechanical signal of exemplary upper limb freezing episodes from different patients (iPD1, iPD2, and iPD16). The dashed vertical line represents the peak maximum of the last regular tap before freezing at time-point '0'. *X*-axis: time (s); *Y*-axis: pressure force (N).

Coherence is a normalized measure and takes on a value of one if there is an ideal correlation between the two signals and takes on a value of zero if there is no correlation between the two signals (Mima and Hallett, 1999). We calculated the intermuscular coherence between EMG signals in FD and ED, and the corticomuscular coherence between all EEG channels and EMG of FD and ED, respectively. Significance of the coherence estimates was decided on the 95% confidence limit (Rosenberg et al., 1989).

For 'freezing' vs. 'regular tapping', muscular and cortical activity were analyzed for each ULF trial. Regular tapping trials were adjusted to the length of the ULF trials. Here, the power was calculated with a window-length of one second (because some trials were shorter than two seconds) and the window moved forward in steps of 0.1 s, achieving a frequency resolution of 1 Hz. The spectra were averaged over all windows and over all trials of the subject.

For both 'fingertapping only' and freezing vs. regular tapping both muscular and cortical activity are expressed as relative power by dividing the power amplitude at a distinct frequency bin by the mean power of the whole spectrum (1–100 Hz).

2.6.3. Event-locked time–frequency analysis

To characterize the cortical processing when regular movement turned into an ULF episode, time–frequency analysis was performed. Time-point '0' was set as the peak maximum of the last regular tap of the biomechanical signal before entering the ULF episode. We segmented the data from −1000 to +2000 ms relative to time point '0'. For each epoch, EEG signals were decomposed using a complex Morlet wavelet for frequencies from 3 to 25 Hz with a resolution of 1 Hz (Schneider et al., 2008). The whole epoch was normalized to account for the variability between the ULF episodes. This normalization is done by subtracting from all power values the time-averaged power at a distinct frequency bin, and subsequently by dividing all power values by the time-averaged standard deviation of the epoch (Roach and Mathalon, 2008).

2.7. Statistical analyses

Descriptive findings are reported as mean ± standard deviation when tested with parametric tests and as median [interquartile range] in case of non-parametrical test procedures. We applied one-sided parametric tests for clinical effects and motor performance. Two-sided non-parametric tests were applied for spectral measures as they were mostly not normally distributed. First, we studied the differences between iPD in StimOff and HC using independent samples *t*-tests or a Mann–Whitney U tests. Next, we studied the influence of subthalamic stimulation by comparing StimOff vs. StimOn in iPD with paired samples *t*-tests or Wilcoxon tests.

'Freezing' was compared with 'regular tapping' (both selected from StimOn interference) using the paired samples *t*-test or Wilcoxon test. Correlation between the total duration of the ULF in each subject and question 3 of the FOG-Q was calculated using the non-parametric Spearman-Rho test. Cortical power, corticomuscular coherence, and time–frequency spectra were tested using a non-parametric cluster-based permutation test (Maris and Oostenveld, 2007). This approach addresses the need to correct for multiple comparisons by building clusters without losing sensitivity of spectral changes. First, a *t*-value is calculated for each sample with the predefined test-statistic (for example an independent samples *t*-test). Then the samples with *t*-values higher than threshold are clustered based on spatial adjacency (and additionally temporal adjacency in time–frequency series). For each cluster the sum of the *t*-values is taken. Then the significance probability is calculated by the Monte Carlo method, which approximated the permutation distribution of the test-statistic (maximum of the cluster-level summed *t*-values) by drawing 5000 random partitions. The Monte-Carlo significance probability was considered significant for two-tailed $P < 0.05$.

Time–frequency spectra were analyzed for statistical significance by comparing distinct time intervals in the time range from

0.5 to 1.5 s after the last regular finger tap with the time period one second before the last regular finger tap (−1 to 0 s) using the cluster-based permutation test explained above. For all tests, P values < 0.05 were considered significant. Statistics were performed using IBM SPSS statistics, version 22 (IBM Deutschland GmbH, Ehningen, Germany) and the Fieldtrip toolbox (Oostenveld et al., 2011).

3. Results

3.1. Differences between iPD StimOff and HC, and effects of subthalamic stimulation

3.1.1. Finger tapping performance and clinical outcome

During 'fingertapping only', stimulation improved bradykinesia according to the diadochokinesia UPDRS III subscore (Table 1, StimOff 19.1 ± 7.0; StimOn 14.1 ± 6.5; $t_{15} = 7.93$, $P < 0.001$). The tapping frequency was slower in iPD StimOff compared to HC. StimOn increased the tapping frequency (Table 2).

3.1.2. Antagonistic muscular activity, cortical activity and corticomuscular coherence

Muscular activity spectra showed distinct peaks both at tapping frequency and at 8 Hz (Fig. 2). For statistical comparison, we used the individual tapping frequencies, and 'activity at 8 Hz' was built as mean value from 7.5 to 8.5 Hz.

At individual tapping frequency, agonistic FD muscle activity was similar in HC, iPD StimOff and StimOn (Table 2, Fig. 2A). The antagonistic ED muscle showed decreased activity in iPD StimOff compared with HC ($P = 0.012$, Fig. 2B), and this was unchanged in the StimOn condition (Fig. 2E). Intermuscular coherence showed neither a difference between StimOff and HC, nor between StimOff and StimOn.

Corticomuscular coherence over the left sensorimotor area (SM1) was taken at the individual tapping frequency as prominent coherence peak from either the C3, C1, CP1, or CP3 electrode. Corticomuscular coherence exceeded the 95% confidence limit with both FD and ED in HC, StimOff, and StimOn. Corticomuscular coherence with the FD was not different in StimOff vs. HC, but increased with StimOn compared to StimOff ($P = 0.039$). HC showed higher corticomuscular coherence with ED compared to StimOff ($P = 0.001$), and this was unchanged in StimOn. All findings are summarized in Table 2.

Together, at tapping frequency, the corticomuscular coherence findings indicated that synchronization between SM1 area and agonistic FD spinal motor neurons increased with STN-DBS. In StimOff iPD patients had reduced synchronization between SM1 and ED spinal motor neurons compared to HC, but this was unchanged with STN-DBS.

Around 8 Hz, FD activity was similar in HC, StimOff, and StimOn (Fig. 2A, D). ED showed increased activity in StimOff compared to HC ($P = 0.039$, Fig. 2B), and this was unchanged with StimOn (Fig. 2E). Intermuscular coherence of antagonistic muscles was stronger in StimOff than in StimOn ($P = 0.026$, Fig. 2F), indicating that higher synchronization between antagonistic spinal motor neurons was reduced by STN-DBS. One may argue that particularly StimOff contained some ULF episodes and this may have confounded our findings. Therefore, we ensured that both FD and ED muscular activity, as well as intermuscular coherence did not differ significantly after removing freezing episodes from the StimOff condition (Section B in the Supplementary material). Cortical activity in StimOff revealed increased activity around 8 Hz compared to HC ($P = 0.002$) and this activity peak was most pronounced in the bilateral central and parietal regions. This increased cortical activity around 8 Hz was similar in StimOn with similar topographic distribution (n.s.). Cortical activity was not coherent with FD or ED activity around 8 Hz. All findings are summarized in Table 3.

3.2. Comparison of 'regular tapping' and 'upper limb freezing' (ULF)

The verbal fluency task led to more irregular tapping (tapping regularity in StimOn interference 0.67 ± 0.36; 'fingertapping only' in StimOn 0.24 ± 0.15; $P = 0.001$; paired samples t-test). Spectral measures were compared between 'regular tapping' and ULF in iPD patients in StimOn interference. Two patients were excluded from this analysis owing to tremor in iPD8 (which occurred only in StimOn interference) and inability to adhere to the interference paradigm in iPD10, respectively. According to criteria one (amplitude decrease < 50%) and two (length > 1 s), 93 episodes were identified. After applying criterion three (> 3 Hz), 21 episodes were excluded, which might have been related to 'simple movement stopping'. ULF episodes were detected in 9 of 14 iPD patients. The median number of ULF episodes per subject was 7 [5.5 11] (median [IQR]) with a duration of 2.3 [2.0 3.5] seconds. There was no correlation between the duration of ULF (normalized to the whole data length of dual task interference) and the Freezing of Gait Questionnaire item 3 (n.s.).

3.2.1. Muscular activity and intermuscular coherence during upper limb freezing (ULF)

The frequency ranges of interest for the statistical analysis of muscular activity were drawn from the grand average plots of muscular activity (Fig. 3). During ULF, both FD and ED showed increased activity around 6 to 9 Hz (FD, $P = 0.021$; ED, $P = 0.011$, Fig. 3A, B). During ULF, ED showed reduced activity in the frequency range from 22 to 34 Hz ($P = 0.038$, Fig. 3B), and no such

Table 2
Motor performance and spectral measures at individual tapping frequency.

	HC	StimOff	StimOn	StimOff vs. HC	StimOff vs. StimOn
Motor performance of fingertapping					
Frequency	2.16 ± .93	1.40 ± .69	1.56 ± .71	0.009	0.04
Regularity	.16 ± .07	.31 ± .16	.24 ± .15	0.027	0.187
Power					
FD	.14 [.08 .17]	.10 [.03 .21]	.09 [.04 .17]	.599	.569
ED	.15 [.08 .25]	.05 [.03 .13]	.05 [.03 .14]	.012	.717
Coherence					
Intermuscular coherence	0.67 [0.24 0.82]	0.33 [0.10 0.57]	0.31 [0.04 0.60]	0.056	0.959
Corticomuscular FD	.28 [.12 .32]	.11 [.03 .21]	.14 [.06 .31]	.066	.039
Corticomuscular ED	.27 [.15 .39]	.04 [.03 .13]	.09 [.05 .25]	.001	.070

Statistical comparisons of motor performance and spectral measures at individual tapping frequency of (i) HC vs. iPD StimOff, and (ii) iPD StimOff vs. StimOn. Corticomuscular coherence is calculated over the sensorimotor cortex. Motor performance is given as mean ± standard deviation, spectral measures are expressed as median [interquartile range]. Abbreviations: HC = healthy controls, StimOff = deep brain stimulation turned off, StimOn = deep brain stimulation turned on, FD = flexor digitorum superficialis, ED = extensor digitorum communis.

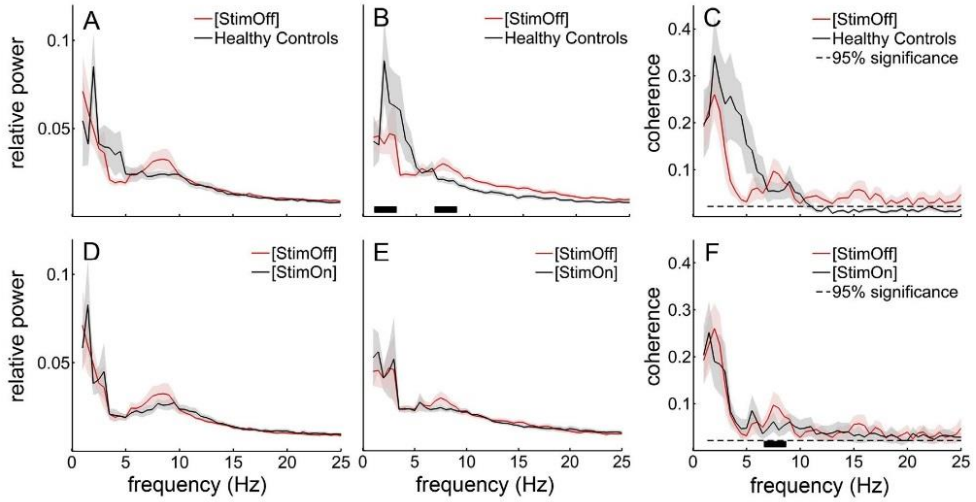

Fig. 2. Activity of the agonist FD and antagonist ED muscles and intermuscular coherence during 'fingertapping only'. The horizontal bold bars display significant differences ($P < 0.05$) at the individual tapping frequency or around 8 Hz. **A–C** compare iPD StimOff vs. HC with respect to **A** FD power, **B** ED power, and **C** intermuscular coherence. **D–F** compare iPD StimOff vs. StimOn with respect to **D** FD power, **E** ED power, and **F** intermuscular coherence. The shaded area represents the standard error of the mean (A–F) and the dotted line represent the 95% confidence limit of intermuscular coherence (C and F). *X*-axis: frequency (Hz); *Y*-axis: relative power (a.u.) or coherence.

Table 3
Spectral measures around 8 Hz.

	HC	StimOff	StimOn	StimOff vs. HC	StimOff vs. StimOn
Power					
FD	.02 [.02 .03]	.02 [.02 .05]	.02 [.02 .03]	.539	.255
ED	.02 [.02 .02]	.03 [.02 .04]	.02 [.02 .03]	.039	.088
Cortical	.02 [.01 .02]	.03 [.02 .03]	.02 [.02 .03]	.002	ns.
Coherence					
Intermuscular	.03 [.02 .04]	.07 [.03 .12]	.03 [.01 .07]	.219	.026
Corticomuscular FD	0.02 [0.01 0.02]	0.01 [0.01 0.01]	0.01 [0.01 0.02]	n.a.	n.a.
Corticomuscular ED	0.02 [0.01 0.02]	0.01 [0.01 0.02]	0.01 [0.01 0.02]	n.a.	n.a.

Statistical comparisons of spectral measures around 8 Hz of (i) HC vs. iPD StimOff, and (ii) iPD StimOff vs. StimOn. The average of cortical power is given over the cluster of significant channels for the comparison StimOff–HC. The comparison StimOff–StimOn did not show significant clusters. Corticomuscular coherence did not exceed the 95% confidence limit, and therefore no statistical comparison is applicable as indicated (n.a.). Data is expressed as median [interquartile range]. Abbreviations: HC = healthy controls, StimOff = deep brain stimulation turned off, StimOn = deep brain stimulation turned on, FD = flexor digitorum superficialis, ED = extensor digitorum communis.

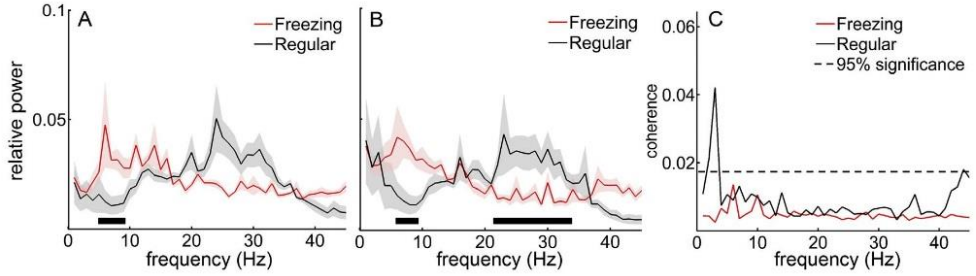

Fig. 3. Activity of the agonist FD and antagonist ED muscles and intermuscular coherence during 'upper limb freezing' (red) and 'regular tapping' (black). The horizontal bars display significant differences ($P < 0.05$) around 6–9 Hz or around 22–34 Hz. **A** power FD, **B** power ED, **C** intermuscular coherence. The shaded area represents the standard error of the mean (A–B). The dotted line represent the 95% confidence limit of the intermuscular coherence (C). *X*-axis: frequency (Hz); *Y*-axis: relative power (a.u.) or coherence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

difference was found in FD activity (Fig. 3). There was no significant intermuscular coherence from 6 to 9 Hz during ULF (Fig. 3C), and the 6 to 9 Hz muscular activity was not coherent to cortical activity in either FD or ED. All data are summarized in Table 4.

3.2.2. Cortical activity during upper limb freezing (ULF)

Cortical activity in the 7–11 Hz range increased during ULF compared to 'regular tapping' over the left frontal cortex, supplementary motor area, left SM1, and left parietal areas ($P < 0.001$, Fig. 4). The time–frequency representations of cortical power from

Table 4
Spectral measures of muscular activity from 6 to 9 Hz and from 22 to 34 Hz.

Measure	6–9 Hz			22–34 Hz		
	'Regular'	'Freezing'	*P*-value	'Regular'	'Freezing'	*P*-value
Power						
FD	.00 [.00 .02]	.03 [.02 .04]	.021	.05 [.02 .05]	.02 [.02 .02]	.066
ED	.00 [.00 .03]	.04 [.02 .05]	.011	.03 [.02 .05]	.02 [.01.02]	.038

Statistical comparisons of spectral measures (i) 6–9 Hz, and (ii) 22–34 Hz in iPD patients. 'Regular tapping' and 'freezing' under dual tasking conditions in StimOn are compared. Data is shown as median [interquartile range]. Abbreviations: FD = flexor digitorum superficialis muscle, ED = extensor digitorum communis muscle.

