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Combined interleaving stimulation of the subthalamic nucleus and the substantia nigra pars reticulata for resistant gait disturbances in Parkinson's disease

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To my family and Marc

TABLE OF CONTENTS

List of Abbreviations

AC	Anterior commissure
ARAS	Ascending reticular activating system
BDI	Beck's Depression Inventory
BG	Basal ganglia
BIS	Barratt Impulsiveness Scale
CAPSIT-PD	Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease
CRF	Case report form
DBS	Deep brain stimulation
FOG	Freezing of gait
FOG-AC	Freezing of Gait Assessment Course
FOG-Q	Freezing of Gait Questionnaire
GABA	Gamma-aminobutyric acid
GPe	External globus pallidus
GPi	Internal globus pallidus
IPG	Internal pulse generator
MCP	Midcommissural point
[MedOff]	Off medication
[MedOffStimOff]	Off medication, off stimulation
MLR	Mesencephalic locomotor region
MMSE	Mini Mental Status Examination
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
NMSS	Non-motor Symptoms Scale
PC	Posterior commissure

PD	Parkinson’s disease
PDQ-39	Parkinson’s Disease Questionnaire
PPN	Pedunculopontine nucleus
PPNc	Pedunculopontine nucleus pars compacta
PPNd	Pedunculopontine nucleus pars dissipata
RBD	REM behaviour disorder
REM	Rapid eye movement
SAE	Severe adverse event
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SPM	Statistical parametric mapping
STN	Subthalamic nucleus
[STNmono]	Conventional DBS of the rostral contacts within the STN
[STN+SNr]	Combined stimulation of two electrode contacts, the rostral ones within the STN and the caudal ones within the SNr
UPDRS	Unified Parkinson’s Disease Rating Scale

List of Figures

List of Tables

1

1. Introduction

"[…] One day we were rehearsing a scene that required both of us to pass through the mayor`s office door simultaneously and in opposite directions. Scripts in hand, we started to walk the scene, but when we both got to the door, instead of passing by Michael, I froze directly in front of him. "You gotta move", I said, rather more bluntly than intended. Michael is one of the nicest guys on the planet, but he was a little confused and taken aback by my direction. "What?" he replied. "You gotta move. I can`t move until you move." He eventually complied, and after the rehearsal, I tried to explain what had just happened. Occasionally, when my brain asks my body to perform simple tasks that involve some degree of judgement regarding spatial relationships, the message gets lost in transmission. It takes some form of outside stimulus, like the movement of an obstacle, curiously, even the introduction of an obstacle, for me to move forward. Some Parkies who freeze when walking can resume again when a ruler is placed in front of their feet and they are forced to step over it. Michael, of course, accepted my explanation and even managed to laugh with me about the strangeness of it all. […]."[1]

*****

Michael J. Fox – actor, author and founder of the Michael J. Fox Foundation describing a situation when he suffered freezing of gait during a rehearsal with his friend Michael Boatman.

[1]Source: Fox, Michael J. (2009): *Always Looking Up*. First edition. New York: Hyperion, p. 11. [Internet] Available from: http://www.amazon.de/Always-Looking-Up-Adventures-Incurable/dp/1401303382#reader_1401303382 [Accessed 01/12/2015].

With these words, the actor Michael J. Fox, described a daily scene on stage during which he experienced freezing of gait (FOG) and his strategy to overcome the feeling being glued to the floor. Like the famous founder of the Michael J. Fox foundation, many Parkinson's patients suffer gait disturbances, especially in advanced disease stages.

To date, there is still no therapeutic strategy to handle gait disturbances like FOG in a satisfying manner. With the introduction of deep brain stimulation (DBS) a precious therapeutic option was found to treat brain disorders like Parkinson's disease (PD) and handle the leading symptoms of this disease, even FOG, in some patients (Vercruysse et al., 2014).

In 1817, James Parkinson (1755 – 1824) described the symptoms of PD in his "An Essay of the Shaking Palsy" for the first time. Tremor, rigidity, bradykinesia as well as postural instability or hypomimia were clinical observations he made in six individuals. Until today, these symptoms define the clinical signs of PD (Jankovic, 2008). Since then, a lot of research on the pathophysiology of PD has been done and different therapeutic strategies were developed to address the symptoms of PD. However, the disease cannot be stopped or even cured. The imbalance of the dopaminergic system caused by the loss of neurons of the substantia nigra (SN) and subsequently the disturbance of activation of the different basal ganglia (BG) components is one major reason behind the motor disorder (Agid, 1991). However, dopaminergic depletion is not the only reason for the occurrence of parkinsonism. It is accompanied by an imbalance of other neurotransmitters as acetylcholine, serotonin, or noradrenalin in consequence of the neuronal death.

Drug therapy can improve the symptoms to a certain degree and in advanced disease stages DBS is an established therapy option. In patients with levodopa-related motor complications and in tremor dominant cases, neurostimulation showed a superior efficacy over best medical management (Schuepbach et al., 2013, Deuschl et al., 2006b). Unfortunately, in spite of a variety of therapeutic options there are still patients who cannot be treated in a satisfying way. New stimulation techniques and new targets for DBS are needed to improve especially

axial symptoms, which currently cannot be treated adequately (Welter et al., 2002).

2

2. Background

With a prevalence of 0.6 % within the group of people aged over 65 years and a prevalence of 3.5 % among people aged over 85 years, PD is one of the most common neurological diseases and besides Alzheimer's Disease the second most frequent neurodegenerative disease in Europe (de Rijk et al., 1997).

In Western Europe's five and the world's ten most populous nations the number of individuals suffering from PD was between 4.1 and 4.6 million in 2005 and is expected to increase to between 8.7 and 9.3 million in 2030 according to the available statistics (Dorsey et al., 2007). These large numbers reflect the worldwide increased life expectancy and the demand for therapeutic options for PD.

As disease progresses, in most patients, bothersome levodopa-induced motor complications emerge. After five to ten years of dopaminergic therapy, a great proportion of patients suffer from levodopa-induced dyskinesia, motor fluctuations or psychosis (Poewe et al., 1986). An optimal treatment in advanced PD patients is often difficult once motor fluctuations emerge.

DBS is a clinical tool used in advanced disease stages to treat levodopa-responsive parkinsonian symptoms when medically intractable fluctuations and dyskinesia occur (Deuschl et al., 2006b, Williams et al., 2010, Okun et al., 2012). Typically, it is induced after eleven to 13 years of disease duration, when motor impairment and quality of life are severely affected. Interestingly, neurostimulation was also superior to medical treatment alone in earlier disease stages with emerging motor complications (Schuepbach et al., 2013).

The first implantable stimulation system for DBS was engineered by the company Medtronic (Minneapolis, USA) in 1987. In the last three decades, DBS developed to an established routine therapy in advanced PD (Schuepbach et al., 2013, Deuschl et al., 2006b). Owing to the pioneering work of Benabid and colleagues (Benabid et al., 1991) and broad research done on DBS since then, the Food and Drug Administration (FDA) approved the application of DBS of the thalamus for PD and essential tremor in 1997. Further approvals followed for DBS of the subthalamic nucleus (STN) and the globus pallidus (GP) for PD in 2002 and for dystonia in 2003[1].

Stimulation techniques improve continuously and DBS is under consideration for diverse neuropsychiatric disorders. With respect to PD, the therapeutic challenge is to manage symptoms resistant to standard therapy. As such, axial symptoms including FOG cannot be addressed with conventional STN-DBS. In this thesis, we probe on novel stimulation techniques and stimulation targets to overcome these limitations.

2.1. Idiopathic PD – overview

Idiopathic PD is a chronic and progressive neurodegenerative disease that affects the dopaminergic transmission resulting in disabling motor symptoms and several non-motor symptoms. It develops gradually, sometimes starting with a slight shaking in one hand (tremor), with body stiffness (rigidity), slowing of movements (bradykinesia) or difficulty with walking and gait (postural instability) (Ng, 1996).

In early disease stages, these motor symptoms are the most obvious ones and the four symptoms mentioned are considered as the cardinal symptoms in PD (Jankovic, 2008). Presumably, neurodegeneration even starts years before any clinical symptoms are noticed (DelleDonne et al., 2008, Koller, 1992) and prodromal non-motor symptoms were established to this end (Gaenslen et al., 2011, Berg et al., 2015).

[1]Source: Homepage University of Wisconsin [Internet] Available from: http://www.uwhealth.org/neurosurgery/deep-brain-stimulation-dbs-frequently-asked-questions/12764 [Accessed 03/01/2015]

PD is diagnosed on the basis of the clinical symptoms and from the time the diagnosis is made, the course of the disease is progressive (Cheng et al., 2010). After emergence of the first motor symptoms and diagnosing PD, medical therapy with levodopa is typically introduced. This early phase, the so-called “honeymoon” period, is characterised by a good and constant response to the dopaminergic treatment. It usually lasts up to five to six years after disease onset (Krack et al., 2003, Rodriguez-Oroz et al., 2005). However, few years later the intermediate stage incorporates progression of motor symptoms. This generally necessitates a combination of diverese antiparkinonian drugs and presents with emergence of first levodopa-related side effects in terms of motor fluctuations, e.g. uncontrolled off phenomena or dyskinesias. Hence, at the intermediate disease stage, efficacy and safety of oral medication may be limited, and this could be the entrypoint for neurostimulation, which improves levodopa-responsive parkinsonian symptoms (Limousin et al., 1998, Kleiner-Fisman et al., 2006). Studies could show a superior effect of STN-DBS to medical therapy alone in intermediate disease stages when the response to dopaminergic therapy is still preserved, but first motor complications are about to emerge (Schuepbach et al., 2013, Deuschl et al., 2006b).

In late disease stages a decline not only of the therapeutic response to dopaminergic mediacation can be observed but also of the response to conventional subthalamic stimulation (Krack et al., 2003, St George et al., 2010, Castrioto et al., 2011). Unfortunately, the improvement achieved by DBS and the second “honeymoon” phase following surgery often fail to control for axial motor impairment on the long-term (Nutt et al., 2011, Castrioto et al., 2011). Presumably, disease progression plays a major role for this limited long-term STN-DBS effect (Rizzone et al., 2014). The question is whether and how DBS itself could help to address such late stage symptoms. Fine-tuning concerning the stimulation parameters showed inconsistent results for the improvement of especially axial motor signs (di Biase and Fasano, 2016). Different long-term therapeutic outcomes of STN-DBS concerning segmental and axial motor symptoms may mirror an origin and involvement of those symptoms in different functional neuronal pathways (Potter-Nerger et al., 2008, Kuriakose et al., 2010,

Weiss et al., 2012a, Chastan et al., 2009, Ferraye et al., 2010, Moro et al., 2010b, Tsang et al., 2010, Thevathasan et al., 2011b). The aim of this study was to interfere on the level of these different neuronal pathways via neurostimulation of different stimulation targets.

2.1.1. Clinical symptomatology of PD

The most easily recognised symptom of PD is **tremor.** Usually it is a rest tremor affecting the distal part of the limb. At disease onset, it mostly appears in a single arm or leg. It is maximal when the limb is at rest and disappears when the limb is moved voluntarily or while asleep. The typical frequency of this rest tremor is between four and six hertz. The tremor is the most apparent and the most common symptom of the cardinal symptoms in PD (Jankovic, 2008). Another very disabling symptom and mandatory sign for PD is **bradykinesia**, the slowness of movements. Bradykinesia is mostly diagnosed by examining the diadochokinesia (e.g. pronation and supination of a hand in rapid succession). Normal daily life tasks like writing or dressing are difficult to perform for patients. The increased muscle tone in PD causes **rigidity**, a resistance or stiffness of the muscles during movements. The muscles are continuously contracted and can cause joint pain. In early disease stages, the muscles of the neck and the shoulders are the most affected. Along progression of PD, the whole body is affected, resulting in a reduced ability to move (Berardelli et al., 1983, Jankovic, 2008). **Postural instability** is a symptom in the late stages of idiopathic PD and is due to deficient postural reflexes. This axial symptom leads to impaired balance, and potentially falls as a common cause of bone fractures, hospitalisation and mortality in PD (Bloem et al., 2001, Stolze et al., 2004). Postural instability is the least treatable motor feature of the four cardinal symptoms in PD (Bloem, 1992, Jankovic, 2008). The axial motor symptoms (postural instability, gait disorders, dysarthria) largely contribute to the motor handicap in PD patients (Klawans, 1986, Bonnet et al., 1987, Bloem et al., 2001). They poorly respond to levodopa treatment and probably emanate from the increasing prominence of non-dopaminergic lesions influencing brain areas outside the BG (Agid, 1991, Bejjani et al., 2000).

FOG is one of the most disabling gait disorders of Parkinson's Disease and around 50% of all PD patients experience FOG in the course of the disease (Lamberti et al., 1997, Shoulson et al., 2002, Amboni et al., 2015, Macht et al., 2007, Giladi et al., 1997)It is a mysterious clinical phenomenon that is common not only in advanced disease stages of idiopathic PD but also in other parkinsonian syndromes or microvascular ischemic lesions (Giladi et al., 2001, Macht et al., 2007). FOG may lead to falls, impair self-dependent mobility (Latt et al., 2009, Kerr et al., 2010, Okuma and Yanagisawa, 2008) and reduce quality of life (Moore et al., 2007, Rahman et al., 2008). The pathophysiology of FOG is poorly understood and even clinical operationalisation of the FOG phenomenon is highly non-trivial. FOG consists of several clinical subforms, however, the main linking feature is the absence or reduction of effective forward progression while walking (Nutt et al., 2011).

It is an episodic gait disturbance consisting of features such as problems to initiate walking, the so-called start hesitation, episodes with arrest of forward progression while walking (so-called akinetic freezing or alternatively 'trembling-in-place like freezing'), or the so-called 'turn' and 'destination' hesitation (Nutt et al., 2011). The feeling of the 'feet being glued to the floor' or a 'trembling of the legs' can accompany FOG (Nutt et al., 2011). As a consequence of these various features, no universal clinical description exists for FOG and therefore one may postulate the underlying mechanisms are heterogeneous. The freezing episode mostly lasts a few seconds, but can sometimes persist up to more than 30 seconds (Schaafsma et al., 2003). It occurs more frequently when patients are in a dopaminergic off state, connoting an important but not exclusive role of dopamine in FOG (Plotnik and Hausdorff, 2008). FOG is mostly related to advanced disease stages but it does not seem to be correlated to the cardinal symptoms of PD, as it occurs also in syndromes without parkinsonism (Factor, 2008). The therapeutic options for axial symptoms and - in particular - FOG are still limited: despite optimal dopaminergic medication and STN-DBS a satisfying amelioration of the symptoms cannot be achieved in all patients (Castrioto et al., 2011). Only about 50% of PD patients who suffer from freezing experience an alleviation of FOG six and twelve months after DBS surgery (Vercruysse et al.,

2014). Thus, a huge number of patients stays resistant to best medical treatment and STN-DBS concerning FOG and axial symptoms. For this reason, advanced stimulation techniques, such as combined stimulation of both STN and the pars reticulata of the substantia nigra (SNr), are probed to address these unmet symptoms (Chastan et al., 2009, Weiss et al., 2011a).

2.2. BG structure and functions

The importance of the BG for brain functions such as the control of voluntary movements, eye movements, learning, routine behaviours, emotion or cognitive function becomes obvious by the numerous neurological diseases associated with BG dysfunction. The integration in cortical loops, which involve the limbic cortex and the prefrontal association cortex, is the reason for the loss or at least the pathological transformation of these functions by a modified storage in these cortical areas (Middleton and Strick, 2000, Obeso et al., 2008).

2.2.1. BG components

The BG consist of several subcortical nuclei situated in the forebrain (mostly in the diencephalon and in the mesencephalon). The main structures belonging to the BG are the striatum, the largest nucleus consisting of the caudate nucleus, and the putamen, the GP, consisting of an internal (GPi) and an external part (GPe), the SN, consisting of the pars compacta (SNc) and the SNr and lastly the STN. These nuclei interact strongly with other brain structures, such as the thalamus, the cerebral cortex or the brain stem (Feger, 1997, Middleton and Strick, 2000, McHaffie et al., 2005).

Historically, the BG were thought to be mainly involved in motor control and thereby, when impaired, the reason for movement deficits. However, today we know that the BG are associated with a variety of other functions, as mentioned above. Figure 1 illustrates the anatomical structures of the BG.

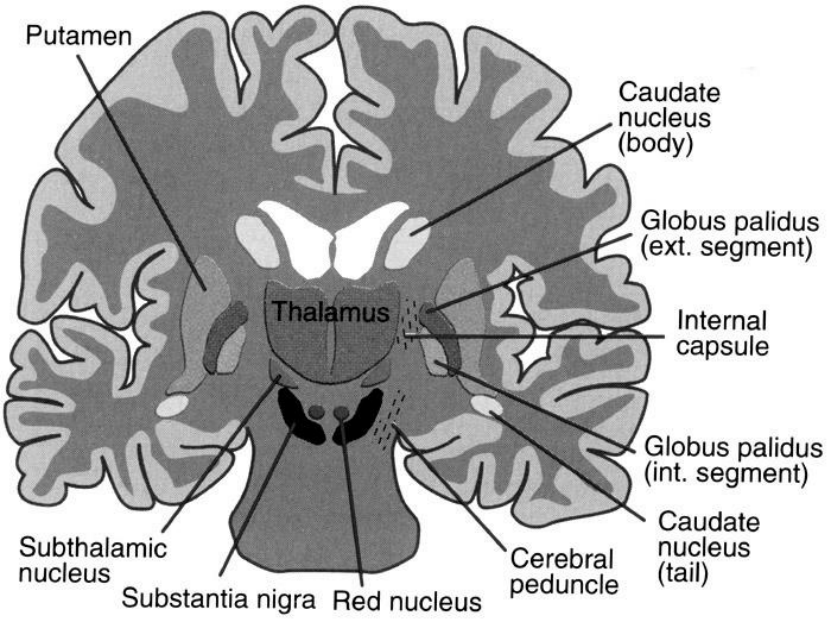

Figure 1 Basal Ganglia components illustrated by Leisman and Melillo (Leisman and Melillo, 2013). Int. = internal, Ext. = external.

2.2.2. Pathogenesis and pathophysiology of PD

It is known, that the parkinsonian symptoms are caused by a progressive degeneration of melanin containing neurons of the SNc (Schapira and Jenner, 2011). The mechanism and cause for the degeneration is not totally clear at present, but genetic predisposition (Xiromerisiou et al., 2010, Kruger et al., 1998, Sharma et al., 2012) and environmental factors (Tanner and Langston, 1990) are probably involved in the process.

The BG structures are not only interconnected (Joel and Weiner, 1994), they also build connections to a variety of other brain areas through different pathways such as connections to the thalamus or the brainstem motor centres (Obeso et al., 2008). Due to the manifold interconnections, almost all pathways in the brain are affected by the neuronal loss of the SNc and the lack of dopamine. As one major function, the BG adjust neuronal excitability in order to control motor output, i.e. executive functioning in terms of inhibition or execution (Leisman and Melillo, 2013). The function of dopamine is to balance inhibitory and excitatory influences on the motor system (Obeso et al., 2008).

Beside the four major pathways in the brain, the oculomotor, the associative, the limbic and the orbitofrontal circuit, the motor circuit is the best characterised pathway (Obeso et al., 2008). Almost all afferent projections of the BG have their

origin in the cortex and project to the striatum (Utter and Basso, 2008). The efferent output nuclei GPi and SNr have inhibitory GABAergic connecting pathways to their neighbouring targets. The GPi projects to the thalamus whereas the SNr projects to the GPi, to the superior colliculus, which is involved in eye movement, to the thalamus and to the pedunculopontine nucleus (PPN) (McHaffie et al., 2005).

There are two main and parallel pathways within the BG for the transfer and the processing of the signals from the striatum to the output nuclei GPi and SNr (Albin et al., 1989, Graybiel, 2000).

The **direct pathway** starts in the neurons of the striatum (D1 receptors), which have inhibitory GABAergic projections to the GPi. The GPi cells themselves also make inhibitory connections on the thalamic neurons (Lang and Lozano, 1998, Alexander and Crutcher, 1990). Excitation of the direct pathway activates the thalamic neurons (DeLong and Wichmann, 2007).

The **indirect pathway** takes course from the neurons of the striatum (D2 receptors) and consists of two inhibitory pathways: one between the striatum and the external globus pallidus (GPe) and the other one GABAergic between the GPe and the STN (DeLong and Wichmann, 2007). The STN has an excitatory glutatmatergic drive to both output nuclei, the GPi and the SNr (Parent and Hazrati, 1995). Excitation of the indirect pathway results in an inhibition of thalamic neurons (DeLong and Wichmann, 2007). Thus, the two pathways have opposite effects on motor output. An imbalance of those two pathways caused by the degeneration of nigro-striatal projections results in an increased activity of GABA and acetylcholine in striatal neurons and finally leads to the typical parkinsonian symptoms (DeLong and Wichmann, 2007).

2.3. The role of the SNr concerning movement and locomotion

The SNr is located in the mesencephalon and belongs to the BG structures. It is the largest nucleus belonging to the midbrain. The SNr serves primarily as an output nucleus within the BG system and the neurons of the SNr produce mainly GABA and acetylcholine as neurotransmitter. Via the axons of the SNr neurons, signals are transferred to various other brain structures, such as the thalamus or

the PPN. The main input to the SNr comes from the striatum via the indirect and direct pathway of the BG routes. The STN is another important input nucleus to the SNr. Inhibitory effects on the SNr neurons are mediated via the neurotransmitter GABA and Substance P from the striatum. Excitatory input to the SNr comes mainly from the glutamatergic projections of the STN (Deniau et al., 2007).

Efferents from the SNr project to the thalamus, to the superior colliculus, to the GPi and to caudally localised nuclei, such as the PPN and motor brainstem areas (Deniau et al., 2007). Several studies showed that the SNr is part of the nigropontine pathway, consisting of neuronal descending projections from the SNr to the pontomesencephalic area, which is known to be involved in locomotion and postural control (Takakusaki et al., 2003, Chastan et al., 2009).

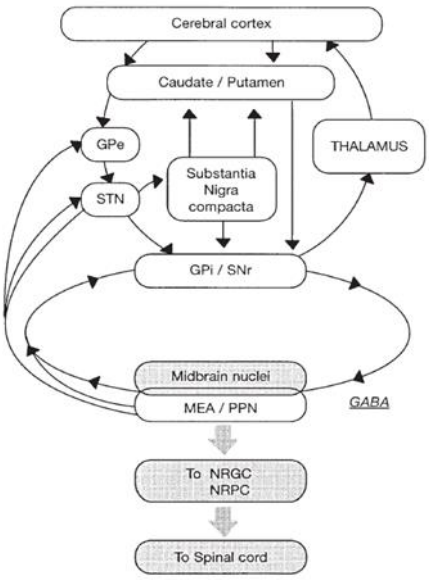

Figure 2 Illustration of the anatomical relationship between the BG nuclei, including the cortico - basal ganglia - thalamo-cortical loop and the basal ganglia - tegmentum - basal ganglia loop.
MEA = midbrain extrapyramidal area, NRGC = nucleus reticularis gignato-cellularis, NRPC = nucleus reticularis pontis caudalis, PPN = pedunculopontine nucleus, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, GPi = internal globus pallidus, GPe = external globus pallidus, GABA = gamma-aminobutyric acid. From Delwaide et al. (Delwaide et al., 2000)

The pathophysiology of locomotion and the specific functions of the pontine nuclei and the descending pathways and loops in which the SNr and the BG are involved is still only fragmentarily understood. However, the current model of the interconnections between the different nuclei is illustrated in Figure 2: the SNr has GABAergic neuronal pathways to the midbrain tegmentum, consisting of the midbrain extrapyramidal area (MEA) and the PPN. From there, the subcortical route leads to the spinal motor neurons via the reticulospinal tract and the nuclei of the reticular formation (Delwaide et al., 2000). An inhibitory efferent GABAergic output from the SNr to the PPN could be displayed in animal studies on cats (Noda and Oka, 1986), rats (Childs and Gale, 1983, Grofova and Zhou, 1998) and non-human primates (Carpenter et al., 1981).

Based on the BG model with activity imbalances of the interconnected nuclei, the symptoms of PD are the coherent result. Given the monosynaptic efferent GABAergic neuronal transmission from the SNr to the PPN (Nandi et al., 2008), the idea of a nigral overinhibition on the PPN locomotor activity is conclusive. The consequence is a lack of postural control and locomotion. Consecutively, electric stimulation of the SNr may reduce the GABAergic drive from the SNr to the PPN and thus normalizes the neuronal activity of the PPN and the mesencephalic locomotor region (MLR).

A modulation of the activity of the SNr by electric stimulation (Sutton et al., 2013), or by microinjection of GABAergic substances into the SNr (Wichmann et al., 2001) could be seen in animal studies (Burbaud et al., 1994). A pharmacological inhibition of the SNr with infusions of GABA agonists, such as muscimol, results in a turning contralateral to the treated site and to a general hyperactivity after bilateral pharmacological SNr - inhibition in cats (Wolfarth et al., 1981, Boussaoud and Joseph, 1985) and rats (Scheel-Kruger et al., 1977, Olpe et al., 1977). This supports the idea of a GABAergic pathway from the SNr to the PPN and underlines the regulatory role of the SNr on PPN locomotor activity. Investigations on the postural muscle tone and locomotion by modulation of the GABAergic influence from the SNr to the PPN are the basis for exploring the effective sites in the SNr and for finding a possible functional topography of the SNr.

Effects of nigral stimulation on locomotion and postural stability in PD patients was explored in several studies in the last years. Chastan found that bilateral SNr stimulation improved axial motor signs but had no effect on the distal parkinsonian motor symptoms (segmental symptoms like akinesia, rigidity and tremor) (Chastan et al., 2009). In contrast, STN stimulation reduced both axial and distal motor symptoms, however, axial sign to a lesser degree (Chastan et al., 2009). An improvement of axial motor signs in levodopa responsive PD patients with STN-DBS could also be shown by Bejjani et al. (Bejjani et al., 2000). The interconnections of the different components belonging to the BG network may be the reason why therapeutic addressing of different sites can result in similar clinical outcomes or may at least be the reason for affecting more than just one site.

2.4. DBS in Parkinson's Disease

DBS is an established treatment option in many diseases concerning movement disorders like PD (Moro and Lang, 2006), essential tremor (Zhang et al., 2010) or dystonia (Krauss, 2002). Furthermore, it is approved for obsessive-compulsive disorder (Lakhan and Callaway, 2010) and epilepsy (Fisher et al., 2010).

During the last 25 years, DBS became an internationally established and accepted therapy concept and it is not the success of this method, which is questioned nowadays, but how it exactly works and how one intervention can improve different diseases and symptoms. A lot of research has been done on this topic resulting in a better understanding of the pathophysiology of the involved structures and in an improvement of the stimulating techniques (Breit et al., 2004). We know that the electrode design and the manner of application of the electric impulses have to be adjusted to the different brain structures, which are used as the stimulating targets as they are differently shaped, sized and physiologically characterized. The attention in this study is on the SNr as stimulation target (in combination with the STN) and interleaving stimulation as manner of stimulation technique.

2.4.1. Hardware and programming

The necessary hardware for DBS consists of an internal pulse generator (IPG), electrode leads and an extension, which connects the IPG to the leads.

