The Effects of Deep Brain Stimulation of the Subthalamic Nucleus on Vascular Endothelial Growth Factor, Brain-Derived Neurotrophic Factor, and Glial Cell Line-Derived Neurotrophic Factor in a Rat Model of Parkinson's Disease

Katharina Faust^a Peter Vajkoczy^a Bai Xi^a Daniel Harnack^b

^aDepartment of Neurosurgery, Charité University Hospital, Berlin, Germany; ^bBeelitz Neurology, Rehabilitation Clinic, Berlin, Germany

Keywords

Neurotrophic factors · Vascular endothelial growth factor · Brain derived growth factor · Glial derived growth factor · Deep brain stimulation · Parkinson's disease

Abstract

Objective: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has evolved as a powerful therapeutic alternative for the treatment of Parkinson's disease (PD). Despite its clinical efficacy, the mechanisms of action have remained poorly understood. In addition to the immediate symptomatic effects, long-term neuroprotective effects have been suggested. Those may be mediated through neurotrophic factors (NFs) like vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF). Here, the impact of DBS on the expression of NFs was analysed in a rat model of PD. **Methods:** Unilateral 6-hydroxydopamine (6-OHDA) lesioned rats received DBS in the STN using an implantable microstimulation system, sham DBS in the STN, or no electrode placement. Continuous unilateral STN-DBS (current intensity 50 μ A, frequency 130 Hz, and pulse width 52 μ s) was conducted for 14 days. Rats were then sacrificed and brains

shock frozen. Striata and motor cortices were dissected with a cryostat. Levels of VEGF, BDNF, and GDNF were analysed, both by quantitative PCR and colorimetric ELISA. **Results:** PCR revealed a significant upregulation of only BDNF mRNA in the ipsilateral striata of the DBS group, when compared to the sham-stimulated group. There was no significant increase in VEGF mRNA or GDNF mRNA. ELISA analysis showed augmentations of BDNF, VEGF, as well as GDNF protein in the ipsilateral striata after DBS compared to sham stimulation. In the motor cortex, significant increases after DBS were observed for BDNF only, not for the other 2 NFs. **Conclusions:** The upregulation of trophic factors induced by STN-DBS may participate in its long-term therapeutic efficacy and potentially neuroprotective effects.

© 2020 The Author(s)

Published by S. Karger AG, Basel

Introduction

All pharmacological treatments of Parkinson's disease (PD) primarily aim at controlling motor symptoms, without reversing or retarding disease progression in a relevant manner. Long-term treatment with dopaminergic drugs may produce wearing side effects and fluctuations

in effectiveness. Over recent decades, deep brain stimulation (DBS) of the subthalamic nucleus (STN) has evolved as a powerful treatment alternative [1]. Initially, DBS was reserved as second-line treatment for late-stage, medical therapy-refractory cases of PD. However, recently, it is being offered earlier in the course of the disease [2]. There is evidence that in addition to the symptomatic alleviation of motor symptoms like rigor, tremor, and bradykinesia, there may be a disease-modifying or even neuroprotective effect of DBS. Several animal models of PD in rodents and monkeys (both pharmacological, toxin-based, and genetic) have demonstrated the attenuation of dopaminergic neurons in the substantia nigra pars compacta (SNc) and of dopamine levels [3–9]. Any treatment that delays or modifies disease progression may be considered to either directly or indirectly influence disease pathogenesis.

How the assumed neuroprotective effect of DBS is mediated on a molecular basis remains unclear. Neurotrophic factors (NFs) constitute one of the potential candidates to be such mediators. In PD specifically, there is evidence that trophic and perfusion deficits in the midbrain may play a role in its pathophysiology [10].

NFs are important promoters for neurogenesis, neuronal maintenance, neural regeneration, and repair, not only in the developing but also in the adult brain [11]. Recently, the administration of NFs has emerged as a potential target for neuroprotective therapies [12].

Vascular endothelial growth factor (VEGF) modulates brain function at the neurovascular interface. It has strong angiogenic activity [13]. Yet, in addition, it is a neurotrophin with direct influence on neural cells [14]. It is expressed during neural development and promotes neuronal differentiation and synaptic plasticity. VEGF has been shown to also play a role in adult neurogenesis, as it stimulates neural stem cells [14]. There is evidence that in PD patients, VEGF expression is increased in the substantia nigra and certain basal ganglia regions [15]. VEGF has been shown to have neuroprotective property in both in vitro [16] and in vivo [17, 18] models of PD. For example, intrastratial delivery of VEGF has rescued Parkinson phenotypes in animal models [19, 20].