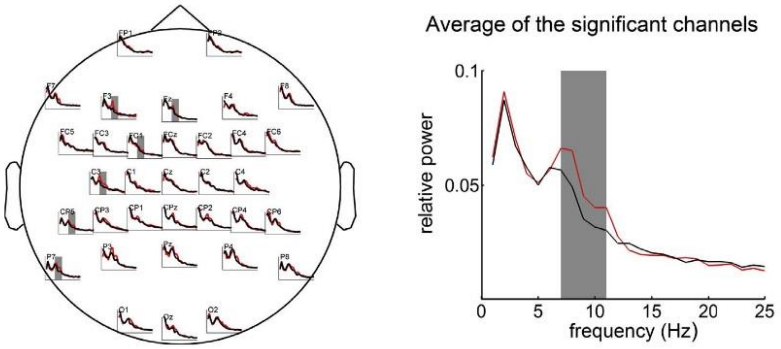

Fig. 4. Cortical activity (relative power) during 'upper limb freezing' (red) and 'regular tapping' (black). Upper limb freezing is associated with increased cortical activity in the 7–11 Hz range, grey boxes mark the significant cluster of channels overlying the left frontal cortex, supplementary motor area, left sensorimotor area, and left parietal cortex ($P < 0.001$). *X*-axis: frequency (Hz) scaled from 0 to 25 Hz in both multi-plot and single-plot, *Y*-axis: relative power ranging from 0 to 0.1 (a.u.). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

7 to 11 Hz event-locked to the onset of the ULF episode showed pronounced activation over the left SM1 area 0.7 s after the last regular finger tap. There was activation spread to the left frontal cortex and to the right parietal area from 0.85 to 1.2 s (Fig. 5).

4. Discussion

During 'fingertapping only', we found that – at individual tapping frequency – STN-DBS increased the corticomuscular coherence with the agonist FD muscle. Additionally, STN-DBS reduced the intermuscular coherence around 8 Hz, whereas STN-DBS did not change the muscular activity. Muscular activity around 8 Hz was not coherent to cortical activity. ULF compared to 'regular tapping' showed increased muscular activity in the low-frequency range from 6 to 9 Hz, and reduced antagonist activity in the frequency range from 22 to 34 Hz. Cortical activity was increased in the frequency range from 7 to 11 Hz during ULF, and the time–frequency decompositions of cortical activity showed emergence of left-hemispheric SM1 activity and spread to the left frontal and right parietal areas event-locked to ULF.

4.1. Subthalamic stimulation reduces low-frequency agonist–antagonist synchronization during repetitive movement

EMG activity in healthy persons occurs predominantly in the beta band (15–30 Hz) or Piper rhythm (35–60 Hz) (Brown, 2000). In untreated iPD patients, however, muscle discharge can occur at abnormally low frequencies below 10 Hz, which can be modulated by dopaminergic treatment and STN-DBS (Brown, 1997; Salenius et al., 2002; Weiss et al., 2012). Here, we found pronounced muscular activity around 8 Hz in iPD patients. Antagonist ED activity was significantly increased in StimOff as compared to HC, but was not modulated by subthalamic stimulation (Fig. 2B, E). Interestingly, however, activity around 8 Hz showed synchronization between agonists and antagonists in iPD in the StimOff condition, and this was significantly reduced with STN-DBS (Fig. 2F). iPD patients have defective agonist–antagonist activation patterns, which was expressed as lack of inhibition to extensor coupled interneurons during flexion compared to healthy persons (Meunier et al., 2000). This defective 1a-inhibition in iPD seems to be modulated at both cortical (Bertolasi et al., 1998) and subcortical level (Meunier et al., 2000), in particular in the subthalamic nucleus (Raoul et al., 2012). Accordingly, common supraspinal input was described to synchronize the antagonistic spinal motor neuron pools to a common frequency. Previous work demonstrated a loss of beta band synchronization between antagonistic muscles in PD 'off state' which was restored with subthalamic stimulation. This was referred to as facilitation of common corticospinal inputs at the physiological corticomuscular beta frequency (Brown et al., 2001; Marsden et al., 2001). In our study, we found a different and inverse pattern with enhanced agonist–antagonist synchronization in the low-frequency range at around 8 Hz in the StimOff condition, and attenuation in StimOn. Interestingly, no cortical oscillator was linearly synchronized to this 8 Hz activity. Mainly, two potential explanations might account for the lack of corticomuscular coherence at 8 Hz. Coupling between cortex and muscle is generally bidirectional with afferent and efferent interactions. A relevant contribution of the afferent component was recently demonstrated in corticokinematic coherence, particularly at frequencies below 10 Hz (Bourguignon et al., 2015). Accordingly, a jitter in conduction times of afferent and efferent contributions (as is present in non-linear coupling) might result in annihilation of corticomuscular coherence if mathematically expressed as ordinary linear coherence. Alternatively, the lack of corticomuscular coherence at 8 Hz gives

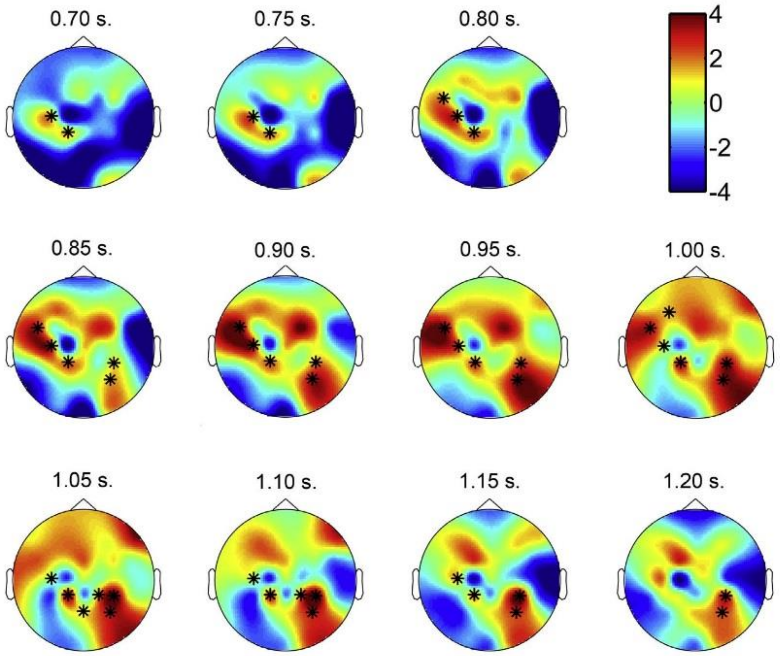

Fig. 5. Time–frequency representation of significant differences between 'upper limb freezing' (after the last regular finger tap) and the pre-freezing period (one-second period prior to the last regular finger tap at time '0') from 7 to 11 Hz. Time '0' indicates the last regular finger tap, before entering a freezing episode. Significant activity increases were found from +0.7 to +1.20 s after the last regular finger tap. Asterisks indicate a higher activity in a cluster over the left SM1, and subsequent spread to left frontal and right parietal cortex ($P = 0.012$). The color bar represents statistical units.

rise to discuss potential motor inputs to the spinal motor neuron pool apart from the corticospinal tract. Motor output from the basal ganglia was demonstrated for the nigro-ponto-reticulospinal pathway and is well known to occur in the startle response. Interestingly, this 'subcortical' descending pathway is characterized by unselective drives to the antagonistic spinal motor neuron pool and can be modulated by both L-Dopa (Delwaide et al., 1993) and subthalamic stimulation (Potter-Nerger et al., 2008). Accordingly, a task-related increase of the 'efferent' subthalamomuscular motor command was identified during isometric fine-motor performance compared to rest in iPD (Weiss et al., 2012). The relevance of such pathways for pathological agonist–antagonist synchronization can be addressed in future with combined subthalamic local field potential and EMG recordings.

Another interesting aspect is the long-range synchronization of cortex and muscle at the individual tapping frequency itself. Our data show that cortical synchronization with the spinal agonist motor neurons is increased at tapping frequency with StimOn compared to StimOff and this adds to previous findings on increased corticospinal contributions resulting from subthalamic stimulation (Brown et al., 2001; Kuriakose et al., 2010; Salenius et al., 2002; Weiss et al., 2012). To summarize, we identified two distinct network processes that parallel motor impairment in the PD 'off state': (i) decrease of corticomuscular synchrony at individual tapping frequency, and (ii) emergence of intermuscular synchronization around 8 Hz without coupling to the cortex.

4.2. Is transient suppression of cortical motor processing a mechanism of upper limb freezing?

During ULF, we found enhanced cortical activity from 7 to 11 Hz in the left SM1, left frontal and left parietal areas (Fig. 4). Similarly, we found in the time–frequency analyses that activity from 7 to 11 Hz increased during ULF, emerging 0.7 s after the last regular finger tap. This increased cortical activity showed a temporal-topographic evolution from the left SM1 (i.e., contralateral to the moving hand) to the left frontal and right parietal areas (Fig. 5).

Here, we discuss our findings in the context of previous models on freezing of gait (Nieuwboer and Giladi, 2013): Our paradigm was designed according to the interference hypotheses (inducing a cortical processing conflict by a second cognitive task). Nevertheless, ULF in our study was rather complemented by further mechanisms. In this sense, the so-called 'threshold model' (accumulation of motor deficits until the threshold for freezing is reached) could be considered relevant to our findings, because the tapping was more irregular during StimOn interference compared to StimOn. The 'decoupling model' considers disruption of the pre-planned/automatic movement and emergence of an alternate rhythm in freezing leading to ineffective movement ('block'). A role of the 'decoupling' mechanism to freezing in our study is supported by the findings: the original tapping rhythm and associated corticomuscular coherence at individual tapping frequency collapsed during ULF. Instead, abnormal low-frequency muscle activation from 6 to 9 Hz was observed as alternate rhythm. Again, this low-frequency muscular activity was not coherent to cortical activity. Similarly, altered cortical activity from 7 to 11 Hz emerged.

In general, enhanced alpha activity is increasingly recognized as an electrophysiological correlate of cortical motor suppression (Klimesch et al., 2007) and a spatial distribution similar to our study was found during active inhibition of preplanned movement (Sauseng et al., 2012). Interestingly, activity modulations around 10 Hz are not limited to cortical motor suppression, but also occur at the level of the pedunculopontine nucleus. A transient reduction

of the pedunculopontine alpha-band power was observed in parallel to freezing of gait (Thevathasan et al., 2012) and coherence modulation between the pedunculopontine nucleus and cortex in the alpha band was identified in phasic wrist movement (Tsang et al., 2010). Whether alpha rhythm modulation might constitute a 'binding mechanism' to balance the contribution of cortico-spinal and nigro-ponto-reticular transmission to the spinal motor neuron might be addressed in future with combined electrophysiological recordings from the midbrain and brainstem nuclei together with MEG/EEG and EMG. Together, we consider the abnormal increase in cortical activity in the alpha frequency range in ULF most likely as an inhibitory mechanism in the active sensorimotor cortex. The disruption of corticomuscular synchronization in ULF at the freezing rhythm itself further argues into this direction and attracts interest on alternate (presumably subcortical) drivers.

4.3. Methodological considerations

Several methodological considerations have to be taken into account when interpreting our findings on ULF. We aimed to operationalize detection and data segmentation of the ULF episodes. Hitherto, there is no 'gold standard' in identifying ULF episodes and in defining an accurate onset. This is inherent to research on freezing phenomena that develop with variable time delay after a regular movement and may be preceded by a short period of movement slowing and amplitude decrease before 'trembling-in-place-like-freezing' emerges (Nutt et al., 2011). Therefore, the most reproducible and reliable way to standardize the segmentation of our time–frequency analyses was to time-lock to the last regular finger tap before ULF emerged. We aimed to stabilize this natural variability by combining several earlier constraints on freezing pathophysiology. By selecting only ULF episodes with frequency > 3 Hz, we assumed that freezing behavior has a generic pathophysiological mechanism, because this criterion was demonstrated as common spatiotemporal characteristic in ULF (Vercruysse et al., 2012) and freezing of gait (Delval et al., 2010). Following this constraint, we rejected 21 out of 93 episodes ULF episodes in StimOn interference that might have related to 'simple movement stopping'.

Moreover, ULF episodes were only selected during StimOn interference, although ULF episodes also occurred in StimOff and to a lesser extent in StimOn. However, the concatenation of freezing episodes of all therapeutic conditions would have yielded less consistent results from a clinical standpoint. Furthermore, we primarily intended to capture ULF episodes resistant to subthalamic neurostimulation. On the other hand, we considered that during dual task interference ULF is provoked due to a cortical processing conflict and limited attentional resources (Bloem et al., 2001; Yogev et al., 2005). This includes that the patients transiently might 'forget to tap', i.e., stop their attempt to overcome the motor suppression. However, we found increased low-frequency motor output of the antagonistic muscles during the ULF episodes. This is not compatible with 'motor inactivity' as could be expected if patients shifted their attention away from the motor task and towards the verbal fluency task.

We further selected for patients with akinetic-rigid symptom dominance, and therefore the results should not be generalized to the whole iPD population. Nevertheless, this selection criterion was necessary to prevent a potential and confounding influence of tremor on our findings. Accordingly, we inspected each individual EMG data set for tremor and, consecutively, one patient had to be excluded when tremor solely occurred during StimOn interference. Generally however, the ULF phenomenon in this study and in rich previous work is separable from action tremor despite of its 'trembling-like' nature for several major reasons that were also conceived in our study (Barbe et al., 2014; Nieuwboer et al., 2009; Nutt et al., 2011): during ULF the power spectrum of the biomechanical signal traces did not show a distinct peak at tremor frequency (unlike the excluded measurement of iPD8, who showed a tremor peak). Characteristic for ULF, the power spectrum of the biomechanical signal showed broader low-frequency activity (exemplary data provided as Supplementary material, Section A). This was referred to as 'unevenness of the energy spectrum' (Nutt et al., 2011) and considered as typical feature of freezing phenomena, and therefore constitutes a motor phenomenon different from PD resting or action tremor that generally occurs at a constant and circumscribed frequency (Nutt et al., 2011). Typically, PD tremor also occurs with significant corticomuscular coherence at single and double tremor frequency, which was not observed in our spectral analysis of ULF (Hirschmann et al., 2013; Timmermann et al., 2003). Finally, it should be recognized that the mechanisms on ULF identified here should only carefully be translated to freezing of gait (Barbe et al., 2014). Nevertheless, our work generates important motor network hypotheses for future research on freezing of gait pathophysiology.

5. Conclusion

In conclusion, we propose that subthalamic stimulation strengthens the synchronization of cortical and agonist muscle activity at individual tapping frequency, and reduces increased low-frequency agonist–antagonist synchronization around 8 Hz. Upper limb freezing is reflected by muscular low-frequency activation around 6 to 9 Hz and cortical overactivity in the frequency range from 7 to 11. Together, our findings bring to light several network hypotheses that are valuable for future research to address freezing of gait, an unmet therapeutic need in Parkinson's disease.

Funding

This work was supported by a research grant of St. Jude Medical Inc.

Acknowledgements

Marlieke Scholten, Rosa Klotz, Christian Plewnia, Bastiaan R. Bloem, Christoph Braun, Ulf Ziemann, and Rathinaswamy B. Govindan report no competing financial interest.

Tobias Wächter received financial support for research from and conducted commissioned research for Medtronic.

Carina Mielke was supported by a research grant of St. Jude Medical.

Alireza Gharabaghi is supported by a grant from Medtronic.

Rejko Krüger has received speaker's honoraria and/or travel grants from St. Jude Medical, and Medtronic.

Daniel Weiss received research funding, speaker's honoraria, and a travel grant from Medtronic and a research grant from the German Research Council (DFG, WE5375/1-1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinph.2015.02.012.

References

Barbe MT, Amarell M, Snijders AH, Florin E, Quatuor EL, Schonau E, et al. Gait and upper limb variability in Parkinson's disease patients with and without freezing of gait. J Neurol 2014;261:330–42.