The IPG is a battery-powered neurostimulator positioned under the skin below the collarbone. Continuous electric pulses are delivered by the IPG via the extensions to the leads and thus to the electrode in the brain at a set amplitude, frequency and pulse width. The extension is an insulated wire running subcutaneously from the IPG via the backside of the ear, up the side of the neck to the head and down to the upper ends of the leads in the brain. The electrode leads are implanted on both sides of the brain and are located in one or two different nuclei of the brain when they reach their final position. They consist of a coiled and insulated wire and four platinium/iridium contacts at the distal end of the lead. The special characteristic about the implantation is the need of the patient's feedback to find the optimal localisation for the electrodes. The awake

procedure is done in local anaesthesia. The IPG and the extension can be implanted in general anaesthesia (Coffey, 2009). As already mentioned, different modes of application concerning the electric pulses exist and have proved to function effectively. The conventional stimulation settings include the **monopolar stimulation,** in which the active electrode contact is set as the cathode and the IPG case is set as the anode. With monopolar stimulation, the electric field is relatively wide and the stimulated area spreads equally in all directions (Volkmann et al., 2006).

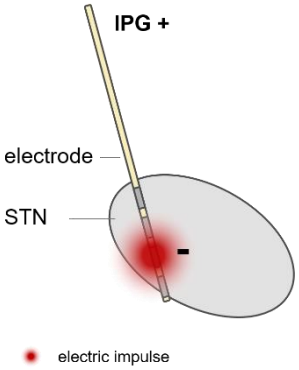

Figure 3 Schematic illustration of monopolar stimulation.
IPG = internal pulse generator, STN = subthalamic nucleus.

With **double monopolar stimulation** two adjacent negative contacts on the electrode are stimulated at similar amplitude and pulse width (Miocinovic et al., 2014).

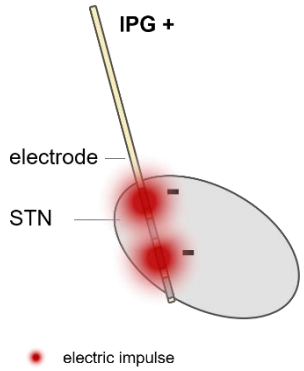

Figure 4 Schematic illustration of double monopolar stimulation.
IPG = internal pulse generator, STN = subthalamic nucleus.

Using **bipolar stimulation**, the anode is not the IPG case but another electrode contact. Thereby, the spread of current is minimized (Volkmann et al., 2006). In general, monopolar stimulation requires lower stimulation intensities, namely lower amplitudes, to achieve similar clinical effects as bipolar stimulation (Volkmann et al., 2006). The current spread is broader with monopolar stimulation because the positive and the negative potentials are relatively distinct from each other compared to bipolar stimulation, in which the current is more focused and the surrounding tissue, in particular the internal capsule, is less affected by the potential distribution. Hence, with a monopolar stimulation setting side effects occur on lower stimulation intensities compared to bipolar stimulation settings (O'Suilleabhain et al., 2003).

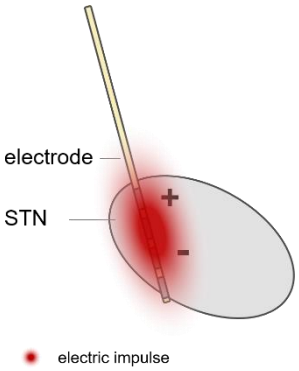

Figure 5 Schematic illustration of bipolar stimulation.
STN = subthalamic nucleus.

Before finding the best stimulation parameters in each individual, the optimal electrode localisation is determined by clinical testing of effects and side effects of a single electrode contact. In addition, neuroanatomical representation can be analysed by fusion of the preoperative magnetic resonance imaging with postoperative computed tomography, as was done in this study. Finding the optimal stimulation parameters for every patient is empiric with the aim to induce the maximum of therapeutic effect and to prevent or achieve a tolerable minimum of adverse effects (Volkmann et al., 2006). Each electrode contact has to be tested with various combinations of stimulation parameters according to standardised clinical algorithms to find the best individual stimulation parameters (Deuschl et al., 2006a). Side effects result from the flow of current to the surrounding areas of the stimulated sites (Grill, 2005, McIntyre et al., 2004).

2.4.1.1. Stimulation techniques for DBS - interleaving stimulation

Another programming option besides the commonly applied single monopolar or bipolar stimulation mode is the stimulation with interleaved pulses. It is available in the latest clinically approved IPGs (Activa series, Medtronic, Minneapolis, USA) and allows for switching between two different sets of stimulation parameters on two electrode contacts (Kovacs et al., 2012, Weiss et al., 2011a, Wojtecki et al., 2011). In cases where stimulation at one contact cannot alleviate motor symptoms sufficiently or when simultaneous stimulation of multiple contacts at one amplitude cannot be tolerated due to side effects, interleaved stimulation can be useful (Wojtecki et al., 2011). Interleaving pulses are achieved by a rhythmic and rapid automatic switching of current flow between two groups of stimulation parameters on the same electrode but on different contacts (Miocinovic et al., 2014). Each contact can be stimulated independently at its individual best amplitude and pulse width at a common frequency resulting in distinct current spreading at each of the contacts and thus in different tissue activation. Hence, one of the advantages of interleaved stimulation lies in modelling the field of current spread (Kovacs et al., 2012).

Thereby, it provides an optional programming tool necessary for this study, as it allows to stimulate two different sites (STN and SNr) at their best individual

stimulation settings. Hence, it is possible to modulate different functional motor loops by co-stimulation of these two sites, which are probably integrated in different locomotor pathways (Miocinovic et al., 2014).

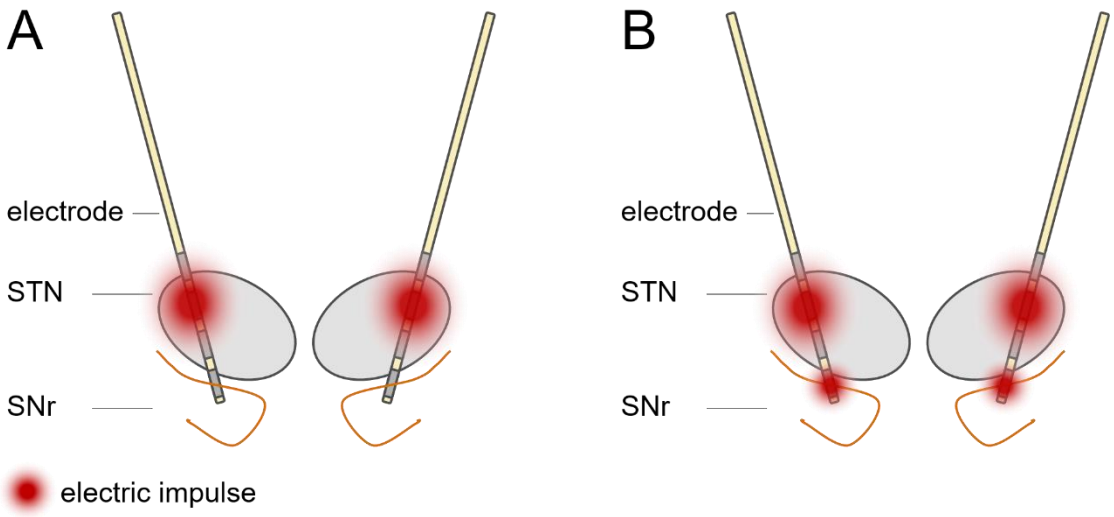

Figure 6 Schematic illustration of the conventional monopolar STN-DBS (A) and interleaving stimulation of two electrode contacts (B)

2.4.2. Mechanisms of DBS

The mechanism of DBS is currently not completely understood and there exist several strongly debated ideas on the mechanism and effect of DBS.

The earliest supposition was concluded from the similar clinical outcome of lesioning specific nuclei and DBS, hypothesising that high-frequency stimulation inhibits local neuronal activity and thus attenuates the output of the stimulated nuclei. An inhibition of neurons by high-frequency stimulation of the STN was first described in rats (Benazzouz et al., 2000). These findings could be confirmed in monkeys and humans by stimulation of the STN or the GPi (Meissner et al., 2005, Welter et al., 2004, Dostrovsky et al., 2000). Reduced activity of the GPi and the SNr was induced by STN-DBS (Benazzouz et al., 1995, Maltete et al., 2007). It was proposed that electric stimulation of the brain tissue causes an activation of presynaptic inhibitory afferents to the stimulated site, which is supposed to be the main mechanism underlying the observed inhibition (Boraud et al., 1996, Wu et al., 2001, Gradinaru et al., 2009). Another approach to explain the assumption that DBS inhibits the stimulated site is the depolarisation block of voltage-gated

currents in the neurons of the stimulated nucleus, which leads to a block of the neuronal output near the electrode (Beurrier et al., 2001, Burbaud et al., 1994). High-frequency DBS induces high-frequency firing rates in the cells of the stimulated site, but the membrane potential does not have enough time to repolarise, which leads to the depolarisation block (Dostrovsky et al., 2002).

Other studies recording neuronal activity in the stimulated nuclei showed an increase of the firing pattern, proposing an increase of the output from the stimulated nucleus. In one study, STN-DBS was applied in parkinsonian monkeys resulting in an increased level of activity of the pallidal neurons (GPi and GPe), seen in the extracellular recordings (Hashimoto et al., 2003). An increased firing pattern of the stimulated site was not only found in excitatory efferents, but also in inhibitory efferents. An inhibition of thalamic neurons was observed in normal monkeys during DBS of the GPi (Anderson et al., 2003) and DBS of the GPe inhibited STN neurons in monkeys (Vitek et al., 2012).

The activation theory is furthermore supported by imaging data. An increased blood flow could be detected in the GPi during STN-DBS and in the cortex during thalamic DBS in human positron emission tomography studies (Perlmutter et al., 2002, Hershey et al., 2003). These findings are consistent with the idea of an activation of the output from the stimulated nucleus. Equally, in an MRI-study with STN-DBS in humans, an increase of the blood oxygen-level could be detected in the GPi (Jech et al., 2001).

Despite the successful use of DBS and the astonishing clinical improvement of parkinsonian symptoms the exact mechanism of action remains unclear. There are probably additional processes taking part in the mechanism of action in DBS besides the mentioned. Recent works have shown that not only direct effects of high-frequency stimulation of the stimulated site play a role in the mechanism of DBS but also the effects of fibre tracts and glia surrounding the stimulated nucleus (Jantz and Watanabe, 2013). In summary, it can be stated that the mechanisms activation and inhibition both probably contribute to the functional principle of DBS, resulting in a complex model of excitatory and inhibitory effects on the whole BG network.

2.4.3. Benefits of DBS in PD patients

The international gold standard in the therapy of PD is the drug therapy with dopaminergic medication (Brooks, 2008).

In advanced disease stages, when the PD medication does not improve the PD symptoms in a sufficient way and when “on/off” fluctuations or dyskinesia occur more frequently, DBS can be a safe and effective therapy for well selected patients (Deuschl et al., 2006b). Furthermore, DBS allows for a reduction of antiparkinsonian medication by about 50 % of the daily levodopa equivalent dosage (Alexoudi et al., 2015, Deuschl et al., 2006a). This is an important fact for patients who suffer from medication-induced side effects, such as psychosis or impulse control disorder.

It could be shown that DBS can improve quality of life in patients suffering from PD (Volkmann et al., 2009). With disease progression, PD is associated with a decline in physical and social functioning caused by motor and non-motor symptoms, such as motor fluctuation, pain, sleep problems or psychiatric symptoms. Neurostimulation was associated with an improvement of 25 % in quality of life-scales (Deuschl et al., 2006b).

Recent studies showed that in some cases, DBS is not the last resort but a superior therapeutic option in earlier disease stages with beginning motor complications (Schuepbach et al., 2013). Thus, it is possible that prospectively more patients will undergo DBS earlier when quality of life and social functions are yet more preserved.

2.4.4. Therapeutic stimulation – the different targets

In PD, the DBS leads can be implanted in different target nuclei. The efficacy on motor deficits and the side effects caused by DBS depend on the targets applied. The STN and the GPi are the two sites in the brain tissue mostly used for DBS in PD and the efficacy of DBS of these two sites is well documented. The PPN as an experimental target for DBS in PD was studied in the last years with heterogeneous findings on axial motor signs, such as gait and balance impairment. As alternative target for resistant axial impairment, the SNr was proposed recently (Chastan et al., 2009, Weiss et al., 2011a, Weiss et al., 2011b).

2.4.4.1. Internal globus pallidus (GPi)

Stimulation of the GPi and pallidotomy have been established treatment options in PD since years. Pallidotomy was a common therapy for PD in the 1960s (Svennilson et al., 1960). Then, levodopa was introduced for the therapy of parkinsonian symptoms (Barbeau, 1969, Cotzias et al., 1969) and the surgical treatment was not the preferred therapy for PD anymore. In 1987, Benabid et al. published results of thalamic-DBS for tremor in patients with PD and the high-frequency stimulation era started (Benabid et al., 1987).

Based on the pathophysiology of the BG and research done on this topic of neurology, we know that the firing rate of the neurons of the GPi is increased in PD, which is caused by the loss of the dopaminergic neurons of the SNc (Dostrovsky et al., 2002). The abnormal inhibitory outflow from the GPi to the thalamus can be blocked by DBS of the GPi, resulting in an alleviation of all cardinal symptoms of PD, a reduction of the drug-induced dyskinesias and an amelioration of quality of life (Volkmann et al., 1998, Pahwa et al., 1997).

Compared to bilateral pallidotomy, DBS of the GPi seems to be more safe and side effects, especially neuropsychological side effects, are rarer with DBS (Volkmann et al., 1998).

2.4.4.2. Subthalamic nucleus

Interest in the STN as a target for DBS evolved from the appreciation of its important role in the BG projections to the GPi, the SNr and the GPe (Obeso et al., 2008). As described earlier, the increased excitation from the STN leads to the motor abnormalities of PD. The hyperactivity of the STN can be blocked, resulting in the inhibition of thalamocortical activity and thereby alleviating the cardinal signs of PD. Bergman et al. demonstrated in 1990 that the typical PD symptoms can be reversed by blocking the STN in terms of lesioning it (Bergman et al., 1990). In their study, they induced parkinsonism in monkeys by treatment with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), lesioned the STN and observed an amelioration of the parkinsonian symptoms.

Lesioning of the STN is irreversible and side effects like hemiballism limit the procedure (Chen et al., 2002, Tseng et al., 2003).

Mimicking the effects of a lesion is possible with DBS (Benabid et al., 1991). Benabid et al. were the first ones to demonstrate that a chronic high-frequency stimulation of the STN could simulate the results of lesioning the STN. After this first study, numerous other clinical trials demonstrated the efficacy and safety of STN-DBS in PD patients (Limousin et al., 1998, Deuschl et al., 2006b). The clinical success of this method and the benefit of avoiding side effects, such as destruction of neighbouring structures, make it preferable to the subthalamotomy (Starr et al., 1998).

Currently, STN-DBS, as a reversible and adjustable method of blocking the increased neuronal activity of the STN without a large destructive brain lesion, is the most widely used approach to address segmental motor symptoms and motor fluctuations in PD (Deuschl et al., 2006b).

2.4.4.3. Comparison between GPi and STN as targets for DBS in PD

There are clinical studies demonstrating a comparable effect of STN-DBS and GPi-DBS (Group, 2001, Burchiel et al., 1999)but also studies showing a superiority of STN-DBS compared to the GPi-DBS, especially concerning akinesia (Krack et al., 1998).

Nowadays, STN-DBS is generally the preferred surgical method regarding DBS in PD. The amount of antiparkinsonian drugs patients have to take to address motor symptoms can be substantially reduced only with STN-DBS. However, drug adjustment may be easier with GPi-DBS (Vingerhoets et al., 2002). STN-DBS has no direct effect on dyskinesia but the decrease of antiparkinsonian medication leads to a reduction of dyskinesia symptoms (Group, 2001).

2.4.4.4. Pedunculopontine nucleus

The increasing awareness that the PPN might play an important role in movement disorders made it interesting as target for medical and surgical intervention.

The PPN is the principle site of the MLR, which is only loosely defined. It is a region in the rostral brainstem consisting of the PPN and the cuneate nucleus and it is involved in locomotion (Nandi et al., 2008),.

The PPN is formed by a collection of neurons within the upper brainstem. Caudally to the PPN, the pontine reticular formation is situated and rostrally, it lies next to the SN. The medial lemniscus is found laterally to the PPN and medially it is limited by the superior cerebellar peduncle. The PPN is composed of two parts on the basis of cell density. The pars compacta (PPNc) consisting of mainly cholinergic neurons and the pars dissipata (PPNd) containing mostly glutamatergic neurons but also cholinergic, dopaminergic, noradrenergic and GABAergic neurons (Mesulam et al., 1989). The PPNc is located in the dorsolateral part of the PPN and it is situated caudally, the PPNd is the rostral part of the PPN (Jones and Beaudet, 1987, Rye et al., 1988, Lavoie and Parent, 1994). Afferents to the PPN arrive from the BG output nuclei, the GPi and the SNr. They consist mainly of GABAergic neurons projecting to the PPNd neurons (Jackson and Crossman, 1983). Ascending efferents from the PPN project mainly to the thalamus and are predominantly cholinergic (Garcia-Rill, 1991). Descending efferent pathways project to diverse brain areas such as the midbrain, the pons, the medulla, the spinal cord and deep cerebellar nuclei. They are partly cholinergic and partly non-cholinergic (Pahapill and Lozano, 2000). However, data for humans is available only rarely. Electrical stimulation and medical interventions such as the application of neuroactive substances to the PPN showed an influence on the locomotor activity in animal experiments. Therefore, the PPN is thought to be potentially involved in locomotion in humans as well. Electrophysiological studies suggest that glutamatergic PPNd neurons are associated with the initiation of movement, whereas cholinergic PPNc neurons may be important for the maintenance of gait (Garcia-Rill and Skinner, 1987a, Garcia-Rill and Skinner, 1987b, Pahapill and Lozano, 2000). A pharmacological inhibition of the PPN leads to akinesia in primates. In preclinical studies it could be shown that lesioning of the PPN by injection of kainic acid leads to akinesia in nonhuman primates. Instead, injection of bicuculline (GABA receptor antagonist) into the PPN resulted in an improvement of akinesia in parkinsonian rendered nonhuman primates (Nandi et al., 2002a). Concerning electric stimulation, parkinsonian primates showed improved motor activity following low frequency PPN-DBS (Jenkinson et al., 2004). With additional

levodopa drug therapy the motor activity was even greater (Nandi et al., 2008). In contrast, stimulation above 45 Hertz produced akinesia (Nandi et al., 2002b). The involvement of the PPN in the control of locomotion could not only be shown in animal studies (Nandi et al., 2002a, Takakusaki et al., 2003), but there is also evidence that PPN-stimulation in humans has a positive effect on axial symptoms which cannot be sufficiently treated with conventional STN-DBS.

Lately, several studies showed a small effectiveness of motor signs such as gait disturbances and FOG by low-frequency PPN-stimulation in PD patients (Moro et al., 2010a, Ferraye et al., 2010, Stefani et al., 2007, Thevathasan et al., 2011a). Electric stimulation of the PPN in humans is currently still investigational as there is only little experience with PPN-DBS. The interindividual benefit varies strongly among treated patients and standard stimulation parameters are not identified yet. Furthermore, neither patient characteristics of potential candidates for PPN-DBS nor the question whether unilateral or bilateral PPN stimulation is more effective is clear.

The apparently strong interconnections between the SNr and the PPN are the reason for the interest in influencing the PPN on the level of the SNr. In a hypothetical model of the involvement of the BG in the control of movement, the projections from the SNr are separated into a projection from the medial part of the SNr to the MLR, which corresponds to the medial PPN and a projection from the lateral part of the SNr to the lateral part of the PPN (Figure 7). In this model, generated on findings in cat experiments, the PPNd is supposed to control locomotion whereas the PPNc is involved in the control of the postural muscle tone (Takakusaki et al., 2003). There is evidence that via cholinergic (Takakusaki et al., 1997, Takakusaki and Kitai, 1997) and non-cholinergic (including glutamatergic, GABAergic and peptidergic) (Mena-Segovia et al., 2008) pathways from the PPN to the reticulospinal tracts, muscle-tone is regulated and locomotion is controlled (Inglis and Winn, 1995). SNr stimulation results in a suppression of the inhibitory effects of the PPN and this suppression could be blocked by application of GABA receptor antagonists into the PPN in cats (Takakusaki et al., 2011). The muscle tone suppression is due to a postsynaptic inhibition of the spinal motor neurons (Takakusaki et al., 2011). These findings

underline the hypothesis that PPN neurons are under the inhibitory control of GABAergic SNr neurons and interventions blocking SNr-activity could restore PPN-function resulting in a normalization of locomotion (Takakusaki et al., 2011). In cats, it could be shown that electric stimulation of the SNr leads to a suppression of PPN inhibitory effects. These effects could be restored by GABA antagonists injected into the PPN (Takakusaki et al., 2011). These findings lead to the following concept: blocking the overactive SNr in PD patients may lead to a normalization of the neuronal function of brainstem centres which are involved in motor control and locomotion and thus may reduce parkinsonian symptoms.

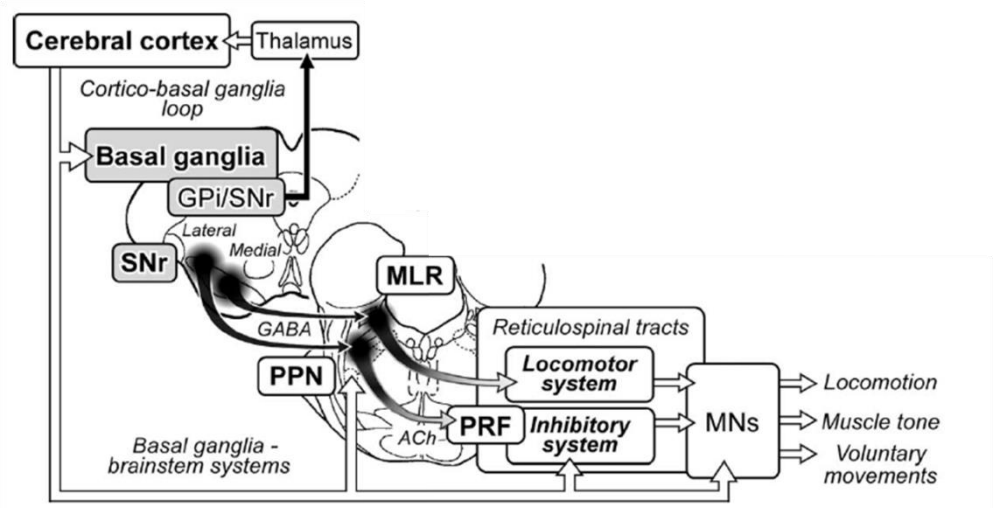

Figure 7 Illustration of the hypothetical model for locomotion and postural stability involving the basal ganglia nuclei, the mesencephalic locomotor region (MLR), the reticulospinal tract and the spinal cord with its motor neurons (MNs).
GPi = internal globus pallidus, SNr = Substantia nigra pars reticulata, PPN = pedunculopontine nucleus, PRF = pontine reticular formation, GABA = gamma-aminobutyric acid, Ach = acetylcholine. From Takakusaki et al. (Takakusaki et al., 2003).

2.4.5. Clinical limitations of conventional STN-DBS

The main treatment goal of DBS in PD patients is a satisfactory control of the burdening motor features. Unfortunately, conventional STN-DBS is limited by a decreasing effectiveness on axial impairment over the course of the years – speech, gait, posture, postural stability are affected(Castrioto et al., 2011, Krack et al., 2003, Nutt et al., 2011). Studies with a follow-up phase up to ten years showed a progressive loss of stimulation benefit concerning axial motor symptoms related to disease progression (Janssen et al., 2014, Castrioto et al.,

2011, Zibetti et al., 2011). Nevertheless, a persistent effect of STN-DBS on the cardinal parkinsonian symptoms could be observed even after long-term stimulation. Especially motor-fluctuations and dyskinesias are still greatly reduced after several years, whereas axial motor symptoms often worsen. The development of levodopa unresponsive PD symptoms might have an important influence on the long-term outcome of STN-DBS (Fasano et al., 2010).

With disease progression, besides the dopaminergic circuits, non-dopaminergic motor-circuits are gradually affected with the result that axial motor symptoms, which are less or not responsive to antiparkinsonian medication, appear. Several reprogramming options of the DBS system were tested with the aim to ameliorate axial symptoms. The so-called 'better side reduction' can positively influence the gait pattern in patients with asymmetric gait patterns via reduction of the stimulation amplitude on the hemisphere controlling the lower extremity with longer step length (Fasano et al., 2011). Hereby, a beneficial effect could be seen in some cases concerning FOG. Another tested strategy to ameliorate axial motor symptoms is low-frequency STN-DBS (≤ 80 Hz). Unfortunately, no significant improvement could be detected in PD patients with resistant axial motor symptoms compared to high-frequency STN-DBS (≥ 130 Hz) (Sidiropoulos et al., 2013, Ricchi et al., 2012).

As there are currently no satisfactory programming and reprogramming options for STN-DBS in PD patients suffering resistant axial motor symptoms, new treatment modalities are needed.

The loss of DBS benefit is ascribed to the natural progression of the disease, but we know that conventional STN-DBS ameliorates mainly segmental symptoms probably via the thalamo-cortico-spinal motor loop, whereas axial motor symptoms could be associated and ascribed to a damaged motor processing of the mesencephalic motor pathways (Moro et al., 2010a, Ferraye et al., 2010) and nigropontine pathways (Potter et al., 2008, Chastan et al., 2009, Weiss et al., 2012a, Tsang et al., 2010). Hence, the additional stimulation of structures with mesencephalic and nigropontine projections, namely the co-stimulation of the SNr, is warranted to study the efficacy on axial signs in advanced disease stages.

2.5. Main questions

The observation that disabling axial motor symptoms occur often in advanced disease stages despite an effective STN-DBS and standard dopaminergic medication concerning segmental symptoms leads us to the idea of different pathological motor network processing.

Given the differing therapeutic responses of segmental and axial motor symptoms to standard STN-DBS and taking the diverse motor loops and the defective motor processing in PD into account, it seems to be conclusive that the different outcomes may reflect the idea of different functional sub-loops in which those symptoms are processed.

With new stimulation techniques and the possibility of very accurate electrode placement in the right target we have the resources to test our ideas in the context of clinical studies.

With this study, we want to explore the following hypotheses, which originate from the already existing neurophysiological and clinical background:

- resistant axial motor impairment in PD, such as FOG, can be ameliorated with the concomitant interleaving high-frequency stimulation of the STN and the SNr in cognitively competent patients.

- additional stimulation of the SNr does not interfere with the effect of the STN stimulation, hence, the motor response of the segmental symptoms remains similar to standard therapy during concomitant nigral stimulation.

- nigral stimulation can be applied safely concerning non-motor issues in well selected patients who tolerated STN stimulation without serious neuropsychiatric side effects.

3

3. Material and Methods

The main purpose of this clinical trial was to investigate the effects of interleaved stimulation on two different targets of the BG concerning axial symptoms in patients with PD.

3.1. Characteristics of the study population

In the time from January 2011 to June 2012 screening for eligibility and enrolment of 28 PD patients with refractory gait disturbances under best individual STN-DBS took place in the Centre for Neurology, Department for Neurodegenerative Diseases of the University of Tuebingen.

