The subthalamic nucleus : a novel motor-associative- limbic interface

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THE SUBTHALAMIC NUCLEUS
A NOVEL MOTOR-ASSOCIATIVE-LIMBIC INTERFACE

Cover by Geertjan Zonneveld

Changing the mood.

The cover illustrates the message of this thesis. Applying current to neuronal elements of the subthalamic nucleus can have dramatic effects on one's behaviour: it can make one sad or happy

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THE SUBTHALAMIC NUCLEUS

A NOVEL MOTOR-ASSOCIATIVE-LIMBIC INTERFACE

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus,
Prof. mr. G.P.M.F. Mols
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op vrijdag 2 februari 2007 om 14.00 uur

Door

Yasin Temel

Geboren op 2 juli 1977 te Sivas, Turkije

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*There is a single light of science,
and to brighten it anywhere is
to brighten it everywhere.*

Isaac Asimov (1920–1992)

Aan mijn ouders
Aan Halime
Aan Sara

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CHAPTER 1

Introduction

INTRODUCTION

Once it was believed that the subthalamic nucleus (STN), a relatively small nucleus in the midbrain, was no more than a relay station serving as a “gate” for cortico-basal ganglia-thalamocortical circuits. Nowadays, the STN is considered to be one of the main regulators of motor function related to the basal ganglia, through its fundamental role within the basal ganglia-thalamocortical motor circuit^{1,2}. Clinically, this is evidenced by its involvement in movement disorders such as Parkinson disease (PD)^{3–5}. In PD, the STN displays a continuous abnormal “bursting” mode of activity whereas in physiological conditions it exhibits more or less a regular pattern of discharge with intervals of burst activity^{6–8}. This so-called STN hyperactivity has been implicated in increasing the activity of basal ganglia output nuclei and, consequently, excessive inhibition of their targets^{9,10}. This mechanism is held responsible for at least part of the cardinal PD symptoms such as hypokinesia/bradykinesia and rigidity¹¹. In order to surgically “silence” the hyperactive STN, chronic stereotactic deep brain stimulation (DBS) has been applied since the past decade. Bilateral STN DBS induces a marked long-term improvement in motor function and activities of daily living^{12–21}. DBS has generally the same clinical effects as a lesion with respect to the improvement of motor disability in movement disorders, but has more advantages such as its adjustability and reversibility⁵. Nevertheless, the cellular effects of DBS and lesions are considerably different: a lesion destroys and DBS modulates the (electro)physiological activity of neuronal elements^{6,22–24}. To this day, fundamental knowledge about the mechanisms involved in applying electrical currents to deep brain structures is far from complete.

HISTORY

The STN was initially described by Jules Bernard Luys, a French neurologist. In 1865 he published his first and most important work *Recherches sur le système nerveux cerebrospinal: sa structure, ses fonctions, et ses maladies*, a 660-page masterpiece accompanied by a 80-page atlas filled with illustrations. It was in this book that the subthalamic nucleus (STN) was first reported. The STN was classically regarded as a nucleus exerting a potent inhibitory effect on the cortico-basal ganglia-thalamocortical motor circuit. This theory was substantiated by histopathological findings in patients with hyperkinesias such as hemiballismus, who had mostly hemorrhagic lesions at the level of the STN. One of the first complete descriptions of a hemiballismus in a patient was by Malcolm B. Carpenter²⁵. He described a case of a man who developed abnormal involuntary jerky movements in his left extremities. At autopsy, a recent hemorrhagic lesion in the right STN was found. Carpenter and colleagues published three other studies confirming that (surgical) lesions of the STN resulted in direct contralateral hemiballismus in primates^{26–28}. In the next decades, studies kept on appearing on the relation of STN lesions with hemiballismus, in some reports also called “subthalamic

dyskinesia” or “hemichorea”^{29,30}. Clinically, these observations resulted in the direct association of the STN with hemiballismus, and as a consequence, researchers speculated that STN exerted a potent inhibitory effect on its efferents by using γ -amino butyric acid (GABA) as neurotransmitter.

In the late 80's, it became clear that STN efferents were not inhibitory but excitatory. Smith and Parent conducted a study in which they demonstrated that the STN was entirely devoid of GABA immunoreactive perikarya and showed strong glutamate immunoreactivity³¹. This study clearly established the excitatory nature of the STN. This makes it the only glutamatergic nucleus in the basal ganglia^{32–37}. In this context, Miller and DeLong showed in the same period that the STN was involved in experimental PD and showed hyperactivity³⁸. These observations were the basis for the pioneering work of Bergman and colleagues³⁹. They showed that lesions of the STN in a primate model of PD reduced all of the major motor disturbances in the limbs. In the meantime, Benabid and co-workers had introduced DBS as an alternative for ablative surgery in movement disorders⁴⁰ and were the first, motivated by the results of the lesion studies and by a crucial stimulation study in primates in the same year⁴¹, to explore the effects of STN DBS in a patient suffering from advanced PD⁴². In 1995, the results of 3 patients were published showing that bilateral STN DBS resulted in marked improvement in motor symptoms⁴³. This report was actually the beginning of the STN DBS era.

CURRENT PROBLEM

Since that STN DBS has become a widely applied surgical procedure, more data have appeared on its surgical and target-related side effects^{44–47}. Concerning the latter, clinicians observed behavioural effects such as stimulation-dependent cognitive alterations^{48,49} and changes in affective and emotional behaviour^{50–52}. The occurrence of these unpleasant and debilitating psychiatric effects is a major burden to patients and their families, and is currently without satisfactory explanation. The next case illustrates the severity of such a complication⁵³. Three years after the implantation of electrodes in the STN for advanced PD, a 62-year-old man was admitted to our hospital because of a stimulation-related manic state that did not respond to treatment with a mood stabiliser and that led to chaotic behaviour, megalomania, serious financial debts and mental incompetence. Although adjustment of the stimulation parameters resulted in a normophoric state with a return of insight and capacity to judge, this was only at the cost of a serious exacerbation of his motor symptoms that left the patient bedridden. There was no therapeutic margin between the two states. Ultimately, there seemed to be only two alternatives: to admit the patient to a nursing home because of serious invalidity, but mentally in good condition, or to admit the patient to a chronic psychiatric ward because of a manic state, but with acceptable motor capacity and ADL functions. Thorough ethical evaluation followed.

When not being stimulated, the patient was considered competent to decide about his own treatment; in this condition the patient chose for the second option. In accordance with his own wishes he was therefore legally committed to a chronic ward in the regional psychiatric hospital.

These clinical observations point towards a strong regulatory function of the STN of associative (cognitive) and limbic functions. From an anatomical point of view, tracing studies in rodents and primates have shown that the STN is composed of parts that project to associative and limbic areas of the pallidal complex and substantia nigra pars reticulata (SNr) and that the basal ganglia associative and limbic circuits are processed through the STN^{1,54–56}. The functionality of these projections, however, remains as yet elusive. Furthermore, to which extent these projections are influenced by changing STN's neuronal activity by DBS also needs clarification. Although the beneficial effects of STN DBS and lesions on motor function are relatively well described, the consequences of these surgical interventions on the associative and limbic circuits and associated neuropsychological functions have received relatively little attention.

AIM OF THE PRESENT STUDY

In the present study, we aimed to test the hypothesis that the STN is a strong regulator of associative and limbic functions and that modulation of the STN by DBS results in changes in these functions. For this purpose, we carried out the following set of experiments and the results are described in the following chapters.

In **CHAPTER 2**, we describe the long-term effects of bilateral STN DBS on motor function, and activities of daily living in patients suffering from advanced PD. In **CHAPTER 3**, we describe the effects of bilateral STN DBS on reaction time (RT) performance, which is a specific cognitive (associative) task, in patients suffering from advanced PD. **CHAPTER 4** presents the results of a meta-analysis on the effects of bilateral STN DBS on associative and limbic behaviour in patients suffering from advanced PD. In **CHAPTER 5**, we report the results of our first STN DBS study in rats in order to establish the correct DBS settings in this animal model. The preclinical experiments have been performed to uncover the mechanisms involved in the behavioural effects of STN DBS. Subsequently, we report for the first time the application of bilateral STN DBS in freely moving rats in **CHAPTER 6**. In **CHAPTER 7**, we describe the results of the effects of different stimulation parameters of bilateral STN DBS in Parkinsonian rats on motor and associative functions. We review this set of new data together with data from the literature on the functional role of the STN in associative and limbic circuits in **CHAPTER 8**. In **CHAPTER 9**, we describe the discovery of a novel functional connection between the STN and the dorsal raphe nucleus (DRN) serotonergic neurons which may be important for the effects of STN DBS on the regulation of mood. Finally, in **CHAPTER 10** we provide an overall conclusion.

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CHAPTER 2

Long-term effects of bilateral subthalamic nucleus stimulation in advanced Parkinson disease: a four year follow-up study

Veerle Visser-Vandewalle, Chris van der Linden, Yasin Temel, Halime Celik,
Linda Ackermans, Geert Spincemaille, and Jacques Caemaert

ABSTRACT

In this study we aimed to investigate the effects of bilateral STN HFS in patients with advanced Parkinson disease (PD) at long-term, with a minimum follow-up of 4 years. Twenty patients (15 men, five women) were included, with a mean age of 60.9 ± 8.1 years. Surgery was performed under local anesthesia. The target was defined on computerized tomography (CT). At 3 months, significant improvements were found on the total Unified Parkinson disease rating scale (UPDRS) III (motor) score, in the medication off (from 42.3 ± 9.3 to 19.5 ± 6.4), as well as the medication on (from 18.6 ± 12.1 to 10.1 ± 5.9) phase. The UPDRS IVa (dyskinesias) and IVb (motor fluctuations) scores decreased significantly. At long-term follow-up, there were still significant improvements on the total UPDRS III motor score (from 42.3 ± 9.3 to 24.2 ± 13.2), as well as in all motor subscores, in the off phase, during stimulation. In the on phase, the only significant improvement was seen for rigidity. Complications included hypomania to mania in four patients. Our results indicate that HFS STN results in long-lasting improvement of the motor symptoms, ADL activities and functional performance in patients suffering from advanced PD. The stimulation induced behavioural changes need special consideration.

INTRODUCTION

Since the beginning of the 19th century, surgery has been performed on patients suffering from Parkinson disease (PD). Lesions of the thalamus had a good effect on contralateral tremor, while lesions of the pallidum or Forel's field had a good effect on contralateral tremor and rigidity. In 1967, oral levodopatherapy was introduced and, in contrast to the results of surgery at that time, seemed to have a beneficial effect, not only on tremor and rigidity, but also on akinesia. This led to a drastic reduction in the number of operations performed on PD patients. After a few years however, complications of chronic levodopa-intake became apparent, mainly dyskinesias and on-off fluctuations. This led to a renewed interest in the surgical treatment. In 1985 the attention was focussed on the globus pallidus. Pallidotomies seemed to have a good effect on contralateral tremor, rigidity, and, dyskinesias. High frequency stimulation (HFS) became a safer alternative for performing lesions, which led to a reduction in the complication rate. The unsatisfactory effects of pallidal stimulations on akinesia, however, even when performed bilaterally, motivated researchers and clinicians to search for a better target. The discovery that monkeys treated with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) formed an almost perfect animal model for PD, shed new light on the pathophysiology of the disease. An important discovery was that striatal dopamine deficiency led to an overactivity of the subthalamic nucleus (STN). In 1991, Aziz¹ reported on the beneficial effects of lesions of the STN in two MPTP-monkeys: there was not only a good effect on tremor and rigidity, but also on posture and spontaneous movements. Both monkeys, however, developed hemiballismus. In 1993, Benazzouz² successfully performed HFS of the STN in 2 MPTP-monkeys, without the development of hemiballismus. In the same year, Benabid³ successfully performed the first STN HFS in a patient with advanced PD. Since then many other centres followed, and nowadays the STN is generally considered to be the best target when surgery for patients with advanced PD is considered, improving all cardinal PD symptoms. More and more reports appear on the effects at long-term, most of them with a follow-up of 1 to 2 years. In this study we prospectively analyzed the effects of bilateral STN HFS in 20 patients, with a minimum follow-up of 4 years.

CLINICAL MATERIAL AND METHODS

PATIENT POPULATION

Between June 1997 and September 1998 patients were selected for STN stimulation if they had clinical findings consistent with idiopathic PD and, despite optimal pharmacological treatment, severe response fluctuations and/or dyskinesias. Good initial levodo-

pa response was an absolute criterion. The one exception to this was the inclusion of patients who demonstrated levodopa-resistant rest tremor. Exclusion criteria consisted of significant atrophy, multiple white matter lesions or other focal brain abnormalities on MRI, parkinsonism due to known causative factors, Hoehn and Yahr stage 5 at the best moment of the day, a score < 24 on the mini-mental state examination (MMSE), psychosis, and general contraindications for surgery such as severe hypertension or blood coagulation disorders.

SURGERY

Stereotactic surgery using the Leksell G frame was performed under local anesthesia after overnight withdrawal of antiparkinsonian drugs. The target was defined on computerized tomography with the following coordinates: 11–12 mm lateral to the AC-PC line, at the mid-commissural point, and 4 mm inferior to the intercommissural line.

After making a precoronal burrhole, a rigid monopolar test stimulation electrode was introduced with a diameter of 1.8 mm and a naked tip of 2 mm (Radionics Model 3101–2, Ghent, Belgium) 6 mm above the presumed target. Stimulation was initiated with a frequency of 100 Hz and a stimulus duration of 0.1 ms and continued stepwise every 2 mm, until 4 mm below the target. At each step the stimulus intensity was increased at increments of 0.5 V until the desired response was achieved or until unwanted side-effects occurred. At each point of test stimulation the clinical response was evaluated by one neurologist (CvdL) blinded to the presence of stimulation. The following clinical parameters using the Unified Parkinson disease rating scale (UPDRS)⁴ were scored: tremor (if present) and rigidity in all four extremities, rapid finger movements, closing and opening of the hands, dorsi- and plantar flexion of the feet and leg movements. If a clinical response was not present at or several millimeters below the target, the electrode was withdrawn and a second target through a different trajectory, 2 mm anterior, posterior, medial or lateral to the initial target, was chosen. We allowed ourselves, as much as possible, to be led by the clinical result: if a motor effect was found at a low voltage ($< 3V$), suggesting stimulation of the internal capsule, the second trajectory was made more medially; if sensory symptoms occurred, we chose a more anterior route. After determination of the optimum stimulation site the monopolar electrode was replaced by the final quadripolar electrode (Medtronic Model 3389, Kerkrade, The Netherlands), with the second deepest pole at the level of the best clinical result. At the level of the burrhole the electrode was fixed with methyl metacrylate and connected to an extension cable, which was externalized about 7 cm from the burrhole and connected to an external pulse generator (Medtronic Model 3625). When one week of evaluation with test stimulation showed a clear effect on parkinsonian symptoms, a second operation was performed under general anesthesia, to implant the pulse generator subcutaneously (Itrel 3, Medtronic Model 7425).

CLINICAL EVALUATION

Activities of daily living (ADL) were evaluated using part II, motor performance was evaluated using part III, dyskinesias using part IVa, and motor fluctuations using part IVb of the UPDRS⁴. Lower scores reflect better performances. Functional performance was evaluated through the Schwab and England score (higher scores correspond with better performances). Preoperative UPDRS II and III assessments and Schwab and England scores were conducted in the best on and practically defined off state as described in the CAPIT-PD⁵, 4 to 6 weeks prior to surgery. Postoperative UPDRS III scores were evaluated in the best on and practically defined off scores with the stimulators on at 3 months, and with the stimulators on, and 12 hrs off, at long term follow-up. For motor subscores the following questions of the part III scores were used: for rest tremor question 20 (Q 20) with a maximum score of 20, for rigidity, Q 22 with a maximum score of 20, for bradykinesia Q 31 with a maximum score of 4, for akinesia Q 23+24+25+26 with a maximum score of 32, for gait Q 29 with a maximum of 4, for freezing Q 14 of part II with a maximum of 4. Dyskinesias, and daily appearance and predictability of off periods were evaluated with UPDRS part IVa (dyskinesias) and IVb (motor fluctuations) scores with a maximum of 13 and 7, respectively, preoperatively and postoperatively with the stimulator on.

Since all patients kept their stimulators on continuously, postoperative data on ADL (UPDRS II) and functional performance (Schwab and England) were only collected in the stimulation on condition, in the best on and practically defined off medication state.

All postoperative data were collected in the same sequence, and the patient as well as the investigator were aware of the medical and stimulator's status.

MEDICATION

Medication intake was defined by the LED (L-dopa equivalent dose), which equals 100 mg L-dopa or 133 mg of L-dopa modified release preparations or 1 mg of pergolide, 10 mg of bromocryptine, 1 mg of cabergoline, 6 mg of ropinirole, 1 mg of lisuride or 20 mg of apomorphine.

A stable level of medication was maintained for at least 2 months prior to surgery.

STATISTICAL ANALYSIS

Data, which approximated a normal distribution, will be presented as means and standard deviations. Changes between preoperative baseline, 3-month, and long-term follow-up were analyzed using the paired Student t-test. This was repeated for both states (off and on period) in each clinical parameter. A *p*-value < 0.05 is considered to be statistically significant. All data have been analyzed by SPSS-pc, version 11.0.1 (SPSS inc., Chicago, Ill., U.S.A.).

RESULTS

Thirty-two patients met the selection criteria and consecutively underwent bilateral STN stimulation. Five patients died before the 4-year follow-up was reached. One patient became stuporous after diathermia was used on his back musculature, and died soon afterwards, 1 year postoperatively. The CT-scan revealed a brain stem infarction. One patient committed suicide one year after surgery. The other 3 patients died due to causes clearly not related to surgery. Seven patients were followed by their local neurologist, and are excluded from this study since we have no data with the stimulators being switched off.

Twenty patients (15 males, 5 females) were included in this study. Their mean age at time of surgery was 60.9 ± 8.1 years (range 45–76 years). Patient profiles are listed in Table 1. Surgery was initially performed on the side opposite to the more affected side (in eight patients on the right hemisphere). On this side, the number of trajectories necessary to obtain the optimal result varied between 1 and 5, with a mean of 1.7 ± 1.0 . On the second side, the number of trajectories varied between 1 and 4, with a mean of 1.5 ± 0.83 . The final coordinates are listed in Table 2.

TABLE 1. Characteristics of the 20 patients receiving bilateral STN

Characteristic	
<i>Gender</i>	
male	15
female	5
<i>Age at surgery</i>	
mean $\pm$ SD	60.9 ± 8.3
range	45–76
<i>Disease duration (yrs)</i>	
mean $\pm$ SD	15.0 ± 4.4
range	10–22
<i>Most affected side</i>	
L	8
R	12
<i>Follow-Up (months)</i>	
mean $\pm$ SD	53.6 ± 2.6
range	49–58

OVERALL EFFECT ON MOTOR FUNCTION (UPDRS III)

At 3 months, significant improvements were found in the total UPDRS III score, in the medication off (from 42.3 ± 9.3 to 19.5 ± 6.4), as well as the medication on (from 18.6 ± 12.1 to 10.1 ± 5.9) phase. All motor subscores improved significantly in the medication off phase with a $P < 0.001$ for rigidity, akinesia, bradykinesia, freezing and gait, and $P = 0.001$ for tremor.

TABLE 2. Coordinates of the final electrode in reference to the AC-PC line in 20 patients who received bilateral stimulation of the STN

Distance to mid AC-PC	Number of patients			
	R		L	
X: 13 mm lat Y: 0 mm post Z: 4 mm inf	1	(16)	2	(8,20)
X: 13 mm lat Y: 2 mm post Z: 4 mm inf	1	(9)	1	(4)
X: 11 mm lat Y: 0 mm post Z: 2 mm inf	1	(2)		
X: 11 mm lat Y: 2 mm post Z: 4 mm inf	1	(3)	4	(3,14,16,18)
X: 11 mm lat Y: 0 mm post Z: 4 mm inf	12	(1,4,6,7,12,13, 14,15,17,18,19,20)	10	(2,5,6,7,10, 11,13,15,17,19)
X: 11 mm lat Y: 0 mm post Z: 6 mm inf	1	(5)		
X: 10 mm lat Y: 0 mm post Z: 4 mm inf	3	(8,10,11)	3	(1,9,12)

AC = anterior commissure; PC = posterior commissure; R = right; L = left; lat = lateral; post = posterior; inf = inferior; between brackets, patient numbers are given.

In the on phase, however, the only motor subscores in which significant changes ($P \leq 0.05$) were seen, were rigidity and akinesia. The UPDRS IVa (dyskinesias) decreased significantly ($P = 0.004$) (from 1.9 ± 1.8 to 0.4 ± 0.8), as well as the UPDRS IVb (motor fluctuations) (from 3.7 ± 1.3 to 0.7 ± 1.1) ($P < 0.001$).

At long-term follow-up, there were still significant improvements in the total UPDRS III motor score (from 42.3 ± 9.3 to 24.2 ± 13.2), as well as all motor subscores, in the off phase, during stimulation, albeit slightly less pronounced compared to the 3 month values. In the on phase, the only significant improvement was seen for rigidity (from 3.0 ± 3.3 to 1.1 ± 1.8). For the total UPDRS III score, as well as for all other subscores, improvements were not significant. For gait, there was even a significant increase (from 0.6 ± 0.8 to 1.3 ± 1.2). When both stimulators were switched off, there was an insignificant increase in the total UPDRS III off score. In the medication on condition, there was a significant increase in the total UPDRS III score, as well as in all subscores except for rigidity, when the stimulators were off. The UPDRS IVa and IVb scores decreased significantly (from 1.9 ± 1.8 to 0.4 ± 0.9 , and from 3.7 ± 1.3 to 0.8 ± 1.1 , respectively).

EFFECT ON ACTIVITIES OF DAILY LIVING (UPDRS II), AND SCHWAB AND ENGLAND SCORES.

At 3 months, there was a significant decrease in the UPDRS II score during chronic stimulation, in the off phase (from 23.9 ± 7.2 to 12.6 ± 5.2), as well as in the on phase (from 9.1 ± 5.3 to 4.6 ± 2.4). Also at long-term, decreases were significant in the off phase (from 23.9 ± 7.2 to 9.7 ± 4.0), as well as in the on phase (from 9.1 ± 5.3 to 3.9 ± 2.0). The respective scores were significantly ($P < 0.001$) lower at long-term than at 3 months (9.7 ± 4.0 compared to 12.6 ± 5.2 in the off phase, and 3.9 ± 2.0 compared to 4.6 ± 2.4 in the on phase).

The Schwab and England scores increased significantly in the off phase, at 3 months (from $23.0 \pm 11.7\%$ to $64.5 \pm 10.1\%$) and at long-term (from $23.0 \pm 11.7\%$ to $62.3 \pm 14.1\%$), as well as in the on phase (from $65.8 \pm 9.3\%$ to $72.3 \pm 6.6\%$ at 3 months, and to $74.0 \pm 9.6\%$ at long-term).

SIDE-EFFECTS

All patients reported weight gain which was most pronounced during the first postoperative year (min 3, max 11 kg).

COMPLICATIONS

None of the patients experienced epileptic attacks, intracranial haemorrhages or infections.

Eight patients became confused immediately postoperatively. Seven of them recovered after 2–3 days. In one patient (male, 67 years), this confused state lasted for 3 weeks.

In four patients at long-term there was a worsening of speech (reduced volume), when the stimulators were on, compared to the stimulation off condition.

One patient suffered from blepharospasms, clearly related to the stimulation. He refused any treatment.

Four patients (all men) presented with hypomanic to manic characteristics. In three of them this became apparent soon after discharge from the hospital. In two of them hyperactivity and verbal aggressiveness were the main symptoms. One patient started to spend an exuberant amount of money. This was, however, in proportion to his budget, and the psychiatrist judged it to be a “normal” euphoric state, i.e. in proportion to his improved clinical condition. In one patient, changes in behaviour were seen only after 1 year. This patient exhibited hypersexuality and started to steal money from his wife for prostitution. Evaluations by a psychologist, blinded to variations in voltage bilaterally, revealed that these effects were related to stimulation.

TABLE 3. Outcome scores on the UPDRS (parts II and III) and medication in 20 patients who receiving bilateral stimulation of the STN.

Scoring factor	Baseline	3 months Follow-Up			Long Term Follow-Up		
		Stim ON			Stim OFF		
		Score	%	P Value	Score	%	P Value
OFF							
UPDRS II	23.9 ± 7.2	12.6 ± 5.2	-47.3	0.004	Not evaluated	9.7 ± 4.0	0.004
UPDRS III total	42.3 ± 9.3	19.5 ± 6.4	-54.0	< 0.001	53.6 ± 20.6	24.2 ± 13.2	< 0.001
tremor	4.2 ± 4.5	0.7 ± 1.1	-83.0	0.001	4.3 ± 4.7	1.7 ± 3.9	0.039
rigidity	9.1 ± 3.3	1.7 ± 2.1	-81.0	< 0.001	9.0 ± 4.5	2.5 ± 3.3	< 0.001
akinesia	15.0 ± 4.9	7.7 ± 3.9	-48.7	< 0.001	20.1 ± 9.3	11.2 ± 5.9	0.046
bradykinesia	3.1 ± 0.8	1.5 ± 0.6	-51.6	< 0.001	2.6 ± 1.3	1.9 ± 1.5	0.015
freezing	2.6 ± 0.9	1.0 ± 0.7	-61.5	< 0.001	1.7 ± 1.8	1.2 ± 1.1	0.013
gait	2.8 ± 0.8	1.4 ± 0.7	-50.0	< 0.001	3.1 ± 1.1	1.8 ± 1.2	0.002
Schwab & England	23.0 ± 11.7	64.5 ± 10.1		< 0.001	Not evaluated	62.3 ± 14.1	< 0.001
ON							
UPDRS II	9.1 ± 5.3	4.6 ± 2.4	-49.5	0.023	Not evaluated	3.9 ± 2.0	0.040
UPDRS III total	18.6 ± 12.1	10.1 ± 5.9	-45.7	0.002	38.3 ± 18.5	14.4 ± 7.5	0.162
tremor	1.3 ± 2.6	0.2 ± 0.7	-84.6	0.063	4.0 ± 5.5	0.2 ± 0.7	0.079
rigidity	3.0 ± 3.3	1.7 ± 2.1	-43.3	0.046	6.1 ± 6.0	1.1 ± 1.8	0.030
akinesia	6.5 ± 4.8	3.9 ± 2.6	-40.0	0.007	15.7 ± 8.6	6.9 ± 5.0	0.961
bradykinesia	0.4 ± 0.6	0.4 ± 0.6	0.0	1.000	2.2 ± 1.4	0.4 ± 0.5	1.000
freezing	0.7 ± 0.7	0.4 ± 0.5	-42.9	0.083	1.8 ± 1.6	0.5 ± 0.8	0.546
gait	0.6 ± 0.8	0.4 ± 0.5	-33.3	0.171	2.5 ± 1.3	1.3 ± 1.2	0.039
Schwab & England	65.8 ± 9.3	72.3 ± 6.6		< 0.001	Not evaluated	74.0 ± 9.6	< 0.001
UPDRS IVa	1.9 ± 1.8	0.4 ± 0.8	-79.0	0.004		0.4 ± 0.9	0.009
UPDRS IVb	3.7 ± 1.3	0.7 ± 1.1	-81.0	< 0.001		0.8 ± 1.1	< 0.001
MEDICATION							
LEU	1133 ± 383	439 ± 237	61.3	< 0.001		598 ± 413	0.023

Values are expressed as means ± SD.% = change in% (+:increase,-:decrease) with respect to baseline.

STIMULATION PARAMETERS

At long-term, stimulation was bipolar in 14 patients on the right side, and in 16 on the left. At 3 months, the mean voltage was 1.8 ± 0.6 right and 1.9 ± 0.9 left, with a mean pulse width of 333.2 ± 126.8 right and 288.7 ± 139.7 left, and frequency 134.5 ± 14.0 right, and 141.6 ± 23.0 left. At long-term, the mean voltage was 2.6 ± 0.8 right and 2.6 ± 0.9 left, with a mean pulse width of 169.3 ± 62.9 right and 177.7 ± 90.6 left and frequency 133.4 ± 13.8 right and 136.9 ± 18.8 left.

MEDICATION

The LED decreased significantly by 61.3% at 3 months (from 1133 ± 383 to 439 ± 237), and by 47.2% at long-term (from 1133 ± 383 to 598 ± 413).

PULSE GENERATOR'S LIFE DURATION

Pulse generators had to be changed because they had reached the end of their life in 22 of 40 implantations, after a mean follow-up of 44.8 ± 14.1 months.

DISCUSSION

This study showed that there was a significant effect of bilateral STN stimulation on ADL (UPDRS II) and motor function (UPDRS III) in the medication off phase, at 3 months and at 4 year follow-up. The positive effect on motor function was significant for the total UPDRS III score, as well as for all the subscores (tremor, rigidity, akinesia, bradykinesia, freezing and gait). The effect on ADL was significantly better at long-term compared to the score at 3 months. The effect on motor functions (total score as well as all subscores), seemed to be less pronounced at long-term than at 3 months. All these latter changes in effects between short- and long-term evaluation were, however, not statistically significant.

In the medication on phase, the effect on ADL was again clearly pronounced at 3 months and at long-term, with a significantly better effect at long-term. The increase in UPDRS III motor score and the decrease in the rigidity subscore at long-term, compared to the 3-month scores, were not statistically significant. The tremor and bradykinesia subscores remained the same. For akinesia, freezing and gait, the effect seems to be worse at long-term than at 3 months. This was, however, only significant for akinesia and gait.

The effects on the UPDRS IVa and IVb were significant, and stable, at 3 months and at long-term (the difference in UPDRS IV b values at 3 months compared to long-term is not significant).

All ratings were performed by two unblinded investigators, with the patient also being unblinded. It has been discussed before that in blinded studies patients as well as investigators are often aware of the on or off status of the stimulation because of the symptom relief experienced by the stimulation or because of symptoms that occur at the onset of stimulation, like paresthesias⁶.

These results are comparable to other studies with long-term follow-up. Table 4 shows the results of the effects of bilateral STN stimulation in advanced PD in studies with a minimum of ten patients and a minimum follow-up of 1 year for all patients. The average decrease on the UPDRS III is 54.9% in the medication off phase. The UPDRS II off score decreases by an average of 45.6%. Dyskinesias and motor fluctuations improve by an average of 76.8%. The decrease in the UPDRS III on is better in our study (45.7% , vs an average of 24.3%). This may be due to a difference in postoperative medication reduction. In this study medication could be reduced at 3 months by 61.3%, and at long-term by 47.2%. The change in LED reduction between 3 months and long-term is not statistically significant. The average medication reduction in the studies described in Table 4, is 57.8%, at long-term.

The increase in voltage from 3 months to long-term (from 1.8 ± 0.6 to 2.6 ± 0.8 at the right side, and from 1.9 ± 0.9 to 2.6 ± 0.9 at the left), is counteracted by the decrease in pulse width (from 333.2 ± 126.8 to 169.3 ± 63.0 at the right, and from 288.7 ± 139.7 to 177.7 ± 90.6 at the left). It was our policy to reduce pulse widths during the postoperative period in these patients in order to achieve a more selective effect of stimulation, and thus reduce stimulation induced side-effects (mainly speech disturbances and behavioural changes).

The less obvious effect on the UPDRS III in the medication on phase can be, at least partially, explained by the significant increase in the subscores for akinesia and gait compared to the 3 months scores, with even a significant increase in the gait subscore compared to baseline. The difference in effect on the UPDRS II compared to the UPDRS III score is even more pronounced in the medication on than in the medication off phase. One possible explanation might be the marked reduction in dyskinesias, which make patients function better during ADL in the on phase. On the other hand, the decrease in effect on UPDRS III on can be explained by progression of symptoms that are not in response to levodopa, like balance disturbances.