Bertolasi L, Priori A, Tinazzi M, Bertasi V, Rothwell JC. Inhibitory action of forearm flexor muscle afferents on corticospinal outputs to antagonist muscles in humans. J Physiol 1998;511:947–56.
Bloem BR, Grimbergen YA, van Dijk JG, Munneke M. The, "posture second" strategy: a review of wrong priorities in Parkinson's disease. J Neurol Sci 2006;248:196–204.
Bloem BR, Valkenburg VV, Slabbekoorn M, van Dijk JG. The multiple tasks test. Strategies in Parkinson's disease. Exp Brain Res 2001;137:478–86.
Bourguignon M, Piitulainen H, De Tiege X, Jousmaki V, Hari R. Corticokinematic coherence mainly reflects movement-induced proprioceptive feedback. Neuroimage 2015;106:382–90.
Brown P. Muscle sounds in Parkinson's disease. Lancet 1997;349:533–5.
Brown P. Cortical drives to human muscle: the Piper and related rhythms. Prog Neurobiol 2000;60:97–108.
Brown P, Marsden J, Defebvre L, Cassim F, Mazzone P, Oliviero A, et al. Intermuscular coherence in Parkinson's disease: relationship to bradykinesia. Neuroreport 2001;12:2577–81.
Camicioli R, Oken BS, Sexton G, Kaye JA, Nutt JG. Verbal fluency task affects gait in Parkinson's disease with motor freezing. J Geriatr Psychiatry Neurol 1998;11:181–5.
Cooper SE, McIntyre CC, Fernandez HH, Vitek JL. Association of deep brain stimulation washout effects with Parkinson disease duration. JAMA Neurology 2013;70:95–9.
Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 2004;134:9–21.
Delval A, Snijders AH, Weerdesteyn V, Duysens JE, Defebvre L, Giladi N, et al. Objective detection of subtle freezing of gait episodes in Parkinson's disease. Mov Disord 2010;25:1684–93.
Delwaide PJ, Pepin JL, Maertens de Noordhout A. The audiospinal reaction in Parkinsonian patients reflects functional changes in reticular nuclei. Ann Neurol 1993;33:63–9.
Deuschl G, Schade-Brittinger C, Krack P, Volkmann J, Schafer H, Botzel K, et al. A randomized trial of deep-brain stimulation for Parkinson's disease. N Engl J Med 2006;355:896–908.
Devos D, Labyt E, Derambure P, Bourriez JL, Cassim F, Reyns N, et al. Subthalamic nucleus stimulation modulates motor cortex oscillatory activity in Parkinson's disease. Brain 2004;127:408–19.
Farmer SF, Bremner FD, Halliday DM, Rosenberg JR, Stephens JA. The frequency content of common synaptic inputs to motoneurones studied during voluntary isometric contraction in man. J Physiol 1993;470:127–55.
Fling BW, Cohen RG, Mancini M, Nutt JG, Fair DA, Horak FB. Asymmetric pedunculopontine network connectivity in parkinsonian patients with freezing of gait. Brain 2013;136:2405–18.
Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
Giladi N, Hausdorff JM. The role of mental function in the pathogenesis of freezing of gait in Parkinson's disease. J Neurol Sci 2006;248:173–6.
Giladi N, Shabtai H, Simon ES, Biran S, Tal J, Korczyn AD. Construction of freezing of gait questionnaire for patients with Parkinsonism. Parkinsonism Relat Disord 2000;6:165–70.
Herman T, Giladi N, Hausdorff JM. Neuroimaging as a window into gait disturbances and freezing of gait in patients with Parkinson's disease. Curr Neurol Neurosci Rep 2013;13:411.
Hirschmann J, Hartmann CJ, Butz M, Hoogenboom N, Ozkurt TE, Elben S, et al. A direct relationship between oscillatory subthalamic nucleus–cortex coupling and rest tremor in Parkinson's disease. Brain 2013;136:3659–70.
Hjorth B. Source derivation simplifies topographical EEG interpretation. Am J EEG Technol 1980;20:121–32.
Jessell TM, Surmeli G, Kelly JS. Motor neurons and the sense of place. Neuron 2011;72:419–24.
Kilner JM, Baker SN, Salenius S, Hari R, Lemon RN. Human cortical muscle coherence is directly related to specific motor parameters. J Neurosci 2000;20:8838–45.
Klimesch W, Sauseng P, Hanslmayr S. EEG alpha oscillations: the inhibition-timing hypothesis. Brain Res Rev 2007;53:63–88.
Krack P, Batir A, Van Blercom N, Chabardes S, Fraix V, Ardouin C, et al. Five-year follow-up of bilateral stimulation of the subthalamic nucleus in advanced Parkinson's disease. N Engl J Med 2003;349:1925–34.
Kuriakose R, Saha U, Castillo G, Udupa K, Ni Z, Gunraj C, et al. The nature and time course of cortical activation following subthalamic stimulation in Parkinson's disease. Cereb Cortex 2010;20:1926–36.
Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. J Neurosci Methods 2007;164:177–90.
Marsden J, Limousin-Dowsey P, Fraix V, Pollak P, Odin P, Brown P. Intermuscular coherence in Parkinson's disease: effects of subthalamic nucleus stimulation. Neuroreport 2001;12:1113–7.
Meunier S, Pol S, Houeto JL, Vidailhet M. Abnormal reciprocal inhibition between antagonist muscles in Parkinson's disease. Brain 2000;123:1017–26.
Mima T, Hallett M. Corticomuscular coherence: a review. J Clin Neurophysiol 1999;16:501–11.
Nieuwboer A, Giladi N. Characterizing freezing of gait in Parkinson's disease: models of an episodic phenomenon. Mov Disord 2013;28:1509–19.
Nieuwboer A, Vercruysse S, Feys P, Levin O, Spildooren J, Swinnen S. Upper limb movement interruptions are correlated to freezing of gait in Parkinson's disease. Eur J Neurosci 2009;29:1422–30.
Nutt JG, Bloem BR, Giladi N, Hallett M, Horak FB, Nieuwboer A. Freezing of gait: moving forward on a mysterious clinical phenomenon. Lancet Neurol 2011;10:734–44.
O'Shea S, Morris ME, Iansek R. Dual task interference during gait in people with Parkinson disease: effects of motor versus cognitive secondary tasks. Phys Ther 2002;82:888–97.
Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011;2011:156869.
Potter-Nerger M, Ilic TV, Siebner HR, Deuschl G, Volkmann J. Subthalamic nucleus stimulation restores corticospinal facilitation in Parkinson's disease. Mov Disord 2008;23:2210–5.
Rahman S, Griffin HJ, Quinn NP, Jahanshahi M. The factors that induce or overcome freezing of gait in Parkinson's disease. Behav Neurol 2008;19:127–36.
Raoul S, Roualdes V, Deligny C, Leduc D, Lamy JC, Lackmy-Vallee A, et al. Subthalamic nucleus stimulation reverses spinal motoneuron activity in parkinsonian patients. Brain 2012;135:139–47.
Roach BJ, Mathalon DH. Event-related EEG time-frequency analysis: an overview of measures and an analysis of early gamma band phase locking in schizophrenia. Schizophr Bull 2008;34:907–26.
Rosenberg JR, Amjad AM, Breeze P, Brillinger DR, Halliday DM. The Fourier approach to the identification of functional coupling between neuronal spike trains. Prog Biophys Mol Biol 1989;53:1–31.
Salenius S, Avikainen S, Kaakkola S, Hari R, Brown P. Defective cortical drive to muscle in Parkinson's disease and its improvement with levodopa. Brain 2002;125:491–500.
Sauseng P, Gerloff C, Hummel FC. Two brakes are better than one: The neural bases of inhibitory control of motor memory traces. Neuroimage. 2012;65C:52–8.
Schneider TR, Debener S, Oostenveld R, Engel AK. Enhanced EEG gamma-band activity reflects multisensory semantic matching in visual-to-auditory object priming. Neuroimage 2008;42:1244–54.
Schuepbach WM, Rau J, Knudsen K, Volkmann J, Krack P, Timmermann L, et al. Neurostimulation for Parkinson's disease with early motor complications. N Engl J Med 2013;368:610–22.
Shine JM, Handojoseno AM, Nguyen TN, Tran Y, Naismith SL, Nguyen H, et al. Abnormal patterns of theta frequency oscillations during the temporal evolution of freezing of gait in Parkinson's disease. Clin Neurophysiol 2014;125:569–76.
Shine JM, Matar E, Ward PB, Bolitho SJ, Pearson M, Naismith SL, et al. Differential neural activation patterns in patients with Parkinson's disease and freezing of gait in response to concurrent cognitive and motor load. PLoS One 2013a;8:e52602.
Shine JM, Moustafa AA, Matar E, Frank MJ, Lewis SJ. The role of frontostriatal impairment in freezing of gait in Parkinson's disease. Front Syst Neurosci 2013b;7:61.
Silberstein P, Pogosyan A, Kuhn AA, Hotton G, Tisch S, Kupsch A, et al. Cortico-cortical coupling in Parkinson's disease and its modulation by therapy. Brain 2005;128:1277–91.
Spildooren J, Vercruysse S, Desloovere K, Vandenberghe W, Kerckhofs E, Nieuwboer A. Freezing of gait in Parkinson's disease: the impact of dual-tasking and turning. Mov Disord 2010;25:2563–70.
Thevathasan W, Pogosyan A, Hyam JA, Jenkinson N, Foltynie T, Limousin P, et al. Alpha oscillations in the pedunculopontine nucleus correlate with gait performance in parkinsonism. Brain 2012;135:148–60.
Timmermann L, Gross J, Dirks M, Volkmann J, Freund H, Schnitzler A. The cerebral oscillatory network of parkinsonian resting tremor. Brain 2003;126:199–212.
Tsang EW, Hamani C, Moro E, Mazzella F, Poon YY, Lozano AM, et al. Involvement of the human pedunculopontine nucleus region in voluntary movements. Neurology 2010;75:950–9.
Vercruysse S, Spildooren J, Heremans E, Vandenbossche J, Wenderoth N, Swinnen SP, et al. Abnormalities and cue dependence of rhythmical upper-limb movements in Parkinson patients with freezing of gait. Neurorehabil Neural Repair 2012;26:636–45.
Vercruysse S, Spildooren J, Heremans E, Wenderoth N, Swinnen SP, Vandenberghe W, et al. The neural correlates of upper limb motor blocks in Parkinson's disease and their relation to freezing of gait. Cereb Cortex 2013;24:3154–66.
Vercruysse S, Vandenberghe W, Munks L, Nuttin B, Devos H, Nieuwboer A. Effects of deep brain stimulation of the subthalamic nucleus on freezing of gait in Parkinson's disease: a prospective controlled study. J Neurol Neurosurg Psychiatry 2014;5:871–7.
Ward NJ, Farmer SF, Berthouze L, Halliday DM. Rectification of EMG in low force contractions improves detection of motor unit coherence in the beta-frequency band. J Neurophysiol 2013;110:1744–50.
Weiss D, Breit S, Hoppe J, Hauser AK, Freudenstein D, Kruger R, et al. Subthalamic nucleus stimulation restores the efferent cortical drive to muscle in parallel to functional motor improvement. Eur J Neurosci 2012;35:896–908.
Weiss D, Klotz R, Rathinaswamy RB, Scholten M, Naros G, Ramos-Murguialday A, et al. Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's Disease. Brain 2015. http://dx.doi.org/10.1093/brain/awu380.
Weiss D, Walach M, Meisner C, Fritz M, Scholten M, Breit S, et al. Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. Brain 2013;136:2098–108.
Yogev G, Giladi N, Peretz C, Springer S, Simon ES, Hausdorff JM. Dual tasking, gait rhythmicity, and Parkinson's disease: which aspects of gait are attention demanding? Eur J Neurosci 2005;22:1248–56.

5.4 Cortical correlates of susceptibility to upper limb freezing in Parkinson's disease

Published as:

Scholten M, Govindan RB, Braun C, Bloem BR, Plewnia C, Krüger R, et al. Cortical correlates of susceptibility to upper limb freezing in Parkinson's disease. Clin Neurophysiol. 2016a;127(6):2386–93.

Clinical Neurophysiology 127 (2016) 2386–2393

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Cortical correlates of susceptibility to upper limb freezing in Parkinson's disease

Marlieke Scholten [a,b,c,d,*], Rathinaswamy B. Govindan [e], Christoph Braun [c,f,g], Bastiaan R. Bloem [h], Christian Plewnia [i], Rejko Krüger [a,b,c,j], Alireza Gharabaghi [c,k], Daniel Weiss [a,b,c,*]

[a] Department of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research (HIH), University of Tuebingen, Tuebingen, Germany
[b] German Centre of Neurodegenerative Diseases (DZNE), University of Tuebingen, Tuebingen, Germany
[c] Centre for Integrative Neuroscience (CIN), University of Tuebingen, Tuebingen, Germany
[d] Graduate School of Neural & Behavioral Sciences, International Max Planck Research School, University of Tuebingen, Tuebingen, Germany
[e] Division of Fetal and Transitional Medicine, Children's National Medical Center, Washington, DC, USA
[f] MEG Center, University of Tuebingen, Tuebingen, Germany
[g] CIMeC, Center of Mind/Brain Sciences, University of Trento, Italy
[h] Radboud University Medical Centre, Donders Institute for Brain, Cognition and Behavior, Department of Neurology, Nijmegen, The Netherlands
[i] Department of Psychiatry and Psychotherapy, University of Tuebingen, Tuebingen, Germany
[j] Clinical and Experimental Neuroscience, Luxembourg Center for Systems Biomedicine (LCSB), University of Luxembourg and Centre Hospitalier de Luxembourg (CHL), Luxembourg
[k] Division of Functional and Restorative Neurosurgery, Department of Neurosurgery, University of Tuebingen, Tuebingen, Germany

See Editorial, pages 2334–2336

ARTICLE INFO

Article history:
Accepted 29 January 2016
Available online 26 March 2016

Keywords:
Upper limb freezing
Parkinson's disease
Subthalamic nucleus deep brain stimulation
Electroencephalography
Cortical activity
Global phase synchronization

HIGHLIGHTS

- Dual tasking increased susceptibility to upper limb freezing.
- This was associated with increased cortico-cortical phase synchronization from 13 to 30 Hz over the left prefrontal area.
- This abnormal phase synchronization was predictive for freezing behavior.

ABSTRACT

Objective: Freezing behavior is an unmet symptom in Parkinson's disease (PD), which reflects its complex pathophysiology. Freezing behavior can emerge when attentional capacity is reduced, i.e. under dual task interference. In this study, we characterized the cortical network signatures underlying the susceptibility to freezing during continuous finger tapping.
Methods: Fourteen PD patients with STN-DBS and thirteen age- and gender-matched healthy controls performed continuous tapping with the index finger as single motor task and during dual tasking. Synchronized EEG and mechanogram of the finger tapping were recorded. Subsequently, we analyzed cortical activity and cortico-cortical phase synchronization. We correlated these spectral measures with the biomechanically confirmed numbers of freezing episodes during finger tapping.
Results: During dual tasking compared to the single motor task, PD patients showed an increase of cortico-cortical phase synchronization over the left prefrontal area from 13 to 30 Hz. This correlated with

Abbreviations: STN-DBS, Subthalamic nucleus deep brain stimulation; PD, Parkinson's disease; HC, healthy controls; EEG, Electroencephalography; FOG, Freezing of Gait; FOG-Q, Freezing of Gait Questionnaire; SI, synchronization index.

* Corresponding authors at: Department of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research (HIH), University of Tuebingen, Hoppe-Seyler-Str. 3, 72076 Tuebingen, Germany.
E-mail addresses: marlieke.scholten@uni-tuebingen.de (M. Scholten), daniel.weiss@uni-tuebingen.de (D. Weiss).

http://dx.doi.org/10.1016/j.clinph.2016.01.028

increased occurrence of freezing episodes. Interestingly, PD patients lacked the increase of prefrontal cortico-cortical synchronization from 4 to 7 Hz during dual tasking as observed in healthy controls.
Conclusion: Dual task interference led to an increase of left prefrontal beta band synchronization (13–30 Hz) in PD and this increment predicted the number of freezing episodes. This increment may underscore the relevance of prefrontal executive dysfunction in freezing susceptibility.
Significance: These findings enhance our understanding of the pathological network mechanisms behind increased susceptibility to freezing behavior.

1. Introduction

Freezing behavior including freezing of upper limb movement and Freezing of Gait reflects an episodic phenomenon that occurs in the majority of advanced PD patients, leading to impairment in quality of life, falls, and morbidity (Bloem et al., 2004; Giladi et al., 2001; Rahman et al., 2008). Current standard treatments such as L-Dopa and deep brain stimulation of the subthalamic nucleus (STN-DBS) often fail to control freezing behavior in the advanced disease stage (Vercruysse et al., 2014; Weiss et al., 2013). This may mirror the incomplete understanding of the complex pathophysiology behind freezing behavior. As important pathophysiological background, maladaptive motor processing was demonstrated at widely distributed neuronal levels including the basal ganglia (Vercruysse et al., 2013; Weiss et al., 2012), brainstem (Peterson et al., 2014b; Shine et al., 2013b; Snijders et al., 2011), as well as distributed cortical processors (Bartels and Leenders, 2008; Scholten et al., 2016). At the cortical level, pronounced Lewy-body pathology (Virmani et al., 2015) and motor network abnormalities paralleled the increased vulnerability of cortical motor processing in freezers. In particular, motor network abnormalities were observed in the frontal and parietal areas. Altered function of these areas parallel executive dysfunctions (Kostic et al., 2012) relating to impaired motor program adjustments and loss of automaticity (Hallett, 2008; Vandenbossche et al., 2012) as observed in freezers. Congruently, interference of freezers with a second cognitive task was described as an effective procedure to provoke freezing events given the susceptibility of freezers to 'cortical processing conflicts' and 'capacity overloads' (Peterson et al., 2014a; Spildooren et al., 2010).

In keeping with this evidence, motor network abnormalities in freezers clearly exist outside the freezing episode itself (Snijders et al., 2011; Vercruysse et al., 2013), and finally the freezing episode might constitute an endpoint of motor network imbalance or even the beginning of compensatory attempts to reset an efficient motor program (Shine et al., 2014; Toledo et al., 2014). Therefore, if an adequate timeframe for therapeutic intervention exists to prevent freezing behavior, it should probably lie outside the freezing episode itself.