Glial cell line-derived neurotrophic factor (GDNF) has been shown to have specific effect on midbrain dopaminergic neurons [21], which preferentially degenerate in PD. High concentrations of GDNF have been found in the striatum [22]. Both intraventricular and intraparenchymal infusion of GDNF into the striatum have produced strong increases in striatal and nigral dopamine levels in rodents [23–26]. Early clinical trials with intra-

putaminal infusion of GDNF produced a clinical improvement as well as a marked increase in tyrosine hydroxylase (TH)-positive fibres in postmortem analyses [27]. A phase 1/2 multicentre study, however, demonstrated a limited diffusion and bioavailability of GDNF after both intraventricular and intraparenchymal infusion [28].

Brain-derived neurotrophic factor (BDNF) is widely expressed throughout the brain and peripheral nervous system [29]. Its strongest neurotrophic relevance is for motor-related neurons including the primary motor cortex, the basal ganglia, and the nigral substance [30]. BDNF enhances the survival of dopaminergic neurons and loss of BDNF leads to PD [31–33]. In the midbrain of patients with PD, reduced levels of BDNF have been detected [34]. It was shown that the level of decrease in BDNF correlated with disease severity, suggesting a potential role of BDNF in disease pathogenesis [35].

DBS can modify the release of neurotransmitters [36, 37]. In addition, it is well known that neurotransmitters regulate the expression of NF [38]. Therefore, the possibility of a modification of NF levels through DBS in certain stimulation-downstream areas in the brain may be self-suggestive. In fact, the potential of DBS being able to increase several trophic factors including VEGF, BDNF, and GDNF in DBS target areas distant from the stimulated site has been demonstrated before in a different context: Gondard et al. [39] were able to detect robust increases in the hippocampal concentrations of all the 3 trophic factors mentioned above, only a few hours after stimulation of fornix.

Also, it has been shown previously by Spieles-Engemann et al. [40] that STN-DBS can alter the trophic environment of STN target regions. However, they focused only on BDNF in the disease model of PD.

Previously, we had developed a fully implantable microstimulation system for the rat [41], for which we could demonstrate the attenuation of TH-positive neurons in the SNc by STN-DBS in the 6-hydroxydopamine (6-OHDA) model of PD [3, 36, 37]. Therefore, we decided to use this well-established model to investigate the effects of STN lesioning and of STN-DBS on the expression levels of NF. Since the striatum and the primary and secondary motor cortex are relevant target structures of the STN and also relatively easy to isolate via microdissection, we decided to analyse their tissue for NF expression. Besides BDNF, we were interested in 2 further NFs: VEGF and GDNF, since there is evidence that these 2 NFs might also play a role in the neuroprotective potential of DBS. Hence, the aim of this study was to analyse the expression

levels of VEGF, BDNF, and GDNF after STN lesioning and after STN-DBS both on mRNA and protein levels, in a rat model of PD in 2 STN target regions.

Methods

Animal Procedures

All experiments were carried out following permission from local authorities and in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC) for the care of laboratory animals. Male Wistar rats (Harlan-Winkelmann, Borcheln, Germany) weighing 300–320 g during the experiments were used. Animals were housed in a temperature- and humidity-controlled vivarium under a 12-h light-dark cycle with food and water *ad libitum*.

Surgical Procedures

Rats were anaesthetized with a mixture of ketamine 90 mg/kg and xylazine 6 mg/kg *i.p.* with supplemental inhalation of isoflurane if needed. Under general anaesthesia, rats were mounted in a stereotaxic head holder (David Kopf Instruments, Tujunga, CA, USA).

6-OHDA Lesions

A hemi-parkinsonian model was created by unilateral injection of 6-OHDA into the left striatum via convection enhanced delivery, as described elsewhere [42]. Twenty microliters of 6-OHDA (1 mg/mL dissolved in 0.2% ascorbic acid in 0.9% saline) was injected stereotactically (*A/P*: +0.7; *M/L*: 2.8; *D/V*: –5.0, according to Paxinos et al. [43]) with a flow rate of 0.2 μ L/min using a microinjection pump.