The insignificant change on the UPDRS scores after 4 years when both stimulators were switched off in the medication off condition, compared to baseline, might be suggestive of a previously hypothesized neuroprotective effect of bilateral STN stimulation⁷. Nigrostriatal degeneration, which occurs in PD, leads to electrical hyperactivity of the STN. The dopaminergic part of the substantia nigra (SNc) receives an excitatory input from the STN. This raised the possibility of NMDA-mediated excitotoxic damage to the SNc as a result of STN hyperactivity. It has been hypothesized through

animal studies that “silencing” the STN by HFS could lead to slowing of or even halting the disease progression^{8,9}. To date, however, no clear clinical evidence has become available to support this hypothesis.

This study confirms that the implantation of electrodes in the brain is a safe procedure. The stimulation-induced behavioural changes do, however, require special consideration. In this study personality changes were obvious in four patients. In one of them, hypomania with hypersexuality became apparent only one year after surgery. This was not preceded by a change in medication or stimulation parameters. One patient with bilateral STN stimulation for advanced PD was not included in this study because he committed suicide before the 4 year follow-up was reached. Of the 349 patients described in Table 4^{6,10–23,35} presented with persistent changes in mental and/or psychic functioning. More and more reports appear with special focus on the effects on mood of bilateral STN stimulation^{24–32}. STN stimulation seems to induce euphoria, an increase in motivation, and a decrease in fatigue, anxiety, and tension. Although this effect might be partly secondary to the improved motor function, PET-studies have shown that STN stimulation directly influences limbic-related structures like the anterior cingulate gyrus³³. Moreover, acute effects on mood with mirthful laughter related to STN DBS have been described that cannot be explained by a change in motor function²⁸. After STN DBS, not only euphoric states, but also depression is described. Depression after STN DBS might due to three factors. First of all, it might be caused by the postoperative decrease in levodopa which is known to have a psychotropic effect. Secondly, a direct influence of STN DBS on limbic-related structures might be responsible, or, current spread to neighbouring structures, like the substantia nigra. The problem with these mood changes is that they go mostly hand in hand with the positive motor effect: in several patients (all hypomanic) we tried to change stimulation parameters to maintain the motor benefit and avoid behavioural changes, but these attempts were always unsuccessful. The disappearance of the behavioural changes were probably not secondary to the motor deterioration, since only acute effects were tested, and the after effect for motor function after the stimulators were switched off, was longer than the after effect for behaviour.

CONCLUSION

The conclusion of this study is threefold. First of all, the beneficial effects of bilateral STN stimulation on motor function (UPDRS III) and ADL (UPDRS II) is maintained after four years of follow-up. Secondly, these positive effects of bilateral STN stimulation can be obtained by a relatively simple technical procedure, with target determination on CT-scan, without depth-recordings. Thirdly, stimulation-induced behavioural changes after STN DBS require special consideration. They may occur late in the postoperative course.

TABLE 4. Results of bilateral stimulation of the STN in Parkinson disease.

Authors, Journal	N	F-U	Results	Complications	Medication reduction
Limousin <i>et al.</i> N Engl J Med, 1998	20	12	UPDRS II off: -60% UPDRS III off: -60% UPDRS III on: -50% Dyskinesias: -60%	1: intracerebral hematoma with persistent paralysis and aphasia 1: infection (pulse generator and extension removed for 6 m) 8: transient changes in mental status 5: mild, transient aphasia 1: worsening of dysphonia and postural instability 18: weight gain (mean 4.2 kg)	-50% (levodopa)
Fraix <i>et al.</i> Neurology, 2000	24	12	UPDRS III off: -68% UPDRS III on: 16% Dyskinesias: -93%	not mentioned	-81% (levodopa)
Volkmann <i>et al.</i> Neurology, 2001	16	12	UPDRS III off: -67% UPDRS III on: "little changes"	transient abulia; common transient anhedonia; common mild dysphonia; common mild hypersalivation; common 2: infection 1: lead dislodgement 1: lead fracture	-65% (levodopa)
Krause <i>et al.</i> . JNNP, 2001	12	12	UPDRS II: 26% UPDRS III off: "stat. sign. improvement" UPDRS III on: "no sign. change" Dyskinesias: 58% Fluctuations: 64%	1: intracerebral hematoma 1: strong increase in libido other mild stim related side-effects	-34% (LED)
Simuni <i>et al.</i> J Neurosci, 2002	12	12	UPDRS III off: -47% UPDRS III on: -2% UPDRS IV: -64%	1: delayed chronic subdural hematoma -->lead infection-->removal 1: subcortical hemorrhage with hemiparesis and aphasia 1: single generalized seizure 1: infection of battery (removed) 1: transient change in mental status 2: infection (system removal) 2: worsening of preop depression/dementia	-55% (LED)
Vesper <i>et al.</i> Surg Neurol, 2002	38	12	UPDRS II off: 38% UPDRS III off: 48% UPDRS III on: 39% UPDRS IV off: 58% UPDRS IV on: 72%	no serious side effects	-53% (levodopa)
Ostergaard <i>et al.</i> Mov Dis, 2002	26	12	UPDRS III off: -64% Dyskinesia duration: -86% Motor fluctuations: 79%	2: weight gain > 2 kg	-22% (LED)
Spottke <i>et al.</i> J Neurol, 2002	16	12	UPDRS II off: 46% UPDRS II on: 31% UPDRS III off: 66% UPDRS III on: 31% UPDRS IV: 86%	transient and usually mild	-51% (levodopa)
Vingerhoets <i>et al.</i> Neurology, 2002	19	12	UPDRS II off: -37% UPDRS III off: -45% Dyskinesias: -92% Fluctuations: -95%	3: acute agitation (transient) 3: depression	-79% (LED)

TABLE 4. Continued.

Thobois <i>et al.</i> J Neurol, 2002	14	12	UPDRS II off: -52.8% UPDRS III off: -55% UPDRS IV: -76%	2: lower limb paresthesia 1: pulmonary embolism 6: depression 1: dysarthria and freezing 1: sialorrhea and drooling 1: postural imbalance	-65.5% (LED)
Romito <i>et al.</i> Neurology, 2002	22	12	UPDRS II off: -48.2% UPDRS III off: -50.2%	weight gain in all pts (average+12.4%) 2: transient hypersexuality 4: transient manic psychosis 1: seizure 4: hypophonia 4: hypophonia and dysarthria 4: eye lid opening apraxia 2: worsening of depression 2: psychic akinesia 1: limb dystonia 1: bilateral buccinator spasm 7: short-lasting psychosis 1: lead migration 1: SAH 3: unexplained switching off of device 2: sudden end of battery	-65.8% (LED)
Herzog <i>et al.</i> . Mov Dis, 2003	48	24	UPDRS II off: -43% UPDRS III off: -57% UPDRS III on: -35% Dyskinesias: 85%	1: subdural hematoma 1: intracerebral bleeding 2: dislocation of pulse generator 7: confusion 2: psychosis 2: hypomania 5: depression 2: dysarthria 5: eye-opening apraxia	-68% (LED)
Pahwa <i>et al.</i> J Neurosurgery, 2003	33	28	UPDRS II off: -27% UPDRS II on: +10% UPDRS III off: -28% UPDRS III on: -13%	10: dysarthria 3: gait abnormalities 2: psychosis 1: depression 1: muscle spasms 12: lead replacement 2: infections	-57% (LED)
Krack <i>et al.</i> New Eng J Med, 2003	49	60	UPDRS II off: -49% UPDRS II on: +92% UPDRS III off: -54% UPDRS III on: +48% dyskinesias: -65%	1: suicide 2: intracerebral hemorrhage 8: asymptomatic bleeding 3: contusion 2: dementia 12: delirium 2: seizures 4: wound healing problem 1: infection 2: repositioning stimulator 15: eye-opening apraxia 1: impulsive aggressive behaviour 4: hypomania	-63% (LED)
off: off-medication		on: on-medication	SAH: subarachnoidal hemorrhage		LED: levodopa equivalent dose

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CHAPTER 3

Differential effects of subthalamic nucleus stimulation in advanced Parkinson disease on reaction time performance

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ABSTRACT

The aim of the present study was to assess the effect of bilateral subthalamic nucleus (STN) stimulation and dopaminergic medication on speed of mental processing and motor function. Thirty-nine patients suffering from advanced Parkinson disease (PD) were operated on. Motor function and RT performance (simple RT (SRT) and complex RT (CRT)) were evaluated under four experimental conditions with stimulation (stim) and medication (med) on and off: stim-on/med-on, stim-on/med-off, stim-off/med-off and stim-off/med-on. In the last condition, the patients received either low medication (usual dose) or high medication (suprathreshold dose). STN stimulation improved the motor performance in the SRT and CRT tasks. Furthermore, STN deep brain stimulation (DBS) also improved response preparation as shown by the significant improvement of the RT performance in the SRT task. This effect of STN DBS on the RT performance in the SRT task was greater as compared with the CRT task. This is due to the more complex information processing that is required in the CRT task as compared to the SRT task. These data suggest that treatment of STN hyperactivity by DBS improves motor function, confirming earlier reports, but has a differential effect on cognitive functions. The STN seems to be an important modulator of cognitive processing and STN DBS can differentially affect motor and associative circuits.

INTRODUCTION

The human subthalamic nucleus (STN), a relatively small disk-shaped structure, is considered to be the “pacemaker” of the basal ganglia¹. In Parkinson disease (PD), the STN becomes hyperactive and exhibits “burst” activity^{2–4}. Modulating STN hyperactivity is probably the key mechanism whereby deep brain stimulation (DBS) improves Parkinsonian features^{3,5}. Various studies have shown that this improvement is long-lasting^{6–9}. Cognitive changes due to STN DBS have primarily been described in small case series^{8,10–21}. On the other hand, other groups did not find any effect of STN DBS on cognitive processing^{22–26}. An ongoing debate is to which extent cognitive functions are directly related to STN DBS. Anatomical tracing studies in rodents and primates have shown that the STN is composed of parts that project to associative and limbic areas and that the basal ganglia associative and limbic circuits are processed through the STN^{27–30}. The functionality of these projections, however, remains as yet elusive. Furthermore, to which extent these projections are influenced by changing STNs neuronal activity by DBS also needs clarification. In the experimental setting, bilateral STN stimulations have been carried out in rodents showing profound effects on cognitive parameters^{31,32}.

One of the cognitive functions that have been described to be changed following STN DBS in PD patients is the speed of cognitive processing. Saint-Cyr and associates studied the general cognitive changes in PD patients following STN DBS²⁰. One of the conclusions was that patients showed signs of mental slowing. This was based on information obtained from caregivers (clinical impression) and patients (self-report). As speed of mental processing was not measured directly, it remained unclear whether there was objectively a slowing. Furthermore, the magnitude of this slowing was unknown and the mechanisms underlying this phenomenon were not explained. This is of importance as it could be an intrinsic effect of STN stimulation but might also be related to medication or even to a combination of these two.

Cooper and colleagues validated a paradigm to evaluate speed of cognitive processing directly in PD patients using the simple and complex reaction time (RT) performance and introduced a subtraction method to evaluate pure cognitive reaction time³³. RT performance, often used to separate cognitive and motor function in PD, allows one to distinguish between perceptual, cognitive and motor actions^{33–37}.

In the present study, central processing was investigated using the simple (SRT) and complex (CRT) RT concept in advanced PD patients in relation to STN stimulation and dopaminergic medication. We evaluated these different possibilities by testing PD patients in conditions with both stimulators on and off, and with medication on and off. In addition, we used two groups of patients that differed in the amount of medication (low versus high). These manipulations allowed us to evaluate specifically the effects of STN DBS and dopaminergic medication on central processing and motor function.

MATERIALS AND METHODS

PATIENTS

Between January 2002 and January 2004, patients were selected for bilateral STN stimulation if they had clinical findings consistent with idiopathic PD and, despite optimal pharmacological treatment, severe response fluctuations and/or dyskinesias. Good initial levodopa response was an absolute criterion. The one exception to this was the inclusion of patients who demonstrated levodopa-resistant resting tremor. Exclusion criteria consisted of significant atrophy, multiple white matter lesions or other focal brain abnormalities observed on MR images, parkinsonism due to known causative factors, Hoehn and Yahr³⁸ stage 5 at the best moment of the day, psychosis, significant cognitive dysfunction, severe affective disorders, and general contraindications for surgery such as severe hypertension or blood coagulation disorders. Informed consent was obtained from all patients and STN DBS was approved by the local Ethical Committee.

SURGERY

In all patients, the entire stereotactic procedure was performed under local anaesthesia after withdrawal of antiparkinsonian drugs for 24 h. The CRW-stereotactic frame was used and stereotactic coordinates were determined on fused CT/MRI-images (Radionics, Belgium). The following coordinates were used in order to target the STN: 11–12 mm lateral to the AC-PC line, at the mid-commissural point, and 4 mm inferior to the inter-commissural line³⁹. After making a pre-coronary burr hole, a semi-micro-electrode was introduced for electrophysiological recordings (Neuromap, Radionics, Belgium) 6 mm above the defined target. Using steps of 1 mm, the neuronal (multi-unit) activity was recorded at a point 4–5 mm below the target. The STN was characterized by a neuronal firing pattern consisting of increased baseline activity and a strong increase of high-voltage spikes, which were usually present over a length of 4–5 mm. Subsequently, intra-operative macrostimulation was conducted using a rigid monopolar test stimulation electrode (Radionics, Belgium). Macrostimulation started at 4 mm above the target and continued stepwise every 2 mm to a point 4 mm below the target. At each point of test stimulation the clinical response was evaluated by the neurologist.

The following clinical parameters using the Unified Parkinson's disease rating scale (UPDRS)⁴⁰ were scored: tremor (if present) and rigidity in the contralateral extremities, fast finger movements, closing and opening of the hands, dorsi- and plantar flexion of the feet and leg movements. When a good effect was found in the absence of side effects, the test electrode was replaced by the final quadripolar electrode (Medtronic Model 3389) with the second deepest pole at the level of the best clinical result. After verification of the exact position of the final electrode by fluoroscopy, it was fixed in the burr hole with methyl metacrylate (Antibiotic Simplex; Howmedica, Ireland) and

connected to an extension cable, which was externalized about 7 cm from the burr hole. The same procedure was performed on the contralateral side. The present surgical procedure has been described elsewhere in more detail⁴¹.

In all patients, an MRI-scan was performed on the second post-operative day to evaluate the position of the electrodes and to detect asymptomatic bleeding. The patients were tested for a period of one week with both electrodes connected to an external pulse generator (Medtronic Model 3625). Once agreement was reached between the neurologist, neurosurgeon, nursing staff and the patient about the beneficial effects of stimulation, a second operation was performed to implant the pulse generator infraclavicularly or abdominally (Medtronic Model 7428/Kinetra). After this operation, patients were systematically screened during the first months to find the optimal stimulation parameters.

EXPERIMENTAL GROUPS AND CONDITIONS

All patients were examined by a neuropsychologist specialized in PD (PB). Patients underwent extensive standardized clinical and psychometric evaluations. Patients with significant cognitive or mood impairment according to previously published criteria⁴², were excluded from the present study.

The patients were randomly assigned to two groups (groups A and B). In order to study the effect of dopaminergic medication, patients of group A were given the usual amount of medication (mean l-dopa equivalent dose (LED) = 158.7 ± 21.1 mg) and patients of group B received a suprathreshold dose plus a peripheral decarboxylase inhibitor exceeding the usual morning dose, which was 151.2 ± 28.6 mg in group B, by 50 to 100 mg of levodopa, in the stim-off/med-on condition. This suprathreshold dose is thought to reflect the maximum effect of dopaminergic medication on motor performance²⁵.

All patients underwent motor and RT examinations in the four experimental conditions. The sequence of these conditions was as follows. Patients came to the hospital in the morning (0900 hours) with their stimulators on and having already taken their usual morning medication (mean LEDs = 158.7 ± 21.1 mg for group A and 151.2 ± 28.6 for group B). Patients were instructed to take the medication around 0700 h. Motor evaluations started around 0930 h which usually took 30 minutes followed by RT tasks which also took 30 min in total for simple and go/no go RT. This was the first condition with stimulation (stim) on and medication (med) on (stim-on/med-on). In all cases, the CRT was performed before the SRT. The next assessments (motor and RT) were after a drug withdrawal of 9 hours at 1600 h (stim-on/med-off). Thereafter, the stimulator was switched off by the investigator and the patient was instructed to take the medication only for the afternoon and evening. The next morning, all patients had a minimum of 9 h drug withdrawal and 12 h stimulation off. This was the third assessment (stim-off/med-off). Immediately after this test, patients of group A were given

the usual amount of medication (low medication) and patients of group B received a suprathreshold dose (high medication). Three hours later the final assessment was performed (stim-off/med-on). This sequence was chosen for two reasons. Firstly, for the patient's comfort as the stim-off/med-off phase, the worst condition was overnight. Secondly, to minimize a possible confounding effect of learning across test sessions. Thus, the sequence of testing was not associated with an increase in the condition of the patients (stim-on/med-on: best; stim-on/med-off: intermediate; stim-off/med-off: worst; stim-off/med-on: intermediate). There were some constraints (i.e., organizational and ethical issues) preventing completely randomization of the stimulation and medication sequence between subjects.

The manufacturer of the RT device (RT task, Wiener Testsystem PC/S, Schuhfried GmbH, Mödling Austria) provided normalized data of age- and sex-matched healthy subjects with respect to RT performance. This control group was not significantly different from our study group with respect to the level of education.

MOTOR EVALUATIONS

Activities of daily living (ADL) were evaluated using part II and motor performance was evaluated using part III of the UPDRS. Disease stage was assessed using Hoehn and Yahr score and functional performance was evaluated through the Schwab and England score. These scores were evaluated in the best medication on and practically defined medication off scores with both stimulators on and off for 12 hrs as described in the CAPIT-PD⁴³, which resulted in the aforementioned four experimental conditions. All data were collected in the same sequence, and the patient as well as the investigator was aware of the status of the medication and stimulation. To minimize the effect of fatigue, the battery of tests was designed to be as brief as possible. Whenever fatigue was a factor, evaluations were extended across multiple sessions.

Medication intake was defined by the LED equalling 100 mg l-dopa or 133 mg of l-dopa modified release preparations or 1 mg of pergolide, 10 mg of bromocryptine, 1 mg of cabergoline, 6 mg of ropinirole, 1 mg of lisuride or 20 mg of apomorphine⁴⁴. In the present study, the optimal clinical effect of medication appeared after approximately 2.5 h, which is related to the use of slow-release medication. Since the experimental session (stim off-med on) took place 3 h after medication, we assume that the patients benefited to a great extent from medication around this time point.

REACTION TIME PERFORMANCE

Reaction time performance (simple and choice RT) was assessed in the four experimental conditions. The SRT and CRT performance were measured using the same automated device (Reaction time task, Wiener Testsystem PC/S, Schuhfried GmbH, Mödling Austria). This device consisted of two buttons positioned in the midline in a vertical

line 7 cm apart. Above these buttons, two lights (left yellow, right red; 7.5 cm apart) were positioned in a horizontal line. In addition, a tone could be presented via this device. In both tasks (SRT and CRT), subjects started a trial by putting the index finger of their preferred hand on the lower button (start button). Subsequently, the subjects had to respond as quickly as possible to the presentation of a stimulus by releasing the start button and pressing the higher button.

In the SRT, the subjects had to respond to the yellow light that was presented with a variable interval (0.2–4 s). After an initial instruction and training session, 64 trials were performed. For the CRT task, two stimulus dimensions were used. This test has been proven to be adequate for detecting cognitive changes in PD³³. In the CRT, six different response conditions could occur with a variable interval (0.2–4 s). These conditions were either a single stimulus (red, yellow or tone), or a combination of these stimuli (i.e., red light + yellow light, red light + tone, yellow light + tone). The patients were instructed only to respond (pressing the response button) to a combination of red and yellow light or the yellow light and a tone. In case of one of the other four possible conditions, the patients were told not to react. In both tests, times to release the start button and press the response button were measured and were taken as reaction time (RT) and motor time (MT), respectively. In addition, omissions (non-responses) and incorrect responses (only in the CRT task) were measured. Only correct RTs were evaluated for SRT and CRT performance.

We have used one task for the simple and one task for the go/no go condition to minimize the effects of fatigue, as the battery of tests was already extensive. In addition, we expected the motor response times across different tasks to be consistent because the tasks were performed using the same device, with the same set-up, requiring the same motor executions. The only difference was the stimuli to which patients had to react. This means that any disproportionate difference between the tasks is the result of the difference in cognitive load.

STATISTICAL ANALYSIS

Data are presented as means and standard errors. A separate analysis did not show any significant effect of follow-up duration, sex, age at surgery, disease duration or stimulation settings. Subsequently, these factors were excluded in the ANOVAs.

For the clinical motor data, a two factorial within-subjects ANOVA (Medication and Stimulation) was performed. For the RT data, a four factorial mixed between-subjects (Dose) within-subjects ANOVA (Task, Medication and Stimulation) was used. To perform a more detailed evaluation of differences between conditions, a Sidak *post-hoc* test ($P < 0.05$) was used. Omissions and incorrect responses were analysed using the non-parametric Friedman test. All data were analysed by SPSS-pc, version 11.5 (SPSS inc., Chicago, Ill., U.S.A.).

RESULTS

DEMOGRAPHIC DATA AND STIMULATION SETTINGS

Thirty-nine PD patients with bilateral STN DBS were included and underwent the battery of clinical and RT evaluations. Nineteen patients (11 males, eight females), were included in group A and 20 (11 males, nine females) in group B. Their mean age at the time of surgery was 60.2 ± 1.5 and 59.8 ± 1.8 years with a mean disease duration of 14.9 ± 0.9 and 16.0 ± 1.4 years in groups A and B, respectively. The mean follow-up durations were 13.4 ± 2.4 (group A) and 13.8 ± 1.8 (group B) months. Patient characteristics are listed in table 1.

Stimulation amplitudes were 2.7 ± 0.4 V and 3.0 ± 0.2 for the left side and 3.2 ± 0.3 V and 3.3 ± 0.2 for the right side, stimulation frequencies 170 ± 5.8 Hz and 174.1 ± 4.0 for both sides and the pulse widths 113.9 ± 11.6 μ s and 125.6 ± 17.2 for the left side and 116.2 ± 11.6 μ s and 133.1 ± 16.2 for the right side in group A and B, respectively. Thirteen patients received bilateral monopolar and six bilateral bipolar stimulation in group A and 12 bilateral monopolar and eight bilateral bipolar in group B. Stimulation settings did not significantly differ between groups A and B.

TABLE 1. Patient characteristics of PD patients with bilateral STN stimulation in groups A and B undergoing motor and reaction time evaluations. The mean values and standard errors of ages at surgery, disease durations, and, follow-ups are presented.

Characteristics	Group A	Group B
Sex		
male	11	11
female	8	9
Age at surgery (yrs)		
mean $\pm$ SE	60.2 ± 1.5	59.8 ± 1.8
Disease duration (yrs)		
mean $\pm$ SE	14.9 ± 0.9	16.0 ± 1.4
Follow-up		
mean $\pm$ SE	13.4 ± 2.4	13.8 ± 1.8

CLINICAL MOTOR EFFECTS

The total motor score (UPDRS III) improved significantly following STN DBS in the med off and on phases in groups A and group B. Activities of daily living (UPDRS II) also demonstrated significant improvements in the med on and off phases in both groups. Hoehn and Yahr and Schwab and England scores were significantly improved

in the med on and off phases in both groups. All patients responded well to bilateral STN DBS (Table 2 and Fig. 1).

Comparing within conditions, UPDRS subscores, Hoehn and Yahr and Schwab and England scores did not significantly differ between groups A and B except for the stim-off/med-on condition. In this condition suprathreshold medication induced significantly better UPDRS II ($F(1,38) = 7.54, P < 0.05$) and III ($F(1,38) = 20.10, P < 0.01$) scores. These findings were supported by a strong two-way interaction (Medication x Dose: $F(1,38) = 112.21, P < 0.01$). The consistent Stimulation by Medication effects demonstrated that in group A and B medication significantly interacted with stimulation for all scoring factors.

TABLE 2. The effect of STN DBS on motor parameters in the four experimental conditions in groups A and B. UPDRS scores indicate activities of daily living (ADL; part II) and Parkinsonian motor symptoms (part III). STN DBS significantly improved motor and ADL scores in the on and off conditions. In addition, the statistical outcome of factors and interactions are provided. All values are expressed as mean $\pm$ SEM.

Stim. (Stimulation)	on	on	off	off	Factors and Interactions		
Med. (Medication)	on	off	on	off	Stim.	Med.	Stim. x Med.
Scoring factor	Score						
Group A							
UPDRS II	8.1 ± 0.6	10.1 ± 1.7	19.2 ± 2.0	23.3 ± 2.3	F(1,18) = 8.58*	F = 0.75, n.s.	F(1,18) = 95.20**
UPDRS III	13.6 ± 1.3	19.2 ± 1.8	37.9 ± 3.3	48.7 ± 3.6	F(1,18) = 30.26**	F(1,18) = 6.40*	F(1,18) = 77.76**
Group B							
UPDRS II	7.1 ± 0.7	12.7 ± 0.9	12.7 ± 1.2	26.1 ± 1.9	F(1,18) = 134.08**	F(1,18) = 26.13**	F(1,18) = 81.05**
UPDRS III	13.8 ± 1.3	21.8 ± 2.4	23.3 ± 1.7	47.8 ± 3.1	F(1,18) = 56.38**	F(1,18) = 16.56**	F(1,18) = 95.49**

n.s. not significant
 $^*P < 0.05$, $^{**}P < 0.01$

REACTION TIME PERFORMANCE

Normalized data of the SRT and CRT task of healthy controls ($N = 372$; mean age 60 years) were available. The mean performance of healthy controls on RT and MT in the SRT task was 370 and 124 ms, respectively. In the CRT task the RT and MT performance levels were 663 ms and 144 ms, respectively.

Based on the normalized data of healthy controls, the patients in this study responded more slowly on the SRT and CRT task. This was based on the differences between means because no statistical analysis could be performed. The data of Group A and Group B are presented in Figs. 2 and 3, respectively. The level of RT and MT performance was comparable in both medication conditions ($F(1,38) < 1.05, n.s.$). Evaluation of the interactions between Dose and the other factors did not reveal statistical reliable effects on RT and MT ($F_s(1,38) < 1.26, n.s.$). As expected, the subjects reacted faster in the SRT compared to the performance in the CRT (Task effect on RT: $F(1,37) = 233.27$,

FIGURE 1. The effects of stimulation and medication on UPDRS part III (motor) score. In both groups stimulation and medication had similar effects, except for the stim-off/med-on condition. In this condition, patients in group B (suprathreshold medication) had significantly better motor results compared to patients in group A (low dose). In both groups, stimulation significantly improved the motor disability. All data represent mean + SEM. * Differences between conditions measured by post-hoc analysis (Sidak, $P < 0.05$)

$P < 0.01$). Although the absolute effect was modest, the MT in the CRT task was slower when compared with the SRT task ($F(1,37) = 10.16$, $P < 0.01$). DBS of the STN clearly improved RT and MT in both task (Stimulation: $F(1,37) = 35.46$, $P < 0.01$ and $F(1,18) = 64.81$, $P < 0.01$, respectively). In addition, For RT there was a Task by Stimulation interaction ($F(1,37) = 40.80$, $P < 0.01$), indicating that the effect of DBS on RT was more pronounced in the SRT than in the CRT task. No interaction between Task and Stimulation was found for the measure MT ($F(1,37) < 1$, n.s.), indicating that the beneficial effect of DBS on MT was independent of the task. There were no effects of medication or dose on the RT performance (Medication and interactions with Task and Stimulation: $F_s(1,37) < 1$, n.s.). However, medication had a modest but statistically beneficial effect on MT ($F(1,37) = 5.98$, $P < 0.05$), which was independent of stimulation and condition ($F_s(1,37) < 1$, n.s.).

There were only a few omissions (about 5% of all trials) in the SRT and CRT tasks. In the SRT task the number of omissions tended to be different between conditions ($\chi^2 = 7.4$, $0.05 < P < 0.10$). The lowest numbers of omissions was in the stim-on/med-off condition (2.03) while most were made in the stim-off/med-on condition (2.89). In the CRT task, the number of omissions differed among conditions ($\chi^2 = 19.5$, $P < 0.01$), the lowest number being in the stim-off/med-off condition (1.89) while most omissions were made in the stim-on/med-on condition (3.42). The number of decision errors was relatively low in both conditions (about 5%) and a comparable number of decision errors was made in the SRT and CRT task (data not shown: $\chi^2 < 3.0$, n.s.).

FIGURE 2. The effects of stimulation and low medication on reaction time and motor time performance of PD patients performing a simple reaction time task (SRT) and a complex reaction time task (CRT), in group A. All data represent mean + SEM. * Differences between conditions measured by post-hoc analysis (Sidak, $P < 0.05$)

FIGURE 3. The effects of stimulation and high medication on reaction time and motor time performance of PD patients performing a simple reaction time task (SRT) and a complex reaction time task (CRT) in group B. All data represent mean + SEM. * Differences between conditions measured by post-hoc analysis (Sidak, $P < 0.05$)

DISCUSSION

The improvements in motor score, ADL-score and functional performance in the present study demonstrate that STN DBS is an effective treatment for patients suffering from advanced PD. Dopaminergic medication significantly enhanced the beneficial effects of stimulation for all clinical scoring factors. In addition, more dopaminergic medication resulted in better clinical results. These data are in accordance with previous reports^{6–9,21,45}.

RT performance is often used in PD to differentiate between motor and cognitive function. Our patients responded more slowly in the RT tasks compared to age matched controls (normalized data) and the RT was significantly higher in the CRT task in comparison to the SRT task. The present results point out that stimulation of the STN can have a substantial effect on response times. STN stimulation significantly improved RT and MT of the SRT and CRT. Despite these improvements, the overall performance remained impaired with respect to the normalized data. There was a significant interaction between task and stimulation with respect to the RT performance. In contrast to the clinical motor scores, neither high nor low medication had an effect on RT performance in general.

One could argue that only one task for the simple and one for the complex condition might not be sufficient to allow this assumption to be made. This is true when the motor response times are inconsistent across the tasks. Although this was not the case in group A, the MT appeared to be slower in the CRT task in group B. The magnitude of this variation was ± 10 – 20 ms, whereas the difference in RT between the SRT and CRT was more than 100 ms which clearly indicates the presence of a cognitive element. It can, therefore, be stated that the observed differences between the SRT and CRT performance are the result of difference in cognitive load.

To minimize the impact of a learning effect across experimental conditions, we applied a counterbalanced design. However, complete randomisation was not possible because of ethical and organisational issues. Within the present approach, the subjects were tested first in the most optimal clinical conditions (stim-on/med-on and stim-on/med-off) followed by the worst clinical conditions (stim-off/med-on and stim-off/med-off). If a learning effect had been present, the subjects would mainly benefit from this in the clinically worst conditions. Our results show that in the clinically optimal conditions (stim-on/med-on and stim-on/med-off), the motor performances in both SRT and CRT are significantly better than in the clinically worst conditions (stim-off/med-on and stim-off/med-off). If a significant learning effect had been present, the patients would have had a better performance in the last conditions. This is not the case in the present study. Nevertheless, we cannot totally exclude a possible learning effect, but even if present, the effects of stimulation and medication appear to be robust enough. Together with the application of counterbalancing, learning effect does not appear to be a major bias in our results.

DIFFERENTIAL EFFECTS OF STN DBS ON REACTION TIME PERFORMANCE

In the present study STN DBS improved the RT performance in the SRT task to a greater extent than in the CRT task. This finding suggests that STN DBS facilitates the motor preparation to respond to an imperative stimulus. Thus, in the SRT only one response could be selected allowing pre-programming of the response before the onset of the imperative stimulus⁴⁶. In the CRT task one of two incompatible responses (go and no-go) needs to be generated which limits the effectiveness of responses preparation. Consequently, the selective effect in the SRT task can be explained on basis of an improved ability to prepare a response^{35,47–49}. We would like to note that we have used a variable interval procedure which makes pre-programming less effective, when compared to a fixed interval procedure.