The inclusion criteria were:

- signed written informed consent
- age between 18 and 80 years
- idiopathic PD, including genetic forms of PD according to the “UK Parkinson’s Disease Society Brain Bank clinical diagnostic criteria” (Hughes et al., 1992) which is a three step path to diagnose idiopathic PD
- disease duration at least five years or more
- treatment with STN-DBS with the Activa® impulse generator (Medtronic, Minnesota, USA)
- implantation of DBS electrodes at least six months prior to study enrolment
- optimisation of subthalamic stimulation parameters before study enrolment to the best of our current knowledge (Weiss et al., 2011b)
- gait disturbances refractory to the best individual DBS programming and to the best individual dopaminergic treatment

- 12 or more points in the best clinical condition on the ‘axial score’ (score consisting of eight items of the anamnestic UPDRS II (items 3-5) and the clinical UPDRS III (items 27-31), rated on a five-point scale (0-4))
- constant dopaminergic medication for at least four weeks prior to the study enrolment
- localisation of the lowermost contacts of the electrode in the border zone between the STN and the SNr
- localisation of at least one of the two rostral contacts within the STN area

Table 1 Patient characteristics.
F = female, M = male, AaO = age at onset, LED = levodopa equivalent dosage.

ID	Age [years]	Gender	AaO [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

Exclusion criteria were:

- less than 25 points in the Mini Mental Status Examination (MMSE)
- psychosis, suicidality and any other severe chronic diseases that might confound the treatment effects or the interpretation of the study results
- any acute adverse effect from stimulation of the caudal border zone of STN and SNr
- pregnancy

- participation in other clinical studies during enrolment in our trial or during the past three months before study enrolment

16 patients were excluded because of a lack of fulfilling the inclusion criteria. One patient declined to participate, five patients showed cognitive impairment (MMSE < 25 points), five patients suffered other diseases that interfered with gait, four patients displayed image-guided localisation of the lower most electrode contact outside the border zone of STN and SNr and one patient was older than 80 years. Twelve patients were enrolled in the study. Nine patients were men, three patients were women, the mean age of the study cohort was 65.0 ± 8.9 years (range 41 – 74), the mean age of disease onset was 47.0 ± 8.3 years (range 31 – 58), and the mean disease duration was 17.6 ± 5.2 years (range 10 – 26). The mean time since DBS implantation averaged 31.3 ± 24.4 months (range 6 – 79). The mean daily cumulative levodopa equivalent dosage of the twelve patients was 650 ± 310 mg (range 150 – 1098) and the mean composite ‘axial score’ at study enrolment was 16.1 ± 3.5 (range 12 – 23) points. The mean MMSE score was 28.7 ± 1.3 (range 25 – 30) points.

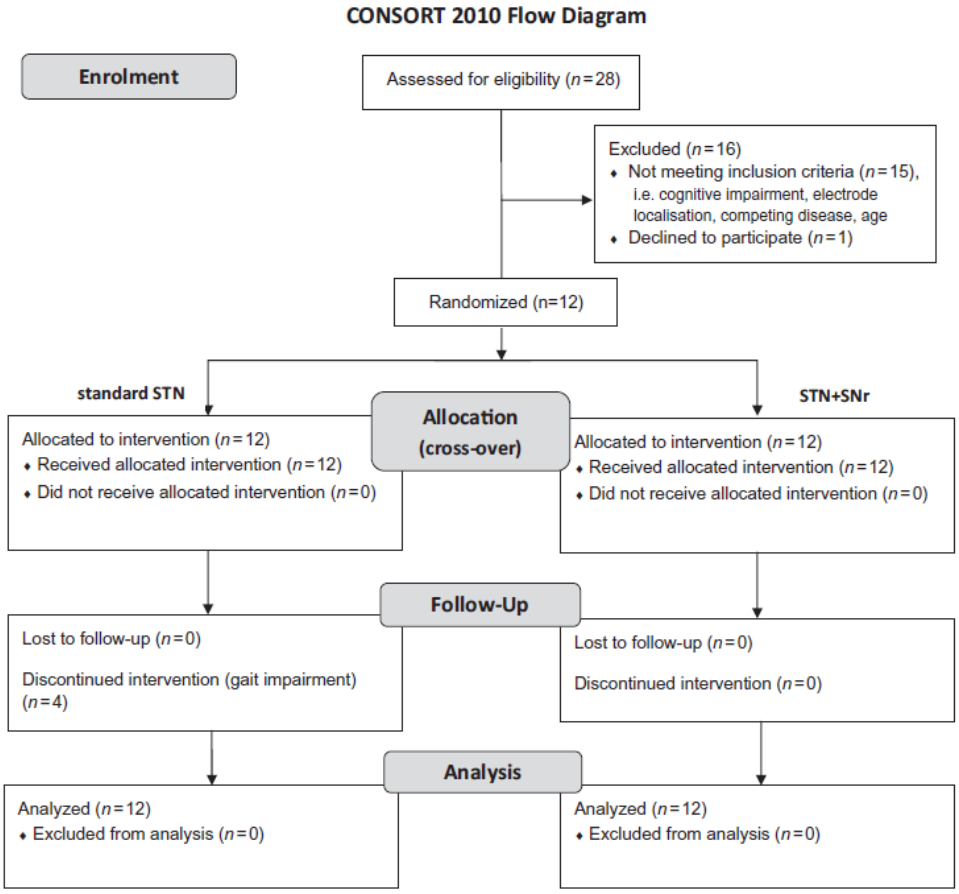

Figure 8 Flow diagram (Schulz et al., 2010) of the phases of this randomised controlled trial. n = number of patients, STN+SNr = combined stimulation of STN and SNr.

All study patients participated after written informed consent. This trial was registered at ClinicalTrials.gov (NCT01355835) and approved by the local Ethics Committee of the Medical Faculty of the Eberhard Karls University Tuebingen, in accordance with the declaration of Helsinki.

3.2. Study design

Considering randomised controlled trials as gold standard for clinical research, we chose a double-blind randomised cross-over study design. This phase II clinical trial consisted of two arms and was performed as single centre clinical trial. Twelve patients were enrolled and randomised on the two treatment arms in a 1:1 ratio. The study can be divided into two parts. The ‘baseline’ and ‘immediate testing’ part and the ‘3-week follow-up’ part. The course of the study phases is visualized in Figure 9.

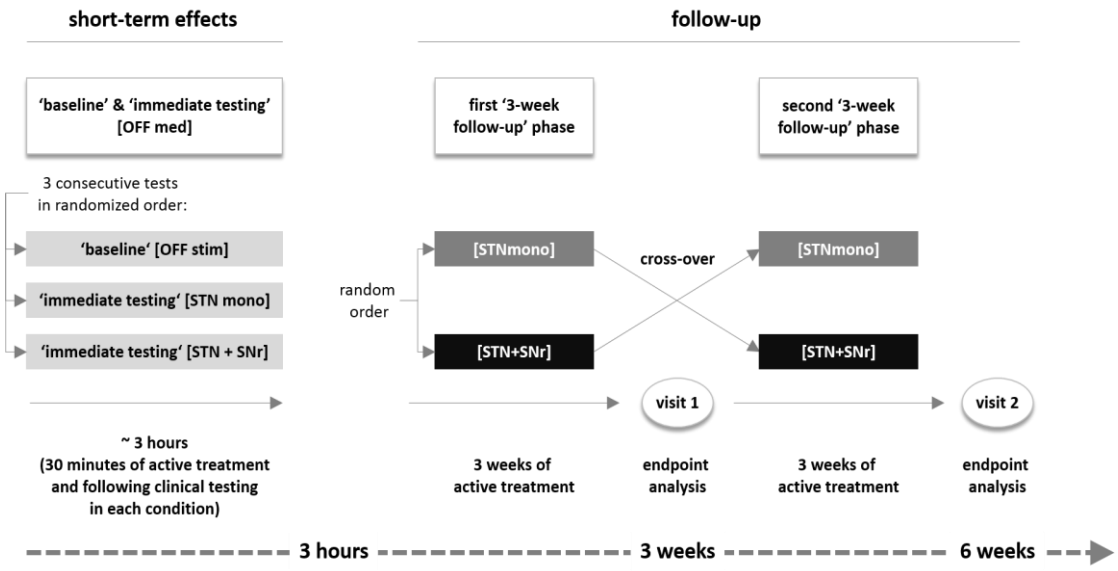

Figure 9 Scheme of the different study phases.
[OFF stim] = off stimulation, [STNmono] = conventional subthalamic stimulation, [STN+SNr] = combined stimulation of STN and SNr.

First, the patient cohort underwent the 'baseline' assessment and the 'immediate testings' to assess short-term effects of the different stimulation settings. After overnight withdrawal of dopaminergic medication, three conditions were tested. The 'baseline' condition without stimulation [StimOff], the conventional subthalamic DBS [STNmono] and the combined interleaved stimulation of the STN and the SNr [STN+SNr]. The two latter ones were also performed in the dopaminergic off state. The reason for testing these three conditions 'off dopaminergic medication' was to assure the optimal subthalamic stimulation parameters and to identify the short term effects of both stimulation settings, the [STNmono] and [STN+SNr] condition. The blinding and the randomised order were kept while testing these three conditions. Additionally, to limit the patients' and the assessors' knowledge about the finally programmed condition, the principle investigator switched between the three conditions [StimOffMedOff], [STNmono] and [STN+SNr] several times before applying the correct randomised stimulating condition. In some cases, the patients and assessors probably noticed when the stimulation was off because segmental symptoms normally occur quickly after switching off the stimulation. We were not able to avoid this but there is no indication that the patients or the assessors could distinguish

between the other two conditions, [STNmono] and [STN+SNr], as they controlled similarly for segmental symptoms and did not cause remarkable side effects or any ‘sensations’ the patients could feel, due to the titration of stimulation parameters. After finally programming the first condition, we waited 30 minutes before starting with the clinical testings to avoid carry-over effects. Then, all the clinical testings were performed. Afterwards, according to the allocation code, the next stimulation setting was introduced. Again 30 minutes of waiting were maintained, the clinical testings were performed for a second time and eventually the last stimulation setting was programmed and the same process was repeated. Finally, besides the clinical endpoint assessment, anamnestic measures were performed one time during this first testing session. The questions referred to motor symptoms, non-motor symptoms and quality of life issues during the past four weeks. The exact content of the anamnestic part of the testing, just as the different clinical tests, are explained in the Clinical tests part of Chapter 3.4. Immediately after finishing the last endpoint assessment on the first testing day, the usual dopaminergic medication was taken by all patients. From then on, the ordinary and regular medication was taken without changes throughout the rest of the study duration, at least in most patients. After termination of the ‘immediate testings’, all twelve patients entered the 2 x 2 cross-over ‘3-week follow up’ phase of the study. The order of each treatment condition was randomised again and treatment was crossed after three weeks, respectively. The stimulation parameters remained, if possible, constant throughout the following three weeks. At the end of the first ‘3-week follow-up’ period, the first endpoint assessment (visit 1) took place. It consisted of the same testings performed during the first part of the study, the ‘immediate testing’. Stimulation settings were crossed after the clinical and anamnestic assessment (visit 1). Patients went home for another three weeks and finally came back for the second and last endpoint (visit 2). The study phase ended here for the participants of the study. They were asked during which of the two three-week phases they felt better and further stimulation settings were applied after consultation with the principle investigator of the study.

3.2.1. Randomisation

The Department of Medical Biometry of the University of Tuebingen developed a computer-generated randomisation list. Randomisation results were kept closed until statistical analyses were finalized.

3.2.2. Treatment

A large number of patients treated with conventional DBS of the subthalamic nucleus develop severe refractory gait disturbances after several years of initially successful therapy (Krack et al., 2003). The first steps to take when gait disturbances emerge consider reprogramming of the stimulation parameters. Concerning the optimisation of the subthalamic stimulation parameters, one option is to increase the stimulation amplitude. Unfortunately, the positive influence of increasing the amplitude is limited and amplitude increases may even worsen gait disturbances (Moreau et al., 2008). Another reprogramming technique refers to the already mentioned concept of so-called 'better side reduction' (Fasano et al., 2011). The reduction of the stimulation amplitude on the hemisphere controlling the lower extremity with longer step length ('better leg') may improve frequency and duration of FOG. The gait pattern becomes more symmetric through 'better side reduction' in patients with asymmetric step lengths and was found to have positive impact on FOG (Fasano et al., 2011). In this study, 'better side reduction' was considered prior to study enrolment.

Moreover, to ensure that every patient had best individual stimulation settings on the subthalamic electrode contacts [STNmono] before entering the study, DBS programming was optimised according to a standardised procedure (Weiss et al., 2011b). This optimisation included an accurate and careful titration of the stimulation parameters of the subthalamic contacts (the second or most upper rostral electrode contacts) in medication off as so-called monopolar review. As such, we started the titration on the very low level of 0.5 V and gradually added 0.5 V until a clinical effect was identified by the neurologist. The stimulation amplitude was further increased until slight and transient side effects occurred, such as blurred vision, paraesthesia or muscle contraction, indicating current-

spreading to the adjacent neuroanatomic structures, for instance the internal capsule.

This procedure is chosen to define the therapeutic width of the stimulation and to ensure well tolerable chronic stimulation parameters. The findings were recorded in the case report form (CRF). After this titration, optimal stimulation settings were defined and maintained during the whole study phase if possible.

An accurate titration of the nigral stimulation parameters was performed in the same session and recorded in the CRF.

Upon signing the informed consent and after performance of the mandatory monopolar review, the clinical testings started.

To our knowledge and based on the current literature, a constant stimulation period of three weeks is sufficient to avoid carry-over effects of a prior stimulation setting (Chastan et al., 2009). Clinical effects of STN-DBS usually occur within a short period of time ranging from a few seconds to a few hours. The quick appearance of motor symptoms after switching off the stimulator could be shown recently in another study (Cooper et al., 2013) and we could notice them also in this trial. Consequently, we performed the endpoint assessments after three weeks of adaption to a special stimulation setting.

We did not consider a ‘3-week follow-up’ period with only nigral stimulation, as other studies demonstrated this would not sufficiently control segmental symptoms, such as tremor, bradykinesia and rigidity (Chastan et al., 2009).

3.2.3. Blinding

In this double-blind randomised controlled clinical trial, the patients, the endpoint assessors and all other participants of the study were kept blinded to treatment allocation. Only the principle investigator (D.W.) was not masked. The principle investigator stored the allocation code and kept it strictly closed until all statistical data analyses were finished. Stimulation parameters were changed several times between [STNmono] and [STN+SNr] before maintaining the allocated treatment parameters as randomised in order to maintain the blinding to the treatment condition for the patients and the endpoint assessors. Programmings were performed by the principle investigator before baseline and immediate testing and

follow-up visits. The principle investigator did not conduct any endpoint assessments. The clinical endpoint assessment was accomplished by an expert neurologist who was trained on PD and DBS treatment.

3.3. Outcome measures

The primary endpoint of this clinical trial was to investigate the impact of combined interleaved stimulation [STN+SNr] on composite axial motor symptoms at '3-week follow-up'. Therefore, a broad-scaled primary outcome measure was defined consisting of a composite 'axial score', built from eight items of the anamnestic UPDRS II and the clinical UPDRS III. The evaluation of the primary endpoint took place after three weeks of constant [STNmono] and [STN+SNr] stimulation respectively in a randomised order (Weiss et al., 2013). The chosen items from the UPDRS II and III cover a broad spectrum of axial motor symptoms such as falling unrelated to freezing, freezing when walking, walking (UPDRS II items 13-15), arising from chair, posture, gait, postural stability, body bradykinesia and hypokinesia (UPDTS III items 27-31). All items were 5-point rated and represented by the numbers 0 to 4. The 'axial score' was the sum of these eight items with a possible range from 0 to 32 points. Higher scores represented higher levels of impairment on different axial motor domains.

The secondary endpoint assessments consisted of a variety of clinical and anamnestic tests, which allowed a differentiated assessment of specific axial motor symptoms. The clinical ratings for these secondary efficacy variables were obtained at 'baseline' and at the 'immediate testings' as well as at the '3-week follow-up' in all treatment conditions (Weiss et al., 2013).

As three of the eight items of the composite 'axial score' (UPDRS II, items 13-15) represent anamnestic information, and as the 'baseline' condition and the 'immediate testings' were separated by only 30 minutes, the score of these items would not respond so quickly and was therefore assessed only once during the 'baseline' and 'immediate testing' session.

The secondary clinical endpoint testings assessed axial motor function by means of the clinical UPDRS III (items 27-31), FOG in terms of the Freezing of Gait Assessment Course (Ziegler et al., 2010), balance with the Berg Balance Scale

(Berg et al., 1992), and gait with the timed walking test from CAPSIT-PD (Defer et al., 1999). Further secondary self-reporting scores assessed gait impairment related to FOG with the Giladi Freezing of Gait Questionnaire (Giladi et al., 2009), quality of life with the PDQ-39 (Jenkinson et al., 1997), non-motor symptoms with the Non-motor Symptoms Scale (Storch et al., 2010) and neuropsychiatric symptoms with the Beck's Depression Inventory (Beck et al., 1961) and the Barratt Impulsiveness Scale (Preuss et al., 2008).

The aim of the secondary endpoint assessment is explorative data analysis and the results were assessed only for descriptive purpose, not for hypothesis testing or for generating evidence for efficacy.

Concerning the statistical evaluation of the primary endpoint only the changes after the '3-week follow-ups' were of importance.

3.4. Clinical tests

In this trial, we evaluated a broad spectrum of axial motor symptoms to detect any amelioration. Therefore, several tests were used to assess different kinds of symptoms.

The primary endpoint was defined as a change in the **'axial score'** after three weeks of constant stimulation. This score was built from three items of the anamnestic **UPDRS II (items 13-15)** concerning FOG and walking and five items of the clinical **UPDRS III (items 27-31)** concerning rising from chair, posture, gait, postural stability, body bradykinesia and hypokinesia.

The differentiated assessment of the secondary clinical endpoints contained the **UPDRS III (items 27-31)** for testing axial motor symptoms, the **Berg Balance Scale** for testing balance, the timed walking test from the Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease (**CAPSIT-PD**) for evaluating gait, the **Freezing of Gait Assessment Course (FOG-AC)** for testing FOG. These mentioned clinical ratings were obtained in all treatment conditions during 'baseline', 'immediate testing' and the '3-week follow-up'. Furthermore, some anamnestic tests were taken during the assessment of the secondary endpoint ('baseline' and '3-week follow-up), such as the **Giladi Freezing of Gait Questionnaire** to evaluate FOG, the **Parkinson's Disease**

Questionnaire (PDQ-39) to assess quality of life, the **Beck's Depression Inventory** (BDI) and the **Barratt Impulsiveness Scale** (BIS) to evaluate neuropsychiatric symptoms and the **Non-Motor Symptoms Scale** (NMSS) to assess non-motor symptoms.

More detailed information about the diverse tests is appended in the Attachment.

3.5. Electrode placement

To assess specifically the effects caused by additional stimulation of the SNr, it was of great importance to characterise the localisation of the electrode contacts. The electrode localisation, namely the correct position of the active STN and SNr contacts, were determined and verified by coregistration analysis. In brief, pre-surgical MR scans were coregistered with post-surgical MR scans and the coordinates of the lowest electrode contacts lying in the border zone of the STN and SNr were determined. From this point, the coordinates of the second most upper electrode contact, lying within the STN region, could be determined. Figure 10 shows the result of the visualisation technique used. The determined coordinates within the STN and SNr were integrated in a coronal image of the Atlas of the Human Brain (Mai et al., 1997) by visualizing the current spread within the activated tissue. Using the Atlas of the Human Brain as reference allows to approximate the correct localisation as this atlas provides accurate delineations of all brain structures. However, we are also aware that such atlas-derived location analysis has immanent limitations, such as interindividual neuroanatomic variability and others (Yelnik et al., 2007).

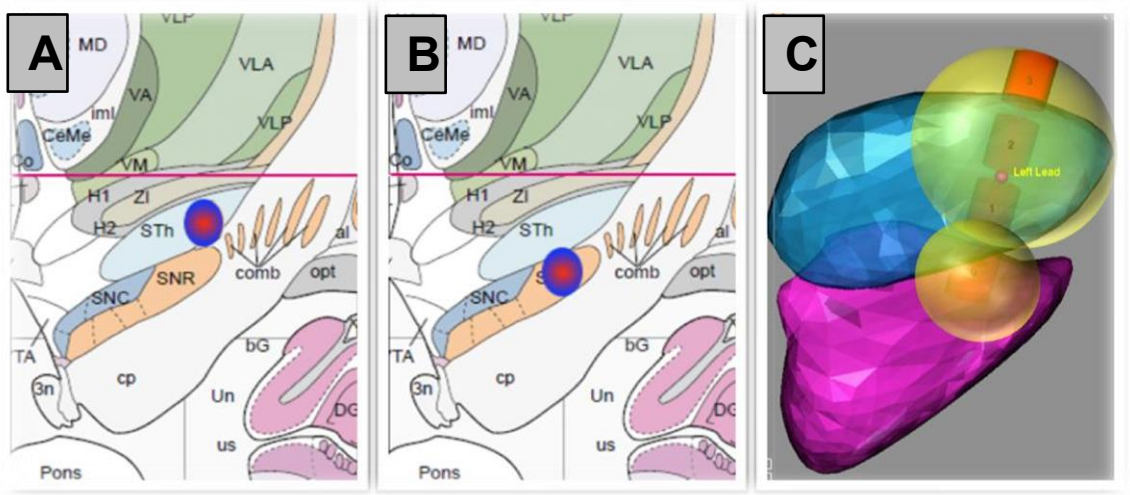

Figure 10 Visualization of the (A) second upper active electrode contact within the dorsolateral STN and of the (B) lowest active electrode contact within the dorsolateral SNr on a coronal view of the Atlas of the Human Brain (Mai et al., 1997). (C) Illustrative image of the STN (blue) and the SNr (purple) with the electrode (orange) and a simulation of the stimulation field (yellow) based on work by Yelnik et al. 2007 and D'Haese et al. 2012 provided by Medtronic.

3.5.1. Imaging methods

The active electrode contacts were localised within the STN and the SNr by coregistration analyses (Matlab 7.0, Nattick, USA) and the open-source toolbox spm5 (Statistical Parametric Mapping). In the following, the main four steps of the electrode determination are explained.

First, pre-surgical 3D T1-weighted MP-RAGE MR scans, as well as post-surgical 3D T1-weighted FLASH MR scans of the patients' brains were uploaded with the spm program for visualisation. As target structures in deep brain areas are usually determined under specification of coordinates, the first step after uploading the MR scans was to determine the point of origin of a coordinate system within the BG structures. The coordinate system itself is defined in the pre-surgical MR scans with the help of distinct field markers within the brain tissue. The midpoint of the line between the anterior commissure (AC) and the posterior commissure (PC) on the sagittal scan is the midcommissural point (MCP). In general, the MCP serves as zero point for the determination of electrode localisations in images of the brain. Figure 11 shows an MR scan with AC, PC and MCP marked in a coordinate system. The AC-PC line is one of the axes of the coordinate system.

The other two axes are determined by the symmetrical plane of the brain and the perpendicular to that plane. The challenge of this step is the fact that the identifiable field markers (AC and PC) are determined manually which may yield some investigator-dependent variability.

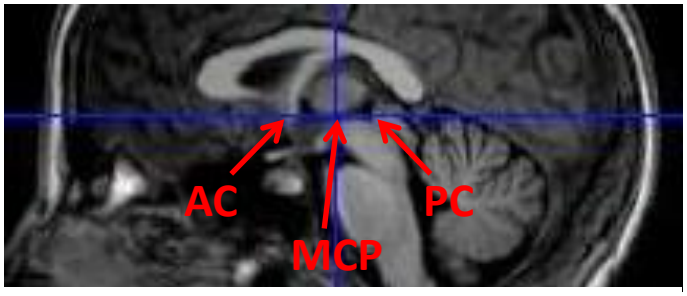

Figure 11 Localisation of AC, PC and MCP in a sagittal T1-weighted MR scan.
AC = anterior commissure, PC = posterior commissure, MCP = midcommissural point.

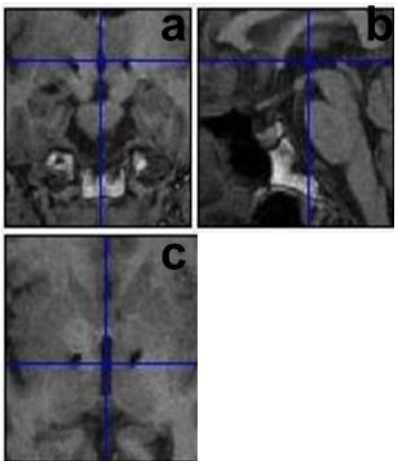

Figure 12 Postoperative MR scan showing MCP in all three planes
a) coronal b) sagittal c) axial.

After defining the coordinate system, the pre-surgical MR scans had to be coregistered with the MR scans of a reference brain with the aim to find and erase skew positions of the head. Hence, the purpose of this coregistration with the reference brain was to eliminate possible tilts and rotations in all three planes of the pre-surgical MR scans. In the next step, the pre-surgical MR scans served as reference data and the post-surgical scans were coregistered. This step guaranteed the correct position of the post-surgical MR scans. Between the steps, it was important to check the position of the MCP and to correct it manually, if necessary. Next, the contacts of the lowest electrode contact were determined on both sides of the brain, namely the contact within the border zone of the STN and the SNr. As the lowest contact of the electrode begins only 0.5 mm from the tip and the electric stimulation field spreads some millimetres around the contact, the tip of the electrode approximates the lowest contact and represents the coordinates of it (McIntyre et al., 2004, Butson et al., 2007).

This was performed on both hemispheres of the brain. Figure 13 illustrates postoperative MR scans with the artefact caused by the electrode localised on the level of the lowest contact within the border zone of STN and SNr.

Based on the coordinates of the lowest electrode contact, the coordinates of the second upper electrode contact lying in the STN were determined by using the mathematic formula to calculate the Euclidean distance between two points. The Euclidean vector was represented by the electrode itself.

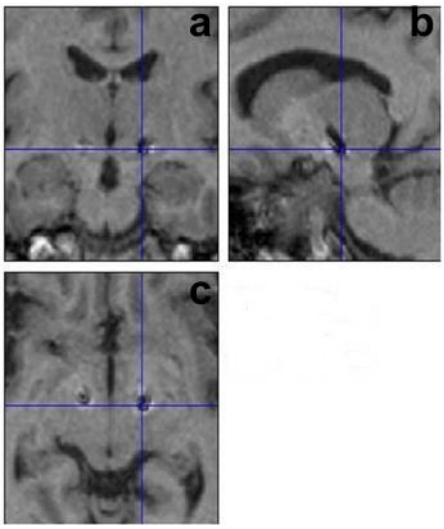

Figure 13 Postoperative MR scan showing the artefact caused by the electrode implantation and the determination of the coordinates of the lowest electrode contact in all three planes. a) coronal b) sagittal c) axial.

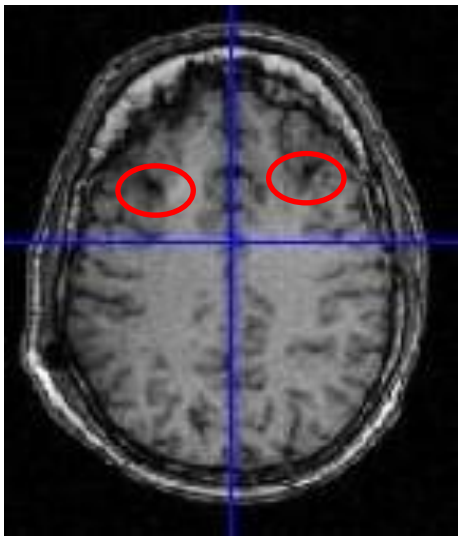

Figure 14 Axial post-surgical MR scan showing two determined points on the top of the electrode.