Electrode Placement and STN-DBS

In the same surgical session, the implantation of a custom-made implantable microstimulation system comprising a monopolar electrode and an impulse generator was performed as previously described [3, 36, 37]. Briefly, the sterile electrode comprising activated iridium was inserted ipsilaterally to the 6-OHDA lesion into the left STN and fixed to the skull with 2 stainless screws and acrylic dental cement (Technovit®; Heraeus Kulzer, Hanau, Germany). Coordinates were taken by averaging bregma and interaural coordinates (*A*: –3.8; *L*: 2.5; *V*: –7.6, according to bregma, and *A*: +5.2; *L*: 2.5; *V*: +2.0 according to the interaural line; Paxinos et al. [43]) as described previously [3, 36, 37]. The electrode was connected by subcutaneous tunnelling of a silicone-isolated lead system to the impulse generator, which was attached subcutaneously on the dorsum of the rat. STN-DBS was initiated 5 days post 6-OHDA lesioning and maintained in constant current mode (frequency 130 Hz, pulse width 52 μ s, and current intensity 50 μ A) continuously for 14 days.

Confirmation of Phenotypes

Functional DBS was verified by recording the current flow in the animal using a custom-made 130-Hz pass filter connected to an oscilloscope (Tektronix Inc., Beaverton, OR, USA) and clinically by reversal of the circular rat movements due to unilateral forelimb akinesia after 6-OHDA lesioning. After sacrifice, 20- μ m cryostat frozen coronal brain sections were obtained from the left

STN and SNc. The STN was stained with neuronal nuclear antigen antibodies to histologically visualize the electrode position [3, 36, 37]. Only animals with correct electrode placement within the STN were included. Animals with signs of tissue damage or bleeding around the electrode canal were also excluded from the study. The SNc sections were subsequently stained with TH antibody to confirm the Parkinson phenotype and the rescue phenotype after DBS. The confirmation of phenotype data used in the model has been published before by our group [3, 36, 37].

Animal Numbers and Grouping

In 42 male Wistar rats, striatal 6-OHDA lesioning was performed. Animals were then divided into 3 groups – (i) ACTIVE: rats received continuous high-frequency DBS after electrode implantation into the left STN over 14 days. (ii) INACTIVE: rats received electrode implantation into the left STN, but the electrode remained unpulsed – modelling a sham stimulation/STN-lesioning condition. (iii) NAIVE: no manipulation to the STN took place.

Ultimately, 22 animals had to be excluded from the study due to (i) incorrect position of the electrode/lesion failure upon post-mortem analysis, (ii) brain tissue damage, (iii) death during surgery or during the 2-week stimulation interval, (iv) severe signs of surgical site infection, or (v) interruption of the stimulation period due to stimulator malfunction/cable disconnection. Group allocation of the remaining 21 study animals was as follows: 9 animals in the ACTIVE group, 5 animals in the INACTIVE group, and 7 animals in the NAIVE group.

Protein Isolation and ELISA

Frozen naive tissue samples were homogenized in cold RIPA buffer (+HALT protease inhibitor cocktail; Thermo Scientific Pierce® Protein Research Products) by repeated passing through sequentially decreasing cannula diameters. After centrifugation, the supernatant was diluted 1:40. Total protein was quantified colorimetrically using the Pierce® BCA Protein Assay Kit (Thermo Scientific) according to the manufacturer's instructions applying the microplate procedure. Concentrations of VEGF, BDNF, and GDNF were measured by optical density at 540 nm using ELISA kits according to the manufacturer's instructions (Duo Set ELISA Development System by R&D Systems). All ELISAs were performed twice and in duplicate samples each.

RNA Extraction and PCR

RNA was isolated from the frozen naive tissue samples using TRIZOL® reagent according to the manufacturer's instructions (Life Technologies GibcoBRL). RNA was then converted to cDNA by reverse transcription with elimination of gDNA using the QuantiTect® Reverse Transcription Kit by Qiagen, according to the manufacturer's instructions. Real-time PCR was performed using the SYBR® Green method (QuantiTect SYBR® Green PCR Kit; Qiagen). Plates were prepared according to the manufacturer's recommendations in triplicate sample vials. GAPDH was used as endogenous control. Sequence-specific oligonucleotide primers for the desired target genes were purchased from TIB Molbiol (Berlin, Germany). After primer testing, the following sequences were used – GAPDH: 5'-GCCATCAACGCCCTTCATTGAC-3' and reverse: 5'-ACGGAAGGCCATGCCAGTGAGCTT-3'; VEGF: 5'-AATGATGAAGCCCTGGAGTG-3' and reverse: 5'-TTTCTTTCGCTTTCGTTTTT-3'; BDNF: 5'-GACTCTGGAGAGCGT-

GAAT-3' and reverse: 5'-CCACTCGCTAATACTGTCAC-3'; and GDNF: 5'-CAGCCCAGAGAATTCCAGAG-3' and reverse: 5'-TTTTGTCATACATTGTCTCGGC-3'.