Here, we aim to identify pathological brain states with respect to freezing susceptibility by analyzing cortical activity and cortico-cortical synchronization signatures in advanced PD patients. Cortical activity is expressed as a measure of local neuronal activity along oscillatory amplitude characteristics, and cortico-cortical phase synchronization is determined as correlation of oscillatory phases between distributed brain regions (Engel et al., 2013; Fell and Axmacher, 2011). Both measures have a pivotal role in both motor and cognitive tasks including the pathological network states of PD (Silberstein et al., 2005; Uhlhaas and Singer, 2006; Weiss et al., 2015).

Therefore, we studied cortical spectral measures in PD patients under dual tasking conditions and compared them to the single motor task (outside freezing episodes and motor arrests, respectively). We explored whether these cortical activity and synchronization modulations were related to freezing susceptibility. Therefore, we correlated the spectral modulations induced by dual tasking with the biomechanically confirmed number of freezing events during finger tapping (referred to as 'upper limb freezing'). Finally, we explored whether the modulations observed in PD were disease-specific by additionally analyzing dual tasking effects in healthy controls (HC).

Table 1
Characteristics of PD patients.

	Gender (M/F)	Age (years)	PD duration (years)	Time since DBS implantation (months)	Segmental UPDRS III (items 20–26)	FOG-Q
PD01*	M	61	7	4	10	2
PD02*	M	53	10	35	35	5
PD03	M	59	15	41	12	2
PD04	M	75	8	2	10	7
PD05	M	67	25	77	16	10
PD06*	M	41	10	6	6	10
PD07	M	63	14	3	18	3
PD08*	F	65	24	35	20	15
PD09	F	65	12	47	25	15
PD10*	F	63	19	34	23	14
PD11*	M	32	2	7	26	6
PD12*	M	74	8	25	22	8
PD13*	M	66	8	11	20	4
PD14*	M	63	24	73	30	15

Abbreviations: M, male; F, female; UPDRS, Unified Parkinson Disease Rating Scale; items 20–26, item 22 included only rating of head and upper limbs (total score = 72), FOG-Q = Freezing of Gait Questionnaire (total score = 24).
* Patients with freezing of upper limb during dual tasking.

2. Methods

2.1. Subject characteristics

We studied 16 patients with idiopathic PD treated with STN-DBS for at least three months and 14 HC matched for age and gender. Exclusion criteria were resting tremor, cognitive impairment (Mini Mental State Examination < 25 (Folstein et al., 1975)), or other competing neurological or neuromuscular disease conditions. Data from three subjects (2 PD and 1 HC) were excluded, because one PD patient suffered from tremor only during dual tasking and one HC and one PD patient could not adhere to the dual task paradigm. Therefore, data is presented from 14 PD patients (PD1–PD14, 11 male, 60.6 ± 11.6 years) and 13 HC (HC1–HC13, 8 male, 63.1 ± 10.1 years (mean ± SD)). The study was approved by the local Ethics committee of the University of Tuebingen and all subjects provided written consent. The disease duration of PD patients was 13.3 ± 7.2 years and had STN-DBS since 2.3 ± 2.2 years. All patients reported FOG according to the Freezing of Gait Questionnaire (FOG-Q, score > 1 (Giladi et al., 2000)). FOG-Q score can be distinct from the actual degree of FOG (Shine et al., 2013a) and all patients are therefore considered 'probable freezers' according to previous classifications (Snijders et al., 2012). PD and HC had the same level of education (PD 13.0 ± 3.6 years; HC 12.4 ± 2.7 years; paired samples t-test; $t_{22} = -0.441$, $p = 0.664$). All subjects were right-handed as confirmed by the Edinburgh Handedness Scale (Oldfield, 1971). PD patient characteristics are summarized in Table 1.

2.2. Experimental set up

Here, we performed additional cortical network analysis of data from an experiment already published (Scholten et al., 2016) with respect to the susceptibility for upper limb freezing under dual tasking conditions.

Together, subjects were recorded (i) during continuous finger tapping as single motor task and (ii) during dual task interference when the subjects performed finger tapping in parallel to a phonemic verbal fluency task. We tested patients in the morning after overnight withdrawal from dopaminergic medication. Both the single motor task and dual tasking were performed in the 'stimulation on' treatment condition. We chose this clinical setting a priori for several reasons. First, our group of PD patients were in an advanced disease stage and most of them would not have been able to perform a dual task in the 'Off' condition (without stimulation and medication). Second, freezing behavior often stays resistant to standard STN-DBS treatment (Scholten et al., 2016; Weiss et al., 2013), and therefore exploration of the network mechanisms behind resistant freezing behavior is warranted and necessary to tailor improved therapy concepts in future. Third, the 'stimulation on' condition allowed to introduce reliably a stable 'motor on' condition without akinesia or dyskinesia. Such motor fluctuations could have confounded data interpretation. We favored 'stimulation on' over a dopaminergic treatment regimen, as in this patient cohort with motor fluctuations, STN-DBS allows to achieve more reliable and stable 'motor on' states as compared to dopaminergic medication. Moreover, a set of previous studies supports that spectral EEG analysis can be reliably interpreted in parallel to active STN-DBS (Scholten et al., 2016; Silberstein et al., 2005; Weiss et al., 2012), including the methods applied in this study (Weiss et al., 2015).

Here, we analyzed the electrophysiological recordings from the 36-channel surface EEG (Brain Products version 1.20, MES Electronics, Gilching, Germany). Synchronized to the EEG, the mechanogram measured the exerted force of finger tapping. EEG and force transducer signals were analyzed with Matlab (R2012b, The Mathworks, Natick, MA, USA) based on the open-source toolboxes EEGlab (v10.2.5.5b) (Delorme and Makeig, 2004) and Fieldtrip (Oostenveld et al., 2011).

2.2.1. Single motor task

PD patients and HC were seated comfortably with the right arm supported by an arm rest. They were instructed to perform continuous finger tapping with the right index finger as quickly and precisely as possible, whilst the index finger was kept in permanent contact with the force transducer. The subjects were instructed to push the force transducer with a predefined strength of 2 Newton, and were provided with ongoing visual real-time feedback of the exerted pressure on a computer-screen placed 1 m in front. This procedure was chosen in order to stabilize the movement characteristics that otherwise could have been critical for spectral estimates (Baker et al., 1999). Subjects performed ten blocks of 20 s and between blocks, a pause of 10 s was given to prevent fatigue.

2.2.2. Dual tasking

After the performance of the single motor task, both PD patients and HC performed the finger tapping together with a phonemic verbal fluency task in which the subjects generated as many words as possible from a given initial letter. All subjects received the same set of letters that consisted of the ten most-used German consonants (b, d, g, h, k, l, m, p, s, and v). We presented one consonant per block of 20 s. The order of the presented letters was randomized across subjects. The subjects were instructed to equally share their attention between the finger tapping and the phonemic verbal fluency task (Bloem et al., 2006) and to talk with a low voice in order to limit facial muscle artifacts of the EEG. We validated the performance on the phonemic verbal fluency task using the number of words generated. Both PD patients and HC were able to generate words on each given letter. The total number of words mentioned did not differ between PD patients (51.5 ± 15.0; mean ± standard deviation) and HC (54.7 ± 13.7; One-way ANOVA with years of education as covariate: $F_{1,\ 17} = 0.383$, $p = 0.898$).

2.3. Analyses

In this work, we were particularly interested to explore neuronal signatures behind susceptibility to upper limb freezing. To this end, we followed the approach to analyze spectral signatures from time series of (uninterrupted) continuous finger tapping. First, we rejected upper limb freezing episodes, which were identified according to (i) a decrease of amplitude deflection of the finger tapping below 50% of the requested force modulation of 2 Newton, (ii) an increase of the frequency of motor output above 3 Hz, and (iii) both constraints lasting for at least one second (Scholten et al., 2016). We discussed elsewhere in detail that these upper limb freezing episodes do not correspond to action tremor despite of their 'trembling-like' nature (Scholten et al., 2016). After the rejection of such upper limb freezing episodes, there might still be motor arrests unrelated to upper limb freezing, e.g. when the subject 'forgot to tap' by focusing attention towards word retrieval and away from the motor performance. We classified and discarded such motor arrests unrelated to upper limb freezing from both single motor task and dual tasking in PD patients and HC if the intertap interval exceeded twice the median intertap interval as obtained from the single motor task (Shine et al., 2013b). The intertap interval was determined as the time between two consecutive finger taps. All PD patients showed motor arrests during dual tasking and data rejected per patient had a median length of 34.4 s (interquartile range: [9.3 83.1]). Motor performance was expressed as 'tapping frequency' and 'tapping regularity' in both HC and PD patients during 'single motor task' and 'dual tasking'. Tapping

frequency was measured as number of peaks over time (Hz). The regularity of tapping was expressed as the coefficient of variation and computed as the standard deviation of the interpeak interval normalized by the mean interpeak interval.

2.3.1. Cortical activity and phase synchronization

As preprocessing, EEG signals were filtered from 1 to 200 Hz with a finite impulse response filter. We removed muscle artifacts by visually inspecting the EEG signal. Then, eye blinks and transient muscle artifacts were extracted using Principle Component Analysis and the remaining components were transformed back to the time domain. Afterwards, EEG signals were converted into reference-free current densities using the Hjorth transform.

After preprocessing, data was analyzed in the following frequency bands of interests based on previous electrophysiological findings and clinical correlations: 4–7 Hz, 7–11 Hz, and 13–30 Hz. We chose those band definitions on pathophysiological rationale, as the 4–7 relates to freezing behavior in gait (Shine et al., 2014), and the 7–11 Hz to upper limb freezing (Scholten et al., 2016). Activity in the 13–30 Hz frequency band is associated to impairments in PD motor performance at both subthalamic (Kuhn et al., 2006) and cortical level (Silberstein et al., 2005; Weiss et al., 2015).

Moreover, these frequency bands relate to several physiological cortical processes that may be relevant to dual tasking. The 4–7 Hz band can facilitate multimodal processing and long-range synchronization, thereby promoting neuronal communication of distributed functionally-related integrators (Fell and Axmacher, 2011), the 7–11 Hz band can disengage task-irrelevant cortical regions (Jensen and Mazaheri, 2010), and a reduction of 13–30 Hz band activity may represent disinhibition of neuronal populations (Brinkman et al., 2014).

Cortical activity was analyzed in the frequency domain using spectral analyses. EEG signals were divided into disjoint segments with a duration of 2 s resulting in a frequency resolution of 0.5 Hz. For data in each window, the square of the magnitude of the Fourier transform was taken and averaged over all windows. We report relative cortical activity after normalizing absolute values by the summed activity from 1 to 45 Hz.

To study the multidimensional cortico-cortical synchronization processes in a comprehensive way, we used the global synchronization index as described elsewhere including its detailed mathematical description (Weiss et al., 2015). Together, in this approach the phase synchronization is studied using Hilbert phase and is based on the premise that two signals in synchrony will maintain a constant phase difference. For each channel pair, we calculated the phase difference and quantified the constancy in the phase difference (synchronization index, SI) using the first mode of the Fourier transform. SI takes on a value of one in case of perfect synchrony and zero for asynchrony. Using the SI obtained for each channel pair, an association matrix was formed. Similarly, an association matrix was formed using the SI obtained for the surrogate realizations of each channel pair (Weiss et al., 2015). These matrices were decomposed for eigenvalues. The eigenvalues of the original association matrix that exceeded the maximum eigenvalue of the surrogate association matrix were identified and added. To this end, the global synchronization index was defined as the ratio of the summed eigenvalues to the total number of eigenvalues. If the global synchronization index was non-zero for a subject in a specific frequency band during both the single motor task and dual tasking, we considered these data for further analysis.

To differentiate the topographical representation of the channels that contributed significantly to the global synchronization index, the eigenvector corresponding to the maximum eigenvalue of the original matrix was obtained. Furthermore, we wanted to confirm that cortico-cortical phase synchronization represented genuine neuronal synchronization and was not influenced significantly by volume conduction. Therefore, we calculated the phase differences and divided them in three categories: (i) phase difference close to zero (<0.1); (ii) phase difference close to pi (pi – pi/10 to pi + pi/10), and (iii) other phase differences, where categories (i) and (ii) are due to volume conduction and category (iii) reflects genuine neuronal synchronization. Spectral signatures (relative cortical activity and phase synchronization) were compared between the single motor task and dual tasking in both PD patients and HC.

2.3.2. Correlation analyses

We correlated the spectral signatures with freezing behavior in PD patients and with the performance of the phonemic verbal fluency task in PD patients and healthy controls. Therefore, the correlation was computed between the spectral measures (significant difference between the single and dual task) and biomechanically confirmed number of freezing episodes. For the performance of the phonemic verbal fluency task, we correlated spectral measures with the number of words mentioned during dual tasking.

2.4. Statistical analyses

Non-parametric tests were used if data were not normally distributed (Kolmogorov Smirnov $p > 0.05$).

We hypothesized that dual tasking compared to the single motor task will provoke upper limb freezing in PD (Giladi and Hausdorff, 2006; Snijders et al., 2012). Therefore, we compared the number of upper limb freezing episodes between the single motor task and dual tasking with a one-sided paired samples *t*-test, including the PD patients who showed upper limb freezing during dual tasking.

Cortical activity and global phase synchronization were compared between the single motor task and dual tasking in both PD patients and HC using a cluster-based permutation test as implemented in the fieldtrip toolbox to address for multiple comparisons (Maris and Oostenveld, 2007). This test is based on the Monte Carlo permutation principle and identifies significant changes between conditions using spatial adjacency by building clusters of channels. We performed 5000 randomizations and considered an adjusted two-sided alpha level of $p < 0.05$ significant.

Correlations were calculated with Pearson tests. All tests were decided on a two-sided significance level of $p < 0.05$. Motor performance data and correlations were analyzed in SPSS 22.0.

3. Results

3.1. Motor performance

The tapping frequency in PD patients was similar in the single motor task (1.62 ± 0.75 Hz (mean ± standard deviation)) and during dual tasking (1.68 ± 0.82 Hz) (n.s.). Tapping was more irregular during dual tasking (.33 ± .17) compared to the single motor task (.21 ± .12; paired samples *t*-test, $p = 0.024$).

HC had similar tapping frequency during the single motor task (2.17 ± 0.92) and during dual tasking (1.87 ± 0.82) (n.s.). In HC, tapping was more irregular during dual tasking (.23 ± .10) as compared to the single motor task (.16 ± .07; paired *t*-test, $p = 0.012$).

3.2. Upper-limb freezing

We identified nine upper limb freezing episodes in three PD patients during the single motor task and 72 episodes in nine PD patients during dual tasking. Upper limb freezing episodes lasted 1.9 [1.5 2.2] s (median [interquartile range]) during the single motor task and 2.3 [1.9 3.5] s during dual tasking. Upper limb freezing occurred more frequently during dual tasking (7 [5.5 11]) compared to the single motor task (5 [2 5]; $p = 0.007$), as expected.

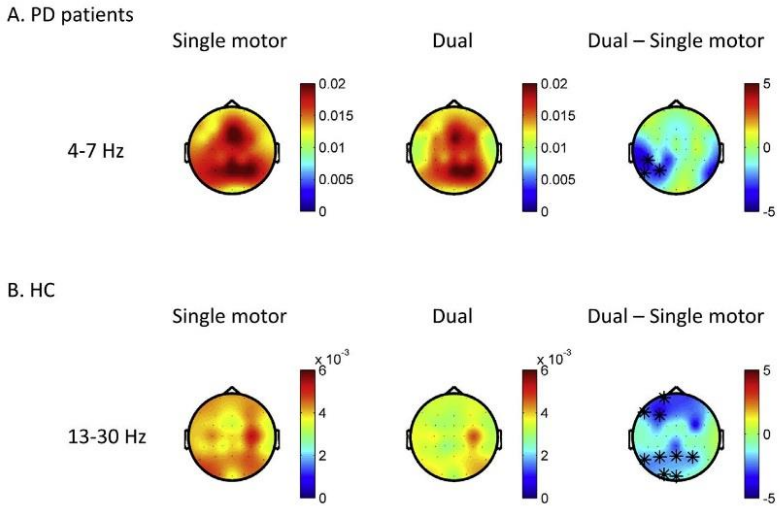

Fig. 1. Topographic overview of cortical activity during single motor task (first column) and dual tasking (second column) in the frequency bands showing significant differences in PD patients (A) and HC (B). The difference between single motor task and dual tasking are indicated by asterisks in the latter column. In PD, during dual tasking cortical activity decreases over the left parietal area in the 4–7 Hz frequency band (electrodes CP5, P3, and P7; $p = 0.0096$). In HC, during dual tasking cortical activity decreases over the left frontal area (electrodes FP1, F3, and F7; $p = 0.0022$) and bilateral parieto-occipital area (electrodes P4, Pz, P3, P7, Oz, and O1; $p = 0.0096$) in the 13–30 Hz frequency band. In the first two columns, color bars indicate the magnitude of relative cortical activity (a.u.) with warmer colors indicating higher relative activity. In the latter column the color bar represent statistical units with warmer colors indicating greater cortical activity during dual tasking compared to the single motor task. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

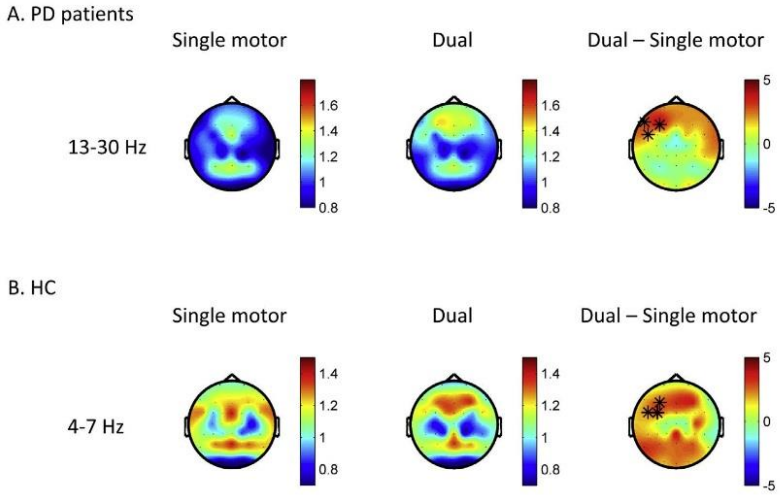

Fig. 2. Topographic overview of the cortico-cortical phase synchronization during single motor task (first column) and dual tasking (second column) in the frequency bands showing significant differences in PD patients (A) and HC (B). The difference between single motor task and dual tasking are indicated by asterisks in the latter column. In PD, during dual tasking phase synchronization increases over the left prefrontal area in the 13–30 Hz frequency band (electrodes F3, F7, and FC5; $p = 0.0054$). In HC, during dual tasking phase synchronization increases in the 4–7 Hz frequency band over the left prefrontal area (electrodes F3, FC3, and FC5; $p = 0.0162$).In the first two columns, color bars indicate the magnitude of global phase synchronization with warmer colors indicating stronger phase synchronization. In the latter column the color bar represent statistical units with warmer colors indicate increased synchronization during dual tasking compared to single motor task. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Cortical activity

In PD, cortical activity was attenuated over the left parietal area in the 4-7 Hz frequency range (electrodes CP5, P3, and P7; $p = .0096$; Fig. 1).