Figure 14 demonstrates a postoperative MR scan with two electrode generated lesions localised at the level of the top of the electrode.

Assuming that point P $\begin{pmatrix} a \\ b \\ c \end{pmatrix}$ equates to the determined lowest electrode contact within the SNr and point Q $\begin{pmatrix} x \\ y \\ z \end{pmatrix}$ equates to a point which is as far away from P as possible, the directional vector of the straight line representing the electrode reads as follows:

$$\overrightarrow{PQ} \begin{pmatrix} x-a \\ y-b \\ z-c \end{pmatrix}.$$

The length (l) of the directional vector is calculated with the following formula:

$$l = \sqrt{(x-a)^2 + (y-b)^2 + (z-c)^2}.$$

As the second upper electrode contact is expected to lie within the STN and as the structure of the electrode is known, the second upper electrode contact must have a distance of 4.75 mm from point P. Figure 15 illustrates a model of an electrode.

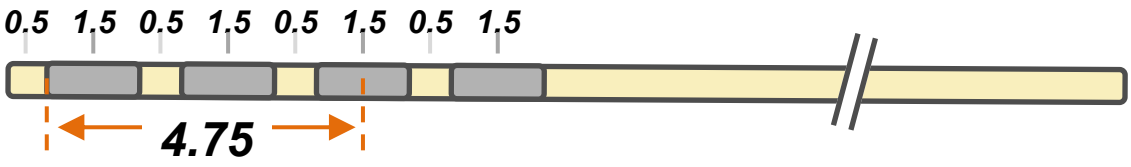

Figure 15 Structure of an electrode; electrode contacts measure 1.5mm, interspaces measure 0.5mm, distance between the beginning of the lowest electrode contact to the middle of the second upper contact measures 4.75 mm.

To finally determine the coordinates of the point S, lying within the STN, the factor *t* has to be determined and multiplied with the directional vector $\overrightarrow{PQ}$:

4,75 = t * l

$$t = \frac{4{,}75}{l}$$

$$S = P + t * \overrightarrow{PQ} = \begin{pmatrix} a \\ b \\ c \end{pmatrix} + t * \begin{pmatrix} x-a \\ y-b \\ z-c \end{pmatrix} = \begin{pmatrix} a + t*(x-a) \\ b + t*(y-b) \\ c + t*(z-c) \end{pmatrix}$$

The MR scans of all twelve patients underwent this process of coordinate determination and the results of the mean coordinates were visualised in a coronal image of the Atlas of the Human Brain (Mai et al., 1997). The coordinates of the right SNr in the three-dimensional space were 12.1 ± 1.3 mm medio-lateral (x-coordinate), -3.3 ± 1.7 mm anterio-posterior (y-coordinate) and -5.8 ± 1.5 mm rostro-caudal (z-coordinate) to MCP. For the left SNr, the x-coordinate was -10.0 ± 0.9 mm, the y-coordinate was -3.4 ± 2.1 mm and the z-coordinate was -6.4 ± 1.8 mm. The coordinates for the right STN were 13.5 ± 1.1 mm (x-coordinate), -0.5 ±1.7 mm (y-coordinate) and -2.2 ± 1.5 mm (z-coordinate). The left STN had the coordinates -11.4 ± 0.8 mm (x), -0.9 ± 2.0 mm (y) and -3.0 ± 1.7 mm (z).
An overview of the computed positions of the electrode contacts within the SNr and the STN in relation to MCP is presented in Table 2.

Regarding the depth of the lowest active contact on both sides within the SNr (y-coordinate in the SNr) and the electric potential spread around the electrode in all directions, which we visualised on coronal images on the Atlas of the Human Brain (Mai et al., 1997) (Figure 10), we can approximate that the lowest active contact of the electrode stimulated the SNr. Hence, the additional effects and clinical benefits of the interleaved stimulation compared to the effects and benefits of the conventional STN-DBS are attributable to the effect the current has on the neuronal tissue of the SNr.

Similar coordinates for the SNr were found by Chastan et al. when testing the effects of nigral stimulation on postural stability and locomotion in Parkinson's patients (Chastan et al., 2009). However, there is a difference concerning the laterality in our study, as the SNr coordinates are more lateral in our patient cohort.

Table 2 Electrode placement in relation to MCP in all twelve patients; SNr representing the localisation of the lowest electrode contact, STN representing the localisation second upper electrode contact. SD = standard deviation, x = medio-lateral, y = anterio-posterior, z = rostro-caudal. STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

	SNr right			SNr left			STN right			STN left		
Patient	**x**	**y**	**z**	**x**	**y**	**z**	**x**	**y**	**z**	**x**	**y**	**z**
1	11.8	-2.5	-5.9	-9.4	0.0	-5.0	13.0	0.6	-2.6	-10.5	-0.3	-1.7
2	10.7	-3.6	-7.7	-9.5	-3.4	-9.8	12.3	-0.8	-3.6	-10.9	-0.5	-6.1
3	11.8	-2.8	-4.7	-10.8	-2.4	-4.3	13.1	0.1	-0.9	-11.5	0.7	-0.8
4	14.3	-3.9	-3.6	-10.5	-2.9	-4.7	15.7	-1.1	-0.1	-12.7	0.0	-1.4
5	13.6	-5.9	-5.7	-11.3	-6.3	-7.3	14.8	-3.6	-1.4	-12.5	-4.2	-3.5
6	10.4	-2.7	-3.6	-9.8	-2.9	-4.8	11.8	0.3	-0.6	-11.5	0.2	-1.2
7	11.4	-1.4	-7.0	-10.4	-2.9	-4.5	13.5	1.7	-3.9	-11.8	0.6	-1.4
8	12.2	-3.6	-8.8	-8.1	-7.6	-7.3	13.8	-0.6	-5.0	-9.8	-5.2	-3.8
9	11.4	-5.8	-6.4	-9.4	-5.0	-6.6	12.9	-2.4	-3.5	-10.8	-1.5	-4.3
10	14.1	-2.9	-5.5	-11.1	-1.8	-7.0	14.6	-0.6	-1.8	-11.9	0.6	-3.0
11	12.9	-4.7	-5.7	-9.8	-1.6	-7.3	13.8	-2.3	-1.8	-11.4	0.8	-3.3
12	11.1	-0.2	-5.4	-10.0	-4.2	-8.5	12.6	2.2	-1.7	-11.7	-1.4	-5.1
SD	1.3	1.7	1.5	0.9	2.1	1.8	1.1	1.7	1.5	0.8	2.0	1.7
Mean	12.1	-3.3	-5.8	-10.0	-3.4	-6.4	13.5	-0.5	-2.2	-11.4	-0.9	-3.0
Rounded	**12**	**-3**	**-6**	**-10**	**-3**	**-6**	**14**	**-1**	**-2**	**-11**	**-1**	**-3**

3.5.2. Stimulation parameters

All patients in this study were implanted with the Activa® impulse generator invented by Medtronic. The Activa® impulse generator allows for interleaved programming due to its advanced technology. It was available at our study site since 2009 and some of the enrolled study patients had the Kinetra® impulse generator implanted first, which was changed to the Activa® impulse generator after battery depletion. All patients were treated with pre-existing intracranial electrode localisation and were not reimplanted in order to participate in this study.

The electric field and especially the spread of the electric field within the neuronal tissue generated by deep brain stimulation depends on several factors, such as the location of the active electrode contacts, the distribution of the electric field and the composition of the tissue surrounding the electrode (Kuncel and Grill, 2004). Besides these factors, the correct setting of the stimulation parameters is important to address the diverse symptoms of PD. As described earlier, there are several stimulation techniques and different possibilities for varying the electrode geometry. In this clinical trial, the patients underwent a careful adjustment of the electrode programming prior to study enrolment. According to the pre-defined study-protocol (Weiss et al., 2011b), the stimulation parameters were defined by following several aspects to ensure the best individual STN-DBS. This included i) an optimal control of the segmental motor symptoms, such as tremor, bradykinesia and rigidity, which was determined by testing different amplitudes and determining the threshold for the clinical effects and side effects. This 'titration' for effects and side effects was done for the SNr as well. Possible effects on the upper contacts within the STN are improvement of segmental motor symptoms, such as a reduction of rigidity. Side effects that can potentially occur are face contraction or dysarthria due to the spread of the electric field to the capsula interna. Possible effects occurring when stimulating only the lowest active electrode contacts within the SNr are reduction of tremor, less rigidity, gait improvement, reduction of FOG, greater step length or no acute effect. Side effects potentially occurring when stimulation is delivered only on the lowest active electrode contacts within the SNr, namely during nigral titration, can

present as diplopia, blurred vision, brachiofacial dystonia, heaviness of eyelids, fatigue, vertigo, dysarthria, globus sensation, face contraction or paresthesia of the face and limbs (Chastan et al., 2009).

Another important aspect to ensure optimal individual stimulation parameters was ii) a sufficient topographical distance between the active contact within the STN and the border zone of the SNr, to avoid current spread to the SNr and consequently incorrect results concerning the clinical effects of conventional STN-DBS. This was ensured on the one hand by checking the patients' postoperative MR-scans. An experienced neurologist of the study group estimated the distance together with a neuroradiologist of the University of Tuebingen. On the other hand, the rostral electrode contacts 2 (second upper contact within the left STN) and 10 (second upper contact within the right STN) were predominantly used for STN-stimulation. iii) The prestudy programming envisaged the concept of 'better side reduction'. This concept provides a programming algorithm for STN-DBS by adjusting the stimulation voltage on both sides of the brain and thus improving symmetry and coordination of gait in PD patients (Fasano et al., 2011). In patients with poor leg symmetry, this programming algorithm was implemented in the programming session before entering the study.

The optimal control for segmental motor symptoms was checked and confirmed again after entering the study in the 'baseline' testing and the 'immediate testing' session after overnight withdrawal of dopaminergic medication. This was done very carefully and according to best clinical practice. The stimulation parameters of the [STNmono] condition, namely the parameters of the active dorsolateral STN, were maintained when introducing the [STN+SNr] condition.

The best individual stimulation settings remained unchanged throughout the whole study in most patients.

Two patients, PD2 and PD9, needed reprogramming of the nigral electrode contacts due to dyskinesias that occurred a few days after introduction of the [STN+SNr] condition in the '3-week follow-up' (Weiss et al., 2013). According to the intention to treat principle, which means that the outcome of these patients was still considered as equal part of the [STN+SNr] '3-week follow-up' condition,

the programming was adjusted. In PD2 the stimulation amplitude was lowered on both active nigral contacts by 0.4 V and thus reduced to 0.7 V. Bothersome dyskinesias disappeared after reprogramming. PD9 reduced his daily levodopa medication dosage by 125 mg based on his own discretion and experience and contacted the study site. The dosage reduction ameliorated the dyskinesias but as they did not disappear completely, the patient was rescheduled by the principle investigator of the study. The stimulation amplitudes of the nigral contacts were lowered by 0.1 V to 1.1 V. With this adjustment of the stimulation settings, bothering dyskinesias were satisfactorily resolved. Both patients subsequently completed the '3-week follow-up' period.

Concerning the electrode geometry, one can see in Table 3 that almost all patients received similar programming settings. In most patients, the best symptom control was reached with a monopolar or bipolar stimulation setting. In PD6, the resting tremor of the right upper extremity was quite insistent and required bipolar programming of the left STN with two negative contacts, the second upper active electrode contact 2 and the most upper active contact 3. With this stimulation setting, high stimulation amplitudes up to 5.7 V could be applied without causing side effects. In this patient, monopolar settings were limited effective as side effects were induced at 3.5 V without maximal tremor suppression. The amplitudes, pulse widths and the diverse polarities on all active contacts within the STN and SNr are presented in Table 3.

Table 3 Stimulation Parameters of the active electrode contacts in all twelve patients.
2- = second upper left electrode contact as cathode, 3- = most upper left electrode contact as cathode, C+ = pulse generator case as anode, 2+ = second upper left electrode contact as anode, 1+ = second lowest left electrode contact as anode, 3+ = most upper left electrode contact as anode, 11- = most upper right electrode contact as cathode, 10- = second upper right electrode contact as cathode, 10+ = second upper right electrode contact as anode, 0- = lowest left electrode contact as cathode, 8- = lowest right electrode contact as cathode, 9+ = second lowest right electrode contact as anode; PD2* and PD9* needed reprogramming of the SNr-voltage due to dyskinesias. Hz = hertz, V = voltage, µsec = microsecond, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

Patient	Frequency (Hz)	Amplitude (V)				Pulse width (µsec)				Polarity			
		Left STN	Right STN	Left SNr	Right SNr	Left STN	Right STN	Left SNr	Right SNr	Left STN	Right STN	Left SNr	Right SNr
PD1	125	4.0	3.2	1.0	1.0	90	60	60	60	2- C+	11- 10+	0- C+	8- C+
PD2*	125	3.3	1.4	0.7	0.7	60	60	60	60	3- 2+	10- C+	0- C+	8- C+
PD3	125	2.2	2.4	3.0	3.0	120	120	60	60	2- C+	10- C+	0- C+	8- C+
PD4	125	3.8	1.4	0.7	0.7	90	90	60	60	2- C+	10- C+	0- C+	8- C+
PD5	125	4.6	2.7	1.3	1.3	90	60	60	60	2- 3+	10- C+	0- C+	8- C+
PD6	125	5.7	3.2	0.8	0.8	180	60	60	60	3- 2-1+	10- C+	0- C+	8- C+
PD7	125	3.7	3.5	1.8	1.8	120	120	60	60	2- C+	10- C+	0- C+	8- C+
PD8	125	4.3	3.6	1.3	1.3	60	60	60	60	3- 2+	11- C+	0- C+	8- 9+
PD9*	125	2.2	1.7	1.2	1.2	60	60	60	60	2- C+	10- C+	0- C+	8- C+
PD10	125	2.7	1.6	2.2	2.2	60	60	60	60	2- C+	10- C+	0- C+	8- C+
PD11	125	3.3	2.8	1.5	1.5	60	60	60	60	2- C+	10- C+	0- C+	8- C+
PD12	125	4.0	2.8	1.5	1.5	120	120	60	60	2- 3+	10- C+	0- C+	8- C+

3.6. Data management

In the time from January 2011 to June 2012, patient screening was performed by the principle investigator. If all inclusion criteria were fulfilled and none of the exclusion criteria applied to the patients, they could be enrolled after signing the written informed consent. Personal data and information about the medical history of the patients as well as the whole data collected during the diverse visits were documented in paper form in the CRF immediately after the assessment. The necessary recordings were documented only by authorized investigators and monitored for completeness and correctness. The study software *koordobas* was used for data management. *koordobas* is an Oracle-based application of the Department of Medical Biometry of Tuebingen. The complete data was entered to *koordobas* by two authorized and independent staff members and

automatically reviewed by the program for completeness, errors and inconsistency. Differences were corrected in a reproducible way. The study data base consisted only of complete and correct data.

All study material will be stored for at least ten years in the archive of the Department of Neurodegenerative Diseases of the Centre of Neurology, Tuebingen. The storage, the processing and the deletion of all person related data was and will be performed according to the German law.

3.7. Data evaluation and statistical analysis

The statistical analyses were carried out by the Department of Biometry of the University of Tuebingen.

For the confirmatory statistical analysis, the primary endpoint was the difference in the ‘axial score’ (UPDRS II items 13-15 and UPDRS III items 27-31) of the two stimulation conditions [STNmono] and [STN+SNr] at ‘3-week follow-up’.

A minimum sample size of ten patients was calculated to be sufficient to detect an effect, namely a difference of four points on the primary outcome measure (the range across the eight items was 0 - 32).

A two-sided paired t-test with $\alpha = 0.05$ was applied on the null hypothesis, based on the assumption that the data was normally distributed and stating that the two stimulation conditions had the same effect (Weiss et al., 2011b).

Statistical power and sample size calculations were determined with the statistical software NQuery Advisor 7.0.

In order to align the sample size to an expected drop-out of two patients, twelve patients were enrolled in this study (Weiss et al., 2011b). To avoid that a possible period effect could confound the results, the primary outcome measures and all secondary outcomes underwent a control for period effects. Therefore, a comparison of the sum of the scores in the two treatment periods was performed using the unpaired t-test (Wellek and Blettner, 2012).

Normal distribution was verified with the Shapiro-Wilk test. Differences which were not normally distributed ($P < 0.05$ of Shapiro-Wilk test) were analysed using the sign test.

Given the small sample size and some clinical heterogeneity concerning the time since DBS implantation and the disease duration in our cohort, non-parametric statistical testing on the primary endpoint was additionally performed using a sign test. Of note, the endophenotypic spectrum of idiopathic PD involves the typically observed variable disease duration and accordingly the variable time since DBS implantation in our cohort.

All analyses of the secondary outcome were descriptive and no confirmatory interpretation was drawn from the results. Secondary endpoints without normal distribution were analysed using a sign test. This was necessary for the 'Non-motor Symptoms Scale', the 'CAPSIT-PD' and the 'Berg Balance Scale'.

In the result section, the measurements are presented using tables and box plots under specification of the mean ± standard deviation for parametric tests and the median with range for non-parametric tests as well as the two-sided p-values without adjustments.

3.8. Safety

Several safety measures were carefully observed in order to protect the participants from any undesired harm. All safety issues were recorded in the CRF. The endpoints of safety were (i) death as a serious adverse event, (ii) severe exacerbation of pre-existing relevant gait disturbances and falls due to aggravated FOG or imbalance. This was evaluated with the items 13 and 14 of the UPDRS II and with item 30 of the UPDRS III. (iii) A worsening of segmental motor symptoms like tremor or rigidity were evaluated with item 20-26 of the UPDRS III. More frequent motor fluctuations were reported by means of the UPDRS IV. (iv) New occurrence or worsening of pre-existing depressive symptoms were evaluated with the BDI, suicidal tendency with item 9 of the BDI, hallucinatory behaviour and psychosis with item 2 of the UPDRS I and impulsivity with the BIS.

4

4. Results

Out of 28 screened patients, twelve were eligible for study participation. These patients were enrolled between January 2011 and June 2012 at the Centre for Neurology, Department for Neurodegenerative Diseases of the University of Tuebingen. In the following, the results for the different endpoints and for all testings are described in detail and illustrated with box plots. A general overview of all study results is given in Table 4 for the 'baseline' and 'immediate testing' and in Table 5 for the results of the '3-week follow-up'.

Table 4 Results of the 'baseline' and 'immediate testing'.
[STNmono] = conventional subthalamic stimulation, [STN+SNr] = combined stimulation of STN and SNr, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, UPDRS = Unified Parkinson's Disease Rating Scale, FOG-AC = Freezing of Gait Assessment Course, CAPSIT = timed walking test from the Core Assessment Program, [a] t-Test, [b] Sign-Test, § Median (Min-Max).

	'baseline'	'immediate testing'		
	[MedOffStimOff]	[STNmono]	[STN+SNr]	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]

Table 5 Results of the '3-week follow-up'.
[STNmono] = conventional subthalamic stimulation, [STN+SNr] = combined stimulation of STN and SNr, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, UPDRS = Unified Parkinson's Disease Rating Scale, FOG-AC = Freezing of Gait Assessment Course, CAPSIT = timed walking test from the Core Assessment Program, FOG-Q = Freezing of Gait Questionnaire, PDQ-39 = Parkinson's disease questionnaire, BDI = Beck's Depression Inventory, NMSS = Non-motor Symptoms Scale, [a] t-Test, [b] Sign-Test, § Median (Min-Max).

	'baseline'	'3-week follow-up'		
	[MedOffStimOff]	[STNmono]	[STN+SNr]	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]
Barratt 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]

4.1. Primary outcome measure

At ‘baseline testing’ (medication off, stimulation off), all enrolled patients presented with severe axial motor symptoms, which is reflected in the ‘axial score’. Patients attained 17.25 ± 4.31 points at ‘baseline testing’. No statistically significant difference could be detected on the ‘axial score’ between the conditions [STN+SNr] and [STNmono] after the ‘3-week follow-up’. After three weeks of constant [STN+SNr] stimulation, patients attained 13.42 ± 6.47 points on the ‘axial score’ and after three weeks of constant [STNmono] stimulation, patients presented with 14.25 ± 5.75 points on the ‘axial score’. This resulted in an effect of 0.83 ± 3.86. The 95% confidence interval of the arithmetic mean of the effect ranged from -1.62 to 3.82 and the p-value resulted in p = 0.47. An additional non-parametric analysis of the primary endpoint, using the sign-test, confirmed the statistical findings of the parametric analysis and reassured that the findings of the primary endpoint were not statistically significant. Figure 16 illustrates the box plots of the primary endpoint.

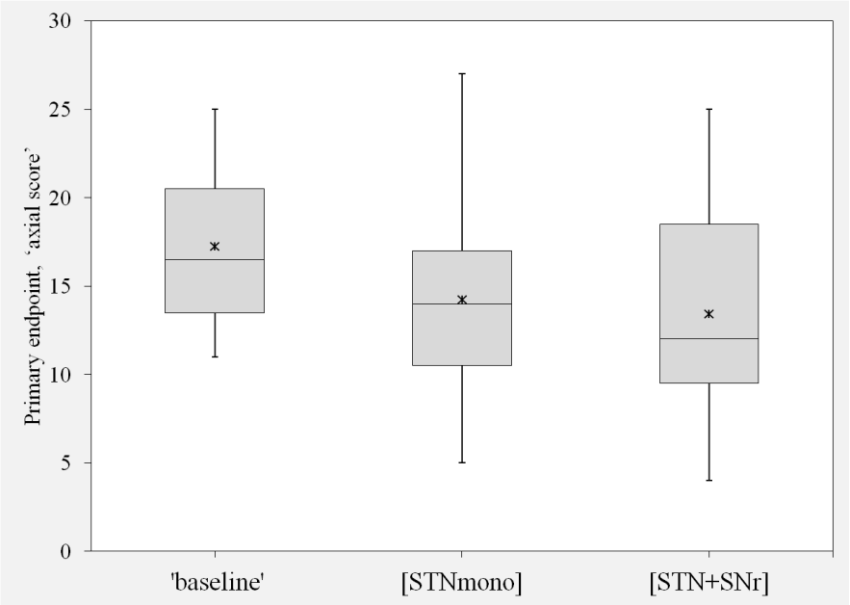

Figure 16 Box plots of the primary endpoint at 'baseline' and '3-week follow-up'.
x-axis = therapeutic condition, y-axis = 'axial score', [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

For the evaluation of the primary endpoint, it is necessary to consider the already mentioned fact that four patients wished to discontinue treatment prematurely during the conventional stimulation of the STN [STNmono]. PD 3 after three hours of standard STN stimulation, PD 7 after 19 days, PD 10 after two days and PD 11 after nine days. All these patients completed the entire [STN+SNr] treatment. Three of these patients (PD 3, PD 10, PD 11) were randomised first to the combined treatment of STN and SNr [STN+SNr]. Table 6 shows the number of points of the individual 'axial score' of these patients after the premature discontinuation of the standard STN treatment [STNmono] and after the completed three weeks of combined stimulation of STN and SNr [STN+SNr].

Table 6 Comparison of the individual 'axial score' of patients who discontinued [STNmono] treatment prematurely.
[STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

	Primary endpoint [STNmono]	Primary endpoint [STN+SNr]
PD3	19	16
PD7	27	25
PD 10	13	9
PD 11	9	4

4.2. Secondary outcome measures

The secondary outcome measures were performed and analysed to detect possible effects on distinct axial motor domains which we did not necessarily expect prior to the study beginning. The results of the secondary endpoints are presented in the following box plots.

4.2.1. Immediate testing

The 'immediate testing' was performed during the same testing session as the 'baseline testing'. All testings were performed off medication [MedOff]. The aim was to detect short term effects of the two different stimulation settings [STNmono] and [STN+SNr].

According to the segmental **UPDRS III items 20-26**, similar results could be detected between [STNmono] and [STN+SNr] in the 'immediate testing'.

At 'baseline testing', patients achieved 38.0 ± 5.01 points. While stimulated with [STNmono], patients achieved 29.17 ± 6.62 points and with [STN+SNr] 27.58 ± 7.96 points. This resulted in an effect of 1.58 ± 3.4. The 95% confidence interval of the arithmetic mean of the effect ranged from -0.57 to 3.74 ($p = 0.13$). The box plots in Figure 17 illustrate the results of the UPDRS III items 20-26.

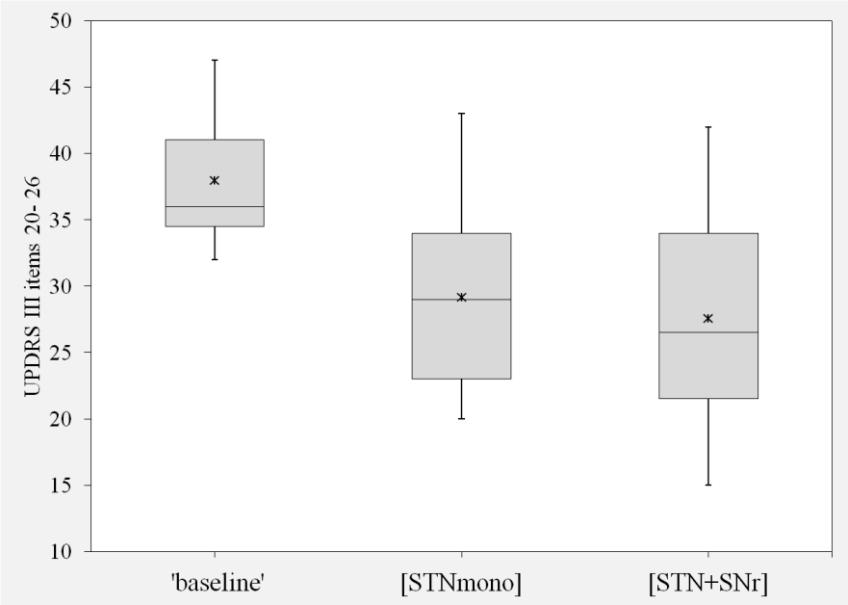

Figure 17 Box plots of the UPDRS III items 20-26 after 'immediate testing'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

Concerning the results of the axial **UPDRS III items 27-31,** a greater improvement could be detected with [STN+SNr] compared to [STNmono], which is reflected in the p-value.

The result of the 'baseline testing' was 11.17 ± 3.56 points. After switching to [STNmono], patients achieved 9.25 ± 4.67 points and with [STN+SNr] stimulation, this reduced to 8.17 ± 4.09 points. This resulted in an effect of 1.08 ± 1.62. The 95% confidence interval of the arithmetic mean of the effect ranged from 0.05 to 2.11 (p = 0.04). The box plots in Figure 18 illustrate the results of the UPDRS III items 27-31.