On the other hand, the differential effect of STN DBS on SRT and CRT performance could also be explained in terms of cognitive slowing. Thus, the greater effect of STN DBS on the RT performance in the SRT task as compared with the CRT task could be explained by the more complex information processing that is required in the CRT task as compared to the SRT task. Although the RT performance in the CRT task was improved after STN DBS, the facilitation was less pronounced as compared with the SRT, suggesting that the benefit of DBS was lost in the CRT task. As mentioned earlier, the more complex cognitive processing in the CRT task may underlie this effect. Thus, the facilitation in RT performance was counteracted by a negative effect of STN DBS.

Slowing of cognitive processing (central slowing) has also been described by Saint-Cyr and colleagues. They have investigated the neuropsychological changes, with emphasis on the frontal striatal circuitry, in patients with advanced PD treated with DBS of the STN²⁰. Despite clinical benefit, a significant decline was seen in working memory, verbal/visuospatial measures and speed of mental processing. The patients were examined in the best medication on and stimulation on condition and were compared to baseline. Hence, it remained unclear whether these changes were the result of a significant decrease in dopaminergic medication, postoperatively, or the intrinsic effect of STN HFS. Reduction of dopaminergic medication could be a plausible explanation since it had been postulated that central slowing could be elicited by a lack of dopamine^{50–52}. Our results, however, demonstrate that central slowing is due to an intrinsic effect of STN stimulation. Turning the stimulator on had a dramatic effect on mental speed whereas medication (low and high) had no effect. Other groups have also found profound worsening of specific cognitive functions after turning on the stimulators. Halbig and colleagues found significant worsening of declarative memory whereas non-declarative memory was improved⁵³. Witt and colleagues described improved cognitive flexibility, but impaired response inhibition⁵⁴. Hershey and colleagues reported reduced working memory and response inhibition when the stimulators were turned on⁵⁵. In all these studies, STN DBS had mainly a differential effect on motor and cognitive functions. Motor functions consistently

improved whereas specific cognitive functions deteriorated. Taken together, the data of the present study can be interpreted in terms of an improved response preparation but also in terms of a central slowing effect of STN DBS.

Motor and cognitive performance related to the basal ganglia are thought to be represented by the basal ganglia-thalamocortical motor and associative circuits^{27,28,56}. These circuits form the elementary pathways through which higher and lower regions of the brain communicate with the basal ganglia. Each of these circuits originates from specific parts of the cortex, motor circuit from the motor cortices and the associative circuit from the prefrontal cortex, are processed in specific thalamic nuclei and project back to at least one of the cortical input areas^{27,56}. This functional subdivision of the basal ganglia into the different basal ganglia-thalamocortical circuits has also been applied to the STN⁵⁷. The dorsolateral part of the STN is involved in somatomotor functions (two thirds of the STN) and the ventromedial part is involved in cognitive functions (one third of the STN)^{27,28}. STN is considered to be one of the main nuclei controlling basal ganglia-related motor functions^{3,58,59}, but its role in the control of cognitive functions needs further clarification. Our data suggest that modulation of STN's neuronal activity by DBS has a substantial effect on the processing speed of basal ganglia-related cognitive information. This means that the STN seems to be an important nucleus in which the basal ganglia associative circuit is further processed towards the output nuclei of the basal ganglia.

STN DBS resulted in differential effects on motor and cognitive function, as shown by the present results. The exact mechanisms of action of DBS are still unknown. However, an increasing amount of data suggests that categorizing DBS as being "inhibitory" and thus equating its effects to those of a lesion, is actually an oversimplification of what is a highly complex and multi-faceted technique^{2,31,60}. For example, groups have obtained opposing results with STN lesions and stimulations in rats on cognitive performance⁶¹⁻⁶⁴. Therefore, the present data cannot (fully) be discussed in the light of lesion studies.

To this day, fundamental knowledge regarding the application of electrical currents to deep brain structures is far from complete. A number of possible mechanisms have, however, been proposed⁶⁵. One of the more popular hypotheses is that DBS causes a reduction of neuronal activity by means of a depolarization block⁶⁶. It has also been proposed that the silencing of target nuclei by DBS is achieved by the stimulation of GABAergic afferents to the target cells and the consequent hyperpolarization of postsynaptic terminals by release of the inhibitory neurotransmitter GABA⁶⁷. The situation becomes even more complex when it is stated that DBS can have opposing effects on structures being stimulated depending on the cellular architecture of the target area. Quantitative models have revealed that DBS inhibits the cell bodies of neurons around the electrode by activation of presynaptic terminals, while stimulating the output of local neurons, by initiation of action potentials in the axon remote from the cell body^{68,69}. Furthermore, this dual effect appeared with high frequencies only⁶⁰. Further studies are warranted to determine precisely how DBS works.

EFFECT OF MEDICATION

In the final experimental condition (stim-off/med-on), patients of group A were given their usual morning medication. As medication intake was reduced by > 50% after surgery, their usual amount of medication ($LED = 158.7 \pm 21.1$ mg) was low compared to the amount which is necessary to obtain maximum clinical benefit in non-operated patients. Therefore, in group B a suprathreshold dose was given.

Both low medication and high medication significantly interacted with STN DBS, which indicates that medication mimicked the beneficial effects of STN DBS. RT performance, however, was not influenced by medication whereas STN DBS substantially improved the motor performance of this task. STN DBS has apparently a dissociative effect on the clinical motor outcome and motor performance of RT responding.

The medication off period in the present study was 9 h, whereas the stimulation off period was 12 h. One could argue that this is not optimal in the purest sense. It was not possible in the present experimental design due to ethical and organisational issues to have a longer medication off period. Nevertheless, we believe that both the stimulation and medication off periods were sufficient as at the end of these periods the motor performances of the patients were significantly deteriorated compared to the on conditions.

CONCLUSION

Our results confirm that STN stimulation has an overall beneficial effect on motor performance. Furthermore, STN DBS differentially improved RT in the SRT and CRT task. These findings could be explained in terms of improved response preparation or STN DBS-related cognitive slowing. Dopaminergic medication did not affect the performance in the SRT and CRT tasks suggesting that the effects are the result of modulation of the STN neuronal activity. These data support the hypothesis that the STN is a potent regulator of the basal-ganglia thalamocortical motor and associative circuits.

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CHAPTER 4

Behavioural changes after bilateral subthalamic nucleus stimulation in advanced Parkinson disease: a systematic review

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ABSTRACT

The long-lasting beneficial effects of subthalamic nucleus (STN) deep brain stimulation (DBS) on motor function have now largely been acknowledged. Whereas behavioural changes have been demonstrated in certain case reports and small case series, some authors have not observed behavioural changes at all. The extent to which these changes occur has not yet been established. The aim of the present study was to systematically analyze behavioural changes of bilateral STN DBS. A structured Medline search was conducted using previously described methods. Studies were selected according to specific in- and exclusion criteria. Data on patients, surgical technique, outcome and complications were collected and pooled.

In total 1398 patients who underwent bilateral STN DBS were included. The total cumulative follow-up period was 1480 patient-years. Cognitive problems were seen in 41%, depression in 8%, and (hypo)mania in 4% of the patients. Anxiety disorders were observed in less than 2%, and personality changes, hypersexuality, apathy, anxiety, and aggressiveness were observed in less than 0.5% of the group studied. About half of the patients did not experience behavioural changes. Caregivers should be aware of the extent of these behavioural changes and a risk/ benefit evaluation should be performed for individual patients.

INTRODUCTION

Bilateral deep brain stimulation (DBS) of the subthalamic nucleus (STN), once considered an experimental treatment, is nowadays widely performed in the treatment of advanced Parkinson disease (PD). Many studies have appeared on the short-term effects¹⁻¹⁰. More recently, four year^{-11,12} and five year-follow up¹³ effects were reported. Bilateral STN stimulation induced a marked improvement in motor function while off medication and in dyskinesia while on medication. These results reinforce the value of bilateral STN DBS in the long-term treatment of advanced Parkinson disease. On the other hand, STN DBS is accompanied by a new set of side effects and complications, which may occur at any time from surgery to several years postoperatively^{14,15}. These negative effects can be related to the hardware such as infections¹⁴, lead fracture¹⁶, dislocation¹⁷, to the electrode insertion (bleedings) or specifically to the region stimulated¹⁸⁻²².

In several case reports and small case series, behavioural changes following STN DBS have been described such as mania^{18,19,23,24}, depression^{6,10,13,21,22,25-37} and cognitive alterations^{6,15,20,24,29,30,38-42}. On the other hand, other authors did not find any negative effect of STN DBS on behaviour⁴³⁻⁴⁷. There is an ongoing debate about which specific behavioural changes are related to bilateral STN DBS and to what extent these occur. In the present study, our aim was to systematically analyze these side-effects in reports describing original data on bilateral STN stimulation in advanced PD.

MATERIALS AND METHODS

SEARCH STRATEGY

A structured Medline (Pubmed) search was performed in articles published up until June 2004, using previously described search methods⁴⁸⁻⁵⁰. The following key words were used: STN, subthalamic nucleus, subthalamic in combinations with DBS, deep brain stimulation, stimulation, HFS, high frequency stimulation. These studies were reviewed independently by three investigators (YT, ST and AT).

STUDY SELECTION AND DATA EXTRACTION

Studies were selected according to the following criteria. Preclinical studies and reviews not containing original data were excluded. Studies not reporting on the effects of STN DBS on behaviour were also excluded. Patients who had underwent unilateral STN DBS, or bilateral STN DBS for another indication than idiopathic PD, were excluded from analysis. From the 515 articles reviewed, 82 met our criteria and were used for the final statistical analysis. During the review process, it was noticed that (parts of) the same study group were described in different journals by the same authors. Corrections were made for these studies according to a previously described method⁵¹. The

study with the largest population was chosen and in case of dual publication, the study was used once. In the reference list, however, all articles are shown.

A standard checklist was used to collect the following data: patient selection, patient characteristics, surgical technique, results (Unified Parkinson Disease Rating Scale⁵² subscores I–IV, and reduction in l-dopa equivalent dose), and behavioural complications including the assessment method and management. Concerning the latter, immediate behavioural complications such as confusion or a transient (hypo)mania that resolved within few days, were not encountered in the final analysis. We were mainly interested in changes that were clinically relevant and not in small quantitative changes on scoring lists/questionnaires.

STATISTICAL ANALYSIS

A meta-analysis was performed on the data using direct pooling⁵³. Since the majority of the studies did not provide the variances of the point estimates, the variances of the populations were considered to be equal. The point estimates were pooled using the number of patients as weights and the frequencies and 95% confidence intervals (CI) were calculated. Data are presented as means (95% CI).

RESULTS

PATIENT CHARACTERISTICS

In total, 1398 patients were implanted with bilateral electrodes at the level of the STN. The male female ratio was 3:2 (table 1). The mean age of the total population at the time of surgery was 55 years (95% CI 41–68) with a mean disease duration of 11 years (95% CI 4–19). The mean follow-up duration was 13 months (95% CI 4–21), and the cumulative follow-up period was 1480 patient-years in the study group.

TABLE 1. The results of the analysis of the patient characteristics (number of patients, age at surgery, disease duration, and follow-up durations) are summarized in this table. The values are expressed as means and 95% confidence intervals (CI).

<i>N of patients</i>	
Total	1398
male-female ratio	3:2
<i>Age at surgery (yrs)</i>	
mean (95% CI)	55 (41–68)
<i>Disease duration (yrs)</i>	
mean (95% CI)	11 (4–19)
<i>Follow-up(mos)</i>	
mean (95% CI)	13 (4–21)

EFFECTS OF BILATERAL STN DBS ON UPDRS SUBSCORES

The Mentation, Behaviour and Mood score (Part I) of the Unified Parkinson Disease Rating Scale (UPDRS) showed a deterioration of 27% (95% CI 7–47) in the on condition (table 2). The Activities of Daily Living (ADL) score of the UPDRS (Part II) improved by 52% (95% CI 34–70) in the off and by 10% (95% CI 0–21) in the on phase. The mean improvement of the UPDRS motor score (Part III) was 58% (95% CI 41–74) in the off and 16% (95% CI 1–31) in the on phase. The complications of therapy (Part IV of the UPDRS) were reduced by 69% (95% CI 53–86). In addition, the levodopa equivalent dose (LED) was decreased by 58% (95% CI 40–75), postoperatively.

TABLE 2. Data were collected on the effects of bilateral STN DBS on the subscores I–IV of the Unified Parkinson Disease Rating Scale (UPDRS)⁵². Subscore I reflects the Mentation, Behaviour and Mood, subscore II the Activities of Daily Living (ADL), subscore III the motor performance, and subscore IV rates the complications of therapy. The reduction in the levodopa equivalent dose (LED) is also displayed in this table. Change in% is the change (+:increase, -:decrease) due to STN DBS at the last follow-up examination with respect to baseline, in the medication *off* and medication *on* conditions.

Scoring Factor	off	on
	%	
UPDRS I (95% CI)	not evaluated	+27 (7–47)
UPDRS II (95% CI)	–52 (34–70)	–10 (0–21)
UPDRS III (95% CI)	–57 (41–74)	–16 (1–31)
UPDRS IV (95% CI)	not evaluated	–69 (53–86)
LED reduction (95% CI)	not relevant	57 (40–75)

BEHAVIOURAL EFFECTS OF BILATERAL STN DBS

First, an overview of all reported behavioural changes was made in the articles studied. The following changes were reported: cognitive alterations^{5,6,9,13,15,20,22,24,29,30,32,33,35,38–42,46,54–70}, depression^{5,6,10,13,25–34,36,37,46,58,60,62,67,71–75}, (hypo)mania^{13,18,19,23,24,37,58,67,72,76}, personality changes^{5,6,31}, hypersexuality^{23,60,77}, apathy^{10,29,71}, anxiety^{29,31}, and aggressiveness⁶⁶.

Cognitive problems varying from moderate deterioration in verbal memory to significant decline in executive functions were seen in 41% (95% CI 19–63) of the patients undergoing bilateral STN DBS. Depression was observed in 8% (95% CI 0–19) of the population investigated and led to suicide in several cases^{13,27,35,60,67,71}. Four per cent (95% 0–12) of the patients had signs of (hypo)mania. Anxiety disorders were observed in less than 2%, and personality changes, hypersexuality, apathy, and aggressiveness in less than 0.5% of the group studied. Management of these behavioural changes was poorly described except for depression, which was usually treated with anti-depressants. The majority of the studies used widely accepted neuropsychological and psychiatric examinations to evaluate the behavioural effects of STN DBS.

The time course and severity of the observed behavioural changes differed. Cognitive alterations were often chronic. In some studies, the patients were substantially affected^{15,20,31,63}, while in others patients had minor symptoms^{38,61,68}. Depression usually resolved after an adequate pharmacological treatment. However, in some cases this was not effective, and in 4% of the patients depression lead to suicide (attempts)^{13,35,60}. The rate of suicide (attempts) in the total study population was 0.4%. Hypo(mania), anxiety disorders, personality changes, hypersexuality, apathy, anxiety and aggressiveness were also often chronic and had usually a substantial effect on patients and their families.

A separate analysis showed no significant association with patient characteristics, surgical technique, assessment method of the behavioural changes, or motor outcome.

TABLE 3. The results of the analysis of the behavioural changes are summarized in this table. The values are expressed as mean percentages and 95% confidence intervals (CI) and show the percentage of patients within the study population (n = 1398) experiencing a certain form of a behavioural change.

Behavioural complications	%
Cognitive alterations	41 (19–63)
Depression	8 (0–19)
(Hypo)mania	4 (0–12)
Anxiety disorders	< 2
Personality changes, hypersexuality, apathy, and agressiveness	< 0.5

DISCUSSION

In the present review, we systematically analyzed patient characteristics, surgical technique, motor outcome and behavioural changes in patients who had undergone bilateral STN DBS for advanced PD. Our results point out that despite substantial improvement of motor disability, patients undergoing bilateral STN DBS can experience different behavioural changes. Three important behavioural changes could be determined. These are cognitive dysfunctions noticed in 41% of the patients, depression in 8%, and (hypo)mania observed in 4% of the subjects. Anxiety disorders, personality changes, apathy, anxiety, and aggressiveness were not frequently observed and only reported in case studies.

In our study, no significant association with patient characteristics, surgical procedure, or motor outcome was found. This may suggest that effective modulation of the STN, irrespective of the surgical approach, can be accompanied by specific changes in behaviour. To avoid possible bias by differences in methodology (not surgical), we applied inclusion- and exclusion criteria which allowed appropriate and well-designed studies to enter the statistical analysis. In all studies, these patients had a manifest improvement of their Parkinsonian features, which is actually the only sign *in vivo* of effective STN DBS⁷⁸. Furthermore, the evaluation of the motor outcome was usually

performed according a standardized protocol⁷⁹. The evaluation of the behavioural parameters was in the majority of the studies conducted using extensive neuropsychological and psychiatric testing. Therefore, it seems unlikely that such differences could have confounded the present results. In addition, similar procedures have been used in the past for the same group of patients with a different neuroanatomical target. DBS of the globus pallidus internus (GPi)^{80–83} and ventral intermediate nucleus of the thalamus (VIM)^{84,85} did not produce behavioural changes to this extent. Again this is suggestive of a target-specific effect. Furthermore, there is currently no convincing data that another structure within the subthalamic region plays a major role in associative and limbic functions except for STN itself.

Another factor that needs to be considered in PD patients undergoing STN DBS is the postoperative drug reduction. It is usually possible to achieve an average reduction of approximately 60% of dopaminergic medication after surgery. The question arises whether this reduction in dopaminergic medication could have contributed to the behavioral changes observed following surgery. Some transient changes in mood and psychotic symptoms can occur when the reduction is performed fast. However, in the majority of the studies presented in this review, the medication reduction was performed slowly. Furthermore, the reduction of dopaminergic medication was usually fully achieved in the first 2–3 months after surgery, whereas the behavioral symptoms were generally also observed at longer followup time points. Secondly, in some studies STN stimulation was switched off to investigate the effects of only stimulation on cognitive parameters^{63,68,86,87}. Results of these studies show that it is actually the modulation of the STN, and not medication or surgery, that has a substantial effect on behavioral parameters.

The question arises whether the rates on the behavioural complications are an under- or overestimation. In our view, these are an underestimation. Especially, in the first period of the DBS era, researchers were mainly focused on the important effects on motor disability, which caused an euphoric state among the patients and clinicians. The behavioral complications were faded into the background and were hardly recognized. Later, more and more groups became focused on these effects and specifically evaluated behavioural performances before and after STN DBS. Our systematic review has included studies from both periods. Therefore, it is more likely that the present rates are an underestimation.

Our data point out that modulating STN's neuronal activity by DBS results in behavioral changes. Several specific cognitive functions were affected: verbal memory and fluency^{6,15,24,29,30,38–42}, executive functioning^{20,29,54,60}, attention^{15,20,41,54}, working memory^{20,54,63}, mental speed 56, 88, and response inhibition^{63,68}. The second most observed behavioral complication was an depression^{5,6,10,13,22,25–37,46,58,60,62,67,71–75}, in some cases leading to suicide (attempts)^{13,27,35,60,67,71}, and thirdly, a (hypo)mania^{13,18,19,23,24,37,58,67,72,76}. Pa-

tients with preoperative cognitive deficits and affective disorders seem to be at risk for further deterioration after surgery^{5,25}. There is also evidence from experimental studies for the involvement of the STN in behaviour. In animal studies, rats and in one study primates, specific behavioral changes were found after STN stimulation and lesions. Rats showed impaired response inhibition^{89–93} and impulsivity⁸⁹, and marmosets had attentional deficits⁹⁴, which are in line with the human literature^{15,18–20,23,24,41,54,63,68}.

Anatomically, cognitive and limbic information related to the basal ganglia is processed by the associative and limbic circuits, respectively. The STN has anatomically a central position within these circuits^{95–103}. In addition, these circuits are processed within the STN by specific groups of neurons located medially (limbic) and ventromedially (associative)^{101,104}. Altogether, the behavioral data presented in this study, point towards a potent regulatory function of the STN in the processing of associative and limbic information towards cortical and subcortical regions. Changes in STN activity can result in changes in behaviour. This hypothesis has also been confirmed (indirectly) by several neuroimaging studies^{105–108}. STN DBS resulted in specific changes at the level of cortical and subcortical associative and limbic regions. Such a potent influence on associative, limbic and motor functions has not been observed with other targets like the GPi^{80–83} or thalamus^{84,85}.

At present, there is a great need for information about potential risk factors (patient characteristics, social factors etc.) which are related to these behavioural complications. The only known risk factor thus far is a preoperative clinically relevant behavioural alteration. Centres that have experienced these behavioural side-effects should put efforts in identifying such risk factors. From the present systematic review, we have remarked that authors do not always provide the information necessary to make valid comparisons between studies with respect to the motor and behavioural outcome. Therefore, authors should be more careful in describing their methods and results. This might allow future studies to identify predictive factors which in turn can be used in the prevention of these complications. Until then, we recommend to perform a risk/ benefit evaluation for individual patients.

CONCLUSION

The results of our study confirm that STN DBS is a very effective in alleviating PD symptoms. It is associated by behavioural changes of which cognitive dysfunctions and emotional changes are the most frequent. Patients with preoperative clinically relevant behavioural alterations can be at risk for further deterioration after surgery. Patients who are candidates for STN DBS should be subjected to a risk/benefit evaluation. For some patients, an alternative could be GPi DBS, in the case of disabling motor fluctuations, or, in the case of therapy resistant disabling tremor, VIM DBS.

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CHAPTER 5

Monopolar versus bipolar high frequency stimulation in the rat subthalamic nucleus: differences in histological damage

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ABSTRACT

The aim of the present study was to determine the effects of monopolar and bipolar high frequency stimulation (HFS) on histological damage and current flow using a commonly applied stimulus amplitude (300 μ A). Bipolar HFS resulted in a large amount of histological damage whereas with monopolar HFS no damage was observed except for the electrode trajectory. Oscilloscopic readings confirmed that this was due to the application of twice as much current to the target with bipolar HFS. Our results demonstrate that there are differences in tissue damage dependent of polarity. In order to create a better comparison to the clinical condition, we suggest that the present rodent models for studying the effect of chronic HFS require further adjustment. This can be achieved by decreasing the present current densities to a level comparable to the human situation.

INTRODUCTION

Deep brain stimulation (DBS) is an established neurosurgical procedure in the treatment of advanced Parkinson disease (PD)¹⁻⁵. Bilateral high frequency stimulation (HFS) of the subthalamic nucleus (STN), in particular, produces long-lasting improvement of cardinal symptoms and reduces medication requirement⁶⁻⁹. However, HFS in the area of the STN may evoke stimulation-dependant behavioural side-effects varying from hypomania to depression^{10,11}. The mechanisms through which DBS induces these behavioural changes or alleviates Parkinsonian symptoms remain unclear. Therefore, DBS is applied in animal models to study the neuronal mechanisms entailed in this procedure.

A major advantage of DBS is that the stimulation parameters can be adjusted, including the polarity, to achieve the optimal clinical condition. Hence, the stimulation applied can be either bipolar or monopolar depending on the clinical effect¹². In the majority of studies with rodents, the polarity is not mentioned. This can particularly be of importance, since with the commonly used parameters (stimulus amplitude = 300 μ A), the current density in the rat is several folds higher in comparison to the human condition¹³. Therefore, the selectivity of monopolar or bipolar stimulation in influencing a brain area or a neuronal element may differ. It is suggested, for example, that low current density only affects large myelinated axons whereas high current density might influence small axons and even cell bodies¹³. This would mean that the effects obtained in rodents can not be extrapolated to the human condition as the current densities are not proportional.

Monopolar stimulation basically produces a current in which electrons diffuse from the negative pole through the “way of the least resistance”. This kind of stimulation might influence a larger area especially when the current density is relatively high. Concentric bipolar stimulation produces a concentrated current around the tip of the electrode in which electrons run from the negative to the positive pole. In the reports published on DBS in rodents, no mention is made of the polarity applied¹⁴⁻¹⁷. In a small pilot study conducted by our group, using concentric bipolar stimulation because of its selectivity, we observed a large amount of coagulative tissue damage resulting from a one hour stimulation using previously reported stimulation parameters (e.g. pulse amplitude 300 μ A)¹⁴⁻¹⁷. The question arises whether monopolar stimulation would also produce such damage. In the present study, we aimed to determine the effect of concentric bipolar stimulation versus monopolar stimulation on tissue damage (trajectory damage versus coagulative tissue damage) and current flow using previously reported stimulation parameters¹⁴⁻¹⁷.

MATERIALS AND METHODS

SUBJECTS

The present experiment received approval from the Animal Experiments and Ethics Committee of Maastricht University (DEC-UM, nr: 2002–62). Lewis male rats ($n = 10$, Maastricht), average body weight 350 g, were kept under light-dark cycles (12 h each phase: lights off 18:00 h) in a temperature-controlled room (Animal Care Department, Maastricht University). The rats were fed *ad libidum* and handled daily for 10 days before experiments. After the induction of anaesthesia using ketamine (10 mg/ml, i.p.) and xylazine (1.6 mg/ml i.p.) injections, the rats were placed in a stereotactic apparatus (Stoelting, USA; model 51653). The target was chosen at the level of STN (coordinates from Bregma: AP -3.8 , ML ± 2.5 , and V -8.0 from skull).

SURGERY AND STIMULATION

Two electrodes (Dixi, France and Technomed, The Netherlands), both concentric and bipolar, with a tip diameter of 200 μm , were used for the experiments. The configuration of both electrodes was similar. Five rats were stimulated with the Dixie-electrode and five with the Technomed-electrode. Rats received unilateral HFS in a concentric bipolar mode (tip of the electrode negative and the insulated cover positive), whereas subsequent stimulation on the contralateral side was performed in a monopolar mode (tip negative, extracranial positive). In four rats, stimulation started with monopolar stimulation. The duration of the stimulation on each side was 1 hour. The duration in other studies varied between 10 minutes to two hours and one hour stimulation was chosen as it was the median duration. The stimulation parameters used in this study were: pulse frequency 130 Hz, pulse width 60 μs and pulse intensity 300 μA , similar to those of other studies^{14–16}. The stimulus was delivered by a World Precision Instruments (WPI, Germany) accupulser (A 310) and a stimulus isolator (A 360), which produced rectangular pulses, a device also used by other groups^{14,15,18}. Real-time reading of the current, during stimulation, was performed with a digital oscilloscope (Agilent Technologies, Agilent 54622D oscilloscope, The Netherlands), using an Agilent 10074C probe, over a 1% resistor of 10 Ω , which produced an adequate pulse train. All control measurements were done using the same oscilloscope, measuring the voltage-drop over the 10 Ω resistor, being independent of the stimulation frequency. Using a resistor of only 10 Ω produces a higher level of noise in the oscilloscope readings. It is more important, however, not to influence the stimulation by a relative high resistor than obtaining a “clean” signal. The low- and high-peaks, shown in the Fig. 1, are the result of the floating characteristics of the WPI stimulator.

HISTOCHEMISTRY

FIGURE 1. Demonstration the current flow during bipolar (a) and monopolar (b) HFS. During monopolar stimulation, the tip of the electrode is the negative pole and the positive pole is the ear (extracranial) whereas during bipolar stimulation the tip of the electrode is negative and the insulated cover is positive. The duration of one pulse in both cases is 60 μ s. In a, the pulse approaches the set pulse intensity (300 μ A) within 20 μ s. In figure b, the maximum pulse intensity is only reached at the end of the pulse duration.

After the stimulation, the rats were perfused transcardially with Tyrode (0.1 M) followed by the fixative containing 4% paraformaldehyde, 15% picric acid and 0.05% glutaraldehyde in 0.1 M phosphate buffer (pH 7.6). The brains were removed from the skull and post fixed for 4 h in the same fixative. Brain tissue was cryo-protected by overnight immersion in 30% sucrose/0.1 M Tris-buffered saline (TBS, pH 7.4) at 4° C. Subsequently, the brains were rapidly frozen and stored at -30° C until further processing. Brains were sectioned in the coronal plane at 30 μ m on a cryostat and mounted on poly-lysine coated slides. These were dried at room temperature and processed for haematoxylin-eosin (HE) (Merck, Germany) histochemical staining.

ANALYSIS

Analysis of histological trajectory damage, which was defined as the amount of damage within the trajectory, was carried out on a computerized image analysis system (SIS, Germany). The area in which the tip of the electrode had been placed was sectioned from beginning to end. The central section represented the maximal size of the tip trajectory (damaged area). All central sections were analyzed. The damaged area and the perimeter were measured from a standardised 1 mm distance (vertical grid) from the ventral border of the tip trajectory. This method of damage analysis was preferential to

a stereologic measurement, as qualitative analysis of the damaged area already showed considerable difference between both modes of stimulation. The oscilloscopic readings were used to measure the amount of current applied per second ($A s^{-1}$) in bipolar and monopolar stimulations. This analysis was undertaken in both conditions following confirmation that the pulse train throughout one hour stimulation was constant. The area below the oscilloscopic plot represented the amount of energy provided to the target and was measured using the area analysing mode on the oscilloscope screen.

STATISTICAL ANALYSIS

Statistical comparisons were made using Wilcoxon signed-rank test (non-parametric) for two-related samples. A value of $P < 0.05$ was considered significant. The data given are medians and standard errors.

RESULTS

The oscilloscope registrations demonstrated that during monopolar stimulation, the pulse amplitude of 300 μA was only reached at the end of the pulse (Fig. 1A). This resulted in less energy being dissipated to the target. Current measurement during concentric bipolar stimulation showed that the maximum pulse amplitude was immediately reached and maintained during the pulse duration, dissipating more energy to the tissue around the electrode (Fig. 1B). These figures, from the same rat, give an indication of the amount of energy delivered into the brain. In Fig. 1A the surface area was $7.80 \times 10^{-9} A s^{-1}$, whereas the surface area in Fig. 1B was $14.25 \times 10^{-9} A s^{-1}$.

Histological damage was defined as the damaged area measured on the central section.

Qualitative microscopic analysis revealed that bipolar stimulation resulted in a large amount of histological damage in contrast to monopolar HFS. This was confirmed by the quantitative analysis of the sections. Monopolar stimulation did not result in any histological damage except for that seen along the trajectory of the electrode. Bipolar concentric stimulation, however, resulted in significant ($P < 0.05$) coagulative damage around the electrode tip and in adjacent tissue (Fig. 2). The area of damage due to monopolar stimulation was $84.44 \times 10^3 \pm 22.96 \times 10^3 \mu m^2$ and $627.92 \times 10^3 \pm 165.22 \times 10^3 \mu m^2$ (Fig. 3) for bipolar stimulation.

There was no significant difference between the electrodes with respect to the current flow and histological damage. Furthermore, the recorded current flow and the observed histological damage was irrespective of the initial mode of stimulation (monopolar or bipolar). The position (ear, skull or body) of the extracranial electrode during monopolar HFS had no effect on the current flow.

FIGURE 2 (A-C). An illustration of the bilateral HFS using two types of polarity. The target was chosen at the level of the STN (a). Illustrative sections of the effect of bipolar (b) and monopolar (c) HFS. (c) Only the tip of the electrode is shown. (b) demonstration of the massive damage resulting from bipolar HFS in the STN and surrounding structures (HE staining of the central section in which the electrodes tip has its maximal diameter, 4x- bright field, Olympus, Japan).

FIGURE 3. Monopolar HFS for one hour produced no additional damage except for the electrode trajectory ($84.44 \times 10^3 \pm 22.96 \times 10^3 \mu\text{m}^2$), whereas bipolar stimulation resulted in significant ($*P < 0.05$) coagulative damage ($627.92 \times 10^3 \pm 165.22 \times 10^3 \mu\text{m}^2$).