In HC, instead, cortical activity was attenuated in the 13-30 Hz frequency range over the left prefrontal (channels FP1, F3, and F7; $p = .0022$) and the bilateral parieto-occipital areas (electrodes P4, Pz, P3, P7, Oz, and O1; $p = .0096$) during dual tasking compared to the single motor task (Fig. 1).

For more information about cortical activity in PD during upper limb freezing as compared to regular tapping under dual tasking conditions, we would like to refer to previous work (Scholten et al., 2016).

3.4. Cortico-cortical phase synchronization

In PD, the topographical differentiation of phase synchronization yielded an increase of synchronization in the left prefrontal area from 13 to 30 Hz during dual tasking compared to the single motor task (electrodes F3, F7, and FC5; $p = .0054$; Fig. 2). All individual PD patients showed significant cortico-cortical phase synchronization. HC showed an increment of phase synchronization in the 4–7 Hz range in the left prefrontal area during dual tasking compared to the single motor task. This was computed from eleven subjects with significant phase synchronization (electrodes F3, FC3, and FC5; $p = .0162$; Fig. 2).

To further characterize the effect of biomechanically confirmed freezing under dual tasking conditions on cortico-cortical phase synchronization, we compared as subanalysis cortico-cortical phase synchronization during freezing episodes to regular tapping (both under dual tasking conditions). Nine patients yielded upper limb freezing during the dual task, and eight of them showed significant cortico-cortical phase synchronization during freezing. Cortico-cortical phase synchronization on these eight patients increased from 13 to 30 Hz over the right premotor area and supplementary motor area during upper limb freezing (electrodes FC2 and Cz, $p = 0.037$) (Supplementary Fig. S1).

In both PD and HC, we ensured that the observed cortico-cortical phase synchronization patterns reflected genuine neuronal synchronization. We found that the three categories (i.e. (i) phase difference close to zero (<0.1); (ii) phase difference close to pi (pi–pi/10 to pi + pi/10), and (iii) other phase differences) occurred with different proportions (Chi-square test; $p < 0.001$). The phase difference occurred more frequently in category (iii) than in categories (i) or (ii) with category (iii) containing at least 95% of the phase differences in every single patient (McNemar's test with Bonferroni correction; $p < 0.0001$). Therefore, we can conclude that our findings on cortico-cortical phase synchronization were largely driven by genuine neuronal synchronization as represented by category (iii).

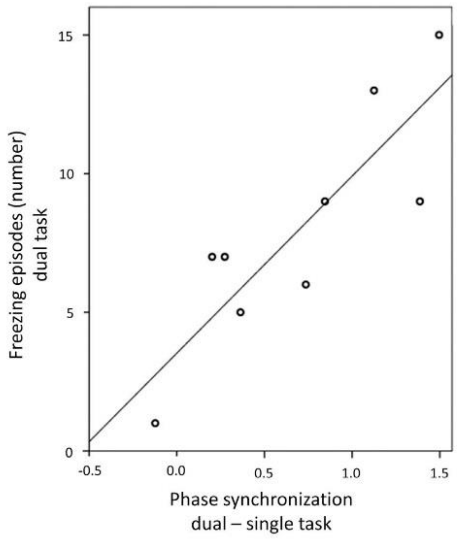

Fig. 3. Indices of the correlation coefficient between the increment of phase synchronization during dual tasking in the 13–30 Hz frequency band of the left prefrontal area (electrodes F3 and F7) and the number of freezing episodes during dual tasking.

3.5. Correlation analyses

In PD patients, the cortical activity decrease from 4 to 7 Hz during dual tasking was not correlated with the number of freezing episodes during dual tasking, nor with the number of words mentioned. The cortico-cortical phase synchronization increase from 13 to 30 Hz over the left prefrontal cortex was correlated with the number of upper limb freezing episodes ($r = 0.858$, $p = 0.003$; Fig. 3), indicating that higher cortico-cortical synchronization over the left prefrontal cortex associated with a more frequent occurrence of upper limb freezing episodes. This phase synchronization increase was not correlated with the number of words mentioned.

In HC, both the cortical activity decrease from 13 to 30 Hz and phase synchronization increase from 4 to 7 Hz during dual tasking were not correlated with the number of words mentioned during dual tasking.

To address whether decreasing movement regularity of finger tapping during dual tasking may have influenced cortical activity or phase synchronization, we correlated the decreased regularity of tapping with dual tasking (as compared to single motor task) and the spectral measures in PD patients and HC. In PD patients, the decreased regularity of tapping during dual tasking did not correlate with the diminished cortical activity from 4 to 7 Hz (n.s.), nor with the increased phase synchronization from 13 to 30 Hz (n.s.). In HC, the decreased regularity of tapping during dual tasking did not correlate with the diminished cortical activity from 13 to 30 Hz (n.s.), nor with the increased cortico-cortical phase synchronization from 4 to 7 Hz (n.s.).

4. Discussion

In this study, we demonstrated that dual tasking increased the susceptibility to upper limb freezing in PD. This was associated with altered long-range cortico-cortical synchronization. Dual task interference increased the cortico-cortical synchronization over the left prefrontal area from 13 to 30 Hz in PD. This synchronization increase correlated with more frequent upper limb freezing episodes. Instead, PD patients lacked some of the cortical network adaptations observed in HC under dual task interference, namely the increase of frontal synchronization from 4 to 7 Hz and the decrease of oscillatory activity in the left prefrontal and bilateral parieto-occipital areas from 13 to 30 Hz.

The observed increase of cortico-cortical phase synchronization from 13 to 30 Hz in PD and its correlation with an increased number of upper limb freezing episodes deserves deeper consideration in the context of previous findings. Increased cortico-cortical beta band (13–30 Hz) synchronization was described to parallel impaired motor processing and segmental PD motor symptoms (Silberstein et al., 2005; Weiss et al., 2015). Similarly, increased beta band synchronization is apparent both on the level of the STN and in subthalamo-cortical projections (Kuhn et al., 2004; Lalo et al., 2008; Stoffers et al., 2008; Weiss et al., 2012). Both L-Dopa and STN-DBS treatment desynchronized this rhythm on the levels of STN (Eusebio et al., 2011) and cortex (Eusebio et al., 2011; Weiss et al., 2015). Interestingly, although our PD patients were recorded in the 'stimulation on' state, dual task interference induced a synchronization increase from 13 to 30 Hz over the left prefrontal area in association to increased susceptibility to upper limb freezing. Therefore, impaired prefrontal processing may parallel freezing susceptibility. This is reflected by cortical beta rhythms that may inhibit the motor output (Brinkman et al., 2014; Weiss et al., 2015). As consequence, less effective motor output might result from impaired prefrontal cortical processing in PD. This might define a pathological brain state premonitory to freezing events that finally constitute the motor 'endpoint' of inefficient motor

integration (Lewis and Barker, 2009). Such executive dysfunction includes impairment in cognitive motor planning and motor automaticity as is characteristic for PD freezers. Moreover, both motor planning and motor automaticity are dependent on prefrontal executive integration. Inability to regulate prefrontal cognitive processes constitute the pathophysiological hallmark of such executive dysfunction (Dirnberger and Jahanshahi, 2013; Hallett, 2008; Nutt et al., 2011), and may critically relate to the impaired processing in subcortico-cortical feedback loops (Jahanshahi et al., 2000; Levy and Dubois, 2006). Therefore, we support the well-established concept by our experimental findings, that the cortical motor network is modulated towards motor inhibition by entraining the prefrontal areas to increased beta band synchronization. Dual task interference may therefore modulate the cortical network state towards a less efficient state of motor processing leading to increased susceptibility to freezing events.

Interestingly, when patients entered freezing of upper limb movement, the left prefrontal beta band synchronization persisted to a similar extent as during regular tapping under dual tasking conditions. However, additional hypersynchronization of the supplementary motor area and right premotor area (ipsilateral to movement) emerged and may mirror deficiency in motor planning (SMA) and motor-inhibitory influences of right-hemispheric structures. The findings are in good accordance with previous work that indicated similar cortical mechanisms (Silberstein et al., 2005; Weiss et al., 2015) including abnormalities of right-hemispheric cortical network architecture in PD patients with freezing (Vercruysse et al., 2013).

In addition to increased synchronization from 13 to 30 Hz, PD patients lacked further cortical network adaptations as observed in healthy controls. PD patients did not show the suppression of cortical activity from 13 to 30 Hz over the left prefrontal and bilateral parieto-occipital areas. We argue that HC have greater capacity to modulate cortical function during dual task interference, and are obviously able to 'down-regulate' beta band activity when cortical working load increases under dual tasking conditions. Again, beta band activity decrease may reflect disinhibition of the associated prefrontal and parietal areas, and thereby might stabilize the maintenance and transfer of the motor planning towards motor output despite of higher cortical computational load. Such motor compensation and increments of automaticity are generally impaired in PD patients (Wu and Hallett, 2005).

As further interesting aspect, we did not observe an increase of frontal synchronization from 4 to 7 Hz in PD patients, as was present in HC during dual tasking. This prefrontal synchronization increase in HC may have its largest impact to promote neuronal communication between long-range distant cortical processors (Connolly et al., 2002). As such, theta band oscillations were described as potent mediators of large-scale cortical activity and excitability modulation (McGinn and Valiante, 2014) and this included the facilitation of executive functioning including motor planning (Demiralp et al., 2007). Interestingly, the relevance of prefrontal theta oscillations increased along increased task complexity and was suggested to support multimodal processing and cognitive control over complex and independent processes (Engel et al., 2013; Fell and Axmacher, 2011; Voytek et al., 2015). More specific to PD, prefrontal theta synchronization was modulated during executive processing in prefrontal-subthalamic projections (Alegre et al., 2013) and during conflict decisions (Zavala et al., 2014) which adds a further piece of evidence that prefrontal both cortico-cortical and cortico-subthalamic theta band coupling is crucial to organize cognitive motor processing in terms of executive functioning. Therefore, additional to 'capturing' the prefrontal area in a motor inhibitory beta band synchronization, the Parkinsonian prefrontal area may also be deficient in entraining large-scale cortico-cortical processes.

4.1. Methodological considerations

We would like to reflect some methodological aspects of this study. First, speaking may lead to EEG signal contamination with muscle artefacts. Therefore, the subjects were instructed to talk with a low voice and to activate as few face muscles as possible to limit facial muscle activation. The remaining artefacts were reduced using principal component analysis. Moreover, the decrease of cortical activity during dual tasking in both PD and HC contradicts relevant confounding of our findings by muscle artefacts. If relevant muscle artefacts were present, we would expect broadband activity increases. Moreover, we measured a group of patients with advanced PD with akinetic-rigid dominance susceptible to freezing behavior and excluded patients with tremor. Further, we observed increases of prefrontal beta band synchronization in PD freezers, and instead a lack of prefrontal theta band increase in PD freezers as opposed to HC. The synchronization changes in both bands did not correlate with word retrieval, however, the beta band synchronization was indicative for increased susceptibility to upper limb freezing. This supports a role of the synchronization changes for freezing behavior but does not entirely exclude additional contributions of word retrieval on cortico-cortical synchronization.

Finally, the experiment considered similar STN-DBS pulse applications both in the 'single motor task' and in the 'dual task'. Therefore, the signatures observed built the correlate of dual task interference in PD patients with STN-DBS, but cannot be explained by STN-DBS per se. No contrast of 'stimulation off' vs. 'stimulation on' was considered in this part of the study presented here. Here, we strived for a neurophysiological basis to develop novel therapeutic strategies for unmet freezing behavior in order to overcome present therapeutic limitations (Scholten et al., 2016; Weiss et al., 2013; Weiss et al., 2015). In this regard, this study shall help to obtain neurophysiological biomarkers premonitory to freezing events. Such electrophysiological biomarkers may open a valuable time window for neuromodulation intervention.

We plan to address with separate experiments in medically treated PD patients in future whether similar signatures would generalize to larger parts of the PD population outside neurostimulation patients.

In conclusion, our electrophysiological recordings pointed to several pathological cortical network processes behind susceptibility to upper limb freezing. Most importantly, we observed increased prefrontal synchronization from 13 to 30 Hz that correlated with increased upper limb freezing. Of similar interest, PD patients lacked cortical network adaptations that were characteristic for HC under dual tasking conditions. The signatures identified in this study are of help to define cortical network states indicative for freezing susceptibility, and this may constitute valuable profiles towards adaptive treatment strategies for episodic freezing phenomena.

Acknowledgement

Funding source: Deutsche Forschungsgemeinschaft (DFG) with grant number: WE5375/1-1.

Conflict of interest: Marlieke Scholten, Rathinaswamy B. Govindan, Christoph Braun, Bastiaan R. Bloem, Christian Plewnia, and Alireza Gharabaghi declare no competing financial interest.

Rejko Krüger has received speaker's honoraria and/or travel grants from Medtronic.

Daniel Weiss has received research funding, speaker's honoraria, and a travel grant from Medtronic.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinph.2016.01.028.

References

Alegre M, Lopez-Azcarate J, Obeso I, Wilkinson L, Rodriguez-Oroz MC, Valencia M, et al. The subthalamic nucleus is involved in successful inhibition in the stop-signal task: a local field potential study in Parkinson's disease. Exp Neurol 2013;239:1–12.

Baker SN, Kilner JM, Pinches EM, Lemon RN. The role of synchrony and oscillations in the motor output. Exp Brain Res 1999;128:109–17.

Bartels AL, Leenders KL. Brain imaging in patients with freezing of gait. Mov Disord 2008;23(Suppl. 2):S461–7.

Bloem BR, Grimbergen YA, van Dijk JG, Munneke M. The, "posture second" strategy: a review of wrong priorities in Parkinson's disease. J Neurol Sci 2006;248:196–204.

Bloem BR, Hausdorff JM, Visser JE, Giladi N. Falls and freezing of gait in Parkinson's disease: a review of two interconnected, episodic phenomena. Mov Disord 2004;19:871–84.

Brinkman L, Stolk A, Dijkerman HC, de Lange FP, Toni I. Distinct roles for alpha- and beta-band oscillations during mental simulation of goal-directed actions. J Neurosci 2014;34:14783–92.

Connolly JD, Goodale MA, Menon RS, Munoz DP. Human fMRI evidence for the neural correlates of preparatory set. Nat Neurosci 2002;5:1345–52.

Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 2004;134:9–21.

Demiralp T, Bayraktaroglu Z, Lenz D, Junge S, Busch NA, Maess B, et al. Gamma amplitudes are coupled to theta phase in human EEG during visual perception. Int J Psychophysiol 2007;64:24–30.

Dirnberger G, Jahanshahi M. Executive dysfunction in Parkinson's disease: a review. J Neuropsychol 2013;7:193–224.

Engel AK, Gerloff C, Hilgetag CC, Nolte G. Intrinsic coupling modes: multiscale interactions in ongoing brain activity. Neuron 2013;80:867–86.