Each reaction contained 0.5 μ L of cDNA with 100 nM primers in a total volume of 25 μ L. The cycling parameters were 50°C for 2 min and 95°C for 10 min, then followed by 45 cycles of 95°C for 15 s and 60°C for 1 min (combining annealing and extension in 1 step at 60°C). Each PCR was repeated twice in triplicate samples each.

Statistical Analysis

For relative expression analysis of the mRNA concentrations, the Δ -CT method was used. Relative quantification (RQ) values were calculated as $2^{-\Delta\Delta CT}$. The control groups (i.e., the naive animals) were defined as the calibrators. All RQ values of each respective control group were gauged to 1 (i.e., 100%). All RQ values of the study groups were then extrapolated conformably into multiples of the calibrator. Comparisons between the groups were done using ANOVA and Tukey-Kramer multiple comparison tests.

For ELISA, mean protein concentrations in relation to the protein standard (bovine serum albumin, BSA) were plotted with standard deviations. Comparison of datasets was again performed by ANOVA and Tukey-Kramer post hoc tests. Differences were considered significant when $p < 0.05$. All graphs were plotted using GraphPad Prism 8.0. Statistical analysis was performed with GraphPad InStat 3.

Results

After exclusion of 22 animals based on the above-mentioned exclusion criteria, 9 rats remained in the ACTIVE group, 5 in the INACTIVE (sham) group, and 7 in the NAIVE group. In all animals analysed below, correct electrode position within the STN had been confirmed.

Impact of STN-DBS on VEGF mRNA Levels

We observed a slight decrease in VEGF mRNA both in striatum and frontal cortex of the STN-lesioned rats (INACTIVE group) as compared to the STN-NAIVE controls both in the striatum and cortex bilaterally, however, without statistical significance ($p > 0.05$). In ACTIVE rats compared to INACTIVE rats, there was an increase in VEGF mRNA both in striatum and frontal cortex, again bilaterally. On the ipsilateral striatum, the mean increase in RQ value was most pronounced (3.85-fold). However, on multivariate analysis, these differences were not statistically significant ($p > 0.05$). Mean striatal RQ of VEGF (mean $\pm$ SE) was $57.9 \pm 17.9\%$ in the INACTIVE group and $223.0 \pm 12.3\%$ in the ACTIVE group ipsilaterally, and $78.6 \pm 26.2\%$ in the INACTIVE group and $166.6 \pm 34.8\%$ contralaterally (see Fig. 1a, b).

Impact of STN-DBS on BDNF mRNA Levels

BDNF was the NF with the greatest increase in mRNA after STN-DBS as compared to sham DBS, and the only one that demonstrated statistical significance on multivariate analysis. The increase was significant in the ipsilateral striatum and in the contralateral motor cortex. In the ipsilateral striatum, BDNF mRNA was increased by 16.7-fold in the ACTIVE group ($1,415.9 \pm 29.8\%$) compared to the INACTIVE group ($84.8 \pm 57.7\%$), $p < 0.01$; $F: 3.42$. In the contralateral hemisphere, there was an increase in striatal mRNA by 4.5-fold. RQs were $172.4 \pm 14.8\%$ in the INACTIVE group versus $772.6 \pm 29.8\%$ in the ACTIVE group, $p > 0.05$. In the frontal cortex, there was a slight increase in BDNF mRNA in the ACTIVE group compared to the INACTIVE group ($140.2 \pm 41.7\%$ vs. $273.3 \pm 35.6\%$) of less than 2-fold, $p > 0.05$. In the contralateral hemisphere, an RQ increase of less than 3-fold was statistically significant, however ($121.8 \pm 45.8\%$ vs. $327.8 \pm 78.5\%$, $p < 0.05$, $F: 3.93$; see Fig. 1c, d).