DISCUSSION

The objective of the present study was to investigate whether monopolar and bipolar HFS would induce different effects with respect to the current flow and amount of tissue damage (trajectory damage versus coagulative damage), using previously reported stimulation parameters. The stimulation procedure and the electrode properties of the present study were similar to others^{14–17}. Two different electrodes were used to exclude electrode-specific reactions during stimulation (internal validity check). The present data show that, using a commonly reported stimulation amplitude (300 μA) for a duration of one hour, concentric bipolar stimulation results in a greater amount of tissue damage in comparison to monopolar stimulation. This difference is due to the application of almost twice as much energy to the target during bipolar concentric stimulation as confirmed by the analysis of the oscilloscopic readings.

The clinical effect of HFS is similar to ablation. However, it has major advantages such as its reversibility and adjustability. The aim of HFS is to modulate a target's electrical activity in order to obtain the same clinical effect as a lesion. PD patients receive chronic HFS. With the stimulation parameters used in the clinical setting no histological damage, except for the electrode trajectory, can be observed. In a recent study, post-mortem analysis of eight PD patients treated with DBS for up to 70 months showed well-preserved neural parenchyma and only mild gliosis due to reactive changes as a result of electrode placement¹⁹. The reason for this is most probably the relatively low current density. The regular electrodes used in PD patients have a large contact area ($\pm 6 \text{ mm}^2$) limiting the current densities that can be applied¹³. The current density used in preclinical studies, however, is several times higher. Therefore, changing the polarity only (from monopolar to bipolar) results in severe damage when a commonly used stimulation amplitude is applied for one hour whereas in the human condition both polarities can be used for chronic HFS without lesioning.

Several groups have published on DBS in rats^{14–18,20,21}. The most frequently applied stimulus amplitude varies from 300 to 600 μA with pulse duration of 60 μs and frequency of 130 Hz. In only two studies, however, an explanation is given as to the use of these particular parameters^{14,15}. The stimulation duration ranges from 10 min to 2 hours. Most of the studies report the use of a concentric bipolar stimulating electrode with a tip diameter ranging from 100 to 500 μm without mentioning the positions of the positive and negative poles. The use of a concentric bipolar electrode does not necessarily imply that the stimulation is bipolar. In all reports, a picture is given confirming the electrode placement in the target area. There was no additional damage observed in any of the reports, except that produced by the electrode trajectory. This may be due to the following. Firstly, concentric bipolar HFS with a stimulus intensity of at least 300 μA may not result in visible damage when stimulated for less than one hour^{14–16,20,21}. Nevertheless, if this mode of stimulation is adopted, the observed changes are most likely not only due to HFS, but are also influenced by coagulative neuronal stress. Therefore, chronic HFS with these settings is not recommended. Secondly, monopolar stimulation with the same stimulation parameters does not cause any visible histological damage, as confirmed by our study. However, due to the diffuse nature of a monopolar stimulation in combination with a relatively high current density, applying this type of stimulation most probably influences a larger region than the immediate area in which the tip has been placed. The obtained results could therefore also be caused by the modulation of surrounding regions. Third, only one group reported the use of an oscilloscope to monitor HFS^{16,20}. When performing HFS, an oscilloscope is essential to confirm that a stimulus has been delivered and also to measure the strength of that stimulus. Many factors such as the electrode, cable, and the stimulation device can influence the stimulation. It is possible that in the studies in which no oscilloscope has been used, the current amplitude which is set is not entirely been delivered to the target, especially when the stimulus duration (60 μs) is so short.

The present data are in line with recent observations¹³. With commonly used stimulation parameters in rodents, no comparison can be made with humans due to the large difference in current density. Therefore, the current models used to study the neuronal mechanisms of HFS in rodents need further modification in order to have a more reliable model. This can partly be achieved by delivering comparable current densities to the target. With the present stimulation amplitude, the effect of both polarities might not reflect the changes due to HFS of a specific region. In future experiments, chronic bipolar and monopolar HFS with stimulus amplitude $\geq 300 \mu\text{A}$ should be avoided.

CONCLUSION

The present study demonstrated that monopolar and bipolar HFS resulted in different effects on both current flow and histological damage when a commonly used stimulation amplitude is applied. Bipolar HFS for one hour caused severe damage due to the application of twice as much energy to the target compared to monopolar HFS. Therefore, the conclusion of the present study is threefold; (i) oscilloscopic monitoring during HFS is necessary to be sure that the set stimulation parameters are delivered to the target; (ii) the polarity which is used should be mentioned in future reports; (iii) a more reliable animal model for the study of the neuronal mechanisms should be designed. This can partly be achieved by applying current densities comparable to the human condition

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CHAPTER 6

Premature responding following bilateral stimulation of the rat subthalamic nucleus is amplitude and frequency dependent

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ABSTRACT

The subthalamic nucleus (STN), a key component of the basal ganglia circuitry, functions as an internal clock that regulates the correct sequence of movements in a motor response. The importance of the STN in motor function is evidenced by its involvement in Parkinson disease (PD). This nucleus has also been associated with the attentional and emotional aspects of motor behaviour through its connections with the limbic and prefrontal areas of the brain. As lesions of the STN have been shown to increase premature responding in a serial reaction time task in rats, indicative of its involvement in cognitive performance, the present study aimed to investigate whether bilateral deep brain stimulation (DBS) of the STN, in non-lesioned rats, affects cognitive functions and whether these are dependent on certain stimulation parameters. Rats were trained in a choice reaction time task and implanted bilaterally with electrodes. Stimulation parameters (amplitude, frequency and pulse width) were varied during the test procedure, after which rats were sacrificed and the brains processed for histochemical staining. Results show no change in reaction times or motor times during stimulation. However, a linear decrease in premature responses, was observed with decreasing amplitudes and at high frequencies only. These results are the first to demonstrate that bilateral STN HFS has a positive effect on cognition in freely moving rats. This latter result is in contrast to findings following lesions of the STN, and suggests that current strength and frequency of stimulation are parameters that are integral to the mediation of stimulation effects. Furthermore, the overall effects of DBS on neuronal cells cannot be classified simply as being “inhibitory” and evidently mediates its effects by more complex mechanisms than lesions of the same brain area.

INTRODUCTION

The subthalamic nucleus (STN) plays a critical role within the basal ganglia, and its contribution to the control of motor and behavioural function is becoming increasingly apparent. The neurons of this nucleus possess an innate pacemaker activity that functions as an internal clock, allowing the correct automatic execution of learned movements in a sequence of motor programs¹. Disruption of this tonic activity within the STN is recognized as the pathophysiological hallmark of Parkinson disease (PD). In PD, the STN displays an abnormal “bursting” mode of activity which has been implicated in driving the overactivity of basal ganglia output nuclei, and consequently, excessive inhibition of their targets^{2,3}. For this reason the STN is a popular target in the treatment of PD, and surgically placed lesions of this nucleus reverse many of the motor symptoms associated with this pathology⁴. An emerging technique, deep brain stimulation (DBS), due to its major advantages, has provided a favourable alternative to ablative surgeries in modulating the hyperactivity of the STN. This technique has been adapted from the treatment of chronic pain syndrome and, clinically, is thought to have an inhibitory effect on neural tissue in the region stimulated⁵. DBS of the STN has been proven to have long-lasting beneficial effects on motor disability, functional performance and quality of life in advanced PD and is nowadays considered to be the treatment of choice^{6,7}. However, high frequency stimulation (HFS) of the STN has also given rise to undesirable behavioural side-effects that range from depression^{8,9} to mania¹⁰, cognitive decline¹¹, and impulsivity¹².

This would suggest that the STN not only regulates motor performance, but also influences associative and limbic circuits¹³. Indeed, it has been shown in both animals and humans that the STN is composed of an associative and smaller limbic territory in addition to the more laterally located motor area¹⁴. In line with this evidence, lesions and pharmacological block of the STN in rats have been reported to induce deficits in attentional tasks as shown by an increase in premature responding in serial reaction time tasks^{15–17}. These attentional impairments have been suggested by the authors to be caused by a disruption of the STN’s ability to suppress competing and unwanted responses. More recently, it has been demonstrated that cortical inputs to the STN, specifically from the medial prefrontal cortex, are integral to cognitive processing within the basal ganglia of the rat¹⁸. Therefore inhibition of the STN, by lesioning, and the consequent disruption of afferent and efferent projections affects the cognitive and emotional aspects of motor behaviour. In the clinical context, STN lesions have been performed in advanced PD. Results of a one-year have recently been described by Su *et al.*¹⁹. Unilateral radiofrequency lesion of the STN markedly improved motor symptoms without influencing cognitive performance. Similar findings were also reported by Patel *et al.*⁴. In contrast to these data obtained by subthalamotomies, Dujardin *et al.*²⁰ and Saint-Cyr *et al.*¹¹ have found significant deterioration of cognitive functions as a result of bilateral STN HFS. These data suggest that the

overall effect of HFS might be mediated by more complex, and still unknown, mechanisms than lesions of the same brain area. Furthermore, it remains unclear whether the cognitive changes due to bilateral STN HFS are dependent on certain stimulation parameters. Therefore, in the present study a first attempt was made to study the effects of stimulation parameters on RT performance. For this purpose, the effects of STN DBS were evaluated in non-lesioned rats in which the STN exhibits a physiological pattern of depolarization²¹.

MATERIALS AND METHODS

SUBJECTS

All subjects were Lewis male rats ($n = 10$, Maastricht), average body weight of 300 grams, and were housed individually in standard Makrolon™ cages on sawdust bedding in an air-conditioned room (about 20° C) under a 12/12-hour reversed light/dark cycle (lights on from 18.00 to 6.00 h). All animals had free access to food and water. During the period of behavioral testing the rats were given 10–12 g standard laboratory chow (Hopefarms) per day and ad libitum food from Friday afternoon to Sunday afternoon. This schedule reduced their weight to approximately 90% of their free feeding weight. This study received approval from the Animal Experiments and Ethics Committee of Maastricht University (DEC-UM, nr: 2002–62).

CHOICE REACTION TIME TASK

Rats were tested in Skinner chambers (inner dimensions: 40 × 30 × 33 cm) equipped with two retractable levers with cue lights just above the levers. A food tray (5 × 5 cm and 2.5 cm above the grid floor), that was positioned equidistant between the two levers could be accessed by pushing a hinged panel. The levers (4 cm wide) projected 2 cm into the conditioning chamber and were located 6 cm from both sides of the food tray and 12 cm above the grid floor. A light and a loudspeaker were fixed in the ceiling of the conditioning chamber. The test procedure was controlled by a personal computer and the data stored on disk at the end of a session. Time was recorded to a resolution of 1 ms. During the CRT, the rat must insert its nose into the central panel until a tone sounds. A high tone (10 kHz; 80 dB) required depression of the left lever and a low tone (2.5 kHz; 80 dB) the right lever. A variable period (randomly chosen from 0.6–1.5 s, steps of 0.1 s) between nose insertion and tone was termed the hold duration. When a rat did not hold the panel for the entire hold duration, the same interval was repeated when the panel was next pushed. Depression of the lever resulted in food reward in only 50% of cases to increase the enthusiasm of the animals for the task²². The reinforcement was given independently of the reaction time. Ten seconds elapsed between each trial. Each session lasted 30 min or until the

completion of 80 trials. Pretraining of the rats was completed when the rats showed a stable performance with respect to the variable reaction time.

BEHAVIORAL PARAMETERS

The following parameters were used to evaluate the response of the rats in this task, as described earlier by our group²².

Reaction Time: The mean latency between the onset of the tone and the release of the panel was taken as the reaction time. It is generally accepted that response latencies shorter than 100 ms are unlikely to be true reaction times but should be considered as nonvalid responses. On the other hand, response latencies longer than 1500 ms should not be considered as a task-related reaction time.

Motor Time: The mean latency between the release of the panel and the lever press was taken as the motor time. It was assumed that motor times longer than 2 s did not reflect 'true' motor time.

Premature Responses: The total number of times the rat released the panel before the hold duration had elapsed. It should be noted that after a premature response the rats had to start the same trial again by pushing the hinged panel. In case of a premature response the response lever was not inserted into the chamber.

Number of Trials: The total number of trials the rats completed in a session of 30 min is a maximum of 80.

For the purpose of evaluating behavioural performances prior to surgery, the rats were retrained until performances (i.e. mean reaction time) stabilised. This was achieved within 6–8 days of training. Subsequently, the rats were subjected to two additional sessions that were used for the statistical analysis.

SURGICAL PROCEDURE

Animals were anaesthetized throughout the entire procedure using a combination of ketamine (10 mg/ml) and xylazine (1.6 mg/ml) that was administered intraperitoneally. Rats were placed in a stereotactic apparatus (Stoelting, USA; model 51653). Two burr holes were made in the skull immediately above the STN, to allow for insertion of electrodes. A construction of two stainless steel electrodes (Technomed, The Netherlands), both concentric and bipolar, with a tip diameter of 50 μm and a shaft diameter of 350 μm , was employed in this experiment (see Fig. 1). The electrodes were fixed in position using dental cement (Heraeus Kulzer, Germany).

EXPERIMENTAL PROCEDURE

Rats were left for one week to recover from surgery after which they were retrained in the Skinner boxes (modified to allow for the attachment of a stimulation cable),

FIGURE 1. A picture of the bilateral electrode construction which is implanted in the brain and attached to the skull. The electrodes are designed to optimally target the STN. A connected cable allows the stimulation of freely moving rats.

until behavioural parameters stabilized. Following this, the average values of the behavioural parameters mentioned above were obtained for each of the conditions mentioned below;

- (i) without attachment of stimulation cable ,
- (ii) with attachment of stimulation cable (off stimulation),
- (iii) with stimulation at various amplitudes (3, 30, 150 μ A), 60 μ s pulse width, 130 Hz frequency
- (iv) with stimulation at various amplitudes (3, 30, 150 μ A), 120 μ s pulse width, 130 Hz frequency
- (v) with stimulation at various amplitudes (3, 30, 150 μ A), 60 μ s pulse width, 30 Hz frequency.

STIMULATION

The order of the various amplitudes was randomized using a Latin square design in which each condition was repeated three times. The maximum amplitude applied was 150 μ A, as higher amplitudes clearly induced bilateral dyskinetic/hyperkinetic movements which has also been documented by others²³. Stimulation was performed with 150, 30 and 3 μ A to investigate the effect of a relatively high, medium and low current density with respect to the human condition²⁴. Two different pulse widths were applied (60 and 120 μ s), both frequently used in PD patients²⁵, as it was recently shown that pulse width was associated with cognitive decline after thalamic stimulation for essential tremor²⁶. The frequency applied was low (30 Hz) and high (130 Hz) in order to determine whether changes were dependant on the pulse frequency.

The stimulus was delivered using a World Precision Instrument (WPI, Germany) accupulser (A310) and a stimulus isolator (A360). Real time verification of the parameters applied during stimulation was obtained for both electrodes of the bilateral

construction, using a digital oscilloscope (Agilent Technologies, Agilent 54622D oscilloscope, The Netherlands). Stimulation started five minutes before the test and lasted for the duration of each session, as it was shown that changes in behaviour can occur immediately after the onset of bilateral STN HFS^{9,10}. Each animal had at least a 24-hour stimulation off period before the next session started.

STAINING

Following stimulation rats were perfused transcardially with Tyrode (0.1M) and fixative containing 4% paraformaldehyde, 15% picric acid and 0.05% glutaraldehyde in 0.1 M phosphate buffer (pH 7.6). Brains were carefully removed and postfixed for 2 hours in fixative followed by immersion overnight in 15% sucrose at 4° C. Brain tissue was then frozen and stored at -80° C and subsequently sectioned on a cryostat at 30 µm in the coronal plane. Following this sections were dried at room temperature and processed for hematoxylin-eosin (HE) histochemical staining (Merck, Germany).

STATISTICAL ANALYSIS

Data were analysed using the general linear model of repeated measures ANOVA in the SPSS statistical package. Variables for analysis were amplitude, frequency, pulse width and surgery. Confidence interval adjustments were performed using Sidak post hoc test to compare main effects. A *p* value lower than 0.05 was considered to be significant.

RESULTS

In this study data were obtained from 8 rats, as it was necessary to exclude 2 from analysis due to complications with electrodes following surgery. Oscilloscopic readings during stimulations verified the bilateral application of the set amplitude, frequency and pulse width.

HISTOLOGICAL EVALUATION OF THE ELECTRODE TIPS

Histological evaluation of brain sections, using HE-staining, confirmed that the bilateral electrodes were implanted in the subthalamic region. In seven rats, bilateral electrodes were placed symmetrically (interelectrode variation of < 0.1 mm). In one rat, a difference of approximately 0.2 mm was noticed in the dorsoventral plane (rat 1). In all rats, except for one rat (rat 3), the electrode tips were situated within the STN. In rat 3, both electrodes were at the level of the zona incerta (ZI). In four rats, the bilateral electrode trajectories were also visible at the level of the substantia nigra pars reticulata (SNr). In rat 1, a part of the ipsilateral electrode trajectory was also seen at the level of the ZI and a part of the contralateral at the level of the SNr. Fig. 2 illustrates the localization of one of the electrode tips at the level of the STN.

FIGURE 2. This figure illustrates an electrode that has entered the STN. The electrodes used in this experiment were needlelike. The lines around the electrode trajectory are drawn to scale.

There was no additional histological damage except for the electrode trajectory. This indicated that repeated stimulation with the present settings did not cause any tissue damage.

REACTION TIME PERFORMANCE

As illustrated in Fig. 3, no significant change was seen in the mean reaction times between the preoperative and postoperative phases. Similarly no effect of stimulation was evident in terms of reaction time responses following the application of 150, 30 and 3 μA for the three stimulation modes (ie. stimulation 1: frequency 130 Hz, pulse width 60 μs , stimulation 2: frequency 130 Hz, pulse width 120 μs , stimulation 3: frequency 30 Hz, pulse width 60 μs).

MOTOR TIME PERFORMANCE

A significant effect of surgery was evident from the increase in mean motor time seen postoperatively ($F = 27.93$, $P < 0.01$). Although there was a slight decrease in motor response times during stimulation, this reduction was not significantly different from preoperative or postoperative values (see Fig. 4).

PREMATURE RESPONSE:

As depicted in Fig. 5 there was a significant linear effect of HFS (stimulations 1 & 2) on premature responding ($F = 9.57$, $P < 0.05$). The number of premature responses was seen to decrease proportionately from 150 to 3 μA during HFS at a pulse width of 60 μs ($F = 6.3$, $P < 0.05$), and at a pulse width of 120 μs ($F = 6.62$, $P < 0.05$). No effect on premature responding was observed following low frequency stimulation of the STN at 30 Hz.

FIGURE 3. Mean reaction times of bilateral STN stimulated rats. There were no significant effects of different STN stimulation settings on reaction times; stimulation 1: frequency 130 Hz, pulse width 60 µs, stimulation 2: frequency 130 Hz, pulse width 120 µs, stimulation 3: frequency 30 Hz, pulse width 60 µs.

FIGURE 4. A significant increase in motor time was observed following stereotactic placement of bilateral electrodes. Stimulation did not affect this increase. Stimulation 1: frequency 130 Hz, pulse width 60 µs, stimulation 2: frequency 130 Hz, pulse width 120 µs, stimulation 3: frequency 30 Hz, pulse width 60 µs. * Significant change (Sidak, $P < 0.05$)

FIGURE 5. STN stimulation on a high frequent mode (stimulations 1 & 2) induced a linear decrease in the level of premature responding, which was significant at the lowest amplitude (3 µA). At 30 µA, there was a tendency to a decrease. Stimulation 1: frequency 130 Hz, pulse width 60 µs, stimulation 2: frequency 130 Hz, pulse width 120 µs, stimulation 3: frequency 30 Hz, pulse width 60 µs. * Significant change (Sidak, $P < 0.05$)

DISCUSSION

The results of this study demonstrate that bilateral DBS of the STN can directly modulate cognitive performance as indicated by the significant effects on premature responding in non-lesioned rats. The present data also suggest that both the amplitude and frequency of stimulation are variables that are integral to the mediation of stimulation effects within the STN, as changes in the level of premature responding are observed only at high frequencies and at low amplitudes of stimulation. No effects of stimulation or surgery were found on reaction times which is in line with the recent observations of Darbaky *et al.*²³. Motor times, unaffected by stimulation, were significantly increased most probably due to the bilateral trajectory damage induced by the implantation of the electrodes.

Several studies have been published on premature responding and the STN^{13,15-17,27,28}. Most of them deal with the effect of STN lesions, either unilateral or bilateral. In 1995, Baunez and colleagues reported the effect of bilateral STN lesions, evoked by the local application of ibotenic acid, in bilateral 6-OHDA depleted rats²⁷. They found a dramatic increase in the number of premature responses and concluded that STN lesions produced long-lasting deficits that might be related to dyskinesia or cognitive impairment. In a five-choice serial reaction time task, the same author extended her previous analysis to choice paradigms¹³. Bilateral excitotoxic lesions of the STN were performed and the rats were subjected to a choice RT test. Significant impairments in discriminative accuracy and again an increase in premature responses were observed. Systemic injections of the mixed dopaminergic D1/D2 receptor antagonist, α -flupenthixol reduced premature responding suggestive of a dopamine-dependent mechanism of action. Similar results were obtained by Phillips and Brown. The authors performed unilateral STN lesions in combination with ipsilateral 6-OHDA injections^{17,28}. Their main finding was that a unilateral STN lesion, irrespective of the dopaminergic denervation, resulted in an increase in anticipatory responding. Up to now, only one study has been published on STN DBS and RT performance²³. They performed unilateral STN HFS in 6-OHDA depleted and non-depleted rats. Unilateral stimulation of the STN reduced 6-OHDA induced motor deficits but had no effect on premature responding. The latter result is in contrast to the changes observed following subthalamic lesions by the same group^{13,15,16,27}.

These inconsistent results raise the question as to whether the net effects of DBS are in fact inhibitory or excitatory. To this day, fundamental knowledge regarding the interactions that occur between the neurons of the central nervous system and applied electrical currents has been lacking. However, a number of possible mechanisms have been proposed⁵. One of the more popular hypotheses is that DBS causes a reduction of neuronal activity by means of a depolarization block. This proposed mechanism involves the suppression of voltage-gated sodium, and T-type calcium currents leading to an interruption of spontaneous activity within the neurons²⁹. It has also been pro-

posed that the silencing of target nuclei by HFS is achieved by the stimulation of GABAergic afferents to the target cells and the consequent hyperpolarization of postsynaptic terminals by release of the inhibitory neurotransmitter GABA³⁰. The situation becomes even more complex when it is stated that HFS can have opposing effects on structures being stimulated depending on the cellular architecture of the target area. Differential effects can occur in the cell body and axons of neurons, resulting in either inhibition or excitation³¹. The excitability of axons is much higher than that of cell bodies³². It has been documented both *in vitro*^{33,34} and *in vivo*³⁵ studies that these different neuronal elements are activated at similar stimulation thresholds with conventional stimuli. Therefore, it is possible that stimulations at high amplitudes and frequencies within the STN are non-selective, activating both local neuronal cells and axons of passage thereby “masking” the effect of HFS at the behavioural level. In the present study, stimulation at lower amplitudes results in a very obvious linear decrease in premature responding. However, how this is achieved is still unclear.

As mentioned previously, the STN plays an important role in the execution of movement, or more specifically in response preparatory processes, which involve the selection of specific actions, in addition to the inhibition of unwanted motor responses. This pre-movement neural activity has also been associated with activation in cortical areas including the supplementary motor area, the cingulate motor area and primary motor cortex, and is known as the “motor readiness potential” or “Bereitschaft potential”³⁶. In PD patients, where the STN is overactive, there is an obvious deficit in these preparatory processes during motor initiation, which has been proposed to be linked to the abnormal output of the basal ganglia, and the consequent change in excitability of the aforementioned cortical areas^{37,38}. Therefore the decrease in premature responses observed in this study may be due to an increased activation of these cortical motor areas, which contributes to the motor readiness potential, ultimately enhancing cognitive processes. In a recent report, such increased activation in the frontotemporal network was found in advanced PD patients treated by bilateral STN stimulation³⁹. The authors measured the contingent negative variation (CNV), which according to the authors was a measure of frontal lobe function, during a go/no go task in ten patients with STN stimulation. Assessments were performed in stimulation on and off conditions with medication on. STN HFS significantly increased the CNV amplitude over the frontal and frontocentral regions suggestive of an improved electrical activity in these regions. This study, however, was undertaken in PD patients. Dopaminergic denervation changes the spontaneous activity of STN neurons resulting in burst activity^{40,41}. HFS is thought to interfere with this hyperactivity resulting in improvement of motor symptoms, in a way still not well elucidated. Our study was performed in non-lesioned rats in which the STN has a physiological depolarization pattern²¹. In future experiments, the effect of bilateral STN DBS should be investigated after dopamine depletion.

Several factors might have influenced the present data. We cannot exclude that other surrounding regions have been influenced by stimulation and could contribute

to the observed changes. It is more likely, however, that these changes are related to the STN for three reasons. First, all electrode tips were partially or fully within the borders of the STN. Second, changes in premature responding in that area are specifically related to changes in STN activity^{15–17}. Third, all rats exhibited comparable behaviour. Furthermore, any habituation to the stimulation procedure was prevented by the randomization of the order of the various amplitudes applied in each subject. Altogether, it can be stated that the observed behavioural changes in this experiment can most probably be attributed to interactions that occur in the STN between the applied electrical current, local neurons and cortico-basal ganglia- thalamocortical pathways.

In conclusion, bilateral DBS of the STN in non-depleted rats can enhance cognitive processing (i.e. decrease in premature responses) in an amplitude and frequency-dependent manner. This effect appears to be independent of the speed of responding (RT and MT). However, further study is warranted to determine precisely how stimulation parameters modulate the neuronal output of target nuclei in the brain, and to clarify the neuronal pathway by which electrical stimulation of the STN gives rise to changes at the behavioural level. This knowledge would limit the number of plausible hypotheses that presently exist to explain DBS effects, and also contribute to the development of a more clinically effective treatment for PD, that maximizes motor improvements while at the same time reducing undesirable cognitive side-effects. We suggest that equating the effects of DBS to those of a lesion in the same target area, and categorizing DBS as being “inhibitory”, is perhaps an over-simplification of what is a highly complex and multifaceted technique.

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CHAPTER 7

Acute and separate modulation of motor and cognitive performance in Parkinsonian rats by bilateral stimulation of the subthalamic nucleus

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ABSTRACT

The subthalamic nucleus (STN) is involved in motor and cognitive performance through its key role in the basal ganglia-thalamocortical circuits, but how these different modalities (motor and cognition) are controlled (similar vs. dissimilar) has not yet been elucidated. In the present study, the effects of bilateral STN deep brain stimulation (DBS) on motor and cognitive performance were investigated in a rat model of Parkinson disease (PD). After being trained in a choice reaction time (CRT) task, rats received bilateral injections of 6-hydroxydopamine (6-OHDA) into the striatum. One group of 6-OHDA animals was implanted bilaterally with stimulation electrodes at the level of the STN. Stimulations were performed at 130 Hz (frequency), 60 μ s (pulse width) and varying amplitudes of 1, 3, 30 and 150 μ A, during the CRT task. Finally, rats were sacrificed and the brains processed for staining to determine the dopaminergic lesion (TH immunohistochemistry) and localization of the electrode tip (HE histochemistry). Bilateral 6-OHDA infusion significantly decreased (70%) the number of dopaminergic cells in the substantia nigra pars compacta (SNc) and increased motor time (MT), proportion of premature responding (PR) and reaction time (RT). Bilateral STN stimulation with an amplitude of 3 μ A normalized 6-OHDA-induced deficits in PR and RT. Stimulation with an amplitude of 30 μ A reversed the lesion-induced deficits in MT and RT. Our data show for the first time that bilateral STN stimulation differentially affected the 6-OHDA-induced motor- and cognitive deficits. This means that basal ganglia-thalamocortical motor and associative circuits responsible for specific motor and cognitive performance, which are processed through the STN, have unique physiological properties that can acutely and separately be modulated by specific electrical stimuli.

INTRODUCTION

Basal ganglia-thalamocortical circuits, covering the motor-, associative-, oculomotor- and limbic circuits, are classically considered to be organized in a parallel manner and remain largely segregated from one another¹. Each pathway is thought to be related to specific regions of the basal ganglia but also shares certain key structures¹⁻³. One of these structures is the subthalamic nucleus (STN), a disk-shaped nucleus located between the cerebral peduncle ventrally and zona incerta (ZI) dorsally in the upper mid-brain². The STN is currently regarded as the “pacemaker” of the basal ganglia⁴. Under normal conditions, it exhibits a typical single spike activity whereas under pathological conditions such as Parkinson disease (PD) STN neurons switch to burst activity^{5,6}. This abnormal “bursting” mode of action has been implicated in driving the overactivity of the basal ganglia output nuclei consisting of the globus pallidus internus (GPI) and substantia nigra pars reticulata (SNr), and consequently, excessive inhibition of their targets (thalamic and cortical areas) which is thought to cause the typical PD symptoms^{7,8}.

For this reason, treating STN hyperactivity by deep brain stimulation (DBS) is nowadays a widely applied procedure in PD. It is in fact considered to be the treatment of choice because of its long-lasting beneficial effects on motor function⁹⁻¹⁴. However, STN DBS has also given rise to undesirable behavioural side-effects such as cognitive impairment¹⁵. This suggests that the STN not only regulates basal ganglia motor performance, but might also influence basal ganglia associative circuits¹⁶. Indeed, it has been shown that the STN also consists of an associative territory¹⁷ and that lesions and pharmacological blockage of the STN can induce attentional impairments¹⁷⁻²¹. Nevertheless, how these different modalities (motor and cognition) are regulated by the STN has still not been elucidated. One possibility is that these modalities are regulated similarly, based on the existence of parallel basal ganglia-thalamocortical circuits. Alternatively, it is hypothesized here that STN DBS affects these circuits separately, dependent of the physiological characteristics of these pathways.

In the present study, to test the hypothesis that the motor and associative circuits have unique electrical properties and can be modulated separately by specific stimuli, we investigated the effects of bilateral STN stimulation on motor and cognitive performance in rats with partial 6-OHDA lesions in a choice reaction time (CRT) task. In this task, several parameters can be measured simultaneously, i.e., the speed of information processing, response inhibition and motor function²². The first two parameters are considered as cognitive parameters, whereas the last parameter is considered to be a measure of motor function^{16,23-25}. In a previous study, in which the use of a bilateral DBS device in freely moving control rats was validated, we have shown that the effects of bilateral STN DBS on cognitive performance depend on the amplitude of the DBS¹⁶. We, therefore also manipulated the stimulation amplitude in the present study to evaluate the effects of bilateral STN DBS on motor and cognitive performance of partially 6-OHDA lesioned rats in the CRT task.

MATERIALS AND METHODS

SUBJECTS AND STUDY DESIGN

All subjects were Lewis male rats ($n = 30$, 12 weeks old, bred and housed at the Central Animal Facility of Maastricht University, Maastricht, The Netherlands), with an average body weight of 300 g. Animals were housed individually in standard Makrolon™ cages on sawdust bedding in an air-conditioned room (about 20° C) under a 12/12-hour reversed light/dark cycle (lights on from 18.00 to 6.00 h). All animals had free access to food and water. The rats were tested 5 days per week. During this time the rats were given 10–12 g standard laboratory chow (Hopefarms, Woerden, The Netherlands) per day. On two days per week (weekend), they were left to rest and were fed *ad libitum*. This schedule reduced their weight to approximately 90% of their free feeding weight. All experiments were approved by the Animal Experiments and Ethics Committee of Maastricht University.