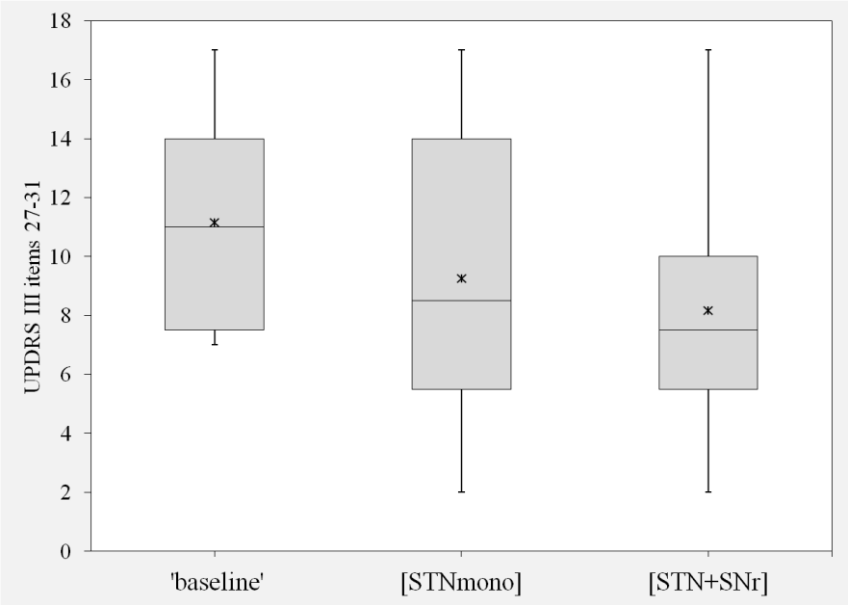

Figure 18 Box plots of the UPDRS III items 27-31 after 'immediate testing'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

The next box plots (Figure 19) show the results of the **Freezing of Gate Assessment Course**. At 'baseline testing', patients presented with severe FOG with 22.17 ± 11.74 points. During [STNmono], they obtained an average of 16.25 ± 12.78 points and during [STN+SNr] of 8.67 ± 10.92 points. This resulted in an effect of 7.58 ± 7.66. The 95% confidence interval of the arithmetic mean of the effect ranged from 2.72 to 12.45 (p = 0.01).

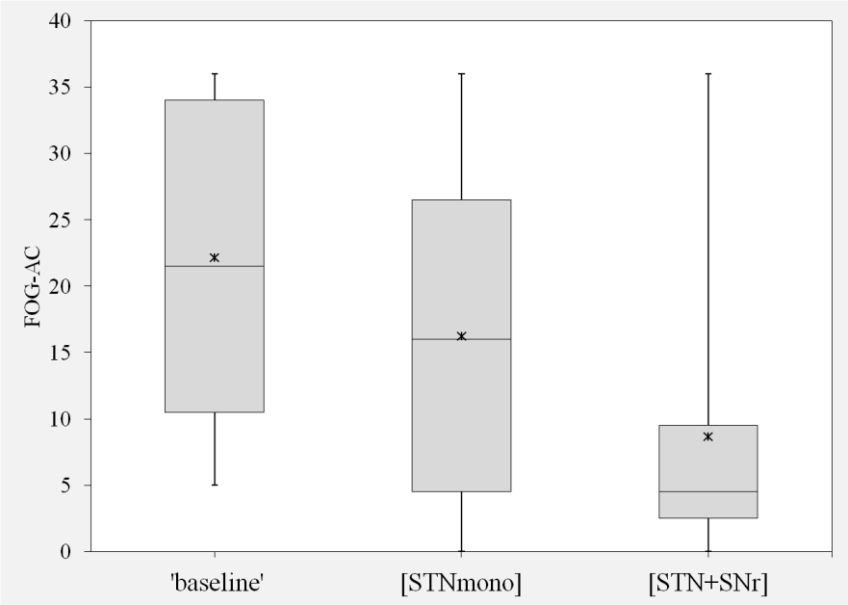

Figure 19 Box plots of the FOG-AC after 'immediate testing'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

The results of the **CAPSIT - PD timed walking test**, with the subdomains 'steps', 'time' and 'freezing' are shown in the next three figures (Figure 20, Figure 21 and Figure 22). In all subdomains, no relevant differences between the conditions could be observed at 'immediate testing'.

The effect concerning 'steps' results in 5.73 ± 10.85. The 95% confidence interval of the arithmetic mean of the effect ranged from -1.56 to 13.02 ($p = 0.55$). In the subdomain 'time', the calculated effect was 7.09 ± 17.64. The 95% confidence interval of the arithmetic mean of the effect ranged from -4.76 to 18.94 ($p = 0.75$). The result of the 'freezing' subdomain had an effect of 0.91 ± 3.02. The 95% confidence interval of the arithmetic mean of the effect ranged from -1.12 to 2.93 ($p > 0.99$).

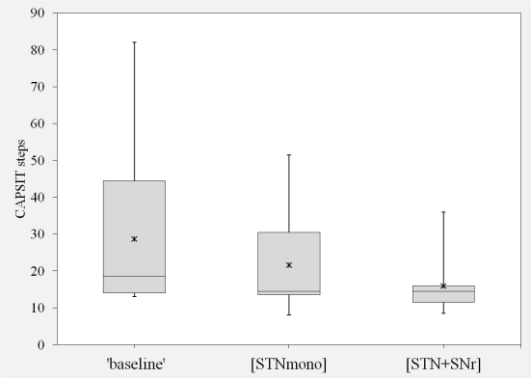

Figure 20 Box plots of the CAPSIT 'steps' after 'immediate testing'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

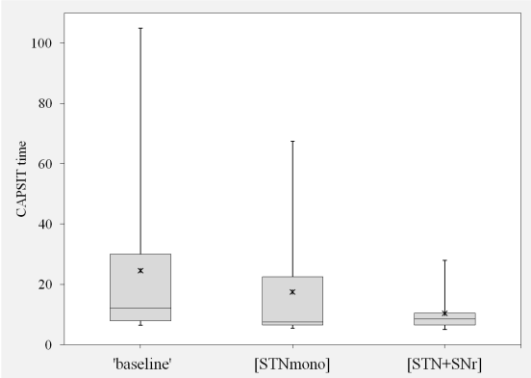

Figure 21 Box plots of the CAPSIT 'time' after 'immediate testing'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

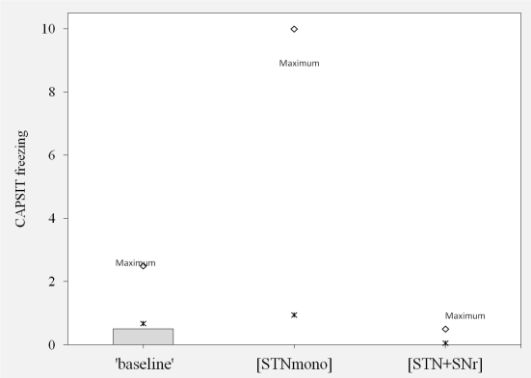

Figure 22 Box plots of the CAPSIT 'freezing' after 'immediate testing'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

In the **Berg Balance Scale**, patients achieved 41.5 (11 - 56)§ points in the 'baseline testing', 47 (15-56)§ points during [STNmono] stimulation and 50 (9 - 56)§ points during [STN+SNr]. This resulted in an effect of -1.5 ± 4.85. The 95% confidence interval of the arithmetic mean of the effect ranged from -4.58 to 1.58 (p = 0.31). Figure 23 shows the box plots for the Berg Balance Scale.

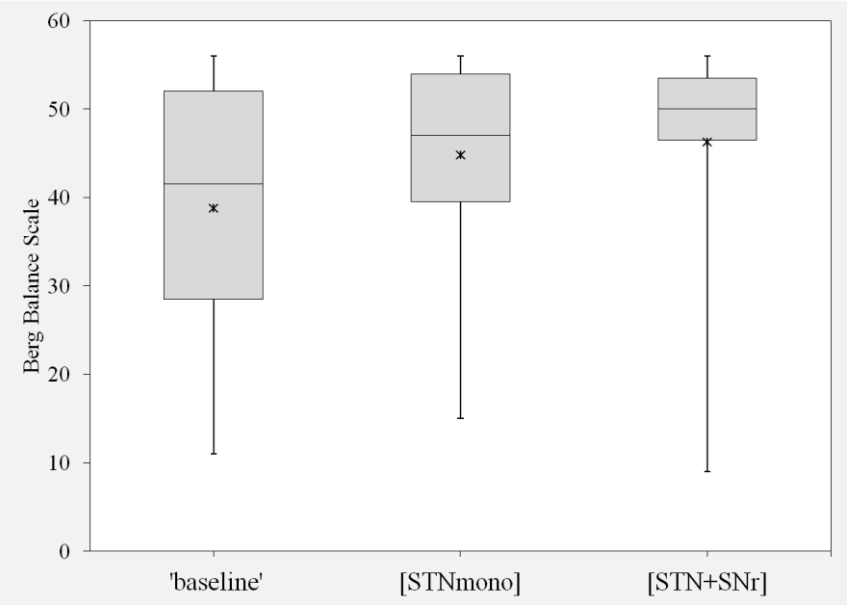

Figure 23 Box plots of the Berg Balance Scale after 'immediate testing'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

4.2.2. 3-week follow-up

4.2.2.1. Axial motor symptoms

In the 3-week follow-up, similar results could be detected between both conditions concerning the **UPDRS III items 27-31**, the subscore for 'axial' motor symptoms. Patients achieved 8.08 ± 4.01 points after three weeks of constant [STNmono] stimulation and 8.08 ± 4.38 points after three weeks of [STN+SNr] stimulation. This resulted in an effect of 0 ± 2.95. The 95% confidence interval of the arithmetic mean of the effect ranged from -1.88 to 1.88 (p = 1.0). Figure 24 illustrates the results of the UPDRS III items 27-31 after the '3-week follow-up'.

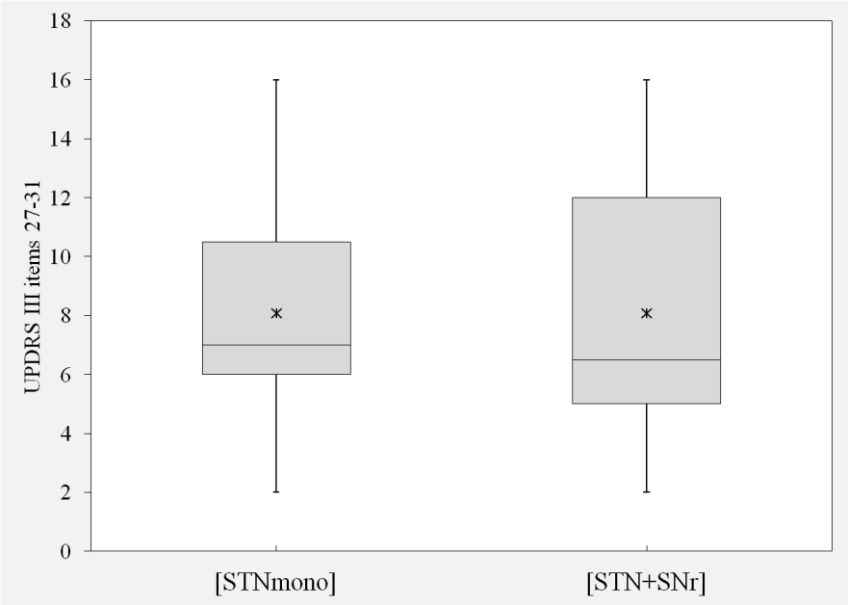

Figure 24 Box plots of the UPDRS III items 27-31 after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

In the **UPDRS III items 20-26**, the subscore for 'segmental' motor symptoms, the outcome was similar in [STNmono] (28.75 ± 6.03 points) and [STN+SNr] (29.75 ± 5.53) (p = 0.52). The effect was -1.0 ± 5.19 and the 95% confidence interval of the arithmetic mean of the effect ranged from -4.3 to 2.3. Figure 25 shows the box plots of the results of the UPDRS III items 20 - 26 after three weeks of constant stimulation in both tested conditions.

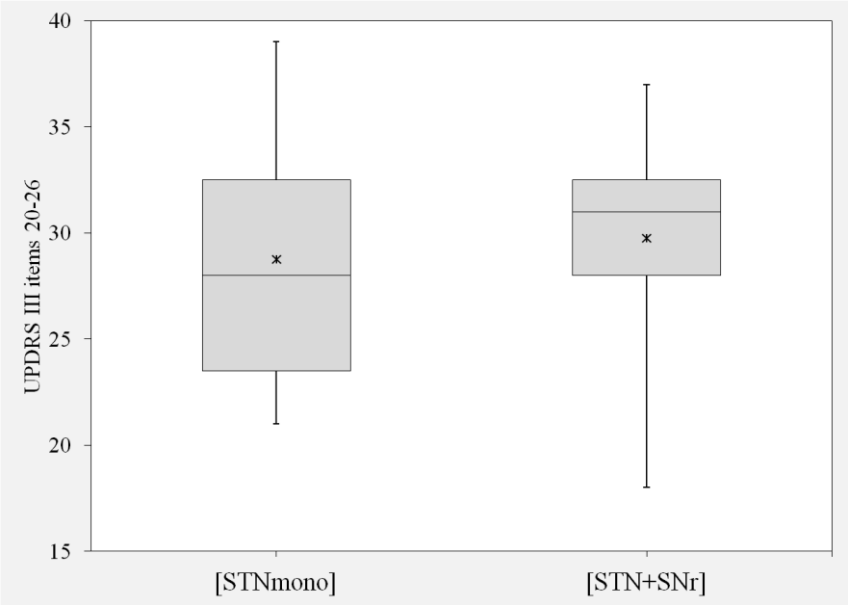

Figure 25 Box plots of the UPDRS III items 20-26 after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

According to the **FOG-AC**, patients presented with severe FOG at 'baseline testing'. The gait disturbances improved more with [STN+SNr] compared to [STNmono]. This improvement is reflected in the p-value ($p = 0.047$). Patients achieved 14.42 ± 13.19 points after three weeks of [STNmono] and 8.33 ± 10.91 points after [STN+SNr]. This resulted in an effect of 6.08 ± 9.41. The 95% confidence interval of the arithmetic mean of the effect ranged from 0.10 to 12.06. The box plots in Figure 26 illustrate the results of the FOG-AC after three weeks of constant stimulation.

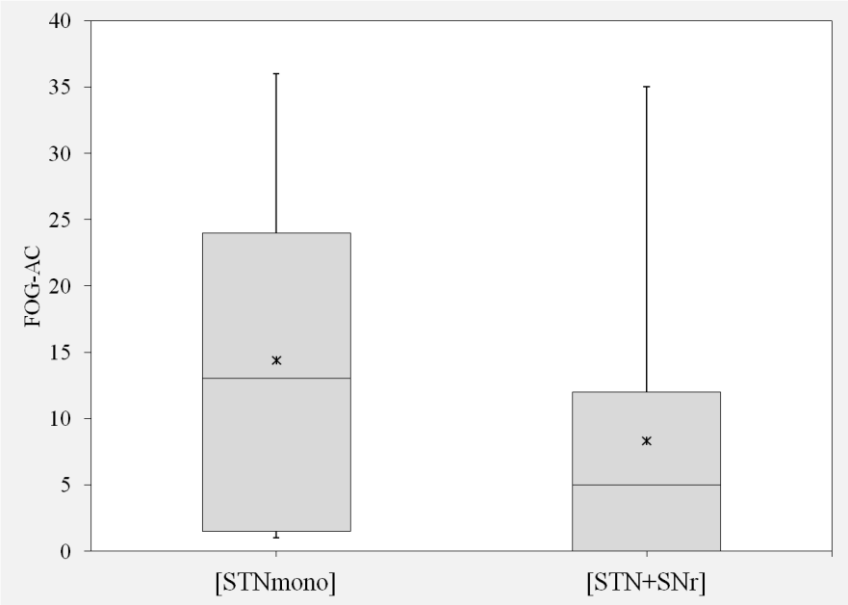

Figure 26 Box plots of the FOG-AC after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

The **CAPSIT-PD timed walking test** showed only little differences between both conditions in all three subdomains after three weeks of constant stimulation. No improvement was observed concerning the conditions 'time' and 'steps' but freezing-episodes occurred less frequently with [STN+SNr] than with [STNmono]. In the subdomain '**steps**', patients needed 14.25 (8 - 115)§ step safter [STNmono] and 13 (8.5 – 28.5)§ steps with [STN+SNr]. This resulted in an effect of 12.05 ± 27.61 The 95% confidence interval of the arithmetic mean of the effect ranged from -6.51 to 30.60 (p = 0.23).

Concerning the **'time**' subdomain, patients needed 7.5 (4.5 - 71)§ seconds with [STNmono] stimulation and 7 (5 - 22.5)§ seconds with [STN+SNr]. The effect was 7.05 ± 17.52 and the 95% confidence interval of the arithmetic mean of the effect ranged from -4.73 to 18.82 (p = 0.34). With the standard [STNmono] stimulation, patients achieved 0.25 (0 – 3.5)§ freezing episodes in the '**freezing**' subdomain and 0 (0 – 0.5)§ freezing episodes with [STN+SNr]. The effect in this domain resulted in 0.55 ± 0.93. The 95% confidence interval of the arithmetic mean of the effect ranged from -0.08 to 1.17 (p = 0.063). The box plots of the CAPSIT-PD timed walking test and its subdomains are shown in Figure 27, Figure 28 and in Figure 29.

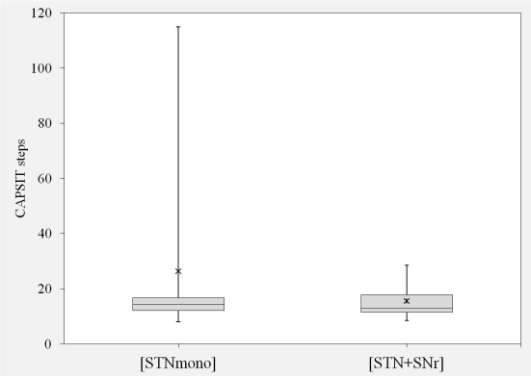

Figure 27 Box plots of the CAPSIT-PD 'steps' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

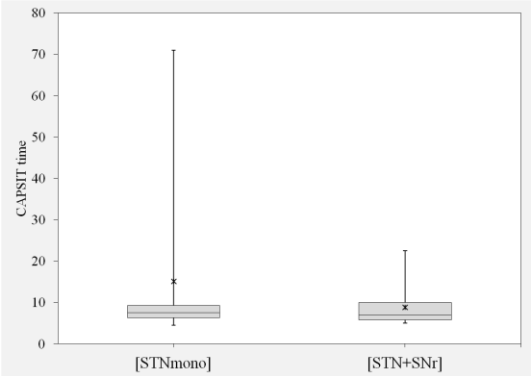

Figure 28 Box plots of the CAPSIT-PD 'time' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

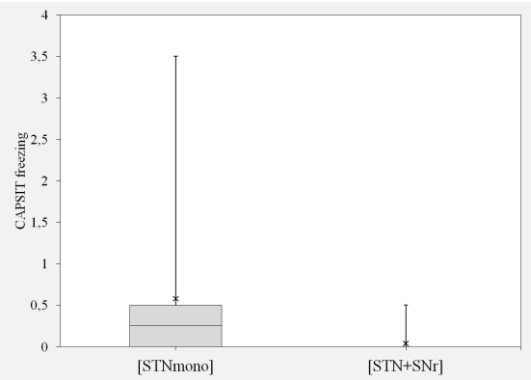

Figure 29 Box plots of the CAPSIT-PD 'freezing' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

Concerning the **Berg Balance Scale**, no differences were observed in the two conditions after three weeks of constant stimulation. Patients achieved 51.5 (19 – 56)§ points after three weeks of [STNmono] stimulation and 51.5 (17 – 56)§ points after three weeks of [STN+SNr] stimulation. This resulted in an effect of -0.58 ± 5.04 points. The 95% confidence interval of the arithmetic mean of the effect ranged from -3.78 to 2.62 (p = 0.70). Figure 30 shows the box plots of the Berg Balance Scale after the '3-week follow-up'.

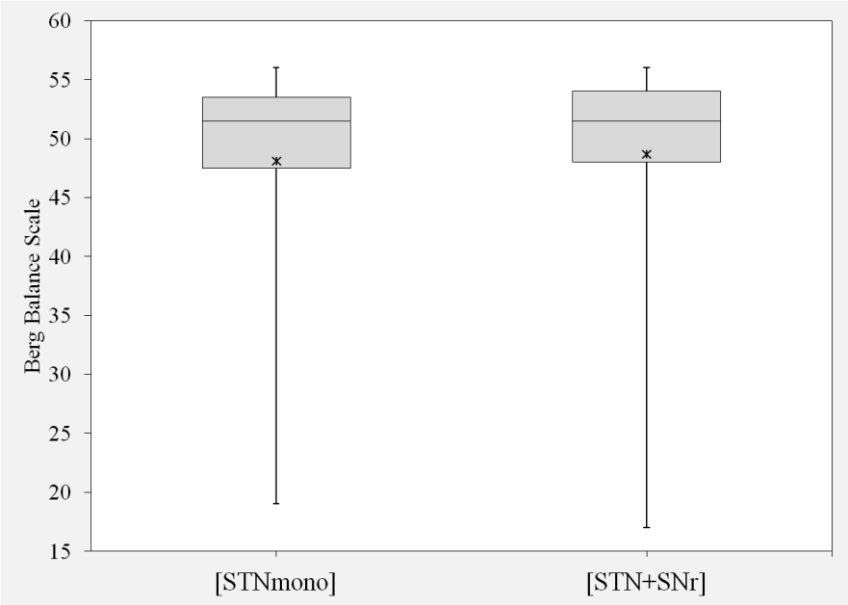

Figure 30 Box plots of the Berg Balance Scale after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

The **FOG-Q** was only performed after the '3-week follow-up' phases, as it is an anamnestic test. Patients had less freezing symptoms after [STN+SNr] than after [STNmono]. At 'baseline testing', patients achieved 14.67 ± 4.7 points, after three weeks of [STNmono] they achieved 16.17 ± 3.83 points and after three weeks of [STN+SNr] 14.50 ± 4.89 points. This resulted in an effect of 1.67 ± 3.23 points. The 95% confidence interval of the arithmetic mean of the effect ranged from -0.38 to 3.72 ($p = 0.10$). The box plots of the results of the FOG-Q are shown in Figure 31.

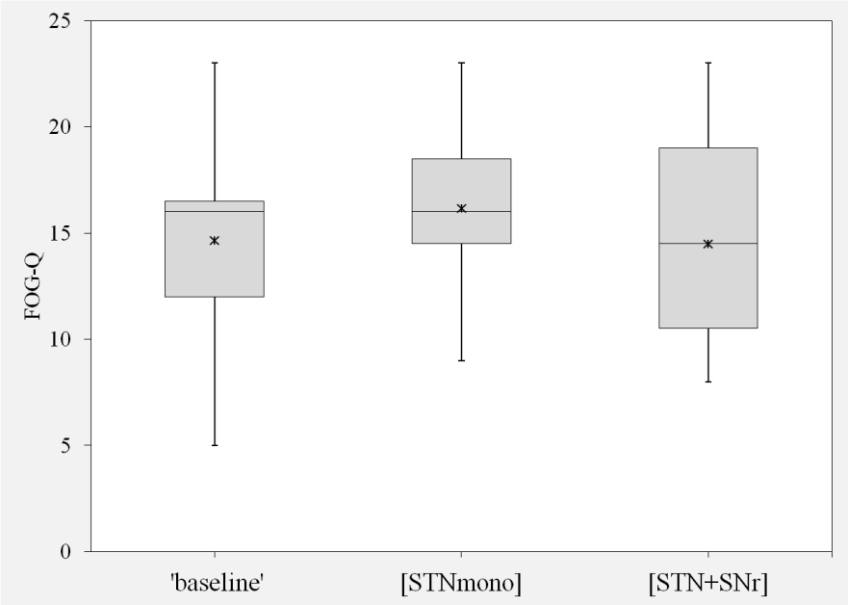

Figure 31 Box plots of the FOG-Q after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

4.2.2.2. Non-motor symptoms

The evaluation of the non-motor symptoms was performed by means of different scores.

The **BDI** was used to measure the severity of potentially existing depressions.

The average score patients reached in this study were not higher than 9.25 points. At 'baseline', patients presented with an average of 8.67 ± 3.37 points. After three weeks of constant [STNmono], the results barely differed from the 'baseline' results (7.91 ± 3.94). At '3-week follow-up' of [STN+SNr], the average score (9.25 ± 5.55) was similar to [STNmono] and 'baseline' ($p = 0.35$). The box plots in Figure 32 illustrate the results of the BDI.

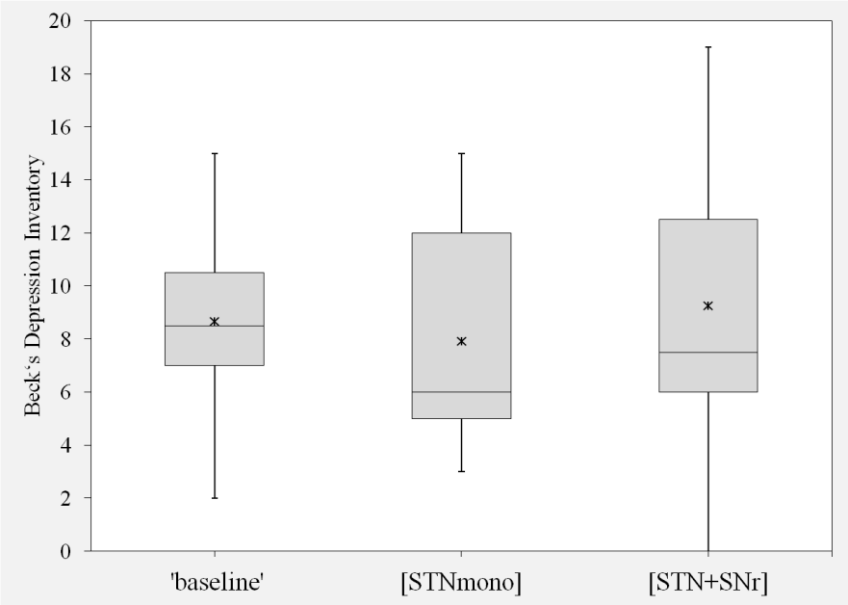

Figure 32 Box plots of the results of the BDI after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

Concerning the range of the results, there was a wider range of points reached after the [STN+SNr] stimulation, as seen in Table 7. Thus, on group level no changes were found but two patients (PD1 and PD7) presented with an increased BDI score during the combined stimulation [STN+SNr] compared to the conventional stimulation [STNmono]. The scores of all patients in all three conditions are given in Table 8.

Of note, PD1 had a longer lasting history of depression. The medical history of PD 7 also showed increased BDI scores in the past. Both patients took antidepressant drugs prior to study enrolment.

Table 7 Range of the points reached in the BDI over all stimulation conditions.
[STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

	'baseline'	[STNmono]	[STN+SNr]
Minimum	2	3	0
Maximum	15	15	19

Table 8 BDI scores for all patients after the three conditions 'baseline', 'STNmono' and 'STN+SNr'. [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

	'baseline'	[STNmono]	[STN+SNr]
PD1	10	7	18
PD2	11	5	6
PD3	10	-	11
PD4	7	5	6
PD5	2	5	0
PD6	12	12	13
PD7	8	15	19
PD8	5	3	5
PD9	8	6	7
PD10	15	13	12
PD11	7	6	6
PD12	9	10	8

Concerning the results of the **UPDRS IV** consisting of eleven items dealing with the complications of the therapy, like dyskinesia or clinical fluctuation, similar results could be identified.

After 'baseline testing' patients achieved 5.75 ± 1.96 points, after three weeks of constant [STNmono] patients achieved 6.27 ± 2.45 points and after three weeks of constant [STN+SNr] patients achieved 5.17 ± 3.04 points, which resulted in an effect of 0.73 ± 1.90. The 95% confidence interval of the arithmetic mean of the effect ranged from -0.55 to 2.01 ($p = 0.23$). Figure 33 illustrates the box plots of the results of the UPDRS IV.