Impact of STN-DBS on GDNF mRNA Levels

There was no significant difference in GDNF mRNA levels in the striatum or motor cortex between the ACTIVE, INACTIVE, and NAIVE groups. As with VEGF, there was a slight reduction in mRNA levels in the electrode-implanted STN as compared to the naive STN both in striatum and cortex, and a minor increase in mRNA of about 2-fold in the ACTIVE versus INACTIVE group, only on the cortical level. Striatal levels of GDNF were $64.6 \pm 25.4\%$ in the INACTIVE group and $96.2 \pm 20.1\%$ in the ACTIVE group ipsilaterally, and $80.6 \pm 20.1\%$ in the INACTIVE group versus $80.0 \pm 21.1\%$ in the ACTIVE group contralaterally. Cortical levels of GDNF were $59.0 \pm 26.1\%$ in the INACTIVE group and $127.4 \pm 24.2\%$ in the ACTIVE group ipsilaterally, and $65.3 \pm 30.7\%$ in the INACTIVE group versus $131.2 \pm 21.0\%$ in the ACTIVE group contralaterally (see Fig. 1e, f). With all NFs, the observed increases in mRNA in the ACTIVE group were more pronounced on the ipsilateral side in the striatal region, and on the contralateral side in the cortical region.

Impact of STN-DBS on Protein Levels

On protein levels, we observed a considerable decrease after electrode implantation/STN lesioning (= INACTIVE groups) as compared to STN-NAIVE animals (Fig. 2). This was the case for all 3 NFs, and in both STN target regions analysed. The decrease was significant in the ipsilateral striatum for all NFs, and in both hemispheres' cortices for BDNF and GDNF. In STN-NAIVE animals, there were generally slightly higher NF protein

Fig. 1. RT-PCR results given as relative quantification ($RQ = 2^{-\Delta\Delta CT}$) values of the 3 neurotrophic factors: VEGF, BDNF, and GDNF measured in the striatum and motor cortex in a 6-OHDA rodent model of PD. Three experimental groups are (1) STN-naive animals (black columns), (2) electrode implanted in the left STN, but INACTIVE (= sham-stimulation group, grey columns), and (3) electrode implanted in left STN and ACTIVE (= stimulation group, white columns). Ipsilateral hemisphere (containing electrode and 6-OHDA lesion) and contralateral hemisphere, each depicted separately. Mean $2^{-\Delta\Delta CT}$ values of the controls (= naive) an-

imals were normalized to 1 (= 100%). All values are expressed as the mean percent of the respective control. Statistical comparison was performed with ANOVA and Tukey-Kramer post hoc test; * $p < 0.05$, ** $p < 0.01$. VEGF (**a, b**), BDNF (**c, d**), GDNF (**e, f**), striata (**a, c, e**), and motor cortices (**b, d, f**). VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; GDNF, glial cell line-derived neurotrophic factor; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; STN, subthalamic nucleus; Ipsi, Ipsilateral; Contra, contralateral.

Fig. 2. Protein concentrations as assessed by ELISA of the 3 neurotrophic factors: VEGF, BDNF, and GDNF measured in the striatum and motor cortex in a 6-OHDA rodent model of PD. Three experimental groups are (1) STN-naïve animals (black columns), (2) electrode implanted in the left STN, but INACTIVE (sham-stimulation group, grey columns), and (3) electrode implanted in left STN and ACTIVE (stimulation group, white columns). Ipsilateral hemisphere (containing electrode and 6-OHDA lesion) and contralateral hemisphere, each depicted separately. Concentra-

tions given as pg per 500 µg of the protein standard (BSA). Statistical comparison was performed with ANOVA and Tukey-Kramer post hoc test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. VEGF (a, b), BDNF (c, d), GDNF (e, f), striata (a, c, e), and motor cortices (b, d, f). VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; GDNF, glial cell line-derived neurotrophic factor; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; STN, subthalamic nucleus; Ipsi, Ipsilateral; Contra, contralateral; BSA, bovine serum albumin.

concentrations within the lesioned striatum as compared to the unlesioned striatum, again, in all 3 NFs, while the motor cortices showed no side differences for any of the NFs. For all 3 NFs, we observed a significant ($p < 0.05$) increase in mean striatal NF protein on the ipsilateral side in the ACTIVE group versus the INACTIVE group. There was also a significant increase in BDNF within the motor cortex of both hemispheres. Absolute NF protein levels in the ACTIVE group, however, never exceeded those of the NAIVE group. The overall increase in NF protein comparing INACTIVE to ACTIVE remained below 2-fold in all experimental groups.