CHOICE REACTION TIME TASK (CRT)

Rats were tested in Skinner chambers (inner dimensions: 40 × 30 × 33 cm) equipped with two retractable levers with cue lights just above the levers. A food tray (5 × 5 cm and 2.5 cm above the grid floor) that was positioned equidistant between the two levers could be accessed by pushing a hinged panel. The levers (4 cm wide) projected 2 cm into the conditioning chamber and were located 6 cm from both sides of the food tray and 12 cm above the grid floor. A light and a loudspeaker were fixed to the ceiling of the conditioning chamber. The test procedure was controlled by a personal computer and the data stored on disk at the end of a session. Time was recorded to a resolution of 1 ms. During the CRT, the rat must insert its nose into the central panel until a tone sounds. A high tone (10 kHz; 80 dB) required depression of the left lever and a low tone (2.5 kHz; 80 dB) the right lever. A variable period (randomly chosen from 0.6–1.5 s, steps of 0.1 s) between nose insertion and tone was termed the hold duration. When a rat did not hold the panel for the entire hold duration, the same interval was repeated when the panel was next pushed. Depression of the lever resulted in food reward in only 50% of cases to increase the enthusiasm of the animals for the task²². The reinforcement was given independently of the reaction time. Ten seconds elapsed between each trial. Each session lasted 30 min or until the completion of 80 successful trials.

BEHAVIORAL PARAMETERS

The following parameters were used to evaluate the response of the rats in this task, as described earlier²².

REACTION TIME (RT)

The mean latency between the onset of the tone and the release of the panel was taken as the reaction time. It is generally accepted that response latencies shorter than 100 ms are unlikely to be true reaction times but should be considered as non-valid responses^{22,26}. On the other hand, response latencies longer than 1500 ms should not be considered as a task-related reaction time^{22,26}. Consequently, all reaction times shorter than 100 ms or longer than 1500 ms were disregarded.

MOTOR TIME (MT)

The mean latency between the release of the panel and the lever press was taken as the motor time. Motor times longer than 2 s did not reflect 'true' motor time. To prevent inclusion of this type of non-valid MTs, we decided to introduce a cut off point, which is also common in human RT research. In general, only 1–2% of all MTs are invalid and these values were disregarded²².

PROPORTION OF PREMATURE RESPONSES (PR)

The number of times the rat released the panel before the hold duration had elapsed were divided by the total number of trials. It should be noted that after a premature response the rats had to start the same trial again by pushing the hinged panel. In the case of a premature response the response lever did not appear in the chamber and could therefore not be pressed by the rat.

NUMBER OF TRIALS

The total number of trials the rats completed in a session of 30 min, with a maximum of 80.

For the purpose of evaluating behavioral performances prior to surgery, the rats were trained until performances (i.e. mean RT, MT and PR) stabilized (no change in performance on three consecutive sessions). Subsequently, the rats were subjected to three additional sessions, which defined the preoperative status of the animals. These data were used in the statistical analysis.

SURGICAL PROCEDURE

After behavioural training, the rats were randomly assigned to one of the following groups: A, sham-operation, B, bilateral 6-OHDA injection, and C, bilateral 6-OHDA injection + bilateral STN electrode implantation. Each group consisted initially of ten animals. First, the animals of groups B and C were operated on. Two rats of group B and

two rats of group C died due to surgery/lesion. These rats were replaced by animals from the sham group. This resulted in the following final group sizes: sham $n = 6$, 6-OHDA $n = 10$, and 6-OHDA + STN $n = 10$.

For surgery, the rats were anesthetized throughout the entire procedure using a combination of ketamine (10 mg/ml) and xylazine (1.6 mg/ml) that was administered intraperitoneally (i.p.). Rats were placed in a stereotactic apparatus (Stoelting, Wood Dale, USA; model 51653). Corneal dehydration was prevented with application of sterile Vaseline album. After making burr holes in the skull, rats of groups B and C received stereotactic injections of 2 μ l 6-OHDA (5 μ g/ μ l dissolved in 0.9% saline and 0.2% ascorbic acid; Sigma, Zwijndrecht, The Netherlands) at four sites (two per hemisphere) in the striatum (coordinates from Bregma: AP 0.7 and -0.4, ML 2.8 and 3.4, and V -5.0 and -5.0, according to the stereotactic atlas of the rat brain, edited by Paxinos). Rats of group A received vehicle injections (0.9% saline and 0.2% ascorbic acid). Injection speed was 0.5 μ l/min, and the cannula was left in place for an additional 2 minutes. One hour before surgery, all rats received desimipramine (20 mg/kg i.p.) to prevent any effect of 6-OHDA on noradrenergic neurons.

Rats of group C underwent implantation of the electrodes in the same surgical session. Two burr holes were made in the skull immediately above the STN (coordinates from Bregma: AP -3.8, ML 2.5, and V -8.0) to allow for the insertion of electrodes. A construction of two gold-plated needle-like electrodes with an inner wire of a platinum-iridium combination (Technomed, Beek, The Netherlands), with a tip diameter of 50 μ m and a shaft diameter of 350 μ m, was employed in this experiment. The inner and outer electrodes were insulated except for the exposed tips. The length of the exposed tips of the electrodes was 75 μ m and the interpole distance was 50 μ m. The electrodes were fixed in position using dental cement (Heraeus Kulzer, Hanau, Germany). This electrode-construction has been validated in our previous studies^{16,27} and more details of the surgical procedure have been published elsewhere¹⁶.

DEEP BRAIN STIMULATION

Rats were left for two weeks to recover from surgery after which they were retrained in the Skinner chambers (modified for the attachment of a stimulation cable), until behavioural parameters stabilized (no change in performance on three consecutive sessions). Then, the average values of the behavioural parameters mentioned above were obtained for each of the following conditions: (i) with attachment of stimulation cable (off stimulation), and (ii) with DBS at various amplitudes (1 μ A, 3 μ A, 30 μ A, 150 μ A), 60 μ s pulse width, and 130 Hz stimulation frequency. The order of the various amplitudes was randomized and each condition was repeated three times. The maximum amplitude applied was 150 μ A, as higher amplitudes clearly induced bilateral dyskinetic/hyperkinetic movements, a finding also documented by others²⁸. The mode of stimulation was bipolar in all cases (inner electrode - and outer electrode +). Stimulation

was performed with 1, 3, 30, and 150 μA to investigate the effect of a relatively very low, low, medium, and high current density¹⁶. Pulse width was set at 60 μs and frequency at 130 Hz, as described earlier¹⁶. Stimuli were delivered using a World Precision Instrument accupulser (A310, WPI, Berlin, Germany) and a stimulus isolator (A360, WPI, Berlin, Germany). Real time verification of the parameters applied during stimulation was obtained for both electrodes of the bilateral construction, using a digital oscilloscope (Agilent 54622D oscilloscope, Agilent Technologies, Amstelveen, The Netherlands). Stimulation started five minutes before the test and lasted for the duration of each session, as it was shown that changes in behaviour can occur immediately after the onset of bilateral STN DBS^{29–31}. Each animal had at least a 24-hour stimulation-off period before the next session started.

HISTOLOGICAL PROCESSING

Following stimulation, rats were perfused transcardially with Tyrode (0.1 M) and fixative containing 4% paraformaldehyde, 15% picric acid and 0.05% glutaraldehyde in 0.1 M phosphate buffer (pH 7.6). Brains were removed and postfixed for 2 hours followed by overnight immersion in 15% sucrose at 4°C. Brain tissue was then quickly frozen with CO₂ and stored at –80 °C. Subsequently, the entire brains were cut serially on a cryostat into 30 μm frontal sections and again stored at –80 °C. Tyrosine Hydroxylase (TH) immunohistochemistry was carried using mouse anti-TH (diluted 1:2000, kindly supplied by Dr. C. Cuello, Canada) as primary antibody^{32–34}. After rinsing steps with TBS, TBS, TBS-T and incubation with the secondary antibody (donkey anti-mouse biotiny; Jackson ImmunoResearch Laboratories, West Grove, USA), the sections were incubated with ABC-kit (Vector laboratories, USA). To visualize the Horse Radish Peroxide (HRP) reaction product, the sections were incubated with DAB. Finally, the sections were mounted, counterstained with standard hematoxylin (HE: Merck, Germany), and coverslipped using Permount (Fisher Scientific, USA). Another series of every tenth sections was histochemically stained with standard hematoxylin-eosin (HE: Merck, Germany) and used to locate the electrode tips.

QUANTITATIVE ANALYSIS OF TH POSITIVE CELLS

Since we expected marked differences in THir, we analyzed 6 animals per group. The immunohistochemically processed sections were used to evaluate the total number of TH immunoreactive (THir) cells within the substantia nigra pars compacta (SNc). All stereological investigations were carried out with a stereological computer microscopy system. On all sections showing the SNc, the region comprising the THir cells within the SNc was delineated and total numbers of THir cells were estimated with the optical fractionator^{35–37}.

PHOTOGRAPHY

All photomicrographs were produced by digital photography using an Olympus U-CMAD-2 digital camera attached to an Olympus AX 70 microscope and image analysis software (analySIS; Imaging System, Münster, Germany).

STATISTICAL ANALYSIS

Data are presented as means and standard errors of means (SEM.). A first analysis was performed only for the 6-OHDA and 6-OHDA + STN groups to evaluate the effect of bilateral electrode implantation on 6-OHDA-induced changes in RT performance. Since no effect of electrode placement was found (ANOVA with Surgery as repeated measures), the data of both lesion groups (6-OHDA and 6-OHDA + STN) were pooled to evaluate the effects of the 6-OHDA treatment. A separate analysis was carried out to test the effects of stimulation within the group of animals that received a 6-OHDA lesion and electrodes using an ANOVA repeated measures analysis (Amplitude as repeated measures) followed by a LSD *post-hoc* test. Since only comparisons were made with the stimulation-off condition, the critical *p* value was adjusted for the four comparisons made (stim-off- 1 μ A, stim-off- 3 μ A, stim-off- 30 μ A, stim-off- 150 μ A) ($P < 0.0125$).

The quantitative data on the total number of THir cells in the SNc were analyzed using a two-way ANOVA using Side (of lesion) as repeated measures with a Bonferroni *post-hoc* multiple comparisons test. A *p* value lower than 0.05 was considered to be significant.

All data were analysed by SPSS-pc, version 11.5.

RESULTS

HISTOLOGICAL EVALUATION OF THE ELECTRODE TIP

Histological evaluation of brain sections, using HE-staining, confirmed that the bilateral electrodes were implanted in the subthalamic region. In all rats, bilateral electrodes were placed symmetrically (interelectrode variation of < 0.1 mm) and the electrode tips were both situated within the STN, with the exception of one rat. In this rat, both electrodes were at the level of the zona incerta (ZI). There was an intrasubthalamic variation in electrode positions. In three rats, both electrodes were localized in the lateral part and in six rats in the medial part of the STN. Fig. 1, illustrates the localization of the electrode tips at the level of the STN.

There was no additional histological damage except for the electrode trajectory. This indicated that repeated stimulation with the present settings did not cause any tissue damage observable with routine HE-staining.

FIGURE 1. On the left side, two representative low-power photomicrograph of a 30 µm-thick frontal section from the brain of a rat subjected to both stereotactic bilateral 6-OHDA injection into the striatum and stereotactic bilateral implantation of concentric and bipolar electrodes to stimulate the STN (tip diameter = 50 µm; shaft diameter = 350 µm) are shown. The tip of the electrode (arrow) is situated within the STN (scale bar = 100 µm). In addition, a schematic illustration of the cumulative data on the bilateral electrode placement is provided. In three rats, the electrodes were positioned in the lateral part of the STN (illustrations 1–3) and in six rats in the medial part of the STN (illustrations 4–9).

6-OHDA LESION

Injections of 6-OHDA into the striatum resulted in a substantial loss of THir cells in the SNc in groups B and C compared to group A (Fig. 2). High-precision design-based stereological analysis revealed a significant 6-OHDA-induced reduction in the total number of THir cells in the SNc of about 70% on both sides (results of two-way ANOVA: factor Group: $F(2,14) = 313.58$, $P < 0.001$; factor Side: $F(2,14) = 0.49$, $P > 0.05$; Fig. 3). Post hoc analysis showed that the amount of TH positive cells did not differ significantly between groups B and C, but both groups were significantly different from group A.

RT PERFORMANCE: EFFECTS OF 6-OHDA LESION

As mentioned in the Methods section, the bilateral implantation of the STN electrodes did not influence the changes induced by the bilateral 6-OHDA treatment. The pooled data indicated that the bilateral 6-OHDA lesion increased the mean RT (Surgery $\times$ Group: $F(1,24) = 8.91$, $P < 0.01$; Fig. 4), the mean MT ($F(1,24) = 5.72$, $P < 0.05$; Fig. 4), and the proportion of PR ($F(1,24) = 4.90$, $P < 0.05$; Fig. 4).

RT PERFORMANCE: EFFECTS OF STN STIMULATION

No significant relationship was found between the localization of the electrode tips (medial or lateral STN) and behavioural outcome. Bilateral stimulation of the STN had an effect on the measure RT (Amplitude: $F(4,36) = 6.28$, $P < 0.01$; Fig 4). Post hoc analysis showed that 3 µA and 30 µA improved the RT performance of the animals in group C

FIGURE 2. Representative low-power photomicrographs of 30 μm -thick frontal sections immunoprocessed for tyrosine hydroxylase (TH), showing the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) in the brain of a rat subjected to stereotactic bilateral vehicle injection into the striatum (group A), a rat subjected to stereotactic bilateral 6-OHDA injection into the striatum (group B), and a rat subjected to both stereotactic bilateral 6-OHDA injection into the striatum and stereotactic bilateral implantation of electrodes to stimulate the STN (group C). The photomicrographs show the same frontal level in the midbrain in which the optic tract divides the THir cells into the VTA (medial) and SNc (lateral) regions. Bilateral 6-OHDA lesion resulted in a substantial reduction in the number of THir cells in the SNc (B, C) (scale bar = 500 μm). The inset in A shows a representative high-power photomicrograph of a THir cell and is representative of the magnification at which the stereological estimates were obtained (scale bar = 30 μm).

FIGURE 3. Total numbers of THir cells in the SNc of rats subjected to stereotactic bilateral vehicle injection into the striatum (group A), rats subjected to stereotactic bilateral 6-OHDA injection into the striatum (group B) and rats subjected to both stereotactic bilateral 6-OHDA injection into the striatum and stereotactic bilateral implantation of electrodes to stimulate the STN (group C). Data are shown as individual values and means per group (lines). Two-way ANOVA revealed significant differences between the groups on both sides ($P < 0.001$). The p values from the corresponding *post-hoc* Bonferroni's multiple comparison tests are provided as *** ($P < 0.001$).

as compared with the stimulation-off condition (postoperative condition). Also the MT performance improved after bilateral STN stimulation ($F(4,36) = 3.39$, $P < 0.05$; Fig 4). Post hoc analysis revealed that the MT performance was only improved after applying a current of 30 μA . The proportion of PR was also reduced after STN stimulation ($F(3,36) = 6.86$, $P < 0.01$; Fig. 4). The post hoc test only showed a reliable reduction in proportion of PR when using a current of 3 μA .

FIGURE 4. Results of the behavioural testing of rats subjected to stereotactic bilateral vehicle injection into the striatum (group A), rats subjected to stereotactic bilateral 6-OHDA injection into the striatum (group B) and rats subjected to both stereotactic bilateral 6-OHDA injection into the striatum and stereotactic bilateral implantation of electrodes to stimulate the STN (group C). Data are shown as mean $\pm$ SEM, and are given as mean reaction time (RT; on the left), mean motor time (MT; middle) and proportion of premature responses (PR; on the right) in a specific choice reaction time task 22. On the x-axis, data are organised in the following order: preoperative data from all rats (Pre), postoperative data from all rats (Post; note that these data also represent the stimulation off data from rats in group C), and stimulation-dependent data from rats in group C as a function of the stimulation amplitude. Repeated measures ANOVA revealed significant differences in the post-operative data between the groups ($p < 0.05$) as well as significant differences between the post-operative data and the stimulation-dependent data of group C. The p values from the corresponding *post-hoc* LSD tests are provided as * ($P < 0.0125$). Note that bilateral STN DBS significantly improved RT at both 3 and 30 μA , MT only at 30 μA , and PR only at 3 μA .

DISCUSSION

In the present study, we have demonstrated that bilateral intrastriatal 6-OHDA infusion significantly decreased the number of THir cells in the SNc of rats. The total number of THir cells counted in the SNc (on average approximately 12 000) and the reduction

in the number of these cells in the SNc (70%) due to bilateral 6-OHDA treatment, are in accordance with previously published data³⁸. Furthermore, our results showed that bilateral striatal 6-OHDA treatment substantially impaired cognitive and motor performance of the rat in a CRT task. This finding is in line with other publications^{18,26,39,40}. It was possible to improve the impaired motor performance in the CRT task by bilateral STN DBS. In addition, the impaired cognitive performance in the same task could also be improved by STN DBS. However, STN DBS affected these modalities with different stimulation amplitudes suggesting that each loop has unique electrical properties which can be modulated independently. This is the first evidence that bilateral STN DBS can differentially affect cognitive and motor performance in an animal model of PD. The motor deficit could only be corrected with 30 μ A, while PR was improved at 3 μ A. In addition, 30 μ A as well as 3 μ A had a significant effect on RT. Higher or lower amplitudes were not effective. In our previous validation study, we observed the same effect on PR 16. The number of premature responses was seen to decrease proportionately from 150 μ A to 3 μ A during STN DBS. The bilateral electrode implantation had no effect on the behavioural performance of the rats in the present study, as the stimulation off data of the rats in group C were not statistically different from rats in group B.

Up to now, no other study has investigated the effects of bilateral STN DBS on motor and cognitive performance in an animal model of PD, but several studies have appeared on the effects of unilateral and bilateral STN lesions on behavioural parameters^{18–21,41,42}. In 1995, Baunez and colleagues reported the effects of bilateral STN lesions in bilateral 6-OHDA depleted rats using a RT paradigm¹⁸. They found a dramatic increase in PR and concluded that STN lesions produced long-lasting deficits that might be related to dyskinesia or cognitive impairment. In a five-choice serial RT task, the same authors extended their previous analysis to choice RT paradigms¹⁹. Significant impairments in discriminative accuracy and again an increase in PR were observed. Similar results were obtained by Phillips and Brown^{41,42}. Their main finding was that a unilateral STN lesion, irrespective of the dopaminergic denervation, resulted in an increase in anticipatory responding in a RT task.

Currently, an increasing amount of data suggests that categorizing DBS as being “inhibitory” and thus equating its effects to those of a lesion, is actually an oversimplification of what is a highly complex and multi-faceted technique^{16,43,44}. An example is the recent study of the effects of unilateral STN DBS in unilateral 6-OHDA-depleted rats on RT performance²⁸. Unilateral stimulation of the STN reduced 6-OHDA-induced motor deficits in the RT task, but had no effect on PR. The latter result is in contrast to the changes observed following unilateral STN lesions in the same task by the same group^{18–21}. Therefore, it remains very difficult to compare data of lesion studies with electrical modulation studies. As lesions are irreversible and can not be adjusted once performed, we suggest that to study functional parameters, DBS is a preferable technique as it is reversible (stimulation can be turned on and off) and adjustable (stimulation parameters can be fine-tuned).

Motor and cognitive performance related to the basal ganglia are thought to be represented by the motor and associative circuits, respectively¹. In the classical concept, both circuits start with projections from specific cortical areas to specific parts of the striatum⁴⁵. The cortical input to the motor circuit originates mainly from the primary motor, premotor and somatosensory areas and is largely directed to the putamen (Fig. 5). The associative circuit originates from the dorsolateral prefrontal cortex (dorsolateral prefrontal circuit) and from the lateral orbitofrontal cortex (lateral orbitofrontal circuit) and projects to the dorsolateral head and ventromedial part of the caudate nucleus, respectively. Both circuits project to the globus pallidus (GP: the equivalent of the globus pallidus externus [GPe] in primates), the entopeduncular nucleus (EP: the equivalent of the GPi in primates), and the SNr. From the GP, a GABAergic pathway projects to the STN. The STN mainly projects to the EP and SNr. Recent evidence suggests that the STN is the main regulator of the basal ganglia output through its extensive connections with the EP and SNr⁴. Finally, after passing specific regions of the thalamus (for details, see Fig. 5), both circuits are closed by the projections to the motor and frontal cortices.

Thus, both circuits responsible for basal ganglia motor and associative performance are processed through the STN. Stimulation of the STN immediately influences this processing and specific stimulation amplitudes result in specific reactions, as shown in the present study. This suggests that the motor and the associative circuits have different physiological characteristics as it has been shown that the level of current amplitude determines which neuronal element is influenced⁴⁴. For instance, the excitability of axons is much higher than that of cell bodies⁴⁹ and large (fast) myelinated axons are likely to be affected by low amplitudes whereas smaller (slow) axons by higher amplitudes⁴⁴. It has been documented both *in vitro*^{50,51} and *in vivo*⁵² studies that these different neuronal elements are activated at similar stimulation thresholds with (high) conventional stimuli. It is possible, therefore, that stimulations at high amplitudes within the STN are non-selective, activating both local neuronal cells and axons of passage thereby “masking” the effect of DBS at the behavioral level. In the present study, stimulation at lower amplitudes (3 μ A) resulted in a very obvious improvement of the PR and RT performance whereas motor performance was improved with medium amplitude (30 μ A). This implies that the threshold of “modulation” of the associative circuit is lower than that of the motor circuit. As explained above, this indicates that the associative circuit has possibly more fast-conducting characteristics than the motor loop with respect to information processing (see for review Ranck⁵²).

To this day, fundamental knowledge regarding the application of electrical currents to deep brain structures is far from complete. A number of possible mechanisms have, however, been proposed⁵³. One of the more popular hypotheses is that DBS causes a reduction of neuronal activity by means of a depolarization block. This proposed mechanism involves the suppression of voltage-gated sodium, and T-type calcium currents leading to an interruption of spontaneous activity within the neurons⁵⁴. It has also been proposed that the silencing of target nuclei by DBS is achieved by the stimulation

of GABAergic afferents to the target cells and the consequent hyperpolarization of post-synaptic terminals by release of the inhibitory neurotransmitter GABA 55. The situation becomes even more complex when it is stated that DBS can have opposing effects on structures being stimulated depending on the cellular architecture of the target area. The similarity in clinical outcomes between DBS and lesion led to the proposition that DBS inhibits the target being stimulated. Recordings made in the stimulated nucleus show inhibition or decreased activity during and after the stimulus train^{56–58}. However, electrical recordings from the efferent nuclei from the stimulated nucleus indicate that DBS increases the output of the stimulated nucleus^{59,60}. Quantitative models have revealed that DBS inhibits the cell bodies of neurons around the electrode by activation of presynaptic terminals, while stimulating the output of local neurons, by initiation of action potentials in the axon remote from the cell body^{61,62}. Furthermore, this dual effect appeared with high frequencies only⁴³. Further studies are warranted to determine precisely how DBS works.

Several factors might have influenced the present data. We cannot exclude that other surrounding regions have been influenced by stimulation and could contribute to the observed changes. It is more likely, however, that these changes are related to the STN for three reasons. First, all electrode tips were partially or fully within the borders of the STN. Second, specific changes in PR in that area are specifically related to changes in STN activity^{16,20,21,42}. Third, all rats exhibited comparable behaviour and there was little variation in our behavioural data (see SEM). Furthermore, any habituation to the stimulation procedure was prevented by the randomization of the order of the various amplitudes applied in each subject. Altogether, it can be stated that the observed behavioural changes in this experiment can most probably be attributed to interactions that occur in the STN between the applied electrical current, local neurons and cortico-basal ganglia- thalamocortical circuits. The intrasubthalamic variation of electrode tips in the medio-lateral plane did not result in different responses. This indicates that with the present current densities in combination with the size of the STN, it is unlikely to influence only specific parts of this nucleus such as the motor or associative part. It is more likely that a general modulation occurs.

Taken as a whole, the present data add new findings to the literature on the effects of STN stimulation on motor and cognitive functions. More specifically, our data indicate that STN DBS can selectively influence behavioural functions dependent on the stimulation amplitude. This means that circuits responsible for specific behavioural functions, which are processed through the STN, have unique physiological properties that can acutely and separately be modulated by specific electrical stimuli. In pathological conditions such as PD, the activities of these basal ganglia-thalamocortical circuits are altered resulting in behavioural deficits. In turn, using specific stimulation settings, each of these behavioural deficits can be corrected when DBS is applied to a key structure within these circuits such as the STN.

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CHAPTER 8

The functional role of the subthalamic nucleus in cognitive and limbic circuits

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ABSTRACT

Once it was believed that the subthalamic nucleus (STN) was no more than a relay station serving as a “gate” for ascending basal ganglia-thalamocortical circuits. Nowadays, the STN is considered to be one of the main regulators of motor function related to the basal ganglia. The role of the STN in the regulation of associative and limbic functions related to the basal ganglia has generally received little attention. In the present review, the functional role of the STN in the control of cortico-basal ganglia-thalamocortical associative and limbic circuits is discussed. In the past 20 years the concepts about the functional role of the STN have changed dramatically: from being an inhibitory nucleus to a potent excitatory nucleus, and from being involved in hyperkinesias to hypokinesias. However, it has been demonstrated only recently, mainly by reports on the behavioural (side-) effects of STN deep brain stimulation (DBS), which is a popular surgical technique in the treatment of patients suffering from advanced Parkinson Disease (PD), that the STN is clinically involved in associative and limbic functions. These findings were confirmed by results from animal studies. Experimental studies applying STN DBS or STN lesions to investigate the neuronal mechanisms involved in these procedures found profound effects on cognitive and motivational parameters. The anatomical, electrophysiological, and behavioural data presented in this review point towards a potent regulatory function of the STN in the processing of associative and limbic information towards cortical and subcortical regions. In conclusion, it can be stated that the STN has anatomically a central position within the basal ganglia thalamocortical associative and limbic circuits and is functionally a potent regulator of these pathways.

1. INTRODUCTION

Once it was believed that the subthalamic nucleus (STN), a relatively small nucleus in the midbrain, was no more than a relay station serving as a “gate” for cortico-basal ganglia-thalamocortical circuits. Nowadays, the STN is considered to be one of the main regulators of motor function related to the basal ganglia, through its fundamental role within the basal ganglia-thalamocortical motor circuit^{1,2}. Clinically, this is evidenced by its involvement in movement disorders such as Parkinson disease (PD)^{3–5}. In PD, which is histopathologically characterized by selective, chronic and progressive nigrostriatal degeneration, the STN displays a continuous abnormal “bursting” mode of activity whereas in physiological conditions it exhibits more or less a regular pattern of discharge with intervals of burst activity^{6–8}. This so-called STN hyperactivity, reflecting the increase in firing rate, has been implicated in increasing the activity of basal ganglia output nuclei and, consequently, excessive inhibition of their targets^{9,10}. This mechanism is held responsible for at least part of the cardinal PD symptoms such as hypokinesia/bradykinesia and rigidity¹¹. In order to surgically “silence” the hyperactive STN, chronic stereotactic deep brain stimulation (DBS) has been applied since the past decade. It has now largely been established that STN DBS is an effective treatment in advanced PD^{12–21} and more recently, four-year^{22,23} and five-year-followup²⁴ effects were reported. Bilateral STN DBS induced a marked long-term improvement in motor function and activities of daily living (ADL).

DBS has generally the same clinical effects as a lesion with respect to the improvement of motor disability in movement disorders, but has more advantages such as its adjustability and reversibility^{5,25}. Nevertheless, the cellular effects of DBS and lesions are considerably different: a lesion destroys and DBS modulates the (electro)physiological activity of neuronal elements^{6,26–28}. To this day, fundamental knowledge regarding the application of electrical currents to deep brain structures is far from complete. A number of possible mechanisms have, however, been proposed⁶. One of the more popular hypotheses is that DBS causes a reduction of neuronal activity by means of a depolarization block. This proposed mechanism involves the suppression of voltage-gated sodium, and T-type calcium currents leading to an interruption of spontaneous activity within the neurons²⁹. It has also been proposed that the silencing of target nuclei by DBS is achieved by the stimulation of GABAergic afferents to the target cells and the consequent hyperpolarization of postsynaptic terminals by release of the inhibitory neurotransmitter GABA³⁰. The situation becomes even more complex when it is stated that DBS can have opposing effects on structures being stimulated depending on the cellular architecture of the target area. The similarity in clinical outcomes between DBS and lesion led to the proposition that DBS inhibits the target being stimulated. Recordings made in the stimulated nucleus show inhibition or decreased

activity during and after the stimulus train^{31–33}. However, electrical recordings from the efferent nuclei from the stimulated nucleus indicate that DBS increases the output of the stimulated nucleus^{34,35}. Quantitative models have revealed that DBS inhibits the cell bodies of neurons around the electrode by activation of presynaptic terminals, while stimulating the output of local neurons by initiation of action potentials in the axon remote from the cell body^{26,28}. Furthermore, this dual effect appeared with high frequencies only²⁷. Further studies are warranted to determine precisely how DBS works.

As STN DBS has become a widely applied surgical procedure, more data have appeared on its surgical and target-related side effects^{36–39}. Concerning the latter, clinicians observed behavioural effects such as stimulation-dependent cognitive alterations^{40,41} and changes in affective and emotional behaviour^{42–44}. Basic researchers applying STN DBS and lesions in animal models to study the neuronal mechanisms entailed in these procedures found profound effects on cognitive and limbic functions^{45–49}. Anatomical tracing studies in rodents and primates have shown that the STN is composed of parts that project to associative and limbic areas of the pallidal complex and substantia nigra pars reticulata (SNr) and that the basal ganglia associative and limbic circuits are processed through the STN^{1,50–52}. The functionality of these projections, however, remains as yet elusive. Furthermore, to which extent these projections are influenced by changing STN's neuronal activity by either lesioning or DBS also needs clarification. Although the beneficial effects of STN DBS and lesions on motor function are relatively well described, the consequences of these surgical interventions on the associative and limbic circuits and associated neuropsychological functions have received relatively little attention. In the present review, we aim to provide a synopsis on the functional role of the STN in the control of the cortico- basal ganglia-thalamocortical associative and limbic circuits.

1.1 STRUCTURE OF THE REVIEW

The present review commences with the history of the STN as in the past 20 years the concepts about the functional role of the STN have changed dramatically. We believe that adequate knowledge about these changes and related historical views will contribute to a better understanding of the current functional concepts of the STN. Subsequently, the current data on the anatomical place of the STN in the cortico-basal ganglia-thalamocortical circuits are summarized with emphasis on the associative and limbic circuits. The intrinsic organization of the STN is also discussed in section 3. After delineating the anatomical position of the STN, original clinical and experimental studies providing data on the involvement of the STN in behavioural processes are systematically discussed. Finally, the role of the STN in the control of cortico- basal ganglia-thalamocortical associative and limbic circuits is outlined.

2. THE HISTORY OF THE STN

The STN was classically regarded as a nucleus exerting a potent inhibitory effect on the cortico-basal ganglia-thalamocortical motor circuit. This theory was substantiated by histopathological findings in patients with hyperkinesias such as hemiballismus, who had mostly hemorrhagic lesions at the level of the STN. Now, we know, for example, that the STN exerts a potent excitatory effect on its efferents. Furthermore, it has been demonstrated that the STN also plays a key role in hypokinetic disorders such as PD. In sections 2.1 and 2.2, reports illustrative for the abovementioned changes are summarized.

2.1 FROM HYPERKINESIA (HEMIBALLISMUS) TO HYPOKINESIA (PD)

One of the first complete descriptions of a hemiballismus in a patient was by Malcolm B. Carpenter⁵³. In this anecdotal publication, Carpenter described a case of a 73-year-old man who developed abnormal involuntary jerky movements in his left extremities. At autopsy, a recent hemorrhagic lesion in the right STN was found. Furthermore, he summarized in this case study other previously published 41 cases of hemiballismus, the first being reported in 1883 by Greiff⁵⁴. Carpenter concluded that STN lesions, mainly hemorrhagic, were a common finding in patients with ballistic movements. At this point, it is worth mentioning that researchers were already aware of the involvement of the STN in motor control in the second half of the nineteenth century. Carpenter and colleagues published in the same decade three other studies confirming that (surgical) lesions of the STN resulted in direct contralateral hemiballismus in primates^{55–57}.