Eusebio A, Thevathasan W, Doyle Gaynor L, Pogosyan A, Bye E, Foltynie T, et al. Deep brain stimulation can suppress pathological synchronisation in parkinsonian patients. J Neurol Neurosurg Psychiatry 2011;82:569–73.

Fell J, Axmacher N. The role of phase synchronization in memory processes. Nat Rev Neurosci 2011;12:105–18.

Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.

Giladi N, Hausdorff JM. The role of mental function in the pathogenesis of freezing of gait in Parkinson's disease. J Neurol Sci 2006;248:173–6.

Giladi N, Shabtai H, Simon ES, Biran S, Tal J, Korczyn AD. Construction of freezing of gait questionnaire for patients with Parkinsonism. Parkinsonism Relat Disord 2000;6:165–70.

Giladi N, Treves TA, Simon ES, Shabtai H, Orlov Y, Kandinov B, et al. Freezing of gait in patients with advanced Parkinson's disease. J Neural Transm 2001;108:53–61.

Hallett M. The intrinsic and extrinsic aspects of freezing of gait. Mov Disord 2008;23 (Suppl. 2):S439–43.

Jahanshahi M, Ardouin CM, Brown RG, Rothwell JC, Obeso J, Albanese A, et al. The impact of deep brain stimulation on executive function in Parkinson's disease. Brain 2000;123(Pt. 6):1142–54.

Jensen O, Mazaheri A. Shaping functional architecture by oscillatory alpha activity: gating by inhibition. Front Hum Neurosci 2010;4:186.

Kostic VS, Agosta F, Pievani M, Stefanova E, Jecmenica-Lukic M, Scarale A, et al. Pattern of brain tissue loss associated with freezing of gait in Parkinson disease. Neurology 2012;78:409–16.

Kuhn AA, Kupsch A, Schneider GH, Brown P. Reduction in subthalamic 8–35 Hz oscillatory activity correlates with clinical improvement in Parkinson's disease. Eur J Neurosci 2006;23:1956–60.

Kuhn AA, Williams D, Kupsch A, Limousin P, Hariz M, Schneider GH, et al. Event-related beta desynchronization in human subthalamic nucleus correlates with motor performance. Brain 2004;127:735–46.

Lalo E, Thobois S, Sharott A, Polo G, Mertens P, Pogosyan A, et al. Patterns of bidirectional communication between cortex and basal ganglia during movement in patients with Parkinson disease. J Neurosci 2008;28:3008–16.

Levy R, Dubois B. Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. Cereb Cortex 2006;16:916–28.

Lewis SJ, Barker RA. A pathophysiological model of freezing of gait in Parkinson's disease. Parkinsonism Relat Disord 2009;15:333–8.

Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. J Neurosci Methods 2007;164:177–90.

McGinn RJ, Valiante TA. Phase-amplitude coupling and interlaminar synchrony are correlated in human neocortex. J Neurosci 2014;34:15923–30.

Nutt JG, Bloem BR, Giladi N, Hallett M, Horak FB, Nieuwboer A. Freezing of gait: moving forward on a mysterious clinical phenomenon. Lancet Neurol 2011;10:734–44.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113.

Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011;2011:156869.

Peterson DS, Fling BW, Mancini M, Cohen RG, Nutt JG, Horak FB. Dual-task interference and brain structural connectivity in people with Parkinson's disease who freeze. J Neurol Neurosurg Psychiatry 2014. http://dx.doi.org/10.1136/jnnp-2014-308840.

Peterson DS, Pickett KA, Duncan R, Perlmutter J, Earhart GM. Gait-related brain activity in people with Parkinson disease with freezing of gait. PLoS One 2014b;9:e90634.

Rahman S, Griffin HJ, Quinn NP, Jahanshahi M. Quality of life in Parkinson's disease: the relative importance of the symptoms. Mov Disord 2008;23:1428–34.

Scholten M, Klotz R, Plewnia C, Wachter T, Mielke C, Bloem BR, et al. Neuromuscular correlates of subthalamic stimulation and upper limb freezing in Parkinson's disease. Clin Neurophysiol 2016;127:610–20.

Shine JM, Handojoseno AM, Nguyen TN, Tran Y, Naismith SL, Nguyen H, et al. Abnormal patterns of theta frequency oscillations during the temporal evolution of freezing of gait in Parkinson's disease. Clin Neurophysiol 2014;125:569–76.

Shine JM, Matar E, Bolitho SJ, Dilda V, Morris TR, Naismith SL, et al. Modeling freezing of gait in Parkinson's disease with a virtual reality paradigm. Gait Posture 2013a;38:104–8.

Shine JM, Matar E, Ward PB, Bolitho SJ, Gilat M, Pearson M, et al. Exploring the cortical and subcortical functional magnetic resonance imaging changes associated with freezing in Parkinson's disease. Brain 2013b;136:1204–15.

Silberstein P, Pogosyan A, Kuhn AA, Hotton G, Tisch S, Kupsch A, et al. Cortico-cortical coupling in Parkinson's disease and its modulation by therapy. Brain 2005;128:1277–91.

Snijders AH, Haaxma CA, Hagen YJ, Munneke M, Bloem BR. Freezer or non-freezer: clinical assessment of freezing of gait. Parkinsonism Relat Disord 2012;18:149–54.

Snijders AH, Leunissen I, Bakker M, Overeem S, Helmich RC, Bloem BR, et al. Gait-related cerebral alterations in patients with Parkinson's disease with freezing of gait. Brain 2011;134:59–72.

Spildooren J, Vercruysse S, Desloovere K, Vandenberghe W, Kerckhofs E, Nieuwboer A. Freezing of gait in Parkinson's disease: the impact of dual-tasking and turning. Mov Disord 2010;25:2563–70.

Stoffers D, Bosboom JL, Deijen JB, Wolters E, Stam CJ, Berendse HW. Increased cortico-cortical functional connectivity in early-stage Parkinson's disease: an MEG study. Neuroimage 2008;41:212–22.

Toledo JB, Lopez-Azcarate J, Garcia-Garcia D, Guridi J, Valencia M, Artieda J, et al. High beta activity in the subthalamic nucleus and freezing of gait in Parkinson's disease. Neurobiol Dis 2014;64:60–5.

Uhlhaas PJ, Singer W. Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. Neuron 2006;52:155–68.

Vandenbossche J, Deroost N, Soetens E, Coomans D, Spildooren J, Vercruysse S, et al. Freezing of gait in Parkinson's disease: disturbances in automaticity and control. Front Hum Neurosci 2012;6:356.

Vercruysse S, Spildooren J, Heremans E, Wenderoth N, Swinnen SP, Vandenberghe W, et al. The neural correlates of upper limb motor blocks in Parkinson's disease and their relation to freezing of gait. Cereb Cortex 2013;24:3154–66.

Vercruysse S, Vandenberghe W, Munks L, Nuttin B, Devos H, Nieuwboer A. Effects of deep brain stimulation of the subthalamic nucleus on freezing of gait in Parkinson's disease: a prospective controlled study. J Neurol Neurosurg Psychiatry 2014;5:871–7.

Virmani T, Moskowitz CB, Vonsattel JP, Fahn S. Clinicopathological characteristics of freezing of gait in autopsy-confirmed Parkinson's disease. Mov Disord 2015. http://dx.doi.org/10.1002/mds.26346.

Voytek B, Kayser AS, Badre D, Fegen D, Chang EF, Crone NE, et al. Oscillatory dynamics coordinating human frontal networks in support of goal maintenance. Nat Neurosci 2015;18:1318–24.

Weiss D, Breit S, Hoppe J, Hauser AK, Freudenstein D, Kruger R, et al. Subthalamic nucleus stimulation restores the efferent cortical drive to muscle in parallel to functional motor improvement. Eur J Neurosci 2012;35:896–908.

Weiss D, Klotz R, Govindan RB, Scholten M, Naros G, Ramos-Murguialday A, et al. Subthalamic stimulation modulates cortical motor network activity and synchronization in Parkinson's disease. Brain 2015;138:679–93.

Weiss D, Walach M, Meisner C, Fritz M, Scholten M, Breit S, et al. Nigral stimulation for resistant axial motor impairment in Parkinson's disease? a randomized controlled trial. Brain 2013;136:2098–108.

Wu T, Hallett M. A functional MRI study of automatic movements in patients with Parkinson's disease. Brain 2005;128:2250–9.

Zavala BA, Tan H, Little S, Ashkan K, Hariz M, Foltynie T, et al. Midline frontal cortex low-frequency activity drives subthalamic nucleus oscillations during conflict. J Neurosci 2014;34:7322–33.

5.5 Effects of subthalamic and nigral stimulation on gait in Parkinson's disease

Will be submitted to Gait & Posture as:

Scholten M, Klemt J, Bunjes F, Bloem BR, Gharabaghi A, Krüger R, Weiss D. Effects of subthalamic and nigral stimulation on gait in Parkinson's disease. *In prep*

Effects of subthalamic and nigral stimulation on gait in Parkinson's disease

Marlieke Scholten[1,2,3,4], Johannes Klemt[1,2,3], Christian Plewnia[3,5], Bastiaan R. Bloem[6], Friedemann Bunjes[3], Rejko Krüger[1,2,3,8], Alireza Gharabaghi[3,7], Daniel Weiss[1,2,3]

[1]Department of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research (HIH), University of Tuebingen, Germany

[2]German Centre of Neurodegenerative Diseases (DZNE), University of Tuebingen, Tuebingen, Germany

[3]Centre for Integrative Neuroscience (CIN), University of Tuebingen, Tuebingen, Germany

[4]Graduate School of Neural & Behavioural Sciences, International Max Planck Research School, University of Tuebingen, Tuebingen, Germany

[5]Department of Psychiatry and Psychotherapy, University of Tuebingen, Tuebingen, Germany

[6]Radboud University Medical Centre, Donders Institute for Brain, Cognition and Behavior, Department of Neurology, Nijmegen, the Netherlands

[7]Division of Functional and Restorative Neurosurgery, Department of Neurosurgery, University of Tuebingen, Tuebingen, Germany

[8]Clinical and Experimental Neuroscience, Luxembourg Center for Systems Biomedicine (LCSB), University of Luxembourg and Centre Hospitalier de Luxembourg (CHL), Luxembourg

Corresponding authors:

Marlieke Scholten, and Daniel Weiss.

Department of Neurodegenerative Diseases, and Hertie Institute for Clinical Brain Research (HIH),

University of Tuebingen, Tuebingen, Germany.

Hoppe-Seyler-Str. 3, 72076 Tuebingen, Germany

Email: marlieke.scholten@uni-tuebingen.de; daniel.weiss@uni-tuebingen.de

Abstract

Conventional subthalamic deep brain stimulation (STN-DBS) for Parkinson's disease (PD) presumably modulates the spatial component of gait. However, temporal dysregulation of gait associates tightly with freezing of gait (FOG). Such temporal locomotor integration may be modulated differentially on distinct levels of the basal ganglia. Owing to its descending brainstem projections, stimulation of the substantia nigra pars reticulata (SNr) area might modulate spatial and temporal parameters of gait integration differentially from the standard subthalamic nucleus (STN) stimulation. In this work, we aimed to characterize the differential effect of STN or SNr stimulation on kinematic gait parameters as primary interest.

Biomechanical parameters were analyzed during unconstrained over ground walking in thirteen PD patients with neurostimulation and FOG. Both tests were performed in three conditions: i) Off stimulation, ii) STN stimulation (alone), iii) SNr stimulation (alone). SNr stimulation was achieved by stimulating the most caudal contact of the electrode. Gait was recorded by three sensors attached on both left and right ankle and lumbar, containing a tri-axial accelerometer, gyroscope, and magnetometer.

STN stimulation improved both spatial features (stride length, stride length variability), as well as temporal parameters in terms of swing time asymmetry. SNr stimulation improved the temporal features of gait in terms of swing time asymmetry. Correlation analysis suggested that more medial coordinates of the SNr contact associate with a stronger regularization of gait. These results suggest that SNr stimulation might complement the effect of standard subthalamic stimulation on temporal gait integration.

Introduction

Standard deep brain stimulation of the subthalamic nucleus (STN-DBS) may improve gait in Parkinson's disease (PD) to certain degree and with interindividual variability [1,2]. STN-DBS robustly improves the spatial parameters (e.g. step length), however, the temporal parameters (e.g. cadence), are generally less amenable to therapy [3–5]. This is critical as temporal regulation of the gait cycle is crucial to PD gait disturbance, in particular to freezing of gait (FOG) that associates closely to temporal abnormalities of locomotor integration [6]. In this sense, several temporal parameters of gait including temporal gait variability and asymmetry correlate with an increased occurrence of FOG [7,8].

Neurophysiologically, rhythmic stepping behavior can be elicited by stimulation of the mesencephalic locomotor region (MLR), with higher stimulation intensities increasing the locomotion cadence in the cat [9,10]. The MLR gives rise to the reticulospinal tract and this tract appears to be involved in eliminating asymmetric gait by modulating the activity level of different groups of muscles during walking [11]. The MLR region appears to be involved in the temporal modulation of gait also in human. In healthy persons, the MLR region is active during mental imagery of gait [12]. Furthermore, an increased firing rate of neurons in the MLR region was observed with an increased cadence of stepping in PD patients [13]. The MLR receives GABA-ergic afferents from the substantia nigra pars reticulata (SNr), a basal ganglia output nucleus [14]. In PD, the output of the basal ganglia including SNr is increased [15] resulting in an over-inhibition of the MLR, and thus, was suspected to diminish the MLR locomotor output.

This over-inhibition can be reduced by high frequency neurostimulation of the basal ganglia. Although several different STN-DBS configuration opportunities exist, FOG still remains an unmet therapeutic need [16,17]. We observed that combined STN+SNr stimulation may have promising effects on FOG reduction [18], the piloting findings from this study are currently under investigation of a multicenter randomized controlled trials (clinicaltrials.gov NCT02588144). However, what the

effect is of SNr stimulation alone on gait kinematics is unknown. From animal experiments, it seems that SNr could be involved in the temporal modulation of the gait cycle and since the temporal modulation in FOG is perturbed, deep brain stimulation of the SNr could reduce FOG in PD patients. Furthermore, on gait initiation SNr stimulation showed an effect on vertical braking [19].

Here, we set out to study the effects of mono STN and mono SNr stimulation on gait kinematics. Please note, that this study was not designed nor powered to study the clinical efficacy of nigral stimulation on FOG (instead, this is subject to the above mentioned trial). In particular, we will contrast the effect of either STN or SNr stimulation (mono, not combined) on both spatial and temporal kinematic gait parameters of unconstrained gait. Furthermore, we aim to associate the anatomical location within the SNr with the gait parameters, since - from animal experiments - it was indicated that SNr sub territories may associate differentially to distinct locomotor integration modalities. In this sense, the medial part of the SNr was suggested to account for the modulation of the gait cycle time and the locomotor speed during locomotion, in contrast to the increase of axial and limbic muscle tone by stimulating the lateral part of the SNr [20–22]. We hypothesize that mono SNr stimulation can regularize the temporal modulation of gait compared to Off stimulation by decreasing the variability of the stride time and temporal gait asymmetry. Moreover, we hypothesize that mono STN stimulation will have stronger impact on the spatial parameters by increasing the stride length and reducing the stride length variability. Furthermore, we hypothesize that the improvement in temporal gait variability and asymmetry may associate with a more medial localization of the SNr contact.

Methods

Subjects

We included 21 patients with idiopathic PD into this study. PD patients were included if STN-DBS electrode contacts of the quadripolar electrode (Medtronic, Minneapolis, MN) reached both the STN and the caudal border zone of the STN and SNr (as determined from a MRI post-operatively by an experienced neurologist). Most caudal electrode contacts were within -5mm ≤ -10mm in the rostro-caudal direction on both sides similar to standard described elsewhere (NCT02588144) [18]. To avoid influence of the stun effect, we included only patients at least three months from STN-DBS implantation. Exclusion criteria were Mini Mental Status < 22, Beck's Depression Inventory > 13), and other neurological or neuromuscular disease except PD. The study was approved by the local Ethics committee of the University of Tuebingen and all subjects provided written consent to participate in the study.

One patient was excluded because of technical problems with the recording hardware and one patient was unable to walk during the recording session. During the measurements, FOG was observed in 15 out of 19 PD patients, confirming these PD patients as 'definitive freezers' [23]. We assured that stimulation of the STN was effective with clinical improvement of at least 30% measured with the UPDRS III score. Thus, three out of 15 PD patients were excluded. These 12 PD freezers were considered for further analyses (PD1-PD12, 11 males, age: 63.7 ± 10.4 years (mean ± standard deviation)). The disease duration was 15.1 ± 3.2 years with implantation of the DBS 34.7 ± 29.0 months ago. The score on the NFOG-Q was 9.4 ± 8.4 points. Electrode coordinates of the caudal contact relative to the mid-commissural point (MCP) were left SNr -10.2 ± 0.4, -3.7 ± 0.5, -6.5 ± 0.4; right SNr 10.4 ± 0.4, -4.0 ± 0.4, -6.3 ± 0.4 (x, y, z; x = medio-lateral, y = anterio-posterior, z = rostro-caudal).