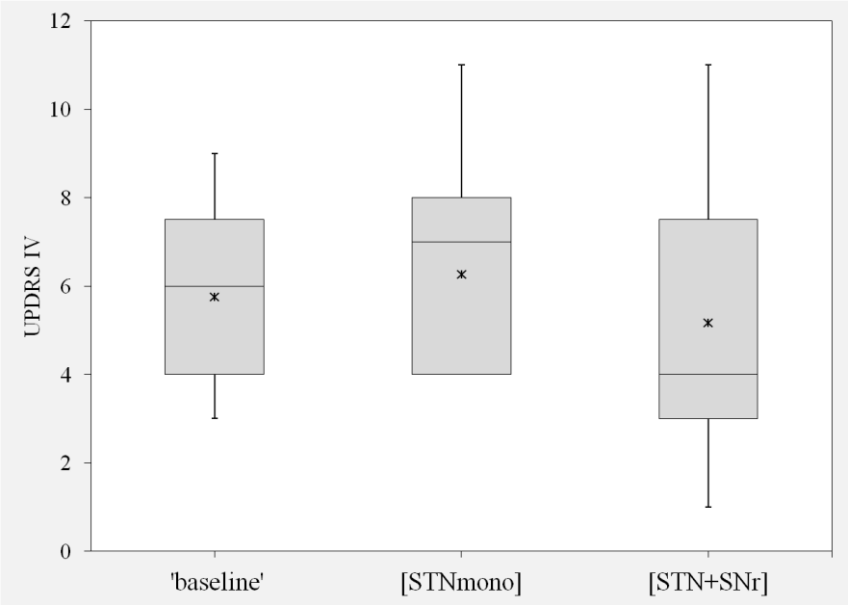

Figure 33 Box plots of the UPDRS IV after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

Another test assessing non-motor symptoms is the **BIS**. The evaluation of the neuropsychiatric symptoms was similar between both treatments. At 'baseline testing', patients achieved 62.6 ± 5.91 points, after three weeks of [STNmono], the testing resulted in 63.55 ± 4.3 points, and after three weeks of [STN+SNr], patients achieved 61.67 ± 5.18 points. The effect was 2.89 ± 7.64 and the 95% confidence interval of the arithmetic mean of the effect ranged from -2.98 to 8.76 ($p = 0.29$). The box plots of these results are illustrated in Figure 34.

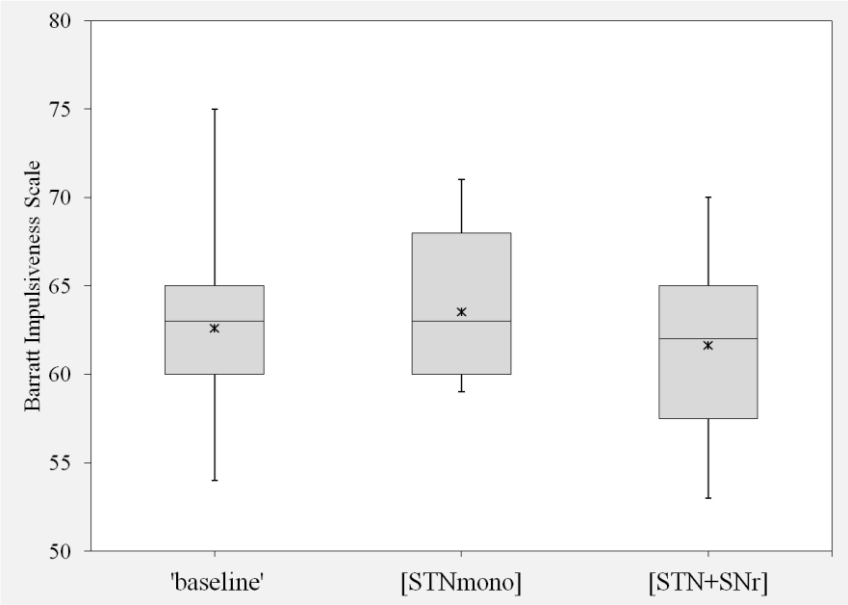

Figure 34 Box plots of the BIS after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

Similar to the other non-motor issues, the **NMSS** detected no relevant differences between both stimulation conditions. None of the nine subdomains of the NMSS improved or worsened substantially with [STN+SNr] stimulation.

Concerning the '**Cardiovascular**' subdomain, patients achieved 1 (0 – 9)§ points after 'baseline testing', 0 (0 – 6)§ points after three weeks of [STNmono] and 0 (0 - 9)§ points after [STN+SNr]. The effect resulted in -0.09 ± 3.18. The 95% confidence interval of the arithmetic mean of the effect ranged from -2.22 to 2.04 (p = 0.38).

In the '**Sleep**' subdomain, patients achieved 9 (0 – 20)§ points after 'baseline testing', 8 (0 – 24)§ points after [STNmono] and 11.5 (0 - 28)§ points after [STN+SNr]. The effect resulted in -3.55 ± 8.77. The 95% confidence interval of the arithmetic mean of the effect ranged from -9.44 to 2.34 (p = 0.18).

The '**Mood**' subdomain showed similar results in both groups. Patients achieved 5.5 (2 - 18)§ points after 'baseline testing', 8 (0 – 28)§ points after [STNmono] and 7 (0 - 49)§ points after [STN+SNr]. The effect resulted in 0.0 ± 10.0. The 95% confidence interval of the arithmetic mean of the effect ranged from -6.72 to 6.72 (p = 0.75).

In the subdomain **‘Cognition’**, the score after the ‘baseline’ examination (0 (0 - 12)§) was similar to the score after three weeks of [STNmono] 0 (0 - 4)§ and after three weeks of [STN+SNr] (0 (0 - 13)§). The effect resulted in -1.91 ± 4.78 points and the 95% confidence interval ranged from -5.12 to 1.31 ($p > 0.99$).

Concerning the ‘**Concentration**’ subdomain, patients achieved 6 (0 – 27)§ points after ‘baseline testing’, 4 (0 – 24)§ points after[STNmono] and 5 (0 - 32)§ points after [STN+SNr]. The effect resulted in -2.82 ± 11.77 points. The 95% confidence interval of the arithmetic mean of the effect ranged from -10.73 to 5.09 ($p = 0.29$).

The assessment of the ‘**Gastrointestinal’** function in this testing showed no difference between the two conditions. After ‘baseline testing’, patients achieved 8 (0 – 25)§ points. After three weeks of [STNmono], the result was 8 (0 – 20)§ points and after [STN+SNr] 6.5 (0 – 20)§ points. The effect resulted in 1.55 ± 3.11. The 95% confidence interval of the arithmetic mean of the effect ranged from -0.54 to 3.63 ($p = 0.73$).

In the subdomain **‘Micturition’**, the score after ‘baseline’ examination (7 (0 - 30)§) was similar to the score after [STNmono] (8 (0 - 28)§) and to the score after [STN+SNr] (8.5 (0 - 18)§). The effect resulted in 0.81 ± 4.38 points and the 95% confidence interval ranged from -2.76 to 3.12 ($p = 1.0$).

Similarly, in the subdomain ‘**Sexual function’** similar results were found between the treatment conditions. After ‘baseline testing’ patients achieved 4 (0 - 18)§ points, after [STNmono] 0 (0 - 12)§ points and after [STN+SNr], patients achieved 1 (0 – 12)§ points. The effect resulted in -0.91 ± 3.94 points and the 95% confidence interval ranged from -3.55 to 1.74 ($p = 1.0$).

Concerning the ‘**Sundries**’ subdomain, patients achieved 7 (0 – 24)§ points after ‘baseline’ testing, 4 (0 – 26)§ points after [STNmono] and 9 (0 - 18)§ points after [STN+SNr]. The effect resulted in 0.27 ± 8.26 points. The 95% confidence interval of the arithmetic mean of the effect ranged from -5.28 to 5.82 ($p = 0.73$).

The results of the subdomains of the NMSS are illustrated in box plots from Figure 35 to Figure 43.

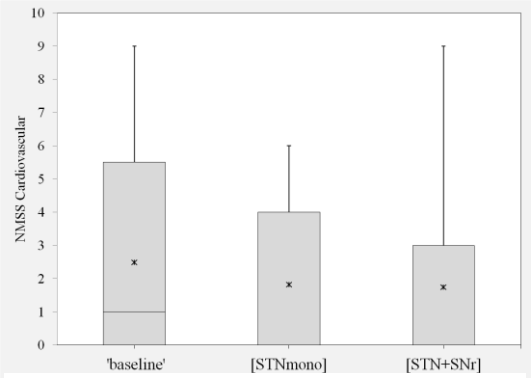

Figure 35 Box plots of the NMSS subdomain 'Cardiovascular' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

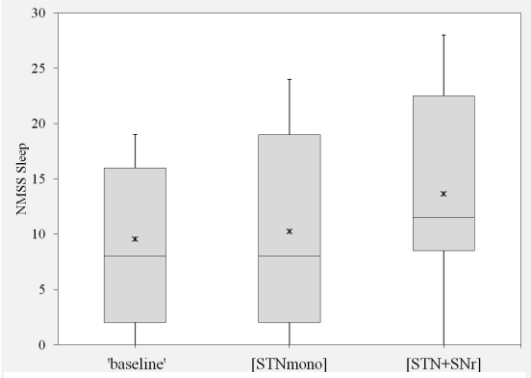

Figure 36 Box plots of the NMSS subdomain 'Sleep' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

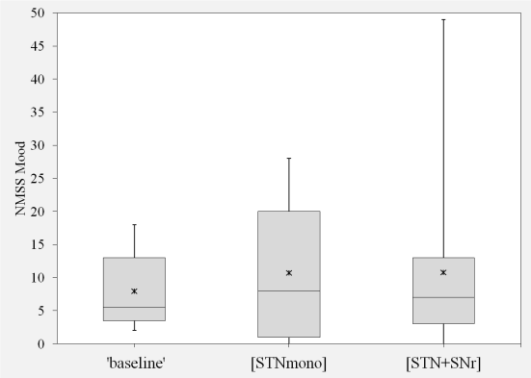

Figure 37 Box plots of the NMSS subdomain 'Mood' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

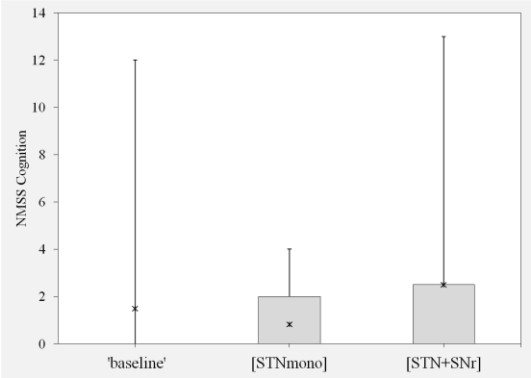

Figure 38 Box plots of the NMSS subdomain 'Cognition' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

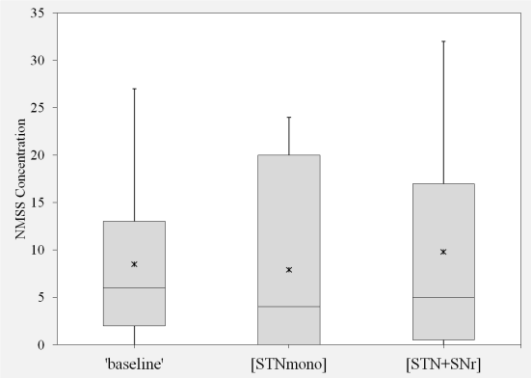

Figure 39 Box plots of the NMSS subdomain 'Concentration' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

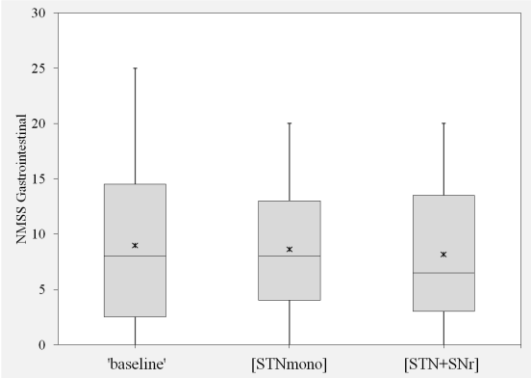

Figure 40 Box plots of the NMSS subdomain 'Gastrointestinal' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

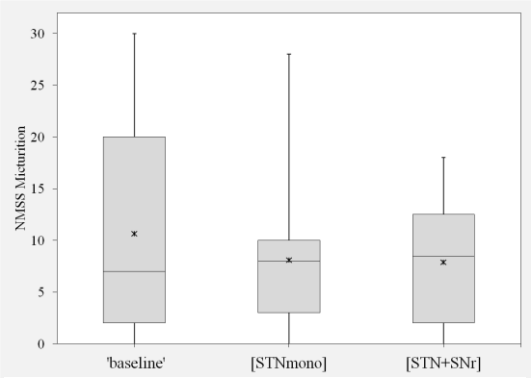

Figure 41 Box plots of the NMSS subdomain 'Micturition' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

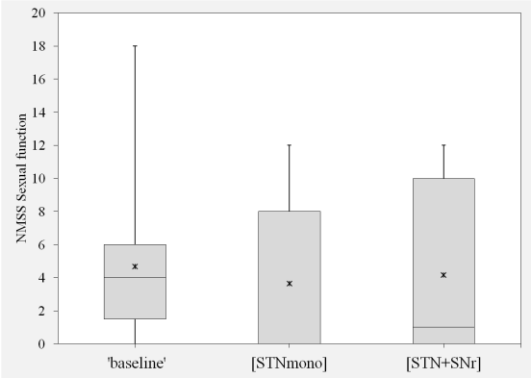

Figure 42 Box plots of the NMSS subdomain 'Sexual function' after '3-week follow-up'.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

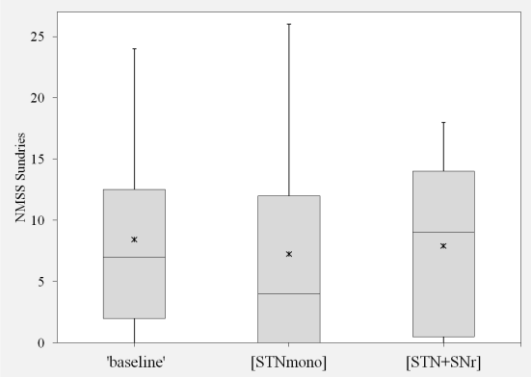

Figure 43 Box plots of the NMSS subdomain 'Sundries' after '3-week follow-up'
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

4.2.2.3. Quality of life

Quality of life was assessed with the PDQ-39 summary index. The PDQ-39 assesses the health status concerning quality of life from the patient's subjective point of view.

None of the subdomains of this index showed serious differences between the two tested conditions at '3-week follow-up'. However, the results of this testing reveal slight differences in several domains after the '3-week follow-up', which are illustrated by box plots and explained by means of concrete figures.

The 'summary index' of the PDQ-39 was unchanged in both treatment arms, as shown in Figure 44. At 'baseline', patients presented with a 'summary index' of 270.28 ± 108.27 points. After the [STNmono] '3-week follow-up', they achieved 256.40 ± 127.51 points and after three weeks of [STN+SNr] 248.68 ± 94.16 points. Thus, barely a difference could be detected which was also reflected in the p-value (p = 0.55).

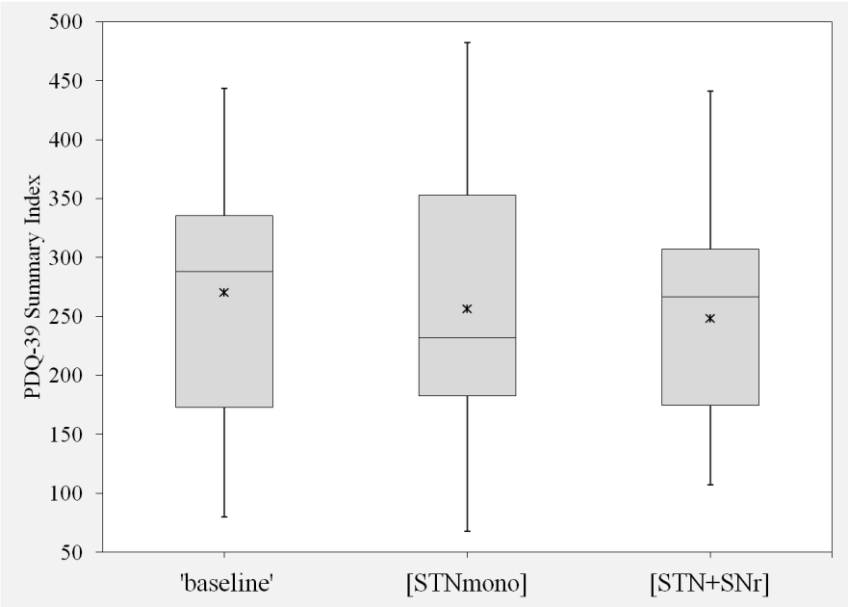

Figure 44 Box plots of PDQ-39 summary index.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

The subdomain **'Mobility'** consists of ten items. At 'baseline', patients presented with a high score in this subdomain (53.96 ± 23.78). After [STNmono], the

outcome was almost the same (54.32 ± 27.23) but it improved slightly with [STN+SNr] (49.38 ± 25.30). The p-value resulted in (p = 0.29).

The subdomain **'Activities of daily living'** consists of six items. At 'baseline', patients presented with a lower score in this subdomain (42.01 ± 20.45) than after [STNmono] (45.08 ± 23.04) or after [STN+SNr] (45.14 ± 22.46). The p-value of these similar outcomes resulted in (p = 0.48).

The score in the **'Emotional well-being'** subdomain was higher after the 'baseline' examination (26.74 ± 15.02) than after [STNmono] (25.38 ± 21.45). After three weeks of [STN+SNr], the score was even lower (23.96 ± 17.87) than after the standard stimulation (p = 0.57).

The subdomain **'Stigma'** consists of four items. After [STNmono], patients had a slightly higher score (22.73 ± 25.35) than after 'baseline' examination (21.88 ± 27.24). The score was marginally lower after [STN+SNr] (20.31 ± 21.01). The p-value resulted in (p = 0.46).

In the subdomain **'Social support'**, a slightly greater improvement could be observed with [STN+SNr] (11.81 ± 10.93) compared to [STNmono] (18.94 ± 23.89), but the difference was marginal (p = 0.28). At 'baseline' examination patients achieved 18.06 ± 23.26 points.

The subdomain **'Cognition'** consists of four items. At 'baseline', patients presented with the highest score (31.25 ± 24.28). The results of [STNmono] (23.30 ± 22.89) were similar to the results of [STN+SNr] (24.48 ± 21.89). Thus, no significant difference could be detected (p = 0.49).

The subdomain **'Communication'** consists of three items. The highest score was reached after 'baseline' examination (40.97 ± 18.62). After [STNmono] the results (31.82 ± 21.99) were lower than after [STN+SNr] (36.81 ± 22.88). The p-value resulted in (p = 0.63).

The subdomain **'Bodily discomfort'** consists of three items. The results hardly differed between the 'baseline' examination (35.42 ± 21.06), [STNmono] (34.85± 22.61) and [STN+SNr] (36.81 ± 16.84). This is reflected in the p-value (p = 0.76).

The results of the subdomains of the PDQ-39 are illustrated in the box plots from Figure 45 to Figure 52.

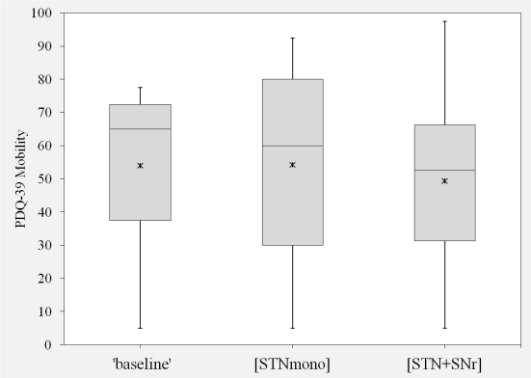

Figure 45 Box plots of PDQ-39, 'mobility' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

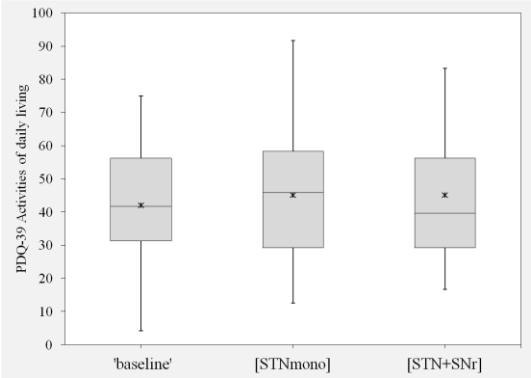

Figure 46 Box plots of PDQ-39, 'activities of daily living' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

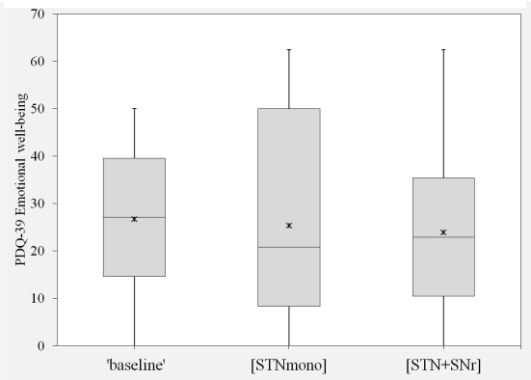

Figure 47 Box plots of PDQ-39, 'well-being' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

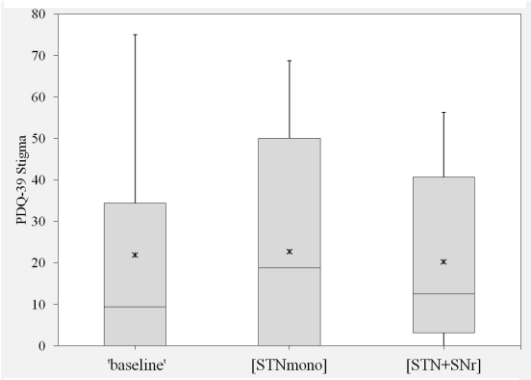

Figure 48 Box plots of PDQ-39, 'stigma' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

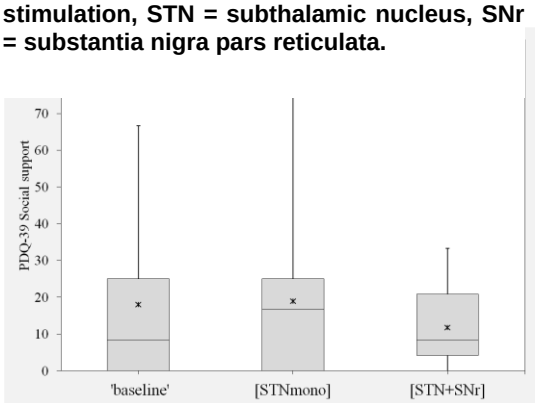

Figure 49 Box plots of PDQ-39, 'social support' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

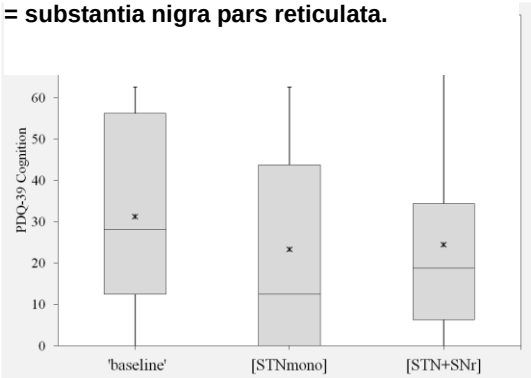

Figure 50 Box plots of PDQ-39, 'cognition' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

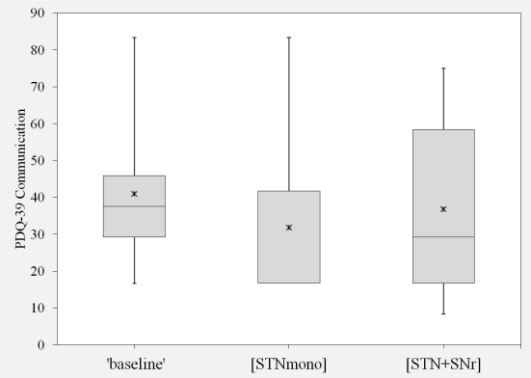

Figure 51 Box plots of PDQ-39, 'communication' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

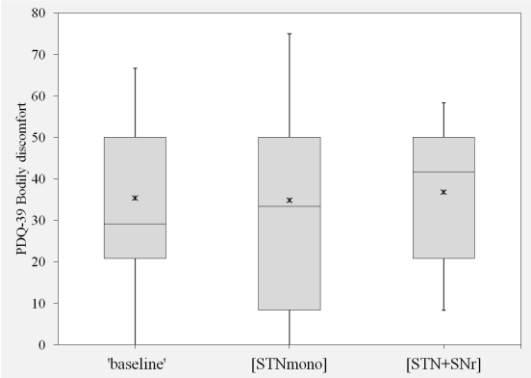

Figure 52 Box plots of PDQ-39, 'bodily discomfort' domain.
x-axis = therapeutic condition, y-axis = score, [STNmono] = standard STN stimulation, [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

4.2.3. Correlation between FOG and electrode coordinates

Exact electrode localisation and precise methods for coordinate identification are important factors to compare the outcome of SNr-DBS. To investigate whether there is an interrelation between the determined electrode coordinates and the FOG outcome, we calculated the correlation between the single electrode coordinates, the x-, y- and z-coordinate, and the change of the FOG outcome of the FOG-AC during the 'immediate testing' phase (Δ StimOff - [STN+SNr]) for all active electrode contacts.

Figure 53 to Figure 64 show that there is no strong positive or negative correlation between our determined electrode coordinates and the FOG outcome. All results were statistically non-significant. The coefficient of correlation (r) ranged from -0,19 to 0.5.