In the next decades, studies kept on appearing on the relation of STN lesions with hemiballismus, in some reports also called “subthalamic dyskinesia” or “hemi-chorea”^{58,59}. Clinically, these observations resulted in the direct association of the STN with hemiballismus, and as a consequence, researchers speculated that STN exerted a potent inhibitory effect on its efferents. In the (neuro)surgical literature of those years, reports can be found on surgical lesions of the subthalamus in patients with movement disorders (mainly PD), also referred to as subthalamic lesions⁶⁰. The latter suggests that already in that time the STN served as a target for surgical interventions in movement disorders and needs therefore clarification. The structure referred to as subthalamus was actually Forel’s tegmental area H₂ (also called fasciculus lenticularis), and not the subthalamic nucleus (Corpus Luysii), which was actually not targeted in the treatment of movement disorders until early 90s (see below).

In 1982, Slater and Dickinson demonstrated that STN lesions could reduce experimental tremor in rats⁶¹. In this study, rats received intraperitoneal (i.p.) injections of the muscarinic cholinomimetic tremorine, which caused resting tremor. Electrolytic lesions of the STN decreased the frequency and intensity of this tremor and the authors concluded that STN was one of the principal mediators of tremorine-induced tremor. However, one year later the same authors reported their results on the effects of STN

lesions on tremorine-induced limb rigidity⁶². Besides causing resting tremor, tremorine was also able to induce rigidity through a cholinergic (muscarinic) mechanism at the level of the striatum. This time they found no effect of STN lesions. It was not until 1989 that evidence became available that the STN was indeed involved in a hypokinetic disorder. Mitchell and co-workers mapped the 2-deoxyglucose uptake in the brain in a primate model of PD⁶³. Six cynomolgus monkeys were exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce parkinsonian symptoms. A profound decrease in 2-deoxyglucose uptake was observed in the STN and this finding was attributed to a decreased activity of the pallidostubthalamic projection. The authors stated that the STN was involved in experimental PD and showed decreased activity in contrast to experimental hemiballismus in which the same authors showed that the STN had increased 2-deoxyglucose uptake⁶⁴.

Miller and DeLong were the first to demonstrate that the STN was not hypo- but hyperactive in an experimental model of PD. In their experiment, which was initially described in an un-peer-reviewed publication, the STN was found to exhibit increased neuronal activity in MPTP-treated primates⁶⁵. These observations were the basis for the pioneering work of Bergman and colleagues⁶⁶. They showed that lesions of the STN reduced all of the major motor disturbances in the limbs, including akinesia, rigidity, and tremor in monkeys rendered parkinsonian by MPTP. Aziz and colleagues confirmed these results one year later using a comparable experimental setup⁶⁷. These results supported the hypothesis of excessive activity in the STN in PD and that ablation of this nucleus was able to improve parkinsonian features. In the meantime, Benabid and co-workers had introduced DBS as an alternative for ablative surgery in movement disorders⁶⁸ and were the first, motivated by the results of the lesion studies and by a crucial stimulation study in primates in the same year⁶⁹, to explore the effects of STN DBS in a patient suffering from advanced PD⁷⁰. In 1995, the results of 3 patients were published showing that bilateral STN DBS resulted in marked improvement in motor symptoms⁷¹. This report was actually the beginning of the STN DBS era.

2.2 FROM INHIBITION (GABA) TO EXCITATION (GLUTAMATE)

Until the late 70s, it was consistently believed that the STN exerted a strong inhibitory effect on its target nuclei since STN lesions were accompanied by hyperkinetic symptoms such as hemiballismus as explained in section 2.1^{53,55,57,72}. However, the 80s were mainly characterized by conflicting reports. For instance, in 1980 Perkins and Stone carried out one of the first studies in which electrical stimulation of the STN was performed in combination with electrophysiological recordings at the level of its efferent⁷³. Their primary aim was to study the connection between the globus pallidus (GP), which is the equivalent of the globus pallidus externus (GPe) in humans, and the STN in the rat. Stimulation resulted in an activation of GP cells suggestive of a net excitatory effect of STN efferents. It has to be noted that it is unclear whether high frequency stimula-

tion (HFS) or low frequency stimulation (LFS) was applied in this study. The authors found this surprising in light of the evidence that destruction of the STN resulted in hyperkinesias^{53,55,57–59}, suggestive of an overall inhibitory effect, and expected, therefore, an inhibition. Three years later, Rouzaire-Dubois and colleagues conducted a similar experiment in which stimulations, single-unit electrophysiological registrations and microiontophoretically applied γ -amino butyric acid (GABA) were used⁷⁴. Stimulation of the STN suppressed the spontaneous firing rate of entopeduncular (EP) cells, which is the equivalent of the globus pallidus internus (GPi) in humans, and local infusion of GABA also had an inhibitory effect on EP neurons. Therefore, these authors concluded that the STN efferents to the EP were inhibitory and that GABA was the neurotransmitter responsible for this effect. However, the same group published another paper in the same year in which STN stimulation inhibited EP neurons but activated substantia nigra pars reticulata (SNr) neurons⁷⁵. In both studies, the authors made no mention of the frequency adopted. It remained unclear whether LFS or HFS was applied. Recent studies indicate that the stimulation frequency determines whether a neuronal element is inhibited or excited^{11,76,77}. This factor could have contributed to the varying results obtained in those years.

It was not until 1988 that the first clear evidence came on this matter⁷⁸. Smith and Parent conducted a study in which the GABA and glutamate immunoreactivity of the primate STN was investigated. They found that the STN was entirely devoid of GABA immunoreactive perikarya and showed strong glutamate immunoreactivity. This study clearly established the excitatory nature of the STN. One year later, Albin and associates confirmed that the STN indeed used glutamate in its efferent neurotransmission⁷⁹. This makes it the only glutamatergic nucleus in the basal ganglia^{80–85}.

These historical data point out that the ideas about the functional role of the STN have changed considerably over the years. The ideas about the anatomical role of the STN have also changed. Past views of the basal ganglia organization were mainly that this set of subcortical nuclei served essentially to integrate converging influences from cortical association and sensorimotor areas during their passage towards the thalamic nuclei¹. Within this concept, the STN was simply considered to be a relay structure. In the past 20 years, more research has focused on the local and regional anatomical characteristics of the STN. The STN is no longer regarded as a relay station but considered to be the clock of the basal ganglia⁸⁶. The current anatomical concepts are discussed in the next session.

3. THE PLACE OF THE STN IN BASAL GANGLIA-THALAMOCORTICAL CIRCUITS

The primary elements of the basal ganglia are the striatum, the pallidum, the SN and the STN 50. In primates, five cortico-basal ganglia-thalamocortical circuits are described consisting of the motor, oculomotor, two prefrontal (dorsolateral and lateral orbitofrontal), and the limbic circuits^{1,2,50,87,88}.

The cortico-basal ganglia-thalamocortical circuits transmit information within the abovementioned basal ganglia elements in a (partially) closed and sequentially arranged way^{50,87–90}. These circuits form the elementary pathways through which higher and lower regions of the brain communicate with the basal ganglia. This communication involves specific functions (e.g., motor, oculomotor, associative and limbic functions) represented by specific circuits. Each of these circuits originates from specific parts of the cortex, are processed in specific thalamic nuclei and project back to at least one of the cortical input areas^{1,2,50,88}. Within each of these circuits the STN is situated on a central position. In this section, the regional and functional anatomy of the basal ganglia-thalamocortical associative, limbic and motor circuits in relation to the primate and rodent STN is summarized. First, the intrinsic organization of the STN is discussed.

3.1 INTRINSIC ORGANIZATION OF THE STN

The primate and rat STN is a relatively small densely populated biconvex-shaped nucleus located between the ZI dorsally and SN posteriorly in the upper midbrain (Fig. 1). It is surrounded by dense bundles of myelinated fibers such as the internal capsule^{91,92}. The STN has approximately 560 000 cells in humans and 25 000 cells in rats^{93–95}. The volume of the STN is circa 240 mm³ in humans and 0.8 mm³ in rats^{91,94}. There are some considerable differences between primates and rats with respect to the dendritic organization of STN neurons. Since the STN is much larger in the primate than in the rat, the dendrites extend to a small part of the primate STN whereas in the rat the dendrites can extend across almost the whole nucleus⁹⁶. In the primate and cat, the STN is considered to be a closed nucleus except for his medial border with the lateral hypothalamic area^{51,92}. This means that the dendritic fields are confined within the nucleus except for the medial border. In the rat, the distal dendrites occasionally cross the dorsal, medial, and ventral borders of the nucleus^{97,98}. These data suggest that the specificity regarding the afferent input might be higher in the primate than in the rat⁹⁶. Concerning the axons of this nucleus, it is thought that most STN neurons are long-axon projection neurons⁹⁹. Most axons emerge directly from the cell body, and some of them form intrinsic collaterals. This collateral system is more frequent in rats, but relatively rare in primates^{100,101}.

The functional subdivision of the basal ganglia into the different basal ganglia-thalamocortical circuits has also been applied to the primate STN⁹¹. These functional domains are largely defined by the afferents and to a lesser extent by the efferent connections. The primate STN has anatomically three subdivisions: the dorsolaterally located somatomotor part (two thirds of the STN), the ventromedially located associative part (one third of the STN), and the medial tip represents the limbic part (Fig. 2)^{50,51,91}. In the rodent STN, two major anatomical domains can be distinguished. The medial part is reciprocally connected with the ventral pallidum (associative and limbic functions) and the lateral part with the dorsal parts of the basal ganglia (sensorimotor functions) and related sensorimotor cortical areas^{96,102}. Because of the difference in dendritic archi-

texture in the rodent and primate STN, the segregation of the STN into functional parts is more evident in primates than in rodents.

The STN receives and projects to a number of different regions inside and outside the basal ganglia. The main afferents arise from the cortex (glutamatergic), GPe (GABAergic), parafascicular (PF) (glutamatergic) and centromedian nuclei (CM) (glutamatergic) of the thalamus, SNc (dopaminergic), PPN (cholinergic, and glutamatergic), and the DRN^{51,91,103–105}. In turn, STN efferents (glutamate mediated neurotransmission) are predominantly directed to GPi, GPe, SNr, SNc, and the PPN^{51,91}.

3.2. ASSOCIATIVE CIRCUIT (FIG. 3A)

Cognitive and other high-level processes related to the basal ganglia are anatomically and functionally represented by the two basal ganglia-thalamocortical associative (also referred to as “prefrontal”) circuits^{1,2,52,88}. These processes are thought to depend on the connections between association cortices (frontal, temporal, and parietal) and striatum^{50,106–108}. In the primate, within the striatum, the associative territory comprises parts of the putamen rostral to the anterior commissure (AC) and most of the head, body and tail of the caudate nucleus^{50,88}. These two associative circuits are the dorsolateral prefrontal circuit (DPC) and lateral orbitofrontal circuit (LOC), which are initiated from distinct cortical areas^{52,88}.

FIGURE 1. This figure illustrates the regional anatomy of the STN in the midbrain of a rat from a midsagittal position. The STN (1) is a relatively small densely populated biconvex-shaped nucleus located between the zona incerta (ZI) (2) dorsally and substantia nigra (SN) (3–5) posteriorly in the upper midbrain. The ZI (grey above the STN) consists of two parts: zona incerta ventralis (ZIV) and zona incerta dorsalis (ZID). The SN consists of three parts: SN pars compacta (SNc) (4), SN pars reticulata (SNr) (3), and SN pars lateralis (SNl) (5). This figure also illustrates the basal ganglia-thalamocortical motor (A), associative (B), and limbic (C) circuits entering the STN and being processed to the SNr which is one of the output nuclei of the basal ganglia. From the output regions, these circuits are finally projected back to the corresponding cortical areas.

FIGURE 2. This figure illustrates the functional subdivisions of the primate STN and shows the medio-lateral orientation of this nucleus. The functional subdivisions of the basal ganglia into the different basal ganglia-thalamocortical circuits have also been applied to the primate STN. The STN has anatomically three subdivisions: the dorsolaterally located somatomotor part (blue), the ventromedially located associative part (green), and the medial tip (red) represents the limbic part. Each circuit is being processed within the corresponding functional part of the STN.

Anatomically, the DPC originates from the dorsolateral prefrontal cortex (DLPC); this is the region around the principal sulcus and on the dorsal prefrontal convexity^{87,88}, and the LOC starts at the level of the lateral orbitofrontal cortex (LOFC), which is actually Brodmann's area 10^{88,109}. The second station is the dorsolateral head and the rostrocaudal axis of the caudate nucleus^{88,106,109}. This corticostriatal projection is glutamatergic. From here, this circuit is directed to the dorsomedial part of the GPi and to the rostral region of the SNr¹¹⁰, using GABA, substance P and dynorphin, and to the anterior parts of the GPe, using GABA and enkephalin as neurotransmitters^{50,88}. The GPi and SNr project (GABAergic) to the VA and CM nuclei of the thalamus, and the DPC is closed by the thalamo-cortical pathway (glutamatergic) back to the DLPC and the LOC back to the LOFC. This pathway is also known as the direct pathway. From the GPe, a reciprocal projection to the STN and a one-way projection to GPi/SNr exists. This pathway is also known as the indirect pathway. Although GPe and GPi have a similar morphological configuration, they form different functional entities⁵⁰. For example, GPi is one of the basal ganglia output nuclei whereas the GPe is a station in the intrinsic circuitry of the basal ganglia^{50,51}.

In rats, there is a similar cortical origin of the associative circuit, but it is processed in the ventral parts of the striatum and pallidum similar to the rat limbic circuit. The associative circuit originates from the prefrontal areas (dorsal agranular insular and dorsal prelimbic areas) and projects to the core of the nucleus accumbens^{111,112}. From here, the projections are directed towards the ventral pallidum (VP) and from here further processed to the STN and SNr⁹⁶. The STN projects back to the VP. From here, the circuit is relayed at the level of the ventral thalamic nuclei and then directed back to the cortical areas¹¹³. Also in the rat there is evidence for the existence of a direct and indirect

pathway, which shows some similarities with primates especially for the motor circuit⁹⁸. However, for the ventral parts of the striatum and pallidum, this scheme of the direct and indirect pathways remains unclear.

The STN is anatomically connected with both direct, through its projection to the GPi and SNr, and indirect, through its projection to the GPe, pathways. This indicates that the STN can regulate the information processing within the basal ganglia at (at least) two levels: at the level of an intermediate station (GPe) and at the level of the basal ganglia output (GPi/SNr). Evidence for such a regulation has been derived from studies in rodents in which STN DBS resulted in changes in neuronal activity of GPi, SNr and GPe^{77,114,115}.

3.3 LIMBIC CIRCUIT (FIG. 3B)

Emotional, motivational and affective processes related to the basal ganglia are represented by the basal ganglia-thalamocortical limbic circuit^{87,88,116}. In the primate, projections from the hippocampus, the amygdala, limbic and paralimbic cortices are primarily concentrated at the level of the ventral striatum^{50,88}. The ventral striatum consists basically of the nucleus accumbens, ventromedial part of the caudate-putamen and the medium-celled portion of the olfactory tubercle^{50,116,117}. The ventral striatum projects in turn to the VP. From here the limbic circuit is directed to the MD nucleus of the thalamus⁸⁸. This circuit is closed by a thalamocortical pathway to the anterior cingulate area and medial orbitofrontal cortex. The STN has reciprocal connections with the VP^{118,119}. The VP is considered to be the major limbic circuit output region. Modulation of the neuronal activity of the STN directly influences the activity of both NMDA and non-NMDA expressing neurons in the VP¹²⁰. Within this concept of the limbic circuit, the STN again has an important role as it is directly connected with the output region of this circuit. Some authors made a subdivision in the limbic circuit into three subcircuits according to the cortical origin^{116,121}. However, the functionality of these subcircuits remains elusive.

In rats, the limbic circuit originates from the limbic areas (infralimbic, ventral prelimbic, and ventral agranular insular areas) and projects to the shell of the nucleus accumbens^{111,122}. From here, the projections are directed towards the subcommissural part of the VP and from here further processed to the MD nucleus of the thalamus⁹⁶. The STN projects to the VP, as explained earlier. Finally, after passing the MD nucleus of the thalamus, the circuit is directed back to the cortical areas¹²³.

3.4 MOTOR CIRCUIT (FIG. 3C)

Motor performance related to the basal ganglia is represented by the basal ganglia-thalamocortical motor circuit^{1,2,52}. In primates, this circuit starts with topographical projec-

tions (glutamatergic) from the primary motor cortex, premotor areas, supplementary motor area, primary somatosensory cortex and somatosensory association cortex, and is principally directed to the putamen^{88,107,124,125}. These projections terminate in almost the entire rostrocaudal axis of the putamen except for its most rostral and caudovertral extensions. The putamen projects topographically to the posterior and ventrolateral parts of both the GPi and GPe and to a posterolateral region within the SNr, using GABA as neurotransmitter. The projections to the GPi and SNr stain intensely for substance P and the projection to the GPe for enkephalin1. From the pallidal and nigral terminals, the motor circuit is further directed to the nucleus ventralis lateralis (VL), the nucleus ventralis anterior (VA), and the centromedian nucleus (CM) of the thalamus^{88,126,127}. This pathway is also known as the direct pathway. From the GPe, a reciprocal projection to the STN and a one-way projection to GPi/SNr exists. This pathway is also known as the indirect pathway^{50,51}. The organizational configuration of the motor circuit has similarities with the associative circuit.

The STN is anatomically connected with both direct, through its projection to the GPi and SNr, and indirect, through its projection to the GPe, pathways. STN is a principal regulator of the output of the motor circuit towards the abovementioned thalamic nuclei¹²⁸. Finally, after passing these specific regions of the thalamus, the motor circuit is closed by the projection to the supplementary motor area, premotor areas, and the primary motor cortex⁸⁸.

In rats, the motor circuit originates from the sensorimotor cortex and projects to the dorsolateral striatum⁹⁶. From here, the projections are directed towards the EP/SNr and the GP. The GP projects to the STN, and the STN sends efferents to the EP/SNr. Finally, after passing the ventral thalamic relay nuclei, the circuit is directed back towards the cortical areas.

In humans as well as in rats, a strong glutamatergic cortico-subthalamic projection exists^{129,130}. It still remains unknown how this projection fits into the existing scheme of the direct and indirect pathway and further research is warranted to establish the clinical importance of this pathway.

These data on the local and regional anatomy in primates and rodents demonstrate that the STN is a central structure within the basal ganglia and that major basal ganglia-thalamocortical circuits are processed through this nucleus. Within this anatomical concept, it has now largely been established that the STN plays an important functional role in motor control^{1,2,98}. With respect to the non-motor functions of the STN, it was initially the basic researchers who applied STN DBS or STN lesions in animal models and found profound effects on cognitive and emotional parameters^{45–49}. This involvement of the STN in functional parameters was later confirmed by clinical studies, mainly by reports on the behavioural effects of STN DBS and lesions in PD patients. In the next section, these data on the functional role of the STN in behaviour are outlined.

FIGURE 3. Schematic illustration of the primate basal ganglia-thalamocortical associative, limbic and motor circuits. The STN has a central position in each of these circuits. Associative circuit: the two associative circuits are the dorsolateral prefrontal circuit (DPC) and lateral orbitofrontal circuit (LOC), which are initiated from the dorsolateral prefrontal cortex (DLPC), and lateral orbitofrontal cortex (LOFC), respectively. The second station is the dorsolateral head and the rostrocaudal axis of the caudate nucleus. From here, this circuit is directed to the dorsomedial part of the GPi and to the rostral region of the SNr and to the anterior parts of the GPe. The GPi and SNr project to the VA and CM nuclei of the thalamus and the DPC is closed by the thalamocortical pathway back to the DLPC and the LOC back to the LOFC. This pathway is also known as the direct pathway. From the GPe, a projection to the STN and GPi/SNr exists. This pathway is also known as the indirect pathway. The STN is anatomically connected with both direct, through its projection to the GPi and SNr, and indirect pathway, through its projection to the GPe. Limbic circuit: projections from the hippocampus, the amygdala, limbic and paralimbic cortices are primarily concentrated at the level of the ventral striatum. The ventral striatum consists basically of the nucleus accumbens, ventromedial part of the caudate-putamen and the medium-celled portion of the olfactory tubercle. The ventral striatum projects in turn to the ventral pallidum (VP). From here the limbic circuit is directed to the MD nucleus of the thalamus. This circuit is closed by a thalamocortical pathway to the anterior cingulate area and medial orbitofrontal cortex. The STN has reciprocal connections with the ventral pallidum. The ventral pallidum is considered to be the major limbic circuit output region. Modulation of the STN neuronal discharge directly influences the activity of both NMDA and non-NMDA expressing neurons in the VP. Within this concept of the limbic circuit, the STN again has a pivotal role as it is directly connected with the output of this circuit. Motor circuit: the cortical input to the motor circuit originates mainly from the primary motor, premotor and somatosensory areas. This somatotopic glutamatergic input is largely directed to the putamen which projects topographically to the motor parts (ventrolateral (VL) and posterior) of the GPe and GPi, and the SNr. From the GPe, a pathway projects to the STN. The STN, mainly projects to the GPi and SNr. The SNr and the GPi serve as the output nuclei of the basal ganglia. The thalamic areas involved in the motor circuit are mainly the VL, ventroanterior (VA) and the centromedian nucleus (CM). This loop is closed by means of the thalamic projection (glutamatergic) to the cortical areas.

4. BEHAVIORAL CONSEQUENCES OF STN STIMULATIONS AND LESIONS

The anatomical data support the hypothesis that the STN plays an important role in cognitive and limbic functions. In this section, clinical and experimental studies on the effects of STN stimulations and lesions confirming this hypothesis are chronologically and systematically discussed.

1.1 CLINICAL STUDIES

STN lesions and stimulation are almost exclusively performed in patients suffering from PD, and, therefore, the studies discussed below involve only PD patients. In addition, only few reports have appeared on STN lesions in PD patients, with mainly a unilateral approach. The vast majority of the articles discussed below are STN DBS studies. In section 4.1.1., we will summarize the observed behavioral effects of STN DBS in patients.

4.1.1. STN STIMULATIONS AND LESIONS (TABLE 1)

–1998

One of the first studies that found behavioural side-effects following STN DBS in patients suffering from advanced PD was by Kumar and co-workers¹⁶. They reported the results of their initial seven patients. Patients underwent bilateral implantation of quadripolar electrodes at the level of the STN. All patients experienced a considerable improvement of their motor symptoms. One patient experienced a mild personality change in combination with disinhibition and one patient with progressive cognitive decline before surgery had an abrupt decline in most areas of cognition, postoperatively. Unfortunately, the authors did not discuss these behavioral complications in their publication. Rodriquez and colleagues reported in the same year their first results of STN DBS in advanced PD with more emphasis on the effects on tremor¹³¹. All patients had a substantial reduction of tremor, but one patient experienced a transient dysthymia and another patient with a history of depression developed a severe depressive state. Also these authors did not discuss these behavioural complications and were mainly focused on the motor outcome.

1999–2000

One year later Kumar and colleagues described three patients in addition to their initial seven subjects¹⁷. Two out of these three new patients experienced behavioural complications. One patient developed decline in verbal memory and one patient experienced acute depression when the most effective electrode contact was activated.

Altogether, four out of their 10 subjects experienced a behavioral complication. This time the authors discussed these side effects and concluded that the neuronal elements affected by STN stimulation may play a more important role than previously was considered in the basal ganglia-thalamocortical circuits. The same group published one year later the neuropsychological outcome of their patients¹³². Patients underwent extensive neuropsychological examinations pre- and postoperatively. Despite manifest improvements in motor performance after surgery, patients had impairments in executive functioning, attention and working memory, and verbal and visual learning. The authors concluded that many aspects of cognitive functioning could decline after STN DBS most probably due to interference with the basal ganglia-thalamocortical associative circuit. Comparable results were described by the same group elsewhere⁴⁰. In the same context, Pillon and co-workers found in a larger cohort of patients a poorer performance on verbal fluency¹³³.

Besides the effects of STN DBS on cognitive performance, others reported emotional consequences. Moro and co-workers observed depression in one patient out of seven¹³⁴. Houeto and colleagues reported four cases of depression out of 23 operated patients³, and Molinuevo and colleagues reported one depressed patient in a series of 15 patients¹³⁵.

In a case report in 2000, Diederich and co-workers described a PD patient in which bilateral STN DBS induced visual hallucinations only when the DBS was turned on¹³⁶. Tuning off the stimulator resolved the hallucinations. The authors concluded that the STN, either directly or indirectly, might be involved in the generation of visual hallucinations.

2001–2002

In the next years, more studies reported behavioural effects of STN DBS. Volkmann and colleagues described the results of STN DBS in 16 patients²¹. Six patients developed signs of depression, postoperatively. The possible mechanism underlying this observation was not discussed. In the same year, Dujardin and associates investigated the influence of chronic bilateral STN DBS on cognitive function in PD¹³⁷. Patients underwent extensive neuropsychological evaluations, pre- and postoperatively. All patients experienced a significant reduction in word fluency and scored significantly worse on the delayed free recall test. Four patients developed overall cognitive decline, two patients became depressed, and one developed an anxiety disorder. Taken together, all patients experienced a behavioural complication despite significant improvements in motor disability. The authors remarked that STN DBS was able to induce a dysfunction of the basal ganglia-thalamocortical associative and limbic circuits by a mechanism not well understood. Alegret and associates published similar results¹³⁸. They described the cognitive outcome of 15 patients with STN DBS. All patients experienced moderate deterioration in verbal memory

and prefrontal and visuospatial functions. Deteriorations in verbal functions were also confirmed by another study in the same year¹³⁹.

Moretti and colleagues studied the debilitating effects of STN DBS on cognitive parameters in two patients with STN DBS in more detail^{140,141}. Patients underwent extensive quantitative and qualitative neuropsychological evaluations. The authors found difficulties in attention focusing, in long-term stability of higher cortical functioning, and a significant reduction of semantic fluency. The authors attributed this finding to STN's relation to the basal ganglia-thalamocortical prefrontal circuits. Valdeoriola and co-workers also remarked a worsening of phonetic and semantic verbal fluency in their stimulated patients¹⁴². In addition, five out of their 26 patients became depressed. In another study in the same year, the adjustment disorders, psychiatric disorders and personality changes were evaluated in 24 patients with STN DBS¹⁴³. Four patients experienced a depression, 18 patients had generalized anxiety, 15 patients showed emotional hyperreactivity, and in eight patients a personality disorder was diagnosed.

One patient with hypersexuality after surgery was reported by Krause and colleagues¹⁴⁴. Krack and associates reported two patients with hypomania as a result of STN DBS and concluded that the STN, with its sensorimotor, cognitive, and limbic parts, was not only involved in motor but also in psychomotor regulation¹⁴⁵. Similar observations of manic episodes following STN DBS were also reported by Kulisevsky and co-workers⁴³. Depressive mood, the other extreme in the span of affective disorders, was found in two out of 26 patients¹⁴⁶, three out of 20 patients¹⁴⁷, three out of 17 patients¹⁴⁸, and six out of 24 patients⁴², three out of 31 patients¹⁴⁹, and in six out of 18 patients¹⁵⁰. Romito and colleagues reported the long-term results of subthalamic nucleus stimulation in 22 patients with advanced PD¹⁵¹. Motor performance and activities of daily living (ADL) improved significantly. However, the most frequently observed complication was behavioural: depression in two patients, manic psychosis in two patients and hypersexuality in four patients.

Finally, Mallet and co-workers reported two patients with advanced PD and a history of severe obsessive-compulsive disorder (OCD) who underwent bilateral STN DBS¹⁵². Surgery improved the PD related motor disability and at the same time the compulsions disappeared and obsessive symptoms improved.

2003–2004

In 2003, Daniele and co-workers published their long-term results on the cognitive and behavioural effects of chronic stimulation of the STN in patients with PD¹⁵³. Twenty patients underwent extensive neuropsychological examination before and after STN DBS. Despite significant clinical improvement in PD symptoms, all patients experienced significant decline in verbal fluency and verbal memory. Three patients had transient manic symptoms with hypersexuality. Impairments in verbal fluency after STN DBS were also described by another study in the same year¹⁵⁴. Kleiner-Fisman and colleagues

observed a variety of behavioural changes in their patients¹⁵⁵. In a study with 25 patients, five patients experienced cognitive impairments, four patients mood changes, one patient had a suicide attempt, and one patient developed hypersexuality.

At the end of the same year, Krack and associates published their five-year followup results of STN DBS²⁴. At present, this is the study with the longest followup duration. In total, 49 patients were examined. As compared with the preoperative condition, the patients' scores at five years for motor function and ADL were significantly improved. Nevertheless, frontal-lobe function in these patients, assessed by neuropsychological tests, tended to be worse. In addition, seven patients became depressed and eight patients developed signs of hypomania. One preoperatively depressed patient committed suicide postoperatively. Depression and hypomania following STN DBS were also observed by others in the same year^{156,157}. With respect to cognition, Moretti and co-workers studied the neuropsychological changes after STN DBS in nine patients with PD after 12 months¹⁵⁸. They found a general cognitive slowing in their patients. The authors concluded that a dysfunction of the fronto-subthalamic pathway was responsible for these results. Central slowing following STN DBS has also been confirmed by our group⁴¹.

One of the few studies describing complete outcome following subthalamotomy was by Patel and associates¹⁵⁹. They performed unilateral lesions of the STN in predominantly hemi-parkinsonian patients and the results of 21 patients were described. All patients underwent pre- and postoperative motor and neuropsychological evaluations. Despite significant improvements in most of the contralateral cardinal symptoms, in some patients deterioration in verbal learning, attention and planning were seen.

In 2004, several other studies appeared on the neuropsychological effects of STN DBS. Morrison and co-workers studied the cognitive functioning following bilateral STN DBS in PD patients¹⁶⁰. The main difference of this study with previous studies was that an age-matched control group was included. The surgical procedure itself mildly adversely affected attention and language functions. STN DBS as a whole resulted in a mild decline in verbal and language functions. In another study, researchers found that STN stimulation reduced working memory and response inhibition performance under conditions of greater challenge to cognitive control despite marked improvement of motor function in PD patients¹⁶¹. The authors concluded that modulation of the associative part of the STN could be responsible for these changes. Impairments in response inhibition after STN DBS in PD patients were confirmed by another group¹⁶².

We have listed above the clinical studies observing behavioural changes in the patients following STN DBS. There are also reports, in the minority however, that have not found any negative effect on the behavioural performance^{163–167}. Therefore, a careful evaluation of differences among studies is crucial. We have carried out a meta-analysis on these data with the inclusion of all available reports on the behavioural effects of STN DBS¹⁶⁸. The results show that behavioural complications are the most commonly observed side-effect after STN DBS. The majority of these patients experience a cog-

TABLE 1. A chronological overview of the clinical reports discussed in section 4.1.1. The table shows the first author of the study, year of publication of the study, the number of subjects involved in the study, the surgical intervention, and the behavioral effects of the surgical intervention. Furthermore, the numbers of patients experiencing these behavioural effects are also shown where possible.