Experimental set up and paradigm

Combined STN+SNr stimulation is under consideration to alleviate freezing of gait [18]. Whereas during clinical routine impulses of STN and SNr are delivered concomitantly, we aimed to gain differentiated pathophysiological insight of either STN or SNr stimulation on locomotor integration in this study. To this end, we contrasted the effects of either stimulation condition alone (i.e. SNr mono as compared to STN mono). We measured all patients in three conditions: STN, SNr, and Off stimulation after a previous overnight withdrawal of dopaminergic medication. The order of the conditions was randomly assigned and each stimulation condition was active at least 20 minutes before recording to limit potential carry-over effect of the previous stimulation condition [24].

STN stimulation represented stimulation of two most rostral contacts, while with SNr stimulation the two most caudal contacts were stimulated (Table 1). Pulse width and frequency of the SNr stimulation were similar to the individual STN stimulation. SNr amplitudes were not be held equivalent to STN amplitudes, since stimulation of nigral contacts generally has lower side effect thresholds and since lower SNr amplitudes were clinically effective in our previous work [18]. Therefore, the voltage was increased in small steps of 0.1 V until a clinical improvement of gait compared to Off stimulation was observed. If a side effect occurred, the highest amplitude possible without the emergence of side effects was chosen (Table 1).

In each condition, subjects walked on a straight overground walkway of nine meters. Subjects were asked to walk forth and back for about three minutes in their self-selected, comfortable pace. In case the PD patients had difficulty walking three minutes, they were asked to walk as long as they could. Before the start of each nine meter walking trajectory, subjects stood still for a few seconds with the feet put together in front of the starting line. Walking was self-initiated by the subjects. Walking aid, such as a cane or walking frame was allowed when used in daily life. The walking aid was used by only two patients and consistently used in all therapeutic conditions to facilitate comparability. In each condition, the clinical motor state was assessed by the UPDRS section III. Clinical subscores

were composed: segmental (sum of items 20-26+31, only upper and lower limbs), gait & posture subscore (sum of items 27-30).

Table 1
Stimulation parameters

	STN			SNr		
	Voltage (V) (left/right)	**Frequency (Hz)**	**Pulse Width (µs) (left/right)**	**Voltage (V) (left/right)**	**Frequency (Hz)**	**Pulse Width (µs) (left/right)**
PD01	5.3/3.0	130	60/60	3.5/3.5	125	60/60
PD02	2.8/3.5	130	60/60	2.5/1.9	130	60/60
PD03	5.5/3.5	130	60/60	2.9/2.9	130	60/60
PD04	4.0/4.5	130	60/60	2.7/2.7	130	60/60
PD05	2.1/2.1	125	60/60	1.6/1.6	125	60/60
PD06	3.2/2.0	130	60/60	2.2/2.2	130	60/60
PD07	5.4/5.1	130	90/90	1.3/1.3	130	90/90
PD08	4.9/3.5	130	90/60	2.5/2.5	130	90/60
PD09	3.5/2.4	125	60/60	1.6/1.6	125	60/60
PD10	1.8/1.7	125	90/90	1.0/1.0	130	90/90
PD11	3.6/1.9	130	60/60	0.3/0.3	130	60/60
PD12	3.8/2.4	125	60/60	0.8/0.8	125	60/60

Abbreviations: STN = subthalamic nucleus deep brain stimulation, SNr = substantia nigra deep brain stimulation

Recordings

During walking, participants wore small, lightweight body-fixed sensors attached to the left and right ankle (about 20 mm above the malleolus), and to the lower back with a belt (Opal, APDM, Portland, OR). Each sensor contained a tri-axial accelerometer, tri-axial gyroscope and a tri-axial

magnetometer with X, Y, and Z axes pointing downward, to the right and forward respectively. Data was sampled at 128 Hz and transferred to Matlab for further offline analysis. In addition, we collected video-tapings fully synchronized to the body-worn sensors for clinical motor state classifications.

Spatial and temporal gait parameters

Gait events were determined using the acceleration in the anterior-posterior direction and the gyroscope in the medial-lateral direction expressing the angular velocity in the sagittal plane. Gait events were based on earlier work showing a good agreement between APDM sensors and the GAITRite system as gold standard [25,26]. Briefly, first we identified the midswing (MS) as peak value exceeding 50 deg/s in the sagittal plane of the gyroscope signal. If multiple peak values with a maximum distance of 750 ms were found, the highest peak was selected and the others were rejected. In the time-interval 750 ms before and after MS, we identified toe-off (TO) and heel-strike (HS). TO was identified as minimum anterior-posterior acceleration in the time interval before MS, and HS was identified in the time interval after MS as the minimum value of angular velocity in the sagittal plane before the maximum anterior-posterior acceleration. A stride was defined as the time span between two consecutive HS of the same leg. All gait cycles were checked for the order of occurrence. For the k^{th} gait cycle of the left leg the gait events were correct when:

$HS_L < TO_R < MS_R < HS_R < TO_L < MS_L < HS_L$.

We discarded gait cycles from further analyses in case of incorrect order of the gait events or if a gait event could not be detected. To exclude acceleration and deceleration during walking, the first and last two steps of each nine meter walkway were rejected. A minimal of 40 gait cycles (left and right together) remained for each participant in each condition for further analyses.

Using these gait events, we computed temporal and spatial gait outcome measures for each condition. The temporal measures consisted of the mean of stride time (in seconds, time between

two consecutive heel strikes of the same leg) and the variability of the stride time. As spatial measures we computed the stride length (as percentage of the leg length (%ll)) and the variability of the stride length. As spatio-temporal measure the peak shank angular velocity (degree/seconds; sagittal maximum angular velocity during the swing phase) and the variability of the peak shank angular velocity were computed. The variability measures were calculated as the coefficient of variation (CV, 100%*standard deviation normalized by the mean). Since outcomes of the left and right legs were highly correlated in all conditions ($0.601 < R < 1.00$, all $p < 0.05$), we report these measures as mean of the left and right legs. As sub-analyses we report the measures of the leg more severely affected and less severely affected leg by Parkinson's disease (referred to as disease 'dominant leg' resp. 'non-dominant leg' in the following). As most objective marker the dominant leg was determined as the leg yielding lower peak angular velocity during the swing phase in the Off condition as compared to the other leg.

Freezers have impaired bilateral coordination [27]. Therefore, we observe the swing time asymmetry (STA) of the swing time defined by [28]:

$$STA = \left| ln\left(\frac{l}{h}\right) \right|$$

Where *l* is the swing time of the leg with the shorter swing time y and *h* is the swing time of the leg with the greater swing time. Swing time asymmetry closer to zero represents a lower grade of asymmetry.

Anatomical location of SNr contact

We determined the electrode position with respect to mid-commissural point of the bilateral lower contacts using a pre-operative and post-operative MRI with Optivise software (Medtronic, Minneapolis, MN). We correlated the medio-lateral coordinate of the electrode (coordinate in x direction) with the spatial and temporal parameters of the dominant leg and swing time asymmetry.

Statistical analyses

We report statistical descriptives as mean ± standard error, unless stated otherwise. We tested for normal distribution of the data using the Kolmogorov Smirnov test ($p < 0.05$). Based on this test, conditions were compared using a repeated measure ANOVA or Friedman test. In case of a significant ANOVA or Friedman test, post-hoc tests were performed comparing Off with STN and Off with SNr stimulation using a paired t-test or Wilcoxon test. All statistical analyses were performed with IBM SPSS statistics, version 22.0 (IBM Deutschland GmbH, Ehningen, Germany).

Results

Clinical outcome

Total UPDRSIII score significantly differed between conditions (χ^2 = 22.167, p = 0.000, Friedman-Test). Both STN and SNr stimulation improved the total UPDRSIII score (STN - Off U = -3.059, p = 0.002; SNr - Off U = -2.671, p = 0.008, Wilcoxon Test, Figure 1). The segmental subscore (χ^2 = 22.167, p = 0.000) and the gait & posture subscore (χ^2 = 15.235, p = 0.000) were significantly changed by stimulation. Both STN and SNr stimulation improved the segmental (STN – Off U = -3.061, p = 0.002; SNr – Off U = -2.515, p = 0.012), and the gait & posture subscore (STN – Off U = -2.980, p = 0.003; SNr – Off U = -2.280, p = 0.023) (Figure 1).

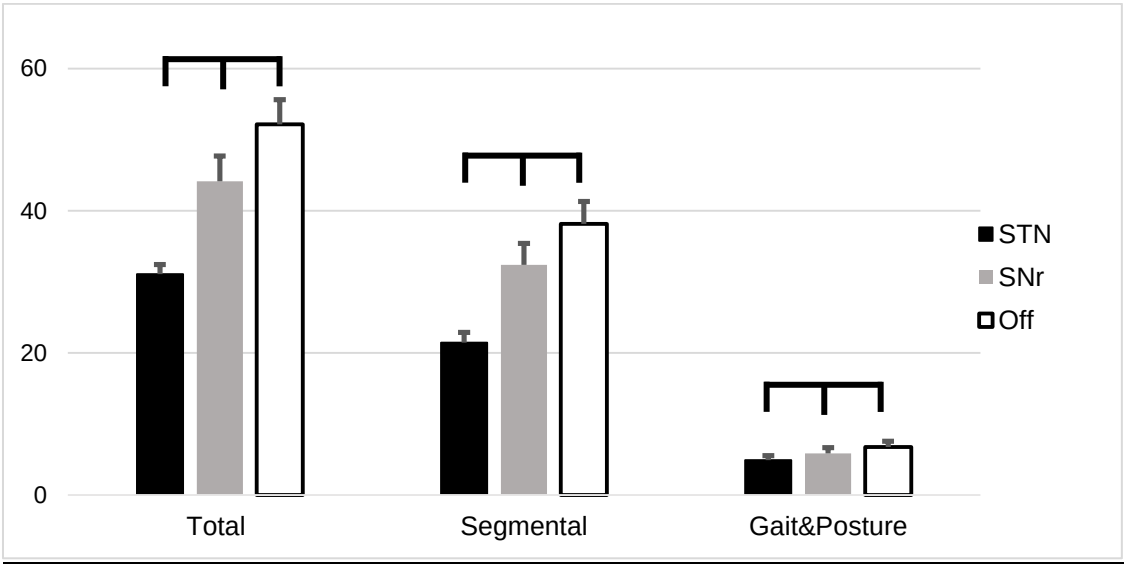

Figure 1 Score of the total UPDRS III (left), segmental score (sum of items 20-26+31, only upper and lower limbs), and gait & posture subscore (sum of items 27-30) during STN, SNr, and Off stimulation. Significant differences (p < 0.05) are denoted by horizontal square brackets. Both STN and SNr stimulation could significantly improve the segmental and gait and posture.

Spatial and temporal gait parameters

STN stimulation influenced only the spatial parameters: we observed a stride length increment and stride length variability reduction compared to Off stimulation (Figure 2). SNr stimulation did not change the spatial parameters. Stride time and peak shank angular velocity as well as the variability

of the stride time and peak shank angular velocity were not influenced by both STN and SNr stimulation. As temporal parameters, swing time asymmetry was improved by both STN and SNr stimulation compared to Off stimulation (Figure 2).

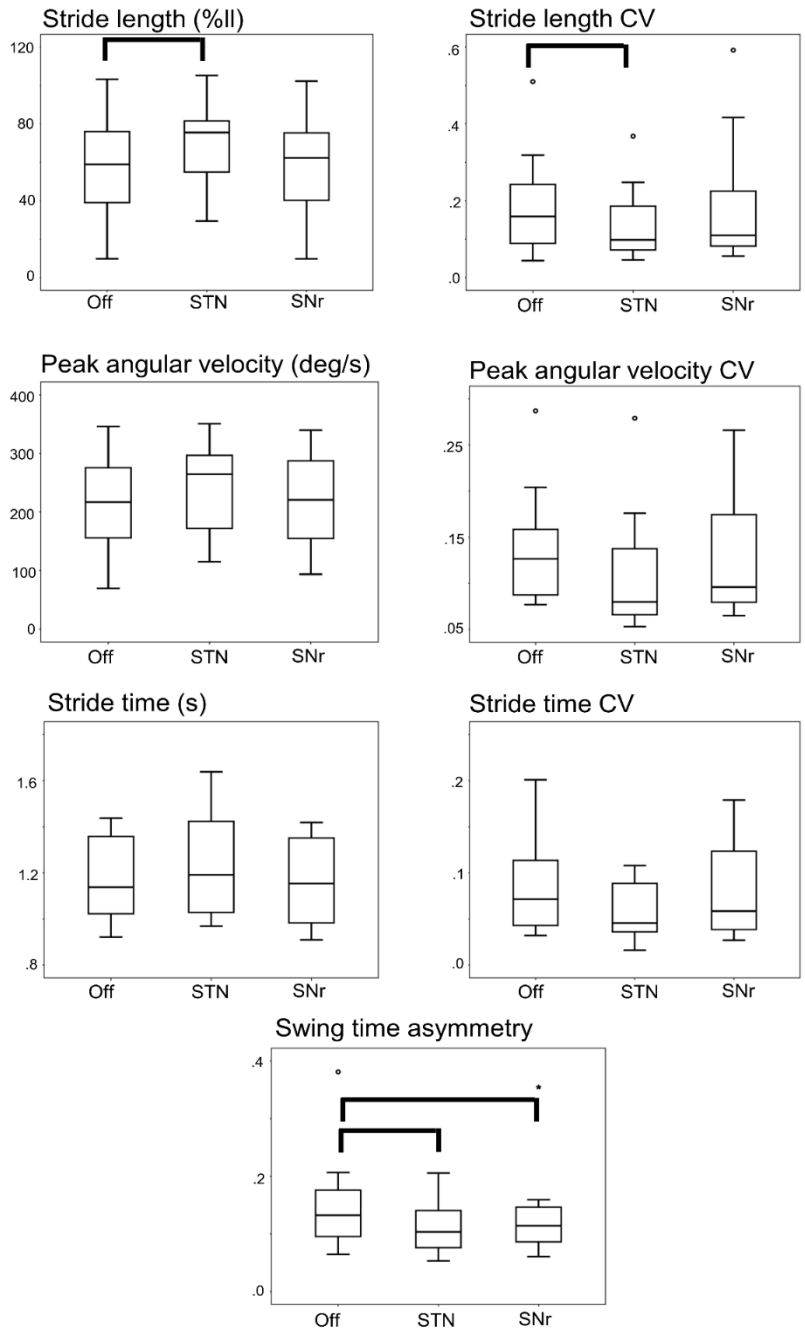

Figure 2 Boxplots representing median values, 25–75% range (box) and min–max range (bars) of spatial and temporal gait parameters. Differences were computed with the Wilcoxon signed rank test and are denoted by horizontal square brackets. Abbreviations: STN = subthalamic nucleus deep brain stimulation, SNr = substantia nigra deep brain stimulation, ll = leg length, CV = Coefficient of Variation.

As sub-analyses, we observed these parameters separately for the disease dominant and non-dominant legs. After breaking down in dominant vs non-dominant side, STN stimulation improved the stride length and stride length variability compared to Off stimulation only for the dominant side (Table 2). SNr stimulation did not improve gait parameters of either the dominant or non-dominant legs.

Table 2
Dominant/non-dominant leg: Spatial and temporal gait parameters

Gait parameter	**Grand average (mean ± sd)**						**P-value**		**P-value**	
	STN		**SNr**		**Off**		**STN – Off**		**SNr - Off**	
	D	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**
Mean										
Stride time (s)	1.2 ± .2	1.2 ± .2	1.2 ± .2	1.2 ± .2	1.2 ± .2	1.2 ±.2	n.s.	n.s.	n.s.	n.s.
PAV (deg/s)	229.4 ± 80.7	264.1± 75.1	207.5 ± 85.6	241.8 ± 74.6	195.6 ± 91.8	237.2 ± 77.0	n.s.	n.s.	n.s.	n.s.
Stride length (%ll)	67.0 ± 24.7	74.9 ± 20.9	56.3 ± 26.5	66.2 ± 25.9	53.1 ± 28.5	63.4 ± 27.8	**.01**	n.s.	.27	n.s.
CV										
Stride time	.05 ±.03	.06 ±.03	.08 ± .05	.07 ±.05	.08 ±.06	.08 ±.04	n.s.	n.s.	n.s.	n.s.
PAV	.12 ± .08	.09 ± .05	.14 ± .09	.14 ± .07	.16 ± .10	.12 ± .04	n.s.	.09	n.s.	0.94
Stride length	.14 ±.12	.13 ± .08	.19 ± .20	.17 ± .13	.22 ± .16	.16 ± .12	**.01**	n.s.	.08	n.s.

Abbreviations: PAV = peak shank angular velocity, sd = standard deviation, STN = subthalamic nucleus deep brain stimulation, SNr = substantia nigra deep brain stimulation, ll = leg length, D = dominant; ND = non-dominant, CV = Coefficient of Variation; P-values are computed with Friedman test and if significant, with Wilcoxon signed rank test.