Left SNr

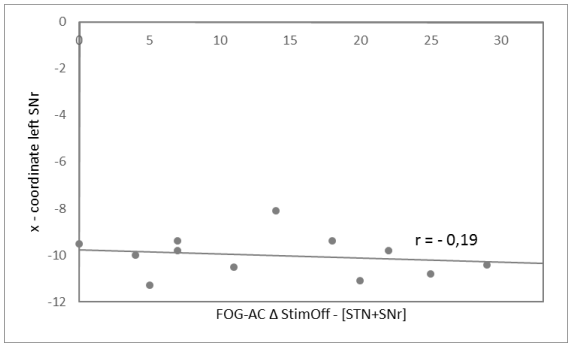

Figure 53 Correlation between the x-coordinate of the left SNr and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

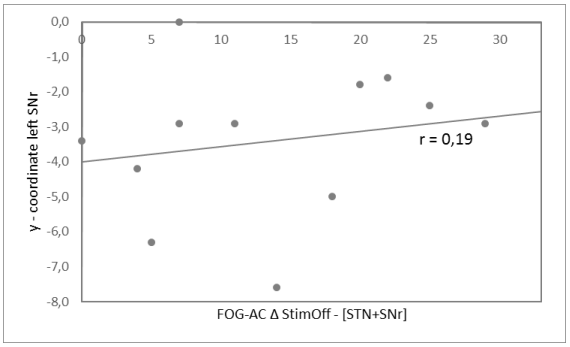

Figure 54 Correlation between the y-coordinate of the left SNr and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

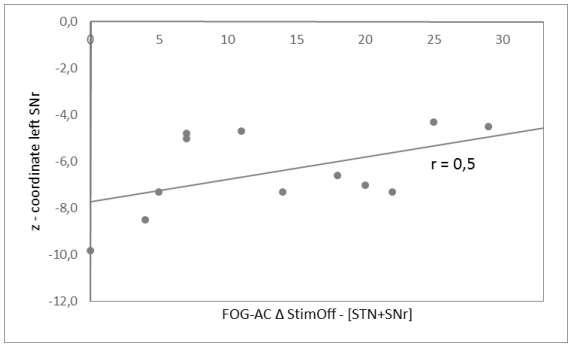

Figure 55 Correlation between the z-coordinate of the left SNr and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

Right SNr

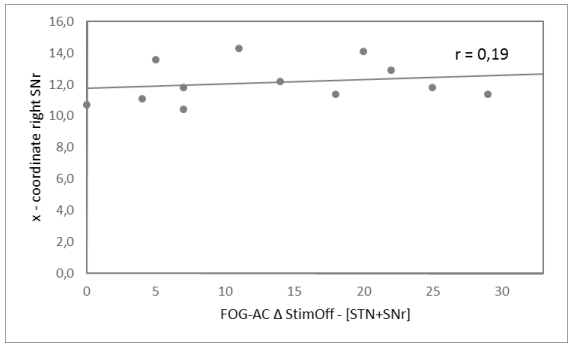

Figure 56 Correlation between the x-coordinate of the right SNr and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

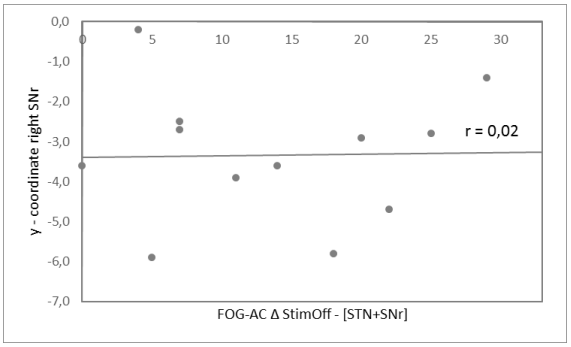

Figure 57 Correlation between the y-coordinate of the right SNr and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

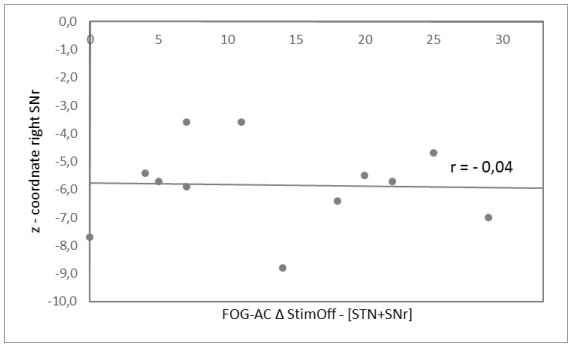

Figure 58 Correlation between the z-coordinate of the right SNr and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

Left STN

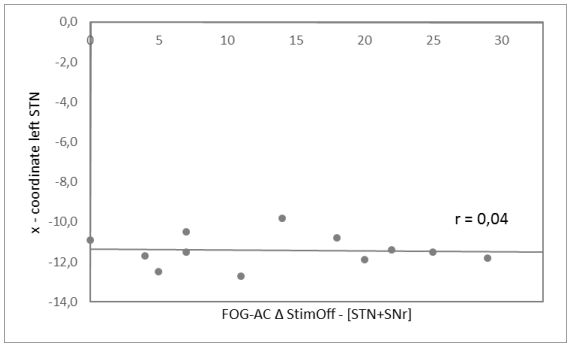

Figure 59 Correlation between the x-coordinate of the left STN and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

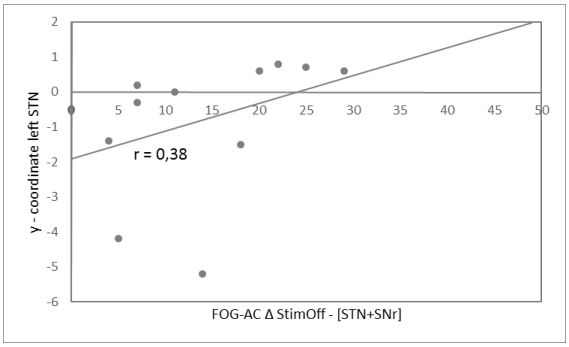

Figure 60 Correlation between the y-coordinate of the left STN and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

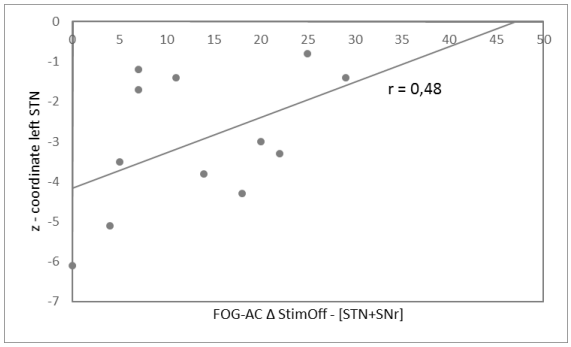

Figure 61 Correlation between the z-coordinate of the left STN and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

Right STN

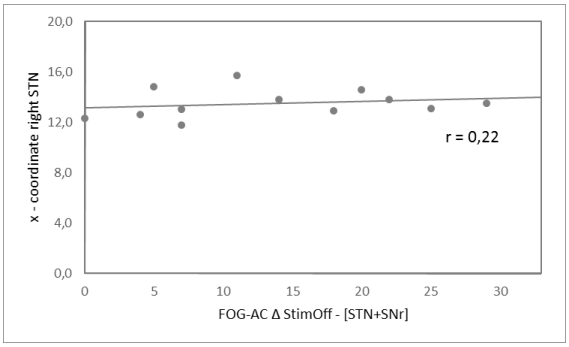

Figure 62 Correlation between the x-coordinate of the right STN and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

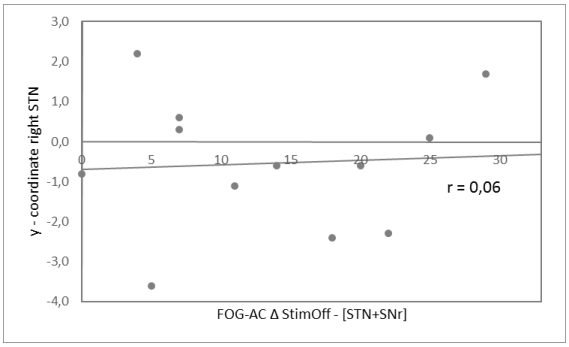

Figure 63 Correlation between the y-coordinate of the right STN and the FOG outcome. [STN+SNr] = combined STN and SN stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

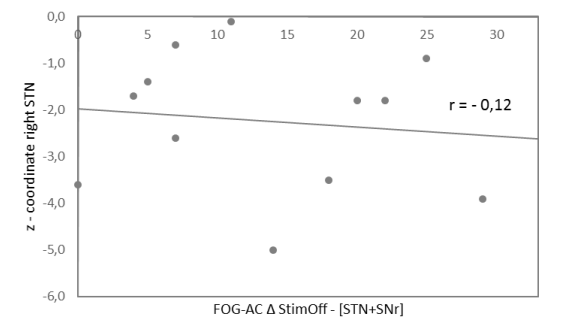

Figure 64 Correlation between the z-coordinate of the right STN and the FOG outcome. [STN+SNr] = combined STN and SNr stimulation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata, r = correlation coefficient.

4.3. Safety measures

No suicidality was reported in any patient (Weiss et al., 2013). On group level, no change was found concerning the BDI. During [STN+SNr], PD1 presented with increased BDI scores compared to [STNmono]. The score after the '3-week follow-up' in the [STN+SNr] condition was 18 and after three weeks of constant [STNmono] stimulation 7. PD7 had a former personal history of hallucinations, which was documented in the preoperative recordings, and he developed visual benign hallucinations with retained insight (item 2, UPDRS I) during the [STN+SNr] condition. At group level, item 2 of the UPDRS I was unchanged

between the two therapeutic conditions. Psychosis did not occur in any patient. Concerning the evaluation of the BIS, the segmental symptoms (items 20-26, UPDRS III) and motor fluctuations (UPDRS IV), the comparison of [STN+SNr] and [STNmono] showed no difference between the two treatments.

4.4. Adverse events

Before entering the study, several strategies were applied to optimise the efficacy of DBS, such as the optimisation of the electrode geometry and the programming of the electrodes by titration of subthalamic and nigral stimulation parameters. Effects and side effects were carefully evaluated. Nevertheless, control over the current spread during the stimulation is limited (Kringelbach et al., 2007).

Despite the best effort of minimizing the chance of occurring adverse events, the probability of the appearance of any side effects is high, hence an accurate observation and reporting of these effects is greatly important for the interpretation of the clinical applicability of the evaluated therapeutic strategy.

Our patients were instructed to be watchful concerning side effects and to report them to the study site as soon as they occur during the ‘3-week follow-up’.

In the final analysis, no serious adverse events were observed in both treatment arms. During [STN+SNr] treatment, no acute side effects were observed in the ‘immediate testing’ but during the ‘3-week follow-up’ four adverse events were reported. Two patients (PD2 and PD 9) suffered from a delayed onset of relevant dyskinesias within the first days after introduction of [STN+SNr]. PD2 came on day two after the introduction of [STN+SNr] and required therapy adjustment according to the intention-to-treat principle. The stimulation amplitudes were lowered on both active caudal contacts, the SNr-contacts by -0.4V and the symptoms resolved completely within a short time. PD9 informed the study site for therapy adjustment after he had already independently conducted a self-administered reduction of the daily levodopa dosage by 125 mg. This reduction ameliorated the dyskinesias but as they did not disappear completely, the patient was rescheduled and the SNr amplitudes were additionally lowered by -0.1V on both sides. After these interventions, the dyskinesias completely

resolved in both patients and they could stick to the schedule and completed the ‘3-week follow-up’ period according to the intention-to-treat-principle.

At the ‘3-week follow-up’ visit, one patient (PD 8) reported that few intermittent episodes of double vision occurred during the [STN+SNr] condition. They lasted for a few seconds and were not highly bothersome for the patient, hence the patient did not contact the study site before the ‘3-week follow-up’ visit.

PD7 and his caregiver reported an initial improvement of FOG during the first two weeks of the [STN+SNr] condition, however increased immobility and recurrent falls occurred during the third week of the ‘3-week follow-up’.

Four patients wished to discontinue standard STN stimulation [STNmono] prematurely (PD3, PD7, PD10, PD11) due to more pronounced gait impairment, immobility and falls. No patient discontinued the ‘3-week follow-up’ prematurely during the [STN+SNr] condition (Weiss et al., 2013).

5

5. Discussion

A lot of research is conducted on the pathophysiology of the BG and on the mode of action and the effectiveness of DBS in PD, however, there are still symptoms, which cannot be addressed in a satisfactory way by standard STN-DBS. Especially in advanced disease stages, the response to STN-DBS concerning axial motor symptoms is limited. The focus in this study was on the treatment of those axial motor symptoms by use of combined stimulation of the STN and SNr. Based on the theoretical background concerning pathological functional sub-loops of BG motor networks, the main question of this work was whether co-stimulation of segregated motor loops, in which the STN and SNr are involved, is more effective than standard stimulation of only the STN concerning debilitating axial motor symptoms.

In the following chapters the results of this phase II trial will be discussed with a special focus on the relevance of the SNr as a possible new and additional target for DBS in PD patients with axial motor impairment.

5.1. SNr as an additional target for DBS in PD

We tested the hypothesis that concomitant stimulation of the STN and SNr [STN+SNr] with interleaved pulses is superior on axial symptoms in patients with advanced PD compared to standard stimulation of only the STN [STNmono]. A broad spectrum of axial motor symptoms was tested to cover a wide range of possible clinical features so that the primary endpoint included a variety of different symptoms reflected as ‘axial score’.

Moreover, the effect of concomitant stimulation was unravelled and the trial focused on specific motor subdomains.

In this study, a general therapeutic effect on axial motor signs with concomitant stimulation [STN+SNr] could not be demonstrated, as reflected by the negative primary endpoint.
Although the study patients were selected carefully with special attention given to gait disturbances, mainly FOG as leading symptom, the primary endpoint of this study obviously covered a too broad spectrum of different clinical features to detect specific motor features. However, the results of the secondary endpoint open the perspective that SNr stimulation in addition to the conventional STN stimulation might improve intractable FOG.

Looking into detail on the exploratory secondary investigation, we noted an improvement of FOG in the FOG-AC in the ‘immediate testing’ and in the ‘3-week follow-up’ with [STN+SNr].
However, the freezing part of the CAPSIT test did not improve with additional nigral stimulation, neither in the ‘immediate testing’ nor in the ‘3-week follow-up’. The provocation of FOG seems to be more distinct in the FOG-AC presumably due to the distraction tasks reliably detecting FOG, whereas the CAPSIT walking test is not primarily designed for detecting FOG but gait parameters in general.

In line with the improvement of FOG in the FOG-AC, the mobility subdomain of the PDQ-39, as part of the exploratory secondary endpoint, showed an improvement with interleaved pulses [STN+SNr] compared to the standard [STNmono] stimulation (Weiss et al., 2013). This non-significant difference amounted to 5 points, which does not allow for a final reasoning because of the small sample size of this clinical trial. Nevertheless, it is worth mentioning that difference of 3.2 points in the mobility subdomain of the PDQ-39 was identified to be relevant concerning the patients’ subjective clinical well-being. Peto et al. showed this in large PD cohorts (Peto et al., 1998). A larger follow-up trial is needed to this end.

Nigral stimulation [STN+SNr] did not show superiority compared to the standard [STNmono] stimulation concerning postural control as a cardinal symptom of PD (Jankovic, 2008) according to the BBS. Impaired balance due to the loss of postural reflexes can cause falls and is the least treatable symptom of PD (Koller et al., 1989). Studies on descending neuronal projections from the SNr to the pontomesencephalic area showed the involvement of the SNr in nigropontine pathways, which are known to influence postural control and locomotion (Chastan et al., 2009, Takakusaki et al., 2003). Based on those neuronal projections, our assumption was that nigral stimulation could interfere with balance difficulties and possibly at least partly restore them. However, the results of the BBS could not reflect our assumption, as the scores remained unchanged regardless of which stimulation settings were applied. Postural instability is generally an intricate symptom regarding a solid and correct diagnosis (Visser et al., 2008). The BBS, just as many other balance tests, is a quite subjective clinical examination measuring impairment in balance function. To limit the bias generated by a subjective judgement of different examiners, only one competent neurologist carried out the test in all patients and during all study conditions. But first and foremost, the randomised and controlled study design contributed to the limitation of the bias.

Cognitive and emotional factors can influence the outcome of balance tests (Bloem et al., 2001). Cognitive impairment, as error source, could partly be eliminated by means of determining a specific range of points, which had to be achieved in the MMSE as an inclusion criterion. Emotional factors, however, could hardly be influenced. Altogether, we tried to minimize possible errors during balance assessment, but nevertheless, no amelioration of postural stability could be detected with interleaved pulses [STN+SNr]. Interestingly, Chastan et al. reported a significant effect of high frequency nigral stimulation on postural control and balance assessed by means of biomechanical analysis during gait initiation in PD patients (Chastan et al., 2009). Step length and step velocity as anteroposterior gait parameters and braking capacity as vertical gait parameter were examined. The reason for the discrepancy concerning our balance results according to the BBS and Chastan’s results regarding postural control and

balance is probably due to the more accurate assessment of postural instability and balance control by use of objective biomechanical methods compared to the clinical assessment with the BBS. However, a reasonable comparison of these two assessment methods is limited.

After termination of the study, all patients were asked to choose one stimulation setting of the two conditions tested, with which they wanted to be treated hereafter. Their choice should be made based on the motor outcome and subjective general well-being during the different stimulation settings. Ten out of twelve study patients wished to continue DBS therapy with combined [STN+SNr] stimulation (Weiss et al., 2013).

It is important to note that four out of twelve study patients discontinued the '3-week follow-up' phase while being treated with the conventional STN stimulation [STNmono] earlier than scheduled, due to intolerable side effects concerning gait impairment, immobility and falls (see chapter 4.4.). Three of those patients were randomised in the group, which was treated first with [STN+SNr] and in the second phase with [STNmono]. This means that in these patients the direct comparison between the two stimulation settings led to a subjectively better outcome with combined nigral stimulation [STN+SNr]. Moreover, in all four of these patients, the primary endpoint score ('axial score') was superior after [STN+SNr] compared to [STNmono] (Table 6). Even if the primary endpoint of our clinical trial did not show significant results, the patients' feedback and their wish to continue DBS therapy with the [STN+SNr] condition after completion of the study gives us a valuable hint and a tendency concerning the subjective clinical efficacy of interleaved pulses on the level of the STN and SNr.

Generally, it has to be taken into consideration that anamnestic outcome measures of the secondary endpoint have to be interpreted with caution as the 33% drop out rate emerged only during standard [STNmono] stimulation.

Furthermore, another important fact that has to be taken into account while interpreting the results of the study is the variability of the endophenotype within the idiopathic PD. In every PD cohort, a large spectrum of different endophenotypes is likely to be represented. These diverse endophenotypes show a clinical heterogeneity concerning the age at disease onset and a

variability concerning disease progression. This was reported for example in LRRK2 mutation carriers (Schiesling et al., 2008). In this context, the heterogeneity could be a factor influencing some of the collected patient characteristics. A rapid disease progression and consequently an early emergence of axial symptoms, like FOG, could be more likely in some inherited forms of Parkinsonism compared to other non-hereditary endophenotypes. Biomarkers, such as the glucocerebrosidase gene (GBA), which was detected to be a risk allele for PD, might be helpful to classify patient subgroups with a special profile concerning disease progression, especially motor impairment and an early onset of FOG (Weiss et al., 2012b, Winder-Rhodes et al., 2013).

Independent of an accurate description of the endophenotype clinical experience with PD patients shows that patients with predominant FOG exist mainly in advanced disease stages (Okuma, 2006). These patients could possibly benefit from an early treatment regime including nigral stimulation. Future studies might help accurately identifying PD patients with prevalent FOG and assessing the potential benefit of nigral neuromodulation in these patients.

Of note, a heterogeneous catalogue of treatment responses was also observed in studies dealing with DBS of the PPN in PD patients with axial motor symptoms (Ferraye et al., 2010, Hamani et al., 2011).

The identification of endophenotype subgroups and the typical related disease features in these patients might help selecting patients more accurately in further studies on gait impairment. The benefit could be a better treatment of resistant axial motor symptoms in those who are affected.

5.2. Therapeutic option for FOG

Although many PD patients are faced with intractable FOG and despite the awareness of FOG to be a dramatic episodic gait pattern the pathogenesis of this phenomenon is not understood so far (Giladi et al., 2001). Therapeutic approaches like medications, DBS and rehabilitation techniques can alleviate gait impairment resulting from this clinical phenomenon, although in patients with advanced FOG, these treatments are of poor efficacy (Nutt et al., 2011). For this reason, FOG is an unresolved problem and a challenge for our understanding of

the normal gait pattern and disorders concerning gait. Clinicians and scientists dealing with FOG phrased a definition of FOG, which was accepted in the year 2010 and reads “brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk” (Giladi and Nieuwboer, 2008). The notion that FOG is an episodic phenomenon suggests that it occurs every once a while, thus, every once a while there is a transient disruption of the locomotion. These disruptions can be provoked by turning, by passing narrow passages or by approaching a destination (Nutt et al., 2011). Furthermore, the likelihood that FOG occurs can be triggered by distraction while walking and stressing situation like being under time pressure (Okuma and Yanagisawa, 2008). This is what we provoked during the FOG-AC. Our patients were distracted by dual-tasking and even triple-tasking and the fact that their performance was assessed created a stressful situation for them. An amelioration of FOG can be observed when auditory cueing at the proper pace is applied, when visual cues are used or during excitement (Plotnik et al., 2014).

In our study, we observed less FOG with additional nigral stimulation compared to the conventional STN stimulation which raises the question of the relationship between the SNr and the clinical phenomenon of FOG. As we know that environmental influence including cognitive and emotional conditions can have an effect on FOG, there might be a linkage between the SNr, mental function and FOG. The interplay of all these components is complex and the pathophysiology of all properties only partly understood at present, for which reason we are still far away from a definite answer to the question whether there is a connection between the SNr and FOG which is controllable by electric pulses. Nevertheless, some of our ideas and thoughts seem to provide potential explanations.

Based on the idea of an overinhibitory drive from the SNr to the PPN (Nandi et al., 2008) and the linkage between the PPN and the reticular formation (Delwaide et al., 2000), it could be possible that the imbalance of the BG nuclei also affects the reticular formation. This could be of interest as the ascending reticular activating system (ARAS) is part of the reticular formation. The ARAS connects the brainstem to the cortex and has multiple functions. It modulates states of sleep and of attention and alertness (Garcia-Rill, 1997). A suppressed PPN in PD

patients might be the reason for a reduced activity of this activating system, resulting in a decreased alertness which subsequently increases the chance of the occurrence of FOG. Consequently, an involvement of mental function is likely in FOG (Giladi and Hausdorff, 2006).

Another hint to the disorder of the reticular formation and the involvement of the ARAS in the development of FOG could be the sleep disturbances many PD patients suffer from (Tandberg et al., 1998, Gjerstad et al., 2008). The prevalence of sleep disturbances in PD patients in general approaches up to 100% (Lees et al., 1988). Some studies could even show that sleep disturbances are a possible early predictor for the development of neurodegenerative diseases like PD (Iranzo et al., 2006). During the so called rapid eye movement (REM) sleep behaviour disorder (RBD) patients act out their dreams and do not have a normal muscle relaxation while sleeping (Iranzo et al., 2009). The RBD seems to be related to neurodegenerative diseases, such as PD (Iranzo et al., 2009). The efficacy of STN-DBS concerning sleep improvement has been studied by sleep poligraphy with the result that there is an association between an extended REM sleep after STN-DBS and the degree of improvement of motor functions (Monaca et al., 2004). Consequently, the loss of neuronal structures and the complex neurotransmitter dysfunction of diverse BG nuclei seem to possibly affect the REM sleep centres of the brain, such as the ARAS (Rye et al., 2000).

Hence, a direct connection between FOG and sleep disorders in PD is not evident, but there is probably a connection between FOG and alertness and between PD and sleep disorders which makes a certain relationship and a common pathophysiology of these components presumably.

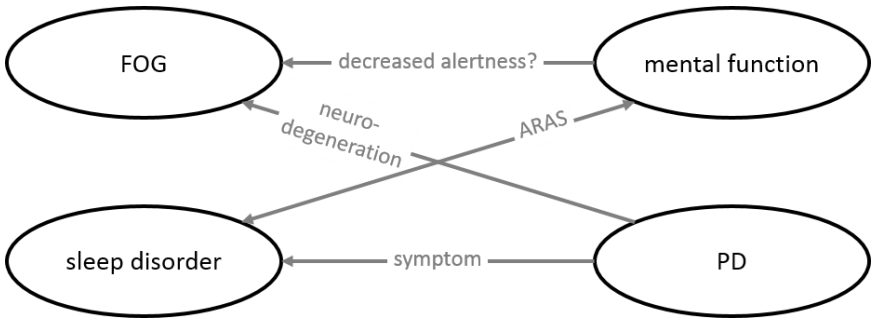

Figure 65 Connection between PD and several features of the disease.
ARAS = ascending reticular activating system, FOG = freezing of gait, PD = Parkinson's disease.

In this study, we did not directly compare the effect of interleaved STN and SNr stimulation [STN+SNr] and PPN stimulation concerning axial signs, and our findings are exploratory, but the positive effects of DBS on both stimulation sites raises the question whether the subcortical downstream circuity from the BG nuclei to the motor neurons of the spinal cord can be modulated with DBS on every junction with a similar effect on axial signs.

Consequently, a better motor response in terms of less FOG might not only be the result of a normalization of the PPN function and its role in locomotion by electric modulation of the overactive SNr, but also the result of an improved ability to concentrate, via a normalization of other downstreaming pathways to brain structures, such as the reticular formation and the ARAS. Accordingly, the SNr can be seen as a junction point of ascending and descending motor and non-motor neuronal pathways. DBS influences FOG probably via both components.

5.3. Influence of nigral stimulation on segmental symptoms and side effects

The segmental UPDRS III showed no improvement with additional SNr stimulation, as expected. This result is in line with the results of other studies demonstrating that STN stimulation mainly improves segmental motor symptoms while SNr stimulation mainly shows positive effects on axial motor symptoms (Chastan et al., 2009). It must be pointed out that concomitant stimulation of the STN and the SNr did not change or even worsen the effect of STN stimulation on segmental symptoms. Likewise, motor fluctuations remained unchanged and were well controlled with additional nigral stimulation. Importantly, concomitant stimulation was well-tolerated and safe. In the [STN+SNr] ‘3-week follow-up’, side effects such as dyskinesias occurring from the current flow to the surrounding areas of the stimulated sites arose in a mild form a few days after introduction of the concomitant stimulation in two patients. These dyskinesias could be completely resolved by reprogramming of the SNr-voltage. We assume that in those patients the topographical distance between the two active contacts within the STN and the border zone of the SNr was probably not sufficient and when

additional nigral stimulation was introduced, the current flow spread to the already stimulated STN area with the effect of an overstimulation. Hence, one leading advantage of concomitant stimulation of the STN and SNr is the possibility to maintain the best individual subthalamic stimulation parameters, even if side effects occur after introduction of nigral stimulation with the need of reprogramming.

Furthermore, concerning the side effects of nigral stimulation, one of the most important results of this clinical trial was the absence of non-motor issues such as behavioural changes and neuropsychiatric disorders, for instance depressive or psychotic symptoms, impulsivity, mood changes or a suicidal tendency (Weiss et al., 2013). These symptoms were observed in some earlier studies (Bejjani et al., 1999, Ulla et al., 2011, Kulisevsky et al., 2002) and are probably the reason for a very careful consideration of the SNr as stimulation target. Earlier, in few selected cases, acute depressive clinical states occurred after introduction of high-frequency stimulation of contacts located within the SNr (Bejjani et al., 1999, Blomstedt et al., 2008). Ulla et al. observed hypomanic clinical states in PD patients after electric stimulation of contacts lying mainly in the ventral part of the SNr (Ulla et al., 2011). The reason for the mood changes after nigral stimulation could be an electric activation of the GABAergic pathway from the SNr to the ventral nuclei of the thalamus, which has neuronal projections to the prefrontal and orbitofrontal cortices. These areas are obviously involved in processing mood disorders (Jackowski et al., 2012, Petrovic et al., 2015). Another trial described an involvement of the ventral STN in mood changes via affection of non-motor circuits the STN is involved in (Okun et al., 2009).
Taking into account the previous findings, we assessed very carefully for neuropsychiatric interference in this study and could observe a safe use of additional nigral stimulation. Nevertheless, recognizing the results of the mentioned case reports, a reliable statement on the causal structure leading to the non-motor side effects of DBS cannot be made. The incidence of neuropsychiatric symptoms after SNr stimulation in random DBS cohorts was never tested and consequently remains undefined. In this trial, it seems plausible that the SNr can be at least involved in the emergence of easily reversible

unwanted effects, as they appeared in some cases after introduction of concomitant nigral stimulation. Taking into account the findings of our study and the previous findings patients with subthalamic and/or nigral stimulation should always be monitored attentively for neuropsychiatric symptoms. For an evaluation of the actual risk of neuropsychiatric interference during nigral stimulation, the cohort of this phase II trial was too small and the follow-up time of three weeks was too short to assess all possible neuropsychiatric side effects of SNr stimulation. Nevertheless, as concomitant SNr stimulation was safe in these twelve patients, neuropsychiatric issues might limit the concept only in a proportion of patients. Treatment vigilance towards neuropsychiatric issues is generally needed in PD treatment including standard medication and neurostimulation regimens, as well as novel treatment strategies.