Author	Year	N of subjects	Intervention	Behavioural consequences (number of patients)
–1998				
Kumar	1998	7	STN DBS	mild personality change and disinhibition (n=1); general cognitive decline (n=1)
Rodriguez	1998	12	STN DBS	severe depression (n=1); dysthymia and mood changes (n=1)
1999–2000				
Kumar	1999	10	STN DBS	mild personality change and disinhibition (n=1); general cognitive decline (n=1); decline in verbal memory (n=1); depression (n=1)
Moro	1999	7	STN DBS	depression (n=1)
Trepanier	2000	9	STN DBS	impairments in executive functioning, attention and working memory, verbal and visual learning (in general)
Saint-Cyr	2000	11	STN DBS	significant deterioration of various aspects of frontal executive functioning (in general)
Pillon	2000	63	STN DBS	poorer performance on verbal fluency (in general)
Houeto	2000	23	STN DBS	depression (n=4)
Molinuevo	2000	15	STN DBS	depression (n=1)
Diederich	2000	1	STN DBS	visual hallucinations (n=1)
2001–2002				
Volkman	2001	16	STN DBS	depression (n=6)
Krause	2001	12	STN DBS	hypersexuality (n=1)
Dujardin	2001	9	STN DBS	reduction in word fluency (in general), overall cognitive decline (n=4), depression (n=2), anxiety (n=1)
Alegret	2001	15	STN DBS	moderate deterioration in verbal memory and prefrontal and visuospatial functions (in general)
Brusa	2001	3	STN DBS	worsening of verbal fluency (in general)
Moretti	2001	2	STN DBS	general amelioration of cognitive performances (in general)
Valdeoriola	2001	26	STN DBS	worsening of phonetic and semantic verbal fluency and worsened line orientation (in general), depression (n=5)
Vingerhoets	2002	20	STN DBS	depression (n=3), decreased verbal fluency (in general)
Martinez–Martin	2002	17	STN DBS	depressive mood (n=3)
Romito	2002	22	STN DBS	hypersexuality (n=4), manic psychosis (n=2), depression (n=2)
Thobois	2002	18	STN DBS	depression (n=6)
Houeto	2002	24	STN DBS	depression (n=4), generalized anxiety (n=18), emotional hyper-reactivity (n=15), personality disorders (n=8)
Ostergaard	2002	26	STN DBS	depression (n=2)
Kulisevsky	2002	15	STN DBS	mania (n=3)
Berney	2002	24	STN DBS	depression (n=6)
Doshi	2002	31	STN DBS	depression (n=3)
Mallet	2002	2	STN DBS	reduction of symptoms of OCD (n=2)
2003–2004				
Daniele	2003	20	STN DBS	significant decline in verbal fluency and verbal memory (in general), transient manic symptoms with hypersexuality (n=3)
Gironell	2003	8	STN DBS	decrease in verbal fluency (in general)
Kleiner–Fisman	2003	25	STN DBS	cognitive decline (n=5), mood changes (n=4), suicide attempt (n=1), hypersexuality (n=1)
Krack	2003	49	STN DBS	frontal-lobe function score tended to be worse (in general), depression (n=7), suicide (n=1), hypomania (n=8)
Romito	2003	33	STN DBS	decline of verbal fluency (in general), hypersexuality (n=4), mania (n=2), depression (n=3)
Herzog	2003	48	STN DBS	hypomania (n=2), depression (n=5)
Moretti	2003	9	STN DBS	slowing of cognitive activity (in general)
Patel	2003	21	STN lesions	deterioration of verbal learning, attention and planning (some patients)
Morrison	2004	17	STN DBS	mild decline in verbal and language functions (in general)
Hershey	2004	24	STN DBS	reduced working memory and response inhibition performance (in general)
Witt	2004	23	STN DBS	impaired response inhibition (in general)

STN, subthalamic nucleus; DBS, deep brain stimulation; UPDRS, Unified Parkinson disease rating scale

nitive impairment. The second most commonly observed behavioural complication is a depression. One could argue, for instance, that differences in patient characteristics, surgical procedures, or the postoperative reduction of dopaminergic medication could have contributed to the differences between reports. An important issue seems to be the electrode localization. In the majority of the studies the postoperative electrode positions were poorly determined. Therefore, it was not possible to carry out a correlation analysis to investigate the relation of the anatomical position of the electrodes with the occurrence of behavioural changes. The stereotactic coordinates were also poorly mentioned. However, all these studies have one thing in common and that is a significant improvement of motor disability in the off condition of patients suffering from PD. This is at present the strongest evidence of effective STN modulation *in vivo*. Furthermore, there is currently no convincing data that another structure within the subthalamic region plays a major role in associative and limbic functions except for STN itself.

Another factor that needs to be considered in PD patients undergoing STN DBS is the postoperative drug reduction. It is usually possible to achieve an average reduction of 60% of dopaminergic medication after surgery. The question arises whether this reduction in dopaminergic medication could have contributed to the behavioural changes observed following surgery. Some transient changes in mood and psychotic symptoms can occur when the reduction is performed fast. However, in the majority of the studies presented in this review, the medication reduction was performed slowly. Furthermore, the reduction of dopaminergic medication was usually fully achieved in the first 2–3 months after surgery, whereas the behavioural symptoms were generally also observed at longer followup time points. Secondly, in some studies STN stimulation was switched off to investigate the effects of only stimulation on cognitive parameters^{161,162,169,170}. Results of these studies show that it is actually the modulation of the STN, and not medication or surgery that has a substantial effect on behavioural parameters.

4.2 EXPERIMENTAL STUDIES

The majority of the experimental studies involved STN lesions and were performed in rats.

4.2.1. STN STIMULATIONS AND LESIONS (TABLE 2)

-2000

In 1995, Baunez and co-workers published their experience with bilateral STN lesions in a rat model of PD 48. The authors remarked that most of the interactions with the STN were found by studying motor parameters in simple behavioural paradigms such as the rotation task. Their aim was to further investigate the effects of bilateral STN lesions in a reaction time task (RT). Rats received bilateral excitotoxic STN lesions and were

made Parkinsonian by bilateral striatal injections of 6-hydroxydopamine (6-OHDA). Rats treated with 6-OHDA showed significant motor and cognitive impairments. Bilateral STN lesions improved the 6-OHDA-induced deficits in motor performance but induced a significant increase in premature responding. The authors concluded that lesioning the STN improved 6-OHDA-induced motor deficits but resulted in an increase in premature responding which could either be a motor symptom (dyskinesia) or a cognitive impairment.

In their next publication, Baunez and associates stated that an increase in premature responding, which was observed in their previous study, could indeed be seen as a part of a more general attentional impairment and not as a sign of dyskinesia⁴⁹. Attentional functions are considered to be in the cognitive dimension and can therefore be seen as a cognitive parameter^{171–175}. To further test this hypothesis, a more complex reaction time task (CRT) was used in this experiment⁴⁹. This task, in contrast to their previous task, required more attentional and information processing skills. Rats, not dopamine depleted, received bilateral excitotoxic STN lesions. Behavioural testing revealed that STN lesions had multiple effects on cognitive functions including discriminative deficits, impulsivity and perseverative behavior.

In 1999, the same group used a pharmacological inactivation model of the STN to confirm their previous results and to investigate the neurotransmitters involved¹⁷⁶. Inactivation was achieved by bilateral injections of DL-2-amino-5-phosphonovaleric acid (APV), a NMDA receptor antagonist, and muscimol, a GABA-A receptor agonist. Rats, not dopamine depleted, were tested in the same CRT task. Intrastubthalamic APV infusion resulted in decreased accuracy of performance, increased latency to make a correct response, and increased number of perseverative responses. Muscimol infusion only affected the accuracy of performance and the latency. Both drugs had no effect on premature responding. The conclusion of this study was that these effects were similar to those seen after bilateral STN lesions, except for premature responding. In another study in the same year, these authors showed that 6-OHDA lesions normalized STN lesion-induced increase in premature responding¹⁷⁷.

Phillips and Brown conducted two studies in which they investigated the effects of unilateral STN lesions in unilaterally striatal 6-OHDA-depleted¹⁷⁸ and in non-depleted rats¹⁷⁹, using the RT task. In both studies, STN lesions resulted in an increase in premature (anticipatory) responding and normalized 6-OHDA-induced motor deficits. They stated that the STN is important in inhibiting unwanted and competing responses, confirming the results of Baunez and colleagues^{48,49,176}.

Henderson and co-workers investigated the effects of unilateral STN lesions in unilaterally 6-OHDA depleted marmosets and rats using tasks other than RT^{180,181}. In their first study, which was carried out on marmosets, unilateral 6-OHDA lesions were made to the medial forebrain bundle. In addition, a group of animals also received ipsilateral excitotoxic STN lesions. The dopaminergic lesion resulted in profound effects on behavioural tasks including rotation, head position, staircase performance, tube

reaching and activity measures. STN lesions improved deficits in head position and staircase performance. However, deficits in skilled movement persisted and STN lesions induced contralateral orientation biases in head turning. The authors concluded that STN lesions could ameliorate some, but not all, parkinsonian-like deficits in the unilateral -6-OHDA lesioned marmoset. In their second study, they evaluated the effects of these lesions in rats using a variety of behavioural tasks. STN lesions significantly reduced rotational asymmetry but had no effect on neglect and skilled paw reaching. Like in their first study, STN lesions induced contralateral orientation biases in head turning and body axis curling, which was, according to the authors, an attentional deficit.

2001-

In 2001, Baunez and associates published their results on the effects of STN lesions in a simple reaction time (SRT) task and a CRT task⁴⁵. Bilateral STN lesions in non-depleted rats increased again premature responding in both tasks. The overall conclusion of Baunez and colleagues was, also based on the abovementioned experiments, that the STN played a critical role in response preparation and more specifically in response selection and inhibition. In a recent anatomical-behavioral study, the same group showed that cortical inputs to the STN, more specifically from the medial prefrontal cortex (mPFC), were involved in the cognitive deficits observed following STN lesions¹⁸². Florio and colleagues reported the effects of unilateral STN lesions in combination with ipsilateral lesions of the pedunculopontine nucleus (PPN) in non-depleted rats¹⁸³. STN lesions increased premature responding, which was not influenced by lesions of the PPN. These data are in line with the results of Baunez and colleagues^{45,182}.

It was not until 2003 that the first study in rats appeared on the effects of STN stimulation on behavioural parameters. Darbaky and colleagues performed unilateral STN stimulations in rats with unilateral striatal injections of 6-OHDA¹⁸⁴. The stimulation parameters were set at a frequency of 130 Hz and a pulse width of 60 μ s. The amplitude of the stimulation was below the dyskinesia-inducing threshold (150 μ A). The effects of other stimulation frequencies, pulse widths or amplitudes were not investigated. In the clinical context, however, it is usual to change the stimulation settings in order to optimize the effect¹⁸⁵. The stimulation was applied during the CRT task. Unilateral STN DBS decreased the apomorphine-induced circling behaviour and reduced catalepsy induced by haloperidol, which are indicative of a beneficial effect of STN DBS on 6-OHDA-induced motor deficits. STN DBS did not result in any effect on cognitive functions. The authors concluded that STN DBS was effective in alleviating motor deficits but not in modulating cognitive functions. Recently, Desbonnet and colleagues reported the first study in which bilateral STN DBS was applied in rats⁴⁶. Using the most suitable DBS settings in the rat¹⁸⁶, bilateral DBS was applied to the STN of non-depleted rat with various stimulation parameters during a CRT task. Results showed a significant linear decrease in premature responding with decreasing amplitudes and at

high frequencies only. It was suggested that a decrease in premature responding could be attributed to a stimulation-dependent increased activation of pre-motor cortical areas associated with the 'motor readiness potential' resulting in modulated cognitive performance. The same group conducted a second study in which bilateral STN DBS was applied in bilateral striatal 6-OHDA depleted rats⁴⁷. Stimulations were performed at 130 Hz (frequency) and 60 μ s (pulse width) since these parameters were shown to be effective in the previous study⁴⁶. In addition, varying amplitudes of 1, 3, 30 and 150 μ A were applied. Bilateral STN DBS with an amplitude of 3 μ A significantly decreased 6-OHDA-induced deficit in premature responding. Stimulation with an amplitude of 30 μ A reversed the lesion-induced motor deficits. These data showed that bilateral STN DBS could acutely and separately influence the 6-OHDA-induced motor- and cognitive deficit. The authors attributed these findings to unique physiological properties of that basal ganglia-thalamocortical motor and associative circuits responsible for specific motor and cognitive performances. The data of the last two stimulation studies confirm that STN is indeed involved in the preparation of response, including selection of responses and inhibition of unwanted actions. Furthermore, the functional properties of the STN can selectively and acutely be modulated by specific stimulation settings.

5. SYNOPSIS

5.1 DELINEATING THE ROLE OF THE STN IN THE BASAL GANGLIA-THALAMOCORTICAL ASSOCIATIVE AND LIMBIC CIRCUITS

The clinical and experimental data demonstrated in sections 4.1 and 4.2 point out that modulating the neuronal activity in STN by ablation or stimulation results in substantial improvement of the pathological motor behaviour, but can be accompanied by behavioural changes. In patients, different aspects of behaviour were altered (Table 3). Apart from clear changes in motor disability, cognitive impairments were the most commonly observed behavioural consequence of STN DBS. Several specific cognitive functions were affected: verbal memory and fluency^{17,133,137–142,153,154}, executive functioning^{40,132,137,155}, attention^{40,132,140,141}, working memory^{40,132,161}, mental speed^{41,158}, and response inhibition^{161,162}. The second most observed behavioural complications were affective disorders: depression^{3,17,21,24,42,131,134,135,137,142,143,146–151}, in some cases leading to suicide (attempts)^{24,149,155}, and (hypo)mania^{43,145,153,187}. Furthermore, changes in personality^{16,143} and anxiety^{137,143}, hypersexuality^{144,155}, and hallucinations¹³⁶ were also observed following STN DBS, albeit only in single cases. Patients with preoperative cognitive deficits and affective disorders seem to be at risk for further deterioration after surgery^{16,131}. In experimental studies, rats and in one study primates exhibited specific behavioural changes after STN stimulation and lesions. Rats showed impaired response inhibition^{45,48,49,178,179} and impulsivity⁴⁹, and marmosets had attentional deficits¹⁸⁰, which are in line with the human literature^{40,43,132,140,141,145,153,161,162,187}.

TABLE 2. A chronological overview of the experimental reports discussed in section 4.2.1. The table shows the first author of the study, publication year of the study, category of subjects involved in the study, the surgical intervention, the nature of the animal model, the assessment method of behavior, and the behavioral effects of the surgical intervention.

Author	Year	Subjects	Intervention	Model	Behavioral evaluation	Results
Baunez	1995	Rats	Bilateral excitotoxic STN lesions	Parkinsonism: bilateral striatal 6-OHDA infusion	Reaction time performance	Improved motor performance, but increased premature responding
Baunez	1997	Rats	Bilateral excitotoxic STN lesions	Non-depleted rats	Reaction time performance	Multiple attentional deficits including premature responding, impulsivity and perseverative behavior
Henderson	1998	Primates	Unilateral excitotoxic STN lesions	Parkinsonism: unilateral medial forebrain bundle 6-OHDA infusion	Rotation, head position, staircase performance, tube reaching and activity measures	Partial improvement of motor deficits, but attentional deficits such as contralateral orientation biases
Baunez	1999	Rats	Bilateral pharmacological inactivation of the STN	Non-depleted rats	Reaction time performance	Multiple attentional deficits including reduced accuracy, reduced latency and perseverative behavior
Baunez	1999	Rats	Bilateral excitotoxic STN lesions	Parkinsonism: bilateral striatal 6-OHDA infusion	Reaction time performance	Multiple attentional deficits including premature responding, impulsivity and perseverative behavior
Phillips	1999	Rats	Unilateral excitotoxic STN lesions	Parkinsonism: unilateral striatal 6-OHDA infusion	Reaction time performance	Improved 6-OHDA-induced deficits in reaction time, but increased anticipatory (premature) responding
Henderson	1999	Rats	Unilateral excitotoxic STN lesions	Parkinsonism: unilateral nigrostriatal 6-OHDA infusion	Head position, curling, rotation, sensorimotor functions, disengagement, and paw reaching	Partial improvement of motor deficits, but attentional deficits such as contralateral orientation biases
Phillips	2000	Rats	Unilateral excitotoxic STN lesions	Non-depleted rats	Reaction time performance	Increased anticipatory (premature) responding
2001-						
Baunez	2001	Rats	Bilateral excitotoxic STN lesions	Non-depleted rats	Reaction time performance	Multiple attentional deficits including reduced accuracy and premature responding
Florio	2001	Rats	Unilateral excitotoxic STN lesions and unilateral PPN lesions	Non-depleted rats	Reaction time performance	Increased anticipatory (premature) responding which was unaffected by PPN lesions
Chudasama	2003	Rats	Unilateral excitotoxic STN lesions	Disconnection: unilateral mPFC lesions	Reaction time performance	Profound attentional and executive impairments
Darbaky	2003	Rats	Unilateral STN stimulation	Parkinsonism: unilateral striatal 6-OHDA infusion	Reaction time performance	Improved motor performance, but no effect on cognitive parameters
Desbonnet	2004	Rats	Bilateral STN stimulation	Non-depleted rats	Reaction time performance	Amplitude- and frequency dependent decrease in premature responding
Temel	2004	Rats	Bilateral STN stimulation	Parkinsonism: bilateral striatal 6-OHDA infusion	Reaction time performance	Amplitude dependent acute and separate modulation of motor and cognitive parameters

mPFC, medial prefrontal cortex; STN subthalamic nucleus; PPN, pedunculopontine nucleus; 6-OHDA, 6-hydroxydopamine

Anatomically, cognitive and limbic information related to the basal ganglia is processed by the associative and limbic circuits, respectively. As described in sections 3.2 and 3.3, the STN has anatomically a central position within these circuits. In addition, these circuits are processed within the primate STN by specific groups of neurons located medially (limbic) and ventromedially (associative). In the rodent STN, anatomical data suggest that the medial part receives information from associative and limbic parts of the VP^{96,102}. Because of the difference in dendritic architecture in the rodent and primate STN as explained above, the segregation of the STN into functional parts is more evident in primates than in rodents. This means that a single STN neuron in the primate might serve more or less a single function (associative, limbic, or motor), whereas in the rat it might be involved in more functions. Altogether, the data presented in this review point towards a potent regulatory function of the STN in the processing of associative and limbic information towards cortical and subcortical regions. Changes in STN activity immediately result in changes in behaviour, without being corrected by regional compensatory mechanisms^{188–191}. This hypothesis has also been confirmed by several neuroimaging studies^{192–195}. Such a potent influence on associative, limbic and motor functions has not been observed with other targets like the thalamic nuclei targeted thus far^{196,197}.

TABLE 3. This table summarizes the different aspects of behaviour that have been reported to be changed after STN DBS or lesions in clinical and experimental studies. More specifically, the affected functions are also shown. Cognitive and affective changes have been reported by various studies. Personality changes, anxiety disorders, sexuality changes and other alterations have been described by case studies.

Aspect of behaviour	Affected functions
<i>Cognitive</i>	verbal memory and fluency executive functions attention working memory mental speed response inhibition
<i>Affective</i>	depression (hypo)mania
<i>Personality</i>	changes in personality
<i>Anxiety</i>	anxiety disorders
<i>Sexuality</i>	hypersexuality
<i>Other</i>	hallucinations

In almost all clinical studies, changes in motor disability were accompanied by unexpected changes in behaviour. This suggests that lesions and stimulations of the STN have generally been non-selective. Lesions were not only confined to the motor part of the STN but usually involved the associative and limbic parts of this nucleus^{45,48,49,178,179,183}. Also with DBS in patients, it is very unlikely with the current technique of chronic stimulation (macrostimulation) and current dimensions of electrodes that the motor part of the STN could be selectively influenced. On the other hand, one could argue that in the case of DBS current spread to surrounding regions might be responsible for the generation of behavioural reactions. Even though we cannot totally exclude the involvement of surrounding regions in the generation of these behavioural effects of STN modulation, currently only one case report supports this hypothesis. In a brief report, Bejjani and co-workers described a patient who developed transient acute depression when the most distal electrode contact was stimulated¹⁹⁸. This contact was 2 mm below the contact that alleviated the signs of PD. Therefore, the authors concluded, supported by postoperative MR imaging, that the most distal electrode was at the level of the SN and that DBS of this nucleus could induce mood changes. On the other hand, various reports^{3,17,21,24,42,131,134,135,137,142,143,146–151} found depression in patients who had clear beneficial effects of STN DBS on their PD signs. For example, a similar case was reported by Kumar and colleagues¹⁷. In this study the most effective contact in relieving PD signs caused depression. From an anatomical point of view, the SNr serves as one of the output nuclei for the limbic circuit. Hypothetically, modulation of SNr activity could therefore also result in changes in affective behaviour.

In experimental studies, changes in premature responses were only seen following lesions and stimulations of the STN in that anatomical region. Baunez and colleagues stated that changes in premature responding were the most sensitive sign of STN modulation in rats⁴⁵. Therefore, the current data is in favor of the hypothesis that the STN itself and not neighboring structures are responsible for the behavioural consequences of STN DBS and lesions. Therefore, it seems that the STN is the pacemaker not only of the basal ganglia motor circuit but also of associative and limbic circuits^{10,86,128}.

5.2 FUTURE PERSPECTIVES

At this time, STN DBS seems to be the only effective surgical treatment in advanced PD with permanent beneficial effects at long-term²⁴. Other surgical techniques such as pallidotomy or pallidal DBS have proven to be less effective^{4,199,200}. Furthermore, the results of sham-surgery controlled trials of embryonic mesencephalic grafting have been disappointing so far^{201,202} and stem cell mediated therapies are still not available^{203,204}. A major drawback of the STN as a target for surgical treatment is the occurrence of behavioural side-effects, which can have a negative effect on the QoL of the patient and family. This is most probably due to the non-selectivity of the surgical approach includ-

ing the targeting, as explained in this review. The challenge is to technically improve the surgical procedure to modulate only the motor part of the STN. The application of micro- instead of macroelectrodes for chronic stimulation, and the introduction of high resolution imaging techniques resulting in accurate visualization of the STN, could lead to more regionally selective modulation.

Secondly, more knowledge about the electrophysiological properties of the basal ganglia-thalamocortical circuits will contribute to a better identification of the functionally different parts within the STN. The hypothesis is that each intra-STN function, and thus each circuit projecting to these intra-STN parts, has a certain threshold of modulation. Evidence for such a possibility has been reported recently⁴⁷. In parkinsonian rats, bilateral STN stimulation was applied during a CRT task. The impaired motor performance and cognitive performance could be improved in the same task by STN DBS. However, STN DBS affected these modalities with different stimulation amplitudes suggesting that each loop has unique electrical properties that can be modulated independently. This suggests that the motor and the associative circuits have different physiological characteristics as it has been shown that the level of current amplitude determines which neuronal element is influenced²⁰⁵. For instance, the excitability of axons is much higher than that of cell bodies²⁰⁶ and large (fast) myelinated axons are likely to be affected by low amplitudes whereas smaller (slow) axons by higher amplitudes²⁰⁵. It has been documented both *in vitro*^{207,208} and *in vivo*²⁰⁹ studies that these different neuronal elements are activated at similar stimulation thresholds with (high) conventional stimuli. It is possible, therefore, that stimulations at high amplitudes within the STN are non-selective, activating both local neuronal cells and axons of passage thereby “masking” the effect of DBS at the behavioural level. Stimulation at lower amplitudes (3 μ A) resulted in a very obvious improvement of premature responding whereas motor performance was improved with medium amplitude (30 μ A)⁴⁷. This implies that the threshold of “modulation” of the associative circuit is lower than that of the motor circuit. As explained above, this indicates that the associative circuit has possibly more fast-conducting characteristics than the motor loop with respect to information processing (see for review Ranck²⁰⁹). Currently, there are no data available on the threshold of modulation of the limbic circuit. Future research should explore the different electrophysiological characteristics of these circuits. This will create the possibility to specifically interfere with the circuit involved by using specific modulation settings. However, besides this “threshold” effect of stimulation the “spatial” effect of stimulation is also of importance. The higher the current density the larger the area that is being modulated (larger spatial effect). To this day, fundamental knowledge regarding the application of electrical currents to deep brain structures is far from complete. A number of possible mechanisms have, however, been proposed including a depolarization block, activation of GABAergic afferents, and dual effects (stimulation and inhibition)^{11,29–35,205,210–214}. However, further studies are necessary to determine precisely how DBS works.

5.3 CONCLUSIONS

The aim of the present review was to provide a synopsis on the functional role of the STN in the control of the basal ganglia-thalamocortical associative and limbic circuits. In conclusion, it can be stated that from a scientific point of view the STN has anatomically a central position within the basal ganglia-thalamocortical associative and limbic circuits and is functionally a potent regulator of these pathways. From a clinical point of view, STN DBS does modulate not only motor but also cognitive and affective functions.

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CHAPTER 9

In vivo modulation of midbrain 5-HT neurones by high frequency stimulation of the subthalamic nucleus

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ABSTRACT

High frequency stimulation of the subthalamic nucleus (STN) improves motor disability in advanced Parkinson's disease but often induces debilitating effects on mood, of unknown neural origin. Using animal models we show that STN stimulation, at clinically relevant parameters, elicits a decrease in 5-HT neuronal activity and an associated increase in behavioural 'despair'. This STN-5-HT link suggests a novel 'motor-limbic interface' that may contribute to the psychiatric effects of STN stimulation.

INTRODUCTION

The use of stimulation electrodes implanted in the brain to control severely disabling neurological and psychiatric conditions is an exciting and fast emerging area of neuroscience. Bilateral, high frequency stimulation of the subthalamic nucleus (STN) has become the surgical therapy of choice for advanced Parkinson's disease¹. The relief of movement disability by STN stimulation is predictable as this nucleus is a critical part of the basal ganglia motor circuitry that is dysfunctional in Parkinson's disease², and similar effects have been observed in animal models³.

Despite having important beneficial motor effects, in almost half of patients bilateral high frequency STN stimulation is associated with the occurrence of unpleasant and debilitating psychiatric effects, including low mood, aggression, and impulsive acts and thoughts which are linked to suicide^{4–6}. These psychiatric effects can be a major burden to patients and their families, and often mitigate the positive effects on motor symptoms. In animals, modulation of the STN also produces a range of non-motor behavioural changes, including increased impulsivity and altered cognitive responses that appear to correlate with the adverse effects experienced by patients⁶. Collectively, these findings associate the STN with the generation of mood disorder symptoms, not only in STN-stimulated subjects but also in mood disorder patients generally.

Currently there is no rational basis for the clinical management of the non-motor effects of STN stimulation because their neural origin is unknown. Candidate neural substrates include altered functioning of the connections between the STN and limbic circuits in the forebrain^{6,7}, but direct evidence is lacking. It is notable, however, that some of the psychiatric effects of STN stimulation, such as low mood and increased impulsivity, have long been associated with reduced 5-hydroxytryptamine (5-HT; serotonin) function^{8–10}. Therefore, in this study we tested the hypothesis that bilateral stimulation of the STN might alter mood-related behavioural outputs by influencing the activity of 5-HT neurones.

RESULTS

Extracellular single-unit recordings of 5-HT neurones in the dorsal raphe nucleus (DRN) of anaesthetised rats (see Supplementary Methods) revealed that bilateral electrical stimulation of the STN at high frequencies (100 or 130 Hz) inhibited the firing rate of the vast majority of 5-HT neurones tested (53 out of 58, $F(1,52) = 31.81$, $P < 0.05$, $-45.1 \pm 3.6\%$ of baseline firing rate). This effect had a rapid onset, and firing rates quickly returned to baseline following cessation of stimulation (Fig. 1a). Analysis of frequency- and current-response relationships (Fig. 1b) revealed that the inhibition of 5-HT neuronal firing occurred at high frequencies (≥ 100 Hz) and low currents

($\geq 30 \mu\text{A}$), but not at low frequencies ($\leq 50 \text{ Hz}$). It is notable that high and not low frequency, bilateral, stimulation of the STN is necessary to induce psychiatric side effects, as well as beneficial motor effects, in Parkinsonian patients⁶.

In the above experiments post-mortem histological inspection of the stimulation sites revealed an electrode location within the STN in cases when the firing rates of 5-HT neurones were decreased (53 neurones, 16 rats) (Fig. 1c), and locations neighbouring the STN when firing rates were not altered (5 neurones, 3 rats) (see Figs. 1 and 2 in Supplementary Methods for details of electrode placements). To investigate further the specificity of the stimulation site, electrodes were stimulated at high frequency at various points during the descent into the STN whilst recording from the same 5-HT neurone in the DRN. This experiment (6 neurones, 4 rats) revealed that inhibition of 5-HT neuronal activity was elicited by electrical stimulation of the STN ($F(1,5) = 17.81$, $P < 0.05$) but not neighbouring structures (zona incerta; Fig. 1d, $P > 0.05$) or remote structures (parietal cortex, ventro-posteromedial nucleus of the thalamus, $P > 0.05$; Fig. 1 in Supplementary Methods). In a final test of specificity it was found that high frequency STN stimulation did not influence the firing rate of DRN neurones that had a relatively fast and irregular firing pattern (detailed in Supplementary Methods), and that are generally recognised to be non-5-HT containing (10 neurones, 10 rats) (Fig. 1d).

Additional experiments showed that the inhibitory effect of STN stimulation on 5-HT neuronal activity was still present in animals with chronic dopamine neurone lesions, thereby modelling the Parkinsonian brain. Thus, high frequency stimulation of the STN also inhibited 5-HT neuronal activity ($-52.4 \pm 4.3\%$ of baseline firing rate; 17 neurones, 5 rats; $F(1,16) = 39.78$, $P < 0.05$) in animals treated with 6-hydroxydopamine (i.c.v. administration)¹¹ as well as sham controls ($-42.7 \pm 7.8\%$ of baseline firing rate; 8 neurones, 3 rats; $F(1,7) = 10.76$, $P < 0.05$). STN stimulation also inhibited 5-HT cell firing in animals after acute depletion of monoamine neurotransmitters by reserpine (see Supplementary Methods).

Although the mechanisms underlying the beneficial motor effects of high frequency stimulation of the STN are not known, recent theories emphasise a role for a reduction of neural activity within the STN and the generation of high frequency activity patterns, which influence the main basal ganglia output structures¹². Bilateral infusion into the STN of muscimol, a GABAA receptor agonist that mimics the action of GABAergic inhibitory inputs, but not saline vehicle, also inhibited 5-HT neuronal activity (5 neurones, 5 rats; $F(1,4) = 8.36$, $P < 0.05$; Fig. 1e). This finding suggests that high frequency stimulation of the STN inhibits DRN 5-HT neurones through a mechanism involving, at least in part, inhibition of neurones in the STN, similar to that perceived to underlie the beneficial motor effects of STN stimulation (see Supplementary Methods for further discussion).

FIGURE 1. Effect of high frequency stimulation of the STN on 5-HT neuronal firing. **a**) Spike train and instantaneous firing rate of a 5-HT neurone in response to stimulation of the STN (left) or zona incerta (right). Horizontal bar indicates stimulation period (100 Hz, 100 μ A, 2 min). **b**) Effect of stimulation frequency and current on 5-HT neurone firing rate. Each point is a mean $\pm$ s.e.m. value ($n = 6$). **c**) Histological verification of the electrode location in the STN (bar = 200 μ m). **d**) Firing rate of 5-HT neurones (left) and non-5-HT neurones (middle) before, during and after STN stimulation (100 Hz, 100 μ A). Also, firing rate of 5-HT neurones (right) before, during and after stimulation of the zona incerta. **e**) Effect on 5-HT neurone firing rate of bilateral infusion of muscimol (0.1 μ g/side) and saline vehicle into STN. * $P < 0.05$ versus relevant pre-stimulus or pre-drug baseline control values. STN stimulation or drug treatment (muscimol) were used as the repeated measures factor in the statistical analysis (one way ANOVA followed by Duncan's post-hoc test).