Anatomical position of SNr contact

We found a negative association between the alteration of peak angular velocity variability by SNr stimulation and the laterality of the electrodes caudal contact (r = -.594, p = 0.042, Figure 3). This indicates that more medial electrode positioning correlates with a more regular gait pattern induced by SNr stimulation. Electrode laterality was not correlated with other gait parameters of general mobility and gait variability, nor with swing time asymmetry or FOG occurrence.

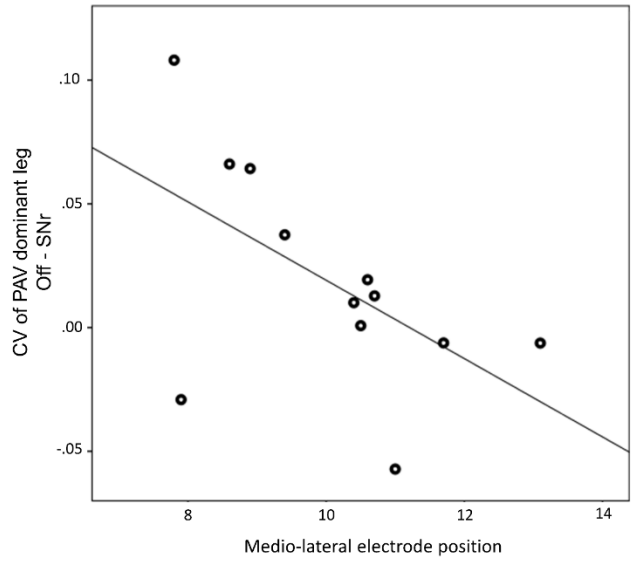

Figure 3 Medio-lateral location of electrode of the most caudal contact is associated with the improvement of peak angular velocity variability by SNr stimulation. Both electrode position and CV of PAV are obtained from the dominant side. A more medial electrode position is associated with more regular gait induced by SNr stimulation (r = -.0594; p = .042).

Abbreviations: CV = coefficient of variation, PAV = peak shank angular velocity.

Discussion

Here, we found that STN stimulation improved both spatial and temporal characteristics of gait with a stride length increment, reduction of the stride length variability, and increased swing time symmetry. Further, mono SNr stimulation improve the temporal characteristic of gait by increasing the swing time symmetry. In addition, we found that a more medial electrode positioning of the caudal contact was associated with an improvement of the gait patterns regularity induced by mono SNr stimulation.

In line with our hypotheses, mono SNr stimulation can modulate the temporal characteristics of the gait cycle by reducing the swing time asymmetry. From animal experiments, we know that the MLR and the resulting reticulospinal tract is involved in gait and the temporal modulation of the gait pattern. One of the target areas of the SNr is the MLR, which is considered the main locomotor center with a GABA-ergic connection between the SNr and the pontomesencephalic tegmentum [22]. Our results point also in the direction of a connection between the SNr and the locomotion controlling MLR area. In this respect, we have further evidence that the MLR modulates the temporal pattern of gait. The PPN, as part of the MLR, can modify the cadence but not step length, as is showed with PPN stimulation in PD patients [29]. The consistence of our kinematic and its concordance to results from PPN stimulation may support the view, that pedunculopontine locomotor integration could be modulated both on the level of PPN [29–31] and SNr. Furthermore, in MRI it has been shown that increasing the gait speed during imaginary walking activates the MLR region [30]. Our results contribute to the hypothesis that mono SNr stimulation can aid to regulate the temporal part of the gait pattern.

The temporal characteristics of gait closely associate with FOG and deficits in the temporal integration can promote FOG [7,8]. Therefore, the additive effect of SNr stimulation on the temporal integration of gait may prevent FOG in combined STN+SNr stimulation. In daily life, FOG is still an unmet therapeutic need. Different reprogramming strategies of STN-DBS for treating FOG have been

put forward including asymmetric stimulation or low-frequency pulses [16,17]. At least for low-frequency STN-DBS, effects were transient [32] and sometimes at cost of segmental motor control (e.g. recurrence of tremor). The neurophysiological concept why combined STN+SNr stimulation may reduce FOG is unknown. Probably, STN and SNr stimulation have synergistic, yet complementary effects on gait. Possibly, SNr stimulation may provide an additional effect on the temporal integration of gait.

Several limitations require careful interpretation of the findings. We analyzed gait during the mono STN and mono SNr stimulation separately, but we chose not during combined STN+SNr stimulation. Our approach comprised to observe the differential effects of mono STN and mono SNr stimulation on gait to provide pathophysiological insight into the distinct mechanisms of STN or SNr pathways towards kinematic features of locomotor integration. Moreover, we did not consider the combined STN+SNr stimulation as further experimental condition as the existing protocol was already lengthy and demanding for most patients.

We conclude that both mono STN improved both the spatial and temporal gait parameters, whereas mono SNr stimulation modulated the temporal pattern of gait by improving the temporal gait asymmetry. A more medial position of the caudal contact of the electrode was associated with a more regular gait pattern induced by mono SNr stimulation. The findings here helped to decipher the enigmatic pathophysiological networks involved in gait and FOG in PD, but further studies are definitely warranted.

References

[1] K.J. Lizarraga, J.R. Jagid, C.C. Luca, Comparative effects of unilateral and bilateral subthalamic nucleus deep brain stimulation on gait kinematics in Parkinson???s disease: a randomized, blinded study, J. Neurol. 263 (2016) 1652–1656. doi:10.1007/s00415-016-8191-3.

[2] J.A. Roper, N. Kang, J. Ben, J.H. Cauraugh, M.S. Okun, C.J. Hass, Deep brain stimulation improves gait velocity in Parkinson's disease: a systematic review and meta-analysis, J. Neurol. 263 (2016) 1195–1203. doi:10.1007/s00415-016-8129-9.

[3] M. Ferrarin, M. Rizzone, B. Bergamasco, M. Lanotte, M. Recalcati, A. Pedotti, L. Lopiano, Effects of bilateral subthalamic stimulation on gait kinematics and kinetics in Parkinson's disease., Exp. Brain Res. 160 (2005) 517–27. doi:10.1007/s00221-004-2036-5.

[4] M. Faist, Effect of bilateral subthalamic nucleus stimulation on gait in Parkinson's disease, Brain. 124 (2001) 1590–1600. doi:10.1093/brain/124.8.1590.

[5] O. Blin, A.M. Ferrandez, J. Pailhous, G. Serratrice, Dopa-sensitive and Dopa-resistant gait parameters in Parkinson's disease, J. Neurol. Sci. 103 (1991) 51–54. doi:10.1016/0022-510X(91)90283-D.

[6] J.G. Nutt, B.R. Bloem, N. Giladi, M. Hallett, F.B. Horak, A. Nieuwboer, Freezing of gait: Moving forward on a mysterious clinical phenomenon, Lancet Neurol. 10 (2011) 734–744. doi:10.1016/S1474-4422(11)70143-0.

[7] J.M. Hausdorff, J.D. Schaafsma, Y. Balash, A.L. Bartels, T. Gurevich, N. Giladi, Impaired regulation of stride variability in Parkinsons disease subjects with freezing of gait, Exp Brain Res. 149 (2003) 187–194. doi:10.1007/s00221-002-1354-8.

[8] M. Plotnik, N. Giladi, J.M. Hausdorff, Bilateral coordination of walking and freezing of gait in Parkinson's disease, Eur. J. Neurosci. 27 (2008) 1999–2006. doi:10.1111/j.1460-9568.2008.06167.x.

[9] S. Mori, Integration of posture and locomotion in acute decerebrate cats and in awake, freely moving cats, Prog. Neurobiol. 28 (1987) 161–195. doi:10.1016/0301-0082(87)90010-4.

[10] S. Grillner, Control of Locomotion in Bipeds, Tetrapods, and Fish, in: Compr. Physiol., John Wiley & Sons, Inc., Hoboken, NJ, USA, 1981: p. Supplement 2: Handbook of Physiology, The Nervous. doi:10.1002/cphy.cp010226.

[11] K. Matsuyama, T. Drew, Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. II. Walking on an inclined plane., J. Neurophysiol. 84 (2000) 2257–76.

[12] K. Jahn, A. Deutschländer, T. Stephan, R. Kalla, M. Wiesmann, M. Strupp, T. Brandt, Imaging human supraspinal locomotor centers in brainstem and cerebellum, Neuroimage. 39 (2008) 786–792. doi:10.1016/j.neuroimage.2007.09.047.

[13] B. Piallat, S. Chabardès, N. Torres, V. Fraix, L. Goetz, E. Seigneuret, E. Bardinet, M. Ferraye, B. Debu, P. Krack, J. Yelnik, P. Pollak, A.L. Benabid, Gait is associated with an increase in tonic firing of the sub-cuneiform nucleus neurons, Neuroscience. 158 (2009) 1201–1205. doi:10.1016/j.neuroscience.2008.10.046.

[14] K. Takakusaki, Neurophysiology of gait: From the spinal cord to the frontal lobe, Mov. Disord. 28 (2013) 1483–1491. doi:10.1002/mds.25669.

[15] M.R. DeLong, Primate models of movement disorders of basal ganglia origin., Trends Neurosci. 13 (1990) 281–5.

[16] A. Fasano, J. Herzog, E. Seifert, H. Stolze, D. Falk, R. Reese, J. Volkmann, G. Deuschl, Modulation of gait coordination by subthalamic stimulation improves freezing of gait, Mov. Disord. 26 (2011) 844–851. doi:10.1002/mds.23583.

[17] C. Moreau, L. Defebvre, A. Destee, S. Bleuse, F. Clement, J.L. Blatt, P. Krystkowiak, D. Devos, STN-DBS frequency effects on freezing of gait in advanced Parkinson disease, Neurology. 71 (2008)

80–84. doi:10.1212/01.wnl.0000303972.16279.46.

[18] D. Weiss, M. Walach, C. Meisner, M. Fritz, M. Scholten, S. Breit, C. Plewnia, B. Bender, A. Gharabaghi, T. Wächter, R. Krüger, Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial, Brain. 136 (2013) 2098–2108. doi:10.1093/brain/awt122.

[19] N. Chastan, G.W.M. Westby, J. Yelnik, E. Bardinet, M.C. Do, Y. Agid, M.L. Welter, Effects of nigral stimulation on locomotion and postural stability in patients with Parkinson's disease, Brain. 132 (2009) 172–184. doi:10.1093/brain/awn294.

[20] T. Wichmann, M.A. Kliem, M.R. DeLong, Antiparkinsonian and behavioral effects of inactivation of the substantia nigra pars reticulata in hemiparkinsonian primates, Exp. Neurol. 167 (2001) 410–424. doi:http://dx.doi.org/10.1006/exnr.2000.7572.

[21] G.C. McConnell, W.M. Grill, Stimulation location within the substantia nigra pars reticulata differentially modulates gait in hemiparkinsonian rats, in: Int. IEEE/EMBS Conf. Neural Eng. NER, IEEE, 2013: pp. 1210–1213. doi:10.1109/NER.2013.6696157.

[22] K. Takakusaki, T. Habaguchi, J. Ohtinata-Sugimoto, K. Saitoh, T. Sakamoto, Basal ganglia efferents to the brainstem centers controlling postural muscle tone and locomotion: a new concept for understanding motor disorders in basal ganglia dysfunction, Neuroscience. 119 (2003) 293–308. doi:10.1016/S0306-4522(03)00095-2.

[23] A.H. Snijders, C.A. Haaxma, Y.J. Hagen, M. Munneke, B.R. Bloem, Freezer or non-freezer: Clinical assessment of freezing of gait, Park. Relat. Disord. 18 (2012) 149–154. doi:10.1016/j.parkreldis.2011.09.006.

[24] S.E. Cooper, C.C. Mcintyre, H.H. Fernandez, J.L. Vitek, Association of Deep Brain Stimulation Washout Effects With Parkinson Disease Duration, (n.d.). doi:10.1001/jamaneurol.2013.581.

[25] A. Salarian, H. Russmann, F.J.G. Vingerhoets, C. Dehollain, Y. Blanc, P.R. Burkhard, K. Aminian, Gait assessment in Parkinson's disease: Toward an ambulatory system for long-term monitoring, IEEE Trans. Biomed. Eng. 51 (2004) 1434–1443. doi:10.1109/TBME.2004.827933.

[26] D. Trojaniello, A. Cereatti, E. Pelosin, L. Avanzino, A. Mirelman, J.M. Hausdorff, U. Della Croce, Estimation of step-by-step spatio-temporal parameters of normal and impaired gait using shank-mounted magneto-inertial sensors: application to elderly, hemiparetic, parkinsonian and choreic gait., J. Neuroeng. Rehabil. 11 (2014) 152. doi:10.1186/1743-0003-11-152.

[27] M. Plotnik, N. Giladi, J.M. Hausdorff, A new measure for quantifying the bilateral coordination of human gait: Effects of aging and Parkinson's disease, Exp. Brain Res. 181 (2007) 561–570. doi:10.1007/s00221-007-0955-7.

[28] M. Plotnik, N. Giladi, Y. Balash, C. Peretz, J.M. Hausdorff, Is Freezing of Gait in Parkinson's Disease Related to Asymmetric Motor Function?, Ann Neurol. 57 (2005) 656–663. doi:10.1002/ana.20452.

[29] W. Thevathasan, M.H. Cole, C.L. Graepel, J.A. Hyam, N. Jenkinson, J.S. Brittain, T.J. Coyne, P.A. Silburn, T.Z. Aziz, G. Kerr, P. Brown, A spatiotemporal analysis of gait freezing and the impact of pedunculopontine nucleus stimulation, Brain. 135 (2012) 1446–1454. doi:10.1093/brain/aws039.

[30] C. Karachi, D. Grabli, F.A. Bernard, D. Tand??, N. Wattiez, H. Belaid, E. Bardinet, A. Prigent, H.P. Nothacker, S. Hunot, A. Hartmann, S. Leh??ricy, E.C. Hirsch, C. Fran??ois, Cholinergic mesencephalic neurons are involved in gait and postural disorders in Parkinson disease, J. Clin. Invest. 120 (2010) 2745–2754. doi:10.1172/JCI42642.

[31] N. Jenkinson, D. Nandi, K. Muthusamy, N.J. Ray, R. Gregory, J.F. Stein, T.Z. Aziz, Anatomy, physiology, and pathophysiology of the pedunculopontine nucleus., Mov. Disord. 24 (2009) 319–28. doi:10.1002/mds.22189.

[32] V. Ricchi, M. Zibetti, S. Angrisano, A. Merola, N. Arduino, C.A. Artusi, M. Rizzone, L. Lopiano,

M. Lanotte, Transient effects of 80 Hz stimulation on gait in STN DBS treated PD patients: A 15 months follow-up study, Brain Stimul. 5 (2012) 388–392. doi:10.1016/j.brs.2011.07.001.

6. Acknowledgement

Forschung macht man nicht alleine, es gibt immer viele helfende Hände. Darum möchte ich mich bei allen bedanken, die geholfen haben, diese Arbeit zu einem guten Ende zu bringen. Daniel, danke dass du mir die Gelegenheit gegeben hast, diese Arbeit zu verfassen. Ich habe sehr viel von dir lernen können und auch wenn du manchmal schon sehr kleinlich warst, konntest du mich immer wieder motivieren. Vielen Dank auch an alle Patienten für's Mitmachen bei meinen Experimenten. Es war sicherlich nicht immer einfach, ich bewundere euer Durchhaltevermögen sehr!

Liebe Kollegen im Büro (unter anderem Anja, Sebastian, Rezzak, Lorenzo, Idil, Rajka), wir waren eine bunte Truppe, zusammengestückelt aus verschiedenen Arbeitsgruppen. Vielen Dank für eure Hilfe, gemeinsames Tee trinken, die Ablenkung und den Austausch. Ohne euch wäre ich nicht jeden Tag gerne zur Arbeit gegangen.

Vielen Dank auch an Christoph Braun und Friedemann Bunjes für die Bereitstellung der benötigten Hardware bei den Messungen und die große Hilfe beim Analysieren, Vieles wäre ohne euch nicht möglich gewesen!

By the way, when we speak about data-analysis, I should not forget to thank Dr. Govindan. Thank you very much for your help, it was great that you could always make some time and had the patience to explain the analysis.

Johannes, vielen Dank für die Hilfe bei den Messungen, auf dich konnte ich mich immer verlassen. Auch in der letzten Phase der Doktorarbeit hat das gegenseitige Motivieren gut getan!

Al die jaren trouw gebleven zijn vele vriendinnen van de studie en de middelbare school in Nijmegen. Dank jullie wel voor jullie bezoekjes in Tübingen, ik heb ervan genoten!

Ook wil ik mijn ouders bedanken voor hun steun. En natuurlijk mijn zusje, voor haar eerlijkheid, die me altijd weer aan het lachen brengt.

Danke auch Gerd und Ingrid für die leckeren Brezeln Sonntagmorgens und für euer Interesse an meiner Forschung.

Lieber Philipp, danke für die Unterstützung. Du hast mir sehr geholfen, auch malabzuschalten. Du konntest mich immer wieder motivieren, auch wenn du manchmal gar nichts von meiner Arbeit verstanden hast.