Further assessment on larger cohorts and an extended follow-up period is necessary for solid results concerning the side effects of nigral stimulation.

5.4. Topography of the SNr and stimulation settings

The SNr is largely unexplored concerning the application of DBS in human beings. Neither the best stimulation parameters and programming nor the most adequate location within the SNr for an optimal symptom control concerning axial motor symptoms is fully unravelled at present. This suggests SNr as experimental target that should be considered only in clinical trials.

The motor-related dorsal putamen of the striatum projects to the lateral SNr, which was observed in primates (Hedreen and DeLong, 1991, Lynd-Balta and Haber, 1994). The SNr itself projects to the thalamic motor region, the VM, and then to the supplementary motor area, which was also observed in primates (Francois et al, 2002). These demonstrated connections underline the involvement of the SNr in motor circuitries and predict an important role of the SNr in the modulation and control of neuronal motor activity.

Several studies describe a specific association between the SNr and axial posture control. Burbaud et al. observed severe postural abnormalities after injection of GABAergic agents into the SNr of primates (Burbaud et al., 1998) while Henderson et al. investigated the effect of SNr lesioning in

hemiparkinsonian primates (Henderson et al., 2005) with the result of no improvement concerning bradykinesia, but an influence on body orientation changes such as the direction of the head position.

This data suggests an important role of the SNr in axial motor symptoms in animals, which is in line with the results of our study performed in humans.

Some studies suggest that there is probably a functional topography within the SNr. Different effects on motor symptoms during the application of electric pulses could be observed concerning the medial and lateral part of the SNr. Monkeys, rendered hemiparkinsonian, showed different motor outcomes depending on the site of the SNr where GABA antagonists were injected (Wichmann et al., 2001). Limb akinesia and bradykinesia were reduced after injection of the GABA antagonist in the centrolateral part of the SNr, while injections in the medial part of the SNr caused a general behavioural activation. Injections in the most lateral and the posterior parts of the SNr showed no motor effects in Wichman et al.'s study. In other studies, a beneficial motor outcome could be detected with a localisation of the active electrode contacts more within the medial part of the SNr in PD patients (Chastan et al., 2009) or more in the caudolateral part in the hemiparkinsonian rat (Sutton et al., 2013). In our study, the active electrode contacts were positioned rather within the dorsolateral part of the SNr as shown by the coordinates (Table 2).

Of course, studies performed in humans provide the most reliable results, but due to the lack of SNr studies in the human, the primate SNr is anatomically best comparable to that of the human. In the topography context of stimulation sites, we compared our identified coordinates of the active electrode contacts within the STN and the SNr with those of other studies. The coordinates Chastan et al. determined in their study for the SNr have the mean values 9.7 mm lateral, 8.1 mm posterior and 6.9 mm inferior in relation to the AC-PC line (Chastan et al., 2009). In this study, the mean values for the active contacts within the SNr are 11.1mm lateral, 3.4 mm posterior and 6.1 mm inferior to the MCP. Hence, in our trial the active contact is laying more lateral compared to Chastan's work. This result is in agreement with the effects of GABA antagonist injections in the

posterior part of the SNr in experimental hemiparkinsonian animals showing no motor amelioration in those primates (Wichmann et al., 2001).

Concerning the coordinates of the STN, similar results could be found compared to other studies. In one study using MRI localisation to determine the correct electrode target within the STN, the mean of the final electrode contacts in relation to the AC-PC line was 11.8 mm lateral, 2.4 mm posterior and 3.7 mm inferior to the MCP (Starr et al., 2002). In another study using the fusion of pre- and postoperative MR images to place the electrode correctly within the STN, the mean of the coordinate-values of the contacts laying within the STN was 11.7 mm lateral, 2.1 mm posterior and 3.8 mm inferior in relation to the AC-PC line (Hamid et al., 2005). In our study, the mean values were 12.4 mm lateral, 0.7 mm posterior and 2.6 mm inferior in relation to the MCP (Weiss et al., 2013).

A very important factor in the comparison of the mentioned studies is the interpatient variability of the STN localisation. Of course, the final target coordinates are dependent on features such as age, sex and the method used for localising the target (ParvareshRizi et al., 2010). Optimal electrode placement is a combination of finding the best point for stimulation, thus the point showing the most characteristic electrophysiological recordings, combined with the possibilities MR image fusions offer. Using only a coordinate-template and fixed reference values is not sufficient to detect the best place for the active contacts. Nevertheless, to evaluate whether there is a correlation between the determined coordinates and the FOG outcome in this study, we calculated the correlation coefficient in chapter 4.2.3. The results show that there is no strong correlation between the results of the FOG-AC and the coordinates of the active electrode contacts. The strongest correlation with a coefficient of $r = 0.5$ was detected concerning the z-coordinate of the right SNr, standing for the rostro-caudal orientation of the electrode. This could give a rough tendency concerning the depth of the contact lying within the SNr and the change of the results in the FOG-AC between the modes [StimOff] and [STN+SNr]. The expectation was that the deeper and the more lateral the lowest active electrode contact was located the better the FOG outcome. This assumption was not confirmed in our study. Reasons could be the small sample size of this study, too small differences

concerning the coordinates in our patients or the interpatient variability concerning the right electrode localisation for the individual optimal clinical outcome. To generate better results and an authentic correlation one important aspect would be to use, for instance, the results of the FOG-AC during the [StimOff] mode as reference value, thus to compare only patients with the same result in the FOG-AC while [StimOff] as basis. Only then, the change in the FOG outcome can really be compared.

Table 9 shows the distance between the z-coordinates of the centres of the active contacts on the electrodes within the STN and the SNr. A sufficient distance between these active contacts is important as the current spread from the stimulated STN to the stimulated SNr and vice versa could influence the adjacent tissue and affect or falsify the study results. Wu et al. showed that neuronal tissue can be activated by a 3 volts monopolar stimulation within a radius of 2.5 mm from the centre of the stimulated electrode contact (Wu et al., 2001). This distance is kept in all patients, except PD 9. The mean distance between STN and SNr in all patients was 3.6 mm for the right electrode and 3.5 mm for the left electrode. Of all 48 stimulated electrode contacts, 41 were stimulated with a monopolar programming, six contacts needed bipolar programming and one contact required bipolar programming with two negative contacts. In the above mentioned study, the 3 volts and 2.5 mm current spread were applied in a monopolar setting. Of note, in bipolar stimulation settings, as we used them for six contacts, the current spread is even smaller than in the monopolar programming. Thus, the 2.5 mm radius should be applicable here as well. In PD 9, the distance between the active contacts is less than 3 mm on both sides. The voltage used on all four active electrode contacts in this patient was at most 2.2 volts, which means that the current spreads probably less than a radius of 2.5 mm and one could assume that side effects were caused by the influence of the current delivered to the STN- or SNr-contact. Anyhow, PD 9 needed reprogramming of the SNr-voltage due to dyskinesias occurring within the first days after introduction of [STN+SNr] (Weiss et al., 2013).

Table 9 Distance between the STN- and SNr-contacts on the 24 electrodes of our seven patients in millimetres.
SD = standard deviation, STN = subthalamic nucleus, SNr = substantia nigra pars reticulata.

Patient	distance between z-coordinates (right STN-right SNr)	distance between z-coordinates (left STN-left SNr)
1	3.3	3.3
2	4.1	3.7
3	3.8	3.5
4	3.5	3.3
5	4.3	3.8
6	3	3.6
7	3.1	3.1
8	3.8	3.5
9	2.9	2.3
0	3.7	4
11	3.9	4
12	3.7	3.4
SD	0.44	0.46
mean	3.59	3.46
rounded	3.6	3.5

Another important fact related to the stimulation of the SNr is the observation that stimulation of only the SNr does not control the wide spectrum of motor symptoms in PD. Chastan et al. could demonstrate an amelioration of axial motor symptoms with nigral stimulation but an insufficient control of segmental symptoms when only the SNr was stimulated (Chastan et al., 2009). For this reason, an additional follow-up phase with only nigral stimulation was not taken into account in this study.

Regarding the stimulation frequency, earlier studies showed a broad spectrum of different frequencies used for different stimulation sites. PPN stimulation for gait therapy turned out to be effective at lower frequencies like 35Hz (Stefani et al., 2007, Ferraye et al., 2010, Moro et al., 2010a, Thevathasan et al., 2011a) and at 50 and 70Hz (Moro et al., 2010a) for unilateral PPN stimulation. Given the pathological overactivity of the SNr in PD (Breit et al., 2006) and the idea of a suppression of the local neuronal activity of the SNr with high frequency stimulation (Lafreniere-Roula et al., 2010), frequencies above 70Hz might be more effective on the level of the SNr. In this trial, we used a frequency of 125Hz.

In contrast to the electrophysiological idea of an activation of the PPN with low frequency PPN-DBS (Zitella et al., 2013, Stefani et al., 2007), the mentioned studies underline the idea of an inhibition of the SNr with electric stimulation of this site, resulting in a more balanced function of the BG.

We could not find examples showing the motor outcome of nigral stimulation with low-frequencies in the human, which certainly would be interesting.

Concerning the STN, changing DBS settings to lower frequencies leads to the fact that segmental symptoms are likely to occur, for which reason a reprogramming of the STN stimulation parameters was not possible and not tested in this clinical trial (Moreau et al., 2008, Ricchi et al., 2012).

Nigral stimulation might be a step in the right direction to approach FOG by reprogramming the stimulation settings to interleaved pulses on the level of the STN and SNr.

5.5. Clinical relevance of the study and future development

The present study underlines the potential role of nigral stimulation concomitant to the conventional subthalamic stimulation in the improvement of specific gait disturbances, especially FOG, in patients with advanced PD (Weiss et al., 2013). Even if the actual mechanism of action of DBS is still not fully understood, and although the pathophysiology of gait disability and FOG is not completely figured out, it is known that DBS modulates neuronal circuitries within the BG, which are involved in controlling locomotion and gait (St George et al., 2010). The central question of this study is whether the SNr takes up a key position in the modulation and control of axial symptoms, especially FOG, and whether the imbalance of the SNr function in patients with PD can be restored by DBS of this stimulation site.

Summarizing the data of this clinical trial and of several previous studies dealing with the effect of DBS on axial motor symptoms in advanced PD, we can approve our key assumption at least partly: there may be a positive effect of interleaved STN and SNr stimulation on specific axial motor symptoms in some patients suffering from advanced PD. The motor feature which improved was FOG.

In conclusion this study demonstrated that: (1) interleaved stimulation of the STN and SNr may lead to a specific subjective amelioration of the distinct axial

symptom FOG in PD patients with predominant FOG as disturbing motor symptom and advanced disease stages; (2) concomitant nigral stimulation does not interfere with the positive effect of conventional STN stimulation on segmental motor symptoms; (3) additional nigral stimulation is well-tolerated and can be applied safely in well-selected patients with advanced PD (Weiss et al., 2013).

For the future development in the field of new applications and programming techniques for DBS, our clinical trial suggests that interleaved pulses of STN and SNr might be utilized as a reprogramming option for patients who develop FOG resistant to conventional STB-DBS along disease progression (Weiss et al., 2013). Currently, a follow-up multicentre randomized controlled trial is active and studies 54 patients for the efficacy and safety of [STN-SNr] compared to standard [STNmono] on refractory FOG as primary endpoint after 3 months of active stimulation[1]. Whether the results of this study can be affirmed and consolidated shall be evaluated in this follow-up trial.

To address gait disturbances in an even more specific way than we can do at present, sensitive biomarkers detecting FOG are needed and would provide objective readout about the emergence of FOG under daily life conditions. As indicator of a characteristic electrophysiological neuromuscular mechanism, biomarkers which can be identified by the neurostimulator, could help switching between two stimulating programmes, for instance between the conventional [STNmono] setting and interleaved pulses [STN+SNr]. In this manner, FOG could be addressed in a symptom-oriented manner, warranting the best therapy for segmental and axial symptoms at the same time. Definite and generally accepted electrophysiological neuromuscular patterns behind FOG are not found to date, but they are investigated, for instance by monitoring of freezing episodes while performing fingertapping tasks (Scholten et al., 2015). Real-time monitoring of neuronal activity and establishing closed-loop neurostimulation control systems could help avoiding frequent adjustment of stimulation settings while considering the nature of a neurochemically changing brain environment (Grahn et al., 2014, Rosin et al., 2011).

[1] Source: https://clinicaltrials.gov/ct2/show/record/NCT02588144 [Accessed 03/12/2016]

5.6. Methodological limitations

In this phase II clinical trial, the effect of concomitant STN and SNr stimulation on FOG was assessed within a patient population consisting of twelve participants. This sample size seems modest, however, small patient populations are characteristic for pilot studies. The primary aim of this pilot study was to evaluate methods and procedures for larger future studies on the same topic and to detect possible connections. Even with a sample size of only twelve patients, we could identify some relevant tendencies and made important observations as basis for further studies on greater sample sizes.

The patient characteristics displayed in Table 1 show a group, which seems quite heterogeneous regarding the duration of STN stimulation prior to the study enrolment (six to 79 months) and the duration of the disease. We chose the wide time frame to detect all patients in our centre with severe axial motor symptoms despite best individual therapy. Obviously, a typical feature of advanced disease stages is the occurrence of these resistant axial motor symptoms which are presumptively the result of a progressive neuronal degeneration (Obeso and Olanow, 2011). Thus, the assumption that criteria like disease duration or age at onset are indicators for late-stage PD and therefore for severe axial motor symptoms is comprehensible (Kempster et al., 2010). Nevertheless, a more homogeneous patient population concerning the mentioned aspects could possibly reveal more specific results. Otherwise, a broader spectrum of patients would increase generalisability of the findings from a clinical trial.

The variability within the endophenotypes of idiopathic PD lead to a natural heterogeneity of the patient population. It was reported that criteria, such as age at disease onset or disease progression, can differ broadly between individuals even with genetic classification (Schiesling et al., 2008). Derived from this fact, disease duration is not the only or most important aspect connected to the development of resistant axial motor symptoms.

As all patients met with the inclusion and exclusion criteria, there was no extrinsic factor interfering with the outcome of the study. Thus, based on the study outcome, there was no evidence for excluding patients from the study analysis

as no differences between patients were found that interfered with the intention-to-treat-principle.

Taking into account that DBS might lead to neuronal plastic changes (St George et al., 2010), the wide time range concerning the duration of STN stimulation prior to the study enrolment could be seen as a methodological limitation of the study. However, derived from the current literature, no concluding statement can be made concerning long term plastic changes caused by DBS.

Certainly, a more selective patient recruitment and more specific inclusion criteria might result in a more distinct outcome in future randomised controlled trials on axial motor symptoms in late-stage PD patients.

Another aspect of this study which could be seen as a limitation of the methods is the duration of the follow-up time. As mentioned in chapter 3.2.2., it is quite improbable that a period of three weeks does not suffice to reveal the full outcome of specific DBS programming. Given the observation that bothersome motor symptoms occur immediately after switching off the stimulator and the fact that the motor effects caused by conventional subthalamic stimulation and nigral stimulation can be differentiated shortly after changing the stimulation settings, the assumptions that a 3-week follow-up phase could be sufficient to detect the effect of a certain programming should be acceptable (Chastan et al., 2009, Cooper et al., 2013). However, it is possible that longer observation periods reveal other results as found in this study and unexpected long-term effects, especially for the nigral stimulation setting [STN+SNr]. As PD is a chronic disease and the therapeutic regime a long lasting matter, the long-term outcome is of utmost interest. This aspect is considered in the ongoing follow-up trial (ClinTrials.gov NCT02588144).

6

6. Summary

In advanced disease stages of Parkinson's disease burdening gait and balance disturbances like FOG occur in many cases. Therapeutic options to address these symptoms are dopaminergic medication and DBS of the STN. Unfortunately, FOG response is quite limited in advanced disease stages. Therefore, we tested a novel approach concerning the option of DBS in this study. An advanced programming technique with interleaved pulses applied on the SNr was introduced additionally to the conventional stimulation of the STN. The main aim of the study was to investigate whether the concomitant stimulation of both nuclei [STN+SNr] is superior to the standard stimulation of only the subthalamic nucleus [STNmono] concerning axial symptoms. Twelve patients were enrolled in this randomised controlled double-blind clinical trial. The two stimulation settings were tested in a 2 x 2 cross-over design. The broad-scaled primary outcome measure of the study was the change of the composite 'axial score', a sum score built from the UPDRS II (items 13-15) and the UPDRS III (items 27-31), at the '3-week follow-up'. The secondary endpoints assessments consisted of clinical and anamnestic tests, which addressed specific axial motor symptoms such as FOG, balance, non-motor-symptoms, neuropsychiatric symptoms and quality of life. The outcome of the primary endpoint revealed no statistically significant difference between the two stimulation settings [STNmono] and [STN+SNr] at the '3-week follow-up'. However, the results of the secondary endpoint pointed to a possible superiority of [STN+SNr] concerning specifically FOG, whereas balance did not improve with combined stimulation. Beside efficacy, safety was evaluated and we observed no longer lasting clinically

relevant adverse effects and no neuropsychiatric side effects during [STN+SNr]. Thus, combined stimulation of both nuclei is safe. Altogether, no overall effect and superiority could be detected with the tested stimulation regime concerning a wide spectrum of axial motor symptoms. Nevertheless, concomitant stimulation of both nuclei mentioned might specifically improve FOG resistant to other therapeutic approaches. This study supports the hypothesis that combined stimulation of STN and SNr might be superior to improve FOG. Hence, the findings from this study translate into an ongoing follow-up multicentre randomised controlled clinical trial (ClinTrials.gov NCT02588144).

7

7. German summary

In fortgeschrittenen Krankheitsstadien der Parkinson-Erkrankung treten häufig belastende Gang- und Gleichgewichtsstörungen auf. Die therapeutischen Optionen, um diese Symptome adäquat zu behandeln sind derzeit limitiert und sprechen inkomplett auf konventionelle dopaminerge Therapie oder die konventionelle Tiefe Hirnstimulation des Nucleus subthalamicus an. Aus diesem Grund wurde in dieser Studie ein neuer Ansatz der Tiefen Hirnstimulation getestet. Eine fortschrittliche Programmierungstechnik, bestehend aus sogenannten „interleaved pulses“, welche zusätzlich zur konventionellen Stimulation des STN eine gleichzeitige Stimulation im Bereich der SNr erlaubt, wurde geprüft. Das Ziel der Studie war zu evaluieren, ob die gleichzeitige Stimulation zweier Hirnkerne [STN+SNr] der gewöhnlichen STN-Stimulation [STNmono] überlegen ist in Bezug auf axiale motorische Symptome.

Zwölf Patienten wurden in diese randomisierte kontrollierte doppelblinde klinische Studie eingeschlossen. Die beiden zu testenden Stimulationseinstellungen [STNmono] und [STN+SNr] wurden in einem 2 x 2 cross-over Design geprüft.

Der breit gefächerte primäre Endpunkt der Studie war festgelegt als Änderung des Ergebnisses des zusammengesetzten „axialen Scores“, eines Summen-Scores bestehend aus Teilen des UPDRS II (Frage 13-15) und des UPDRS III (Frage 27-31). Die ausgewerteten Ergebnisse bezogen sich auf die Resultate nach jeweils 3-wöchiger konstanter Stimulation in den beiden Einstellungen. Die sekundären Endpunkte bestanden aus diversen klinischen und anamnestischen Testungen, welche spezifische axiale motorische Symptome untersuchen, wie

FOG und Balance, aber auch neuropsychiatrische Symptome, Lebensqualität und nicht-motorische Beschwerden. Das Ergebnis des primären Endpunktes zeigte keinen statistisch signifikanten Unterschied des axialen Scores zwischen den beiden getesteten Stimulationseinstellungen nach jeweils 3-wöchiger Stimulationsdauer. Die Ergebnisse der sekundären Endpunkte zeigten jedoch eine mögliche Überlegenheit der kombinierten Simulation [STN+SNr] gezielt bezüglich FOG, wohingegen sich die Balance durch die kombinierte Stimulation nicht besserte verglichen mit der konventionellen Stimulation [STNmono].

Neben der Effektivität bezüglich des motorischen Outcomes wurde auch die Sicherheit der kombinierten Stimulation evaluiert. Es wurden keine länger anhaltenden relevanten klinischen unerwünschten Wirkungen beobachtet, sowie keine neuropsychiatrischen Nebenwirkungen während und nach der [STN+SNr] Stimulation. Folglich kann die kombinierte Stimulation des STN und der SNr als sicher bezeichnet werden. Insgesamt betrachtet konnte mit der [STN+SNr] - Einstellung kein allumfassender positiver axialer motorischer Effekt beobachtet werden und keine Überlegenheit verglichen mit dem konventionellen Stimulationsregime. Nichtsdestotrotz konnte festgestellt werden, dass die kombinierte Stimulation möglicherweise gezielt das FOG verbessern kann, welches durch andere therapeutische Ansätze bislang nicht in einer zufriedenstellenden Weise adressiert werden kann. Die Resultate der Studie bieten eine solide und wertvolle Grundlage für weitere Studien zur geschilderten Thematik, sowie für eine sich anschließende randomisierte Phase-III-Studie (ClinTrials.gov NCT02588144).

8

8. References

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9

9. Declaration of Contributions to the Dissertation

The dissertation work was carried out at the Centre for Neurology, Department for Neurodegenerative Diseases of the University of Tuebingen under the supervision of PD Dr. med. Daniel Weiss.

The study was designed by PD Dr. med. Daniel Weiss und Prof. Dr. med. Rejko Krüger.

I carried out and documented all experiments in cooperation with PD Dr. med. Daniel Weiss, PD Dr. med. Tobias Wächter, Marlieke Scholten and Melanie Fritz.

Statistical analysis was carried out by the Institute for Biometry (Dr. biol. hum. Christoph Meisner).

I confirm that I wrote the manuscript myself under the supervision of PD Dr. med. Daniel Weiss, who is the lead author of the publication (Weiss et al., 2013) and that any additional sources of information have been duly cited.

Signed _____ (Margarete Teresa Walach)

on _____ in Tuebingen

10

10. Publication

Parts of this doctoral thesis have been published elsewhere:

WEISS, D., WALACH, M., MEISNER, C., FRITZ, M., SCHOLTEN, M., BREIT, S., PLEWNIA, C., BENDER, B., GHARABAGHI, A., WACHTER, T. & KRUGER, R. 2013. Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. Brain, 136, 2098-108.

11

11. Attachment

UPDRS II (items 13-15) and UPDRS III (items 27-31)

In the clinical observations and study of Parkinson's disease the UPDRS is the most commonly used scale to asses a broad spectrum of symptoms associated with the disease. The test consists of five sections assessing different kinds of symptoms. Section I evaluates mood, behaviour and mentation. Section II assesses the activities of daily living (ADL), such as speech, swallowing, handwriting, dressing, hygiene or cutting food in an anamnestic way. Section III clinically evaluates motor symptoms. Section IV assess the severity of the disease by means of the Hoehn and Yahr scale. Finally, section V evaluates the activities of daily living by means of the Schwab and England ADL Scale.

All items of the UPDRS assessed in the primary and secondary endpoint of this study are 5-point rated and represented by the numbers 0 to 4. Increasing levels of numbers represent increasing levels of impairment on diverse axial motor symptoms. The 'axial score' is a sum score and ranges from 0 to 32 points (Martinez-Martin et al., 1994).

Berg Balance Scale

The Berg Balance Scale is a clinical test, which evaluates balance abilities of a person. It is the gold standard for functional balance tests. The test consists of 14 items, all 5-point rated and represented by the numbers 0 to 4. The sum of all scores is the final measure. Higher scores represent a better outcome and a more independent accomplishment of the task. The balance tasks differ on the level of

difficulty and range from standing up from a sitting position to standing on one foot (Berg et al., 1992).

Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease (CAPSIT-PD) – timed walking test

The timed walking test from the CAPSIT-PD is a test during which patients have to walk seven metres straight and as fast as possible, turn around and walk back. The number of steps, the time the walk takes and the number of freezing episodes are counted (Defer et al., 1999).

Freezing of Gait Assessment Course

The FOG-AC is a rating instrument, which assesses FOG and festinations by means of a show-jumping course which provokes motor blocks through adding motor and mental tasks like carrying a tray with a cup full of water or counting loudly to the actual walking course. The total score of this course ranges from 0 to 36 points, higher scores corresponding to more festinations and FOG during the tasks (Ziegler et al., 2010).

Giladi Freezing of Gait Questionnaire

The Giladi Freezing of Gait Questionnaire consists of six items assessing gait and falls related to freezing in a detailed and subjective way. Two of those items assess gait and four items assess the severity of FOG. All items are 5-point rated and range from 0 = absence of symptoms to 4 = most severe occurrence of the symptom. The total score ranges between 0 and 24 points, higher scores corresponding to more severe symptoms than lower scores (Giladi et al., 2009).

Parkinson's Disease Questionnaire (PDQ-39)

The PDQ-39 is an anamnestic rating instrument which comprises of 39 questions, including typical motor and non-motor symptoms of Parkinson's disease. The test is designed to evaluate the function and well-being of Parkinson's patients. Scores from 0 to 100 reflect the subjective disease state, lower scores indicating less symptoms and a better health and higher scores indicating more severe

symptoms. The questionnaire can be divided in eight subscales: mobility, activities of daily living, emotional well-being, stigma, social support, cognition, communication, bodily discomfort. The answers include an indication of frequency (never, occasionally, sometimes, often, always) with which an event occurs (Peto et al., 1998).

Barratt Impulsiveness Scale (BIS)

The BIS is a self-report measure to assess impulsiveness. It includes 30 items which are scored on a four-point scale. The questionnaire evaluates impulsive personality traits, such as self-control, attention or cognitive complexity. It is a sum score with higher rates expressing an increased impulsivity (Patton et al., 1995).

Beck's Depression Inventory (BDI)

The BDI is a multiple-choice inventory consisting of 21 questions. It is widely used to measure the severity of depressions by evaluation of different items related to the symptoms of depression such as sadness, hopelessness, feelings of guilt or irritability. Physical symptoms are also identified through questions concerning symptoms, such as weight loss, tiredness or a lack of appetite.

The score for each question ranges between 0 and 3 points, higher scores meaning a larger intensity of the depression. By summing the ratings, a maximum score of 63 points can be reached in this inventory. Patients have no or just a minimal depression when they reach < 10 points, a mild to moderate depression with 10 - 18 points, a moderate to severe depression when 19 - 29 points are reached and a severe depression with 30 - 63 points (Beck et al., 1961).

Non-motor Symptoms Scale

The NMSS is a 30-item-questionnaire which can be divided in nine subdomains, assessing non-motor symptoms in Parkinson's patients. This subjective test allows a measurement of the disease by looking at the severity and frequency of different symptoms. Severity is measured on a scale from 0 to 3, frequency can range between 0 and 4 (Storch et al., 2010).

12

12. Acknowledgement

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