The marked inhibitory effect of STN stimulation on 5-HT neuronal activity predicts that similar stimulation will affect 5-HT-dependent, mood-related behavioural outputs. Therefore, we tested the behavioural effects of STN stimulation in a widely-used, well-validated and 5-HT-dependent model of learned helplessness, the forced swimming test¹³. It was found that bilateral high frequency stimulation of the STN, using parameters that inhibit 5-HT neuronal activity (see above) and reduce motor deficits in an animal model of Parkinson's Disease³ (Fig. 3c, Supplementary Methods), caused a significant increase in immobility and a significant decrease in climbing time compared to non-stimulated controls (F-values > 11.37, $P < 0.05$; Fig. 2), thereby indicative of the induction of behavioural 'despair'¹³. This effect was confirmed in animals with dopamine neurone lesions, and was specific in that STN stimulation did not alter movement in the open field (Figs. 3a and 3b, Supplementary Methods). Importantly, the effects of STN stimulation on immobility and swim time were completely prevented by the selective 5-HT reuptake inhibitor citalopram (Fig. 2) at a dose (10 mg/kg s.c. for 14 days) that by itself had no significant effect in non-stimulated controls ($P > 0.05$) and likely acts by increasing extracellular 5-HT levels in 5-HT terminal regions of the limbic forebrain (see Supplementary Methods).

FIGURE 2. Effect of high-frequency stimulation of the STN on different behavioural measures in the forced swimming test. Four groups of rats (6 per group) were implanted with electrodes in the STN. Group treatments were as follows: i) no STN stimulation during test (No stim), ii) pre-treatment with citalopram followed by no STN stimulation during test (No stim + cital), iii) STN stimulation during test (Stim), and iv) pre-treatment with citalopram followed by STN stimulation during test (Stim + cital). Stimulation parameters were 130 Hz and 150 μ A for the duration of the test. Citalopram pre-treatment comprised 10 mg/kg i.p. for 14 days. Bars represent mean $\pm$ s.e.m. values ($n = 6$). Statistical analysis (2-way ANOVA with repeated measures, post hoc Duncan's test) revealed that STN stimulation was significantly different from other groups with respect to immobility and climbing (F-values > 11.4, $*P < 0.05$).

DISCUSSION

The neural pathways by which high frequency STN stimulation inhibits the firing of DRN 5-HT neurones likely involve polysynaptic connections because the STN does not directly project to the DRN¹⁴. Accordingly, detailed evaluation of peristimulus time histograms for STN-inhibited DRN 5-HT neurones revealed no evidence of antidromic activation, or short-latency (< 10 ms) orthodromic responses (data not shown). Interestingly, several important inputs to the DRN, including the substantia nigra *pars reticulata* and lateral habenula nucleus, are also targets (direct and indirect, respectively) of the STN¹⁵, and may be substrates underlying the observed effects.

Collectively, these data provide the first evidence that electrical stimulation of the STN, at parameters used to relieve the motor deficits of Parkinson's disease, causes a striking inhibition of 5-HT neuronal activity and changes a 5-HT-dependent, mood-related behavioural output. This effect on the 5-HT system may contribute to the psychiatric disturbances observed in STN-stimulated Parkinson's disease patients, and provides an experimental basis for their clinical management using 5-HT targeted antidepressant drugs. Moreover, this powerful link between the STN and 5-HT system supports the existence of a novel 'motor-limbic interface' for the integration and processing of sensorimotor and emotional information.

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SUPPLEMENTARY METHODS CHAPTER 9

In vivo electrophysiology

ANIMALS

Electrophysiological experiments were carried out in Oxford in accordance with the U.K. Home Office Animals (Scientific Procedures) Act (1986) and associated Home Office guidelines. Male Sprague-Dawley rats (270–330 g; Harlan Olac, Bicester, UK) were housed in groups under conditions of constant temperature ($21 \pm 1^\circ\text{C}$) and humidity under a 24 h light/dark cycle (lights on 08:00–20:00 h) with food and water freely available.

ELECTROPHYSIOLOGICAL RECORDING OF DRN NEURONES

Rats were anaesthetised with chloral hydrate (460 mg/kg i.p.) with additional doses as required, supplemented with a single dose of saffan (1.2 mg/kg i.v.) during surgery. Lig-nocaine was applied at the cranial incision site and ear canals prior to placement in the stereotaxic apparatus (Stoelting). A lateral tail vein was cannulated for drug administration. Extracellular single-unit recordings of putative 5-HT and non-5-HT neurones were made essentially as described previously¹. In brief, single barrel glass electrodes (filled with 2 M NaCl; 2% Pontamine sky blue dye; 6–20 M Ω *in vitro*) were lowered into the DRN (coordinates from Bregma²: AP -7.5 , ML 0.0 and DV -4.5 to -5.5 mm) using a hydraulic micromanipulator (Kopf Instruments). Single-unit potentials were amplified and filtered (Gain 1k , 0.5 – 5 kHz band pass; Neurolog system, Digitimer, Ltd.), captured using a 1401plus interface system and analysed offline using Spike2 software (Cambridge Electronic Design, Cambridge, U.K.).

Putative 5-HT neurones satisfied at least 3 of the following established electrophysiological and pharmacological criteria^{3,4}: slow firing rate (0.7 ± 0.1 Hz), regular firing pattern (coefficient of variation, 0.32 ± 0.01), triphasic extracellular waveform with a wide action potential duration (2.33 ± 0.05 msec) and an inhibitory response to administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin ($10 \mu\text{g/kg}$ i.v.). In comparison, putative non-5-HT neurones were identified as faster firing (4.2 ± 1.48 Hz), less regular (coefficient of variation, 0.85 ± 0.11) and had a narrower action potential duration (1.9 ± 0.12 msec). At the end of each experiment, the final position of the recording electrode was marked by iontophoretic dye injection ($20 \mu\text{A}$, negative current, 1 Hz pulses of 300 ms duration for 20 min).

STIMULATION ELECTRODES

Stimulating electrodes comprised two gold-plated needle-like electrodes with an inner wire of a platinum-iridium combination (Technomed, Beek, The Netherlands), with a tip diameter of 50 μm and a shaft diameter of 250 μm . The inner and outer electrodes were insulated except for the exposed tips, which were 75 μm in length, with an inter-pole distance of 50 μm .

Stimulating electrodes were connected to a World Precision Instrument Accupulser (A310, WPI, Berlin, Germany) via a stimulus isolator (A360, WPI, Berlin, Germany). The mode of stimulation was bipolar (negative inner electrode and positive outer electrode) with a 60 μs pulse width. Electrode stimulation parameters were verified during stimulation using a digital oscilloscope.

EXPERIMENTAL PROTOCOLS

Following 3–5 min of recording baseline single-unit activity, stimulations were typically applied for 2–3 min, and recordings continued for another 2–3 min prior to any further stimulations (maximum 6 per neurone). The effect of STN stimulation was tested in several neurones per rat (typically 5–6). It was established that repeated periods of STN stimulation evoked reproducible effects on the firing of an individual 5-HT neurone. In some experiments low frequency stimulation was applied prior to high frequency stimulations (see below) to enable construction of peristimulus time histograms (PSTHs).

We stimulated the STN bilaterally because it is established that this paradigm relieves the motor symptoms of PD and also induces psychiatric side effects in the clinical setting; moreover these effects are observed at high frequency and not low frequency stimulation⁵. Three stimulation protocols were used:

- i. For high frequency stimulation, stimulation was applied bilaterally to electrodes located in the STN whilst recording from a 5-HT or non-5-HT neurone. In some experiments the effects of different stimulation amplitudes (3, 30, 100, and 150 μA) and frequencies (10, 50, 100, 130 Hz) were evaluated.
- ii. Stimulations were applied bilaterally to electrodes at different locations during a descent into the STN whilst continuously recording from a single 5-HT neurone. In this case stimulating electrodes were slowly advanced and 2–3 min stimulations were applied to the following regions (stereotaxic co-ordinates relative to Bregma²); parietal association cortex (AP -3.8, ML 2.5, DV -1.5), ventro-posteromedial nucleus of the thalamus (AP -3.8, ML 2.5, DV -6.0), zona incerta (AP -3.8, ML 2.5, DV -7.0), and then STN (AP -3.8, ML 2.5, DV -8.0). Fig. 1 illustrates the position of stimulation sites during the descent to the STN and corresponding effects on 5-HT cell firing in the DRN.
- iii. To construct PSTHs, low frequency stimulation (1 Hz for 3–5 min) was applied bilaterally to electrodes located in the STN whilst recording from a 5-HT neurone.

HISTOLOGY

To determine the location of stimulation/recording electrodes, at the end of both electrophysiological and behavioural experiments rats were perfused transcardially with phosphate-buffered saline and then fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.6). Brains were removed and post-fixed for 2 h followed by overnight immersion in 20% sucrose at 4°C. Brain tissue was then quickly frozen and stored at -80°C subsequent to the cutting of the coronal tissue sections (30 µm) using a cryostat. Standard hematoxylin-eosin and standard cresyl violet stainings were used to locate the electrode tips.

Analysis of final position of the stimulating electrode revealed a consistent localization in the STN (Fig. 2). The few animals (3/16) failing to show a 5-HT neurone response to STN stimulation had electrodes located either unilaterally or bilaterally outside the STN (Fig. 2).

DOPAMINE LESIONS

The effect of STN stimulation on 5-HT neuronal activity was tested on rats with dopamine neurone lesions, thus modelling the primary pathology of idiopathic PD. For lesions, rats ($n = 8$) were pretreated with desipramine (25 mg/kg i.p.) and anaesthetised with halothane prior to infusion (over 2 min) of 6-hydroxydopamine (250 µg in 10 µl, $n = 5$) or vehicle (1% ascorbic acid/saline, $n = 3$) into the lateral ventricle ((AP -0.9, ML 1.4, DV -4.0)6. Over the 3–4 days immediately following recovery from surgery animals were hydrated at regular intervals (2 ml i.p. 5% glucose solution every 12 h) and provided with dietary supplements to encourage food intake.

Electrophysiological experiments were carried out 14–16 days post-surgery, after which postmortem regional brain extracts were analysed for dopamine and its metabolites, DOPAC and HVA as well as noradrenaline using HPLC with electrochemical detection. Animals treated with 6-hydroxydopamine showed significant ($p < 0.05$) loss of dopamine, but not noradrenaline, compared to vehicle controls in both striatum (dopamine, 14.28 ± 3.78 versus 78.09 ± 10.23 pmol/mg; DOPAC 1.09 ± 0.25 versus 10.00 ± 1.02 pmol/mg; HVA, 1.32 ± 0.46 versus 7.40 ± 2.20 pmol/mg; noradrenaline, 1.22 ± 0.28 versus 1.95 ± 0.37 pmol/mg) and frontal cortex (dopamine, 0.30 ± 0.04 versus 0.58 ± 0.22 pmol/mg; DOPAC 0.09 ± 0.02 versus 0.19 ± 0.03 pmol/mg; HVA, 0.22 ± 0.03 versus 0.35 ± 0.05 pmol/mg; noradrenaline, 2.47 ± 0.24 versus 2.78 ± 0.17 pmol/mg).

The lack of effect of dopamine denervation on STN stimulation-induced inhibition of 5-HT cell firing (see main text) was confirmed in animals subject to acute monoamine depletion using a procedure taken from Garcia *et al.*⁷ Rats were treated with reserpine (5 mg/kg, i.p.) at 20 h, and then α -methyl-*p*-tyrosine (250 mg/kg i.p.) at 4 h before recordings. Rats were akinetic prior to anaesthetic administration. Thus, high-

frequency stimulation of the STN also inhibited 5-HT neuronal activity ($-49.4 \pm 8.3\%$ of baseline firing rate; 6 neurones, 6 rats; $F(1,5) = 20.13$, $p < 0.05$) following co-administration of reserpine (5 mg/kg, i.p.) and α -methyl-*p*-tyrosine (250 mg/kg, i.p.).

INTRA-STN INFUSION OF MUSCIMOL

To test the effect of chemical inhibition of the STN on 5-HT neuronal activity, the GABAA receptor agonist muscimol, or saline vehicle, was infused into the STN during recordings. Prior to commencement of electrophysiological recordings, bilateral cannulae were stereotactically implanted into the STN and left in place for the remainder of the experiment. Cannulae were connected via tubing to a 50 μ l Hamilton syringe driven by a microinfusion pump (Harvard Apparatus, Holliston, USA). Following a baseline recording of 2–3 min, either muscimol (0.1 μ g in 0.5 μ l saline) or saline (0.5 μ l), were infused (0.25 μ l/min), and recordings were continued for up to 20 min.

Previous studies have shown that the dose of muscimol used inhibits the firing of STN neurones *in vivo* and elicits anti-PD effects in animal models^{8–10}. Moreover, several lines of evidence suggest that inhibition of STN neurones may contribute to the anti-PD effects of high frequency STN stimulation; i) STN lesions produce effects in PD comparable to high frequency STN stimulation^{11,12}, ii) high but not low frequency stimulation of the STN improves PD symptoms^{13,14} and most importantly, iii) across a range of species including humans, high frequency stimulation of the STN has been consistently demonstrated to inhibit the firing of STN neurones^{15–18}.

COMPUTATION AND STATISTICAL ANALYSIS OF ELECTROPHYSIOLOGICAL DATA

Stimulation-artefacts (< 0.5 ms in duration) were removed from the spike trains by off-line analysis using Spike 2. Each trace was then visually inspected to ensure the quality of spike identification. The firing rate of each neurone was then quantified in the final 60 s of each baseline, stimulation and post-stimulation period. The regularity of firing was calculated for the same periods by coefficient of variation analysis (standard deviation of inter-spike intervals/mean inter-spike interval). Waveform width was calculated from the average of waveforms recorded during the final 60 s of baseline recording for each neurone, with width determined as the time between a 5% positive deviation from baseline, to the return to baseline following a negative phase⁴. PSTHs were constructed using Spike 2 software and assessed according to published criteria¹⁹.

Electrophysiological data were analysed by the one way ANOVA with repeated measures. STN stimulation or drug treatment (muscimol) were used as the repeated measures factor followed by a Duncan's *post-hoc* test. A P value less than 0.05 was considered significant.

BEHAVIOURAL TESTING

ANIMALS

Behavioural experiments were carried out in Maastricht in accordance with the Animal Experiments and Ethics Committee of Maastricht University. Male Lewis rats (270–330 g; Maastricht University, The Netherlands) were housed individually under conditions of constant temperature ($21 \pm 1^\circ \text{C}$) and humidity under a 24 h reversed light/dark cycle (lights off 05:00–17:00 h) with food and water freely available.

IMPLANTATION OF STN ELECTRODES

Rats were anaesthetised (90 mg/kg i.p. ketamine plus 10 mg/kg i.p. xylazine) and bilateral stimulation electrodes were stereotactically implanted into the STN as described above. Animals were tested following a recovery period of one week.

PROTOCOL FOR FORCED SWIMMING TEST (FST)

Rats were tested in the modified FST according to Detke and Lucki²⁰. Testing was carried out using a partition-wall complex made from 4 transparent Perspex cylinders (50 x 20 cm) separated by black sidewalls, placed on a black base 60 cm from the floor. The cylinders were filled with tap water ($25 \pm 1^\circ \text{C}$) to a depth of 30 cm.

All rats initially underwent a pre-test session in which each rat was placed in the water for 15 min. On the following day and 2 weeks later, the rats were tested by placing in the water for 5 min. STN stimulations commenced 2 min prior to testing and continued for the duration of the 5 min test period. All sessions were recorded using digital camera placed above the cylinders, and the duration of specific behaviours was timed using the recordings by an observer blind to treatments. The behaviours measured were: 'immobility' (no movements or movements solely for the purpose of maintaining the nose above the water), 'swimming' (active swimming with the forepaws), and 'climbing' (actively scratching the walls of the cylinder using both forepaws and hindpaws).

Four groups of 6 rats were tested in the FST and treated as follows: a) No STN stimulation with saline treatment, b) No STN stimulation with citalopram treatment, c) STN stimulation (130 Hz, 150 μA) with saline treatment, and d) STN stimulation (130 Hz, 150 μA) with citalopram treatment. Citalopram treatment comprised 10 mg/kg s.c. once daily for 14 days. Saline treatment comprised once daily injections for 14 days. A challenge dose of 10 mg/kg s.c. citalopram increases extracellular 5-HT in prefrontal cortex of freely moving rats by 2–3 fold²¹.

An additional experiment tested the effect of STN stimulation on immobility time in rats with a dopamine denervation to confirm that the increase in 'behavioural

despair' induced by STN stimulation (see main text) occurred in an animal model of Parkinson's disease. This experiment comprised 2 groups of 6 STN electrode implanted, dopamine denervated rats, one group being stimulated (130 Hz, 150 μ A) during the FST and the other receiving no stimulation. The experiments demonstrated a clear-cut increase in immobility time in the STN stimulated versus non-stimulated animals (Fig. 3a).

OPEN FIELD TEST

Experiments were carried out using an open field test to investigate whether STN stimulation might alter motor activity on thereby confound the results of the FST. Rats with bilateral STN electrodes were placed in a square open-field arena (inner dimensions 50 $\times$ 50 $\times$ 50 cm) and the distance moved over 10 min was recorded by digital camera (connected to Ethovision software; Ethovision Color-Pro version 3.0.15, Noldus Information Technology bv, The Netherlands) placed above the arena. Activity was measured during two sessions, once when electrodes were not stimulated and on a second occasion two weeks later when the electrodes were stimulated (130 Hz, 150 μ A) during the time in the open field. These experiments showed that STN stimulation had no effect on motor activity in the open field (Fig. 3b). For comparison, Fig. 3c shows the ability of STN stimulation at the same parameters to reverse motor-time deficits in dopamine denervated rats from an earlier published study²².

STATISTICAL ANALYSIS OF BEHAVIOURAL DATA

Behavioural data were analysed by two-way ANOVA with Stimulation and Treatment being used as repeated measures, followed by a Duncan's post hoc test. A P value less than 0.05 was considered significant.

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SUPPLEMENTARY FIGURES

FIGURE 1. Effect of high frequency stimulation at various sites during descent to the STN whilst recording a 5-HT neurone in the DRN. a) Illustration of stimulation sites during electrode descent to the STN. Abbreviations: parietal association cortex (PtA), ventroposteromedial thalamus (VPM), zona incerta (ZI) and subthalamic nucleus (STN). b) Typical effects of electrical STN stimulation at these sites on the firing rate of a single 5-HT neurone in the DRN. Horizontal bar indicates stimulation period (100 Hz, 100 μ A, 2 min). Data for zona incerta and STN taken for comparison from Fig. 1 of main paper.

FIGURE 2. Anatomical localisation of high frequency stimulation sites in or close to the STN. Sites were localised by microscopic examination of histologically prepared tissue. Closed circles - stimulation sites that produced consistent inhibition of 5-HT cell firing in the DRN. Open circles - stimulation sites that produced no significant change in 5-HT cell firing in the DRN.

FIGURE 3. Effect of high frequency stimulation of the STN on behaviours in the rat. a) Immobility time of 6-hydroxydopamine-lesioned rats in the forced swim test. b) Locomotor activity in the open field of non-lesioned rats. Note that stimulation had no effect. (c) Motor time in non-lesioned (left) and 6-hydroxydopamine-lesioned (right) rats in a serial choice reaction time task (reproduced from earlier published data (ref. 22). Note that effects of stimulation were only present in lesioned animals. STN stimulation parameters were 130 Hz and 30–150 μ A for the duration of the tasks. Bars represent mean $\pm$ s.e.m. values ($n = 6$). * *Post hoc* analyses revealed that STN stimulation induced a significant effect.

CHAPTER 10

Conclusion

Conclusie

CONCLUSION

In the present study, we tested the hypothesis that the subthalamic nucleus (STN) is a strong regulator of associative and limbic functions and that modulation of the STN by electrical stimulation causes marked changes in these functions. The results of the set of experiments as outlined in this study show that the STN is indeed a strong regulator of associative and limbic functions and that modulation of STN's neuronal activity by deep brain stimulation (DBS) results in affective and cognitive changes. Anatomically, it was already known that STN received input from associative and limbic regions within the basal ganglia such as the anterior part of the globus pallidus externus (GPe) and the ventral pallidum (VP) and also from outside the basal ganglia such as the frontal parts of the cortex¹. However, the functionality of these projections remained elusive. Our clinical and preclinical experiments revealed that the connections of the STN with the associative and limbic regions are clinically very relevant. Furthermore, we discovered a novel functional connection between the STN and the most important serotonergic nucleus in the brain, the dorsal raphe nucleus (DRN).

In the clinical studies, we demonstrated that STN DBS has long-lasting beneficial effects on motor disability in patients suffering from advanced PD (**CHAPTER 2**). Concerning non-motor behaviour, our results showed that STN DBS had substantial but differential effects on cognitive performance of PD patients (**CHAPTER 3**). Stimulation of the STN improved response preparation but impaired the speed of mental processing. In the same study, we evaluated the effects of dopaminergic medication. Dopaminergic medication improved the motor disability, but had no effect on the cognitive performance. Besides changing cognitive performance, STN DBS also changed emotional performance. In our meta-analysis, we showed that PD patients can experience substantial changes in their emotional functions (**CHAPTER 4**).

The mechanisms underlying these non-motor effects of STN DBS remained unknown. Therefore we carried out a set of preclinical experiments to investigate the neuronal mechanisms entailed in this procedure. In our first preclinical experiment, we faced the problem of correct stimulation settings to perform STN DBS in rat models. We observed substantial amount of histological damage when applying previously published stimulation parameters (**CHAPTER 5**). We found out that this was due to the difference in polarity which was responsible for the occurrence of histological damage. We adjusted the stimulation parameters in order to have a more comparable stimulation setting to the clinical situation and prevented histological damage in our rat models. In the next study, we introduced and validated the bilateral STN DBS paradigm in freely moving rats (**CHAPTER 6**). In this study, we found a profound effect of STN DBS on a measure of impulsivity, premature responding. This effect was frequency-, and amplitude-dependent, occurring at high frequency and low amplitude. As reported before in a rat model with STN lesions (**CHAPTER 8**), a change in premature responding is the most sensitive sign of STN modulation. Subsequently, we applied bilateral STN DBS

in Parkinsonian rats (CHAPTER 7). Again we found that when using specific stimulation parameters, it was possible to modulate different behavioural outputs in different ways. We could change motor and cognitive performance acutely and separately by using specific stimulation amplitudes. These findings suggest that basal ganglia-thalamocortical motor and associative circuits responsible for specific motor and cognitive performance have unique physiological properties that can be modulated by specific electrical stimuli.

With respect to the affective changes, we studied the effects of bilateral STN DBS on the activity of 5-HT neurons in the rat dorsal raphe nucleus (DRN) since some of the psychiatric effects of STN stimulation such as low mood and increased impulsivity have long been associated with reduced 5-HT function. We found that STN stimulation at parameters used clinically exerts a powerful, specific, reproducible, reversible and inhibitory effect on 5-HT neurons (CHAPTER 9). These findings strongly suggest that reduced 5-HT output contributes to the psychiatric disturbances induced by STN stimulation, and provide an basis for their clinical management. The data also demonstrate an unsuspected link between the STN and the 5-HT system, which we see as the first critical step towards establishing the existence of a novel 'motor-limbic interface' in the brain.

Altogether, the data presented in this thesis suggest that the STN serves as an interface between the brain's motor and non-motor (cognitive and limbic) circuits. Modulation of the activity of the STN by electrical currents has immediate and substantial effect on motor and non-motor parameters in preclinical and clinical experiments. However, one could argue that postoperative reduction of dopaminergic medication might underlie the psychiatric effects of following DBS. It is usually possible to achieve an average reduction of approximately 60% of dopaminergic medication after surgery. Some transient changes in mood and psychotic symptoms can occur when the reduction is performed fast. However, in the majority of the cases, the medication reduction has been performed slowly. Furthermore, the reduction of dopaminergic medication is usually fully achieved in the first 2–3 months after surgery, whereas the behavioural symptoms were generally also observed at longer follow-up time points. Secondly, in some studies like in chapter 3, STN stimulation was switched off to investigate the effects of only stimulation and medication on motor and cognitive parameters^{2–5}. Results show that it is actually the modulation of the STN and not medication or surgery that has a substantial effect on behavioural parameters.

Furthermore, it can also be argued that current spread to surrounding regions might be responsible for the generation of behavioural changes in the case of STN DBS. Even though that we cannot totally exclude the involvement of surrounding regions in the generation of these behavioral effects of STN modulation, currently only one case report supports this hypothesis. In a brief report, Bejjani and co-workers described a patient who developed transient acute depression when the most distal electrode contact, probably at the level of the substantia nigra, was stimulated⁶. This patient had no

beneficial effects on her motor disability when this contact was stimulated. On the other hand, various clinical reports^{7–23}, found depression in patients who had clear beneficial effects of STN DBS on their PD signs. From an anatomical point of view, the substantia nigra pars reticulata (SNr) serves as one of the output nuclei for the limbic circuit. Hypothetically, modulation of SNr activity could therefore also result in changes in affective behavior. In experimental studies, changes in premature responses were only seen following lesions and stimulations of the STN in that anatomical region. Baunez and colleagues stated that changes in premature responding were the most sensitive sign of STN modulation in rats²⁴. In chapter 9, we performed a trajectory stimulation to evaluate the specificity of the effects of STN DBS. Only stimulation at the level of the STN inhibited 5-HT neuronal firing rate. Therefore, the current data are in favor of the hypothesis that STN itself and not neighboring structures are responsible for the behavioural consequences of STN DBS.

From a mechanistic point of view, our findings identify the STN as an important interface between brain's motor and non-motor (cognitive and limbic) circuits. Modulation of the activity of the STN by electrical currents has immediate and substantial effect on motor and non-motor parameters in preclinical and clinical studies.

From a clinical point of view, the DBS of STN is a very effective therapy in alleviating PD symptoms, but can be associated by behavioural changes of which cognitive dysfunctions and emotional changes are the most frequent. Our recent finding suggests that this may be due to a striking inhibition of 5-HT neuronal activity. This effect on the 5-HT system may contribute to the psychiatric disturbances observed in STN-stimulated PD patients, and provide an experimental basis for their clinical management.

CONCLUSIE

In deze studie hebben we de hypothese getest dat de nucleus subthalamicus (STN), een hersenkern gelocaliseerd in de middenhersenen, de associatieve en limbische functies reguleert en dat modulatie van de STN door middel van elektrische stimulatie een sterke verandering veroorzaakt van deze functies. Onze resultaten, zoals beschreven in deze studie, bevestigen deze hypothese. Anatomische studies hebben reeds aangetoond dat associatieve en limbische gebieden binnen de basale kernen, zoals het anterieure deel van de globus pallidus externus (GPe) en het ventrale pallidum (VP), en buiten de basale kernen, zoals de prefrontale cortex, naar de STN projecteren¹. Echter de relevantie van deze projecties waren grotendeels onbekend. Uit onze preklinische en klinische experimenten komt naar voren dat deze projecties zeer relevant zijn. Verder hebben we een nieuwe functionele verbinding ontdekt tussen de STN en de meest relevante serotonerge kern in de hersenen, de dorsale raphe nucleus (DRN).

In de klinische studies hebben we aangetoond dat bilaterale subthalamische stimulatie (STN DBS) langdurige gunstige effecten heeft op de motorische symptomen van patiënten met de ziekte van Parkinson (**HOOFDSTUK 2**). We hebben ook aangetoond dat STN DBS effecten heeft op non-motorische functies, zoals de cognitie (**HOOFDSTUK 3**). Stimulatie van de STN verbeterde responspreparatie maar vertraagde de mentale snelheid. Behoudens effecten op cognitieve functies, vonden we ook dat STN DBS effecten heeft op affectieve parameters. In het systematische overzichtsartikel hebben we dit laten zien (**HOOFDSTUK 4**).

De onderliggende mechanismen van de non-motorische effecten van STN DBS waren grotendeel onbekend. We hebben een aantal preklinische experimenten uitgevoerd om deze mechanismen te onderzoeken. In onze eerste experiment, zijn we het probleem tegengekomen van de te gebruiken stimulatieparameters. Namelijk, we vonden substantiële histologische schade bij het gebruik van eerder gepubliceerde stimulatieparameters (**HOOFDSTUK 5**). We zijn erachter gekomen dat dit te maken heeft met het verschil in polariteit. We hebben de stimulatieparameters aangepast in onze preklinische experimenten om klinisch relevant te stimuleren. Hiermee hebben we histologische schade voorkomen. In de volgende studie, hebben we het eerste bilaterale STN DBS model in vrij bewegende ratten geïntroduceerd en gevalideerd (**HOOFDSTUK 6**). In deze studie, vonden we een sterke effect van STN DBS op een maat voor impulsiviteit, premature responsen. Dit effect was frequentie-, en amplitude-afhankelijk. Vervolgens hebben we bilaterale STN DBS toegepast in een ratmodel voor de ziekte van Parkinson (**HOOFDSTUK 7**). We vonden dat het mogelijk was om een specifiek gedrag te moduleren door gebruik te maken van specifieke stimulatieparameters. We hebben motorische en cognitieve parameters acuut en onafhankelijk kunnen veranderen door gebruik te maken van specifieke stimulatieamplitudes. Deze bevindingen suggereren dat basale kernen-thalamocorticale motorische en associatieve circuits verantwoordelijk zijn voor specifieke motorische en cognitieve functies en

dat ze unieke elektrofysiologische karakteristieken hebben die gemoduleerd kunnen worden door specifieke elektrische stimuli.

Met betrekking tot de affectieve veranderingen, hebben we de effecten van bilaterale STN DBS op de activiteit van 5-HT neuronen in de DRN onderzocht omdat symptomen zoals veranderde gemoedstoestand of verhoogde impulsiviteit geruime tijd geassocieerd zijn met verlaagde 5-hydroxytryptamine (5-HT = serotonine) functie. We hebben gevonden dat STN stimulatie met parameters die klinisch effectief zijn, een sterke, specifieke, reproduceerbare, reversibele en inhibitoire effect heeft op 5-HT neuronen (**HOOFDSTUK 9**). Deze resultaten suggereren dat een verlaagde 5-HT functie bijdraagt aan de psychiatrische symptomen geïnduceerd door STN DBS. Deze bevindingen demonstreren tevens voor het eerst het bestaan van een potente link tussen de STN en het 5-HT systeem.

De bevindingen zoals gepresenteerd in dit proefschrift suggereren dat de STN als een interface fungeert tussen de motorische en de non-motorische (cognitieve en limbische) circuits van de hersenen. Modulatie van de STN door middel van elektrische stimulatie moduleert direct motorische en non-motorische functies in klinische en pre-klinische condities.

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CURRICULUM VITAE

Yasin Temel was born on 2nd of July 1977, in Yakupoglan (Sivas), Turkey.

He studied medicine at Maastricht University. During his medical study, he worked as a student-assistant at the Department of Cellular Neurosciences, Maastricht University, The Netherlands under the supervision of Prof. Dr. Harry Steinbusch and went to the department of Anatomy of Cambridge University, United Kingdom. He stayed there for six months and wrote his first scientific paper together with Prof. Dr. Joe Herbert. In 2001, he started his training in Neurosurgery at the Department of Neurosurgery, University Hospital Maastricht, The Netherlands. Between 2002 and 2005, he carried out his PhD research at the Department of Cellular Neurosciences, Maastricht University, The Netherlands. Within this period he spent six months at the department of Pharmacology, Oxford University, United Kingdom. He restarted his training in Neurosurgery in 2005. He received two international awards for his research. The first was the best poster award from the European Society for Stereotactic and Functional Neurosurgery (ESSFN) in 2004 at the meeting in Vienna, Austria. The second was the prestigious young investigator award from the World Society for Stereotactic and Functional Neurosurgery (WSSFN) in 2005 at the meeting in Rome, Italy. Besides his clinical activities, he continues to perform scientific work. Together with Dr. Veerle Visser-Vandewalle and Prof. Harry Steinbusch, he received three grants for further scientific work. Currently, three PhD students have been appointed, and he will co-supervise these students.

In 1999 he met Halime and they married in 2001. In 2006 Sara was born.