Impact of STN-DBS on VEGF Protein Levels

Striatal VEGF concentrations (specified always in pg per 500 μ g BSA, mean $\pm$ SE) were on the ipsilateral side: 52.9 ± 4.9 pg in the NAIVE group, 23.9 ± 1.2 pg in the INACTIVE group, and 46.2 ± 3.7 pg in the ACTIVE group; and on the contralateral side 41.4 ± 6.9 pg in the NAIVE group, 26.0 ± 1.1 pg in the INACTIVE group, and 43.6 ± 6.6 pg in the ACTIVE group. On the ipsilateral side, the decreases from NAIVE to INACTIVE ($p < 0.01$) and the increase from INACTIVE to ACTIVE ($p < 0.05$) were significant, $F: 6.14$. Cortical VEGF concentrations were on the ipsilateral side: 51.7 ± 4.7 pg in the NAIVE group, 33.0 ± 8.1 pg in the INACTIVE group, and 50.23 ± 32.4 pg in the ACTIVE group; and on the contralateral side 49.6 ± 5.7 pg in the NAIVE group, 33.2 ± 9.4 pg in the INACTIVE group, and 49.5 ± 8.3 pg in the ACTIVE group (see Fig. 2a, b).

Impact of STN-DBS on BDNF Protein Levels

Striatal concentrations of BDNF protein on the ipsilateral side (specified as pg per 500 μ g BSA) were 385.6 ± 22.9 pg in NAIVE animals versus 155.8 ± 17.4 pg in the INACTIVE animals versus 282.2 ± 20.8 pg in ACTIVE animals. On the contralateral side, BDNF protein concentrations were 268.5 ± 16.1 pg in the NAIVE animals versus 138.4 ± 21.4 pg in the INACTIVE animals versus 256.3 ± 20.2 pg in ACTIVE animals. In the ipsilateral striatum, the decrease in BDNF protein after STN lesioning (NAIVE vs. INACTIVE group) was statistically highly significant, $p < 0.001$. The increase after electrical stimulation (INACTIVE vs. ACTIVE) was also significant, $p < 0.01$.

On the ipsilateral side, cortical BDNF protein concentrations were 306.4 ± 22.6 pg in NAIVE animals versus 112.2 ± 8.2 pg in the INACTIVE animals versus 269.3 ± 14.5 pg in ACTIVE animals. On the contralateral side, cortical BDNF protein concentrations were $307.7.5 \pm 20.2$

in the NAIVE animals versus 145.7 ± 6.2 pg in the INACTIVE animals versus 272.6 ± 7.9 pg in ACTIVE animals. In the cortical region, both the reduction of BDNF protein after STN lesioning and the increase after long-term stimulation were highly significant in both hemispheres after ANOVA and Tukey's multivariate testing, $p < 0.001$ in all groups (see Fig. 2c, d).

Impact of STN-DBS on GDNF Protein Levels

The reduction in GDNF protein concentrations after STN lesioning (NAIVE vs. INACTIVE) was significant in the ipsilateral striatum ($p < 0.01$) and in both motor cortices ($p < 0.05$ in both sides). The increase in GDNF protein after DBS (INACTIVE vs. ACTIVE) was significant only in the ipsilateral striatum ($p < 0.01$), see Figure 2e and f. Striatal concentrations of GDNF protein on the ipsilateral side (specified as pg per 500 μ g BSA) were 153.1 ± 10.6 pg in NAIVE animals versus 58.8 ± 4.1 pg in the INACTIVE animals versus 113.1 ± 8.27 pg in ACTIVE animals. On the contralateral side, BDNF protein concentrations were 100.5 ± 14.1 in the NAIVE animals versus 62.3 ± 4.4 pg in the INACTIVE animals versus 106.7 ± 7.1 pg in ACTIVE animals. Cortical concentrations of GDNF protein on the ipsilateral side (specified as pg per 500 μ g BSA) were 110.7 ± 14.0 pg in NAIVE animals versus 54.2 ± 4.3 pg in the INACTIVE animals versus 98.7 ± 15.8 pg in ACTIVE animals. On the contralateral side, BDNF protein concentrations were 112.5 ± 11.4 in the NAIVE animals versus 54.4 ± 6.3 pg in the INACTIVE animals versus 92.7 ± 4.8 pg in ACTIVE animals. In all experimental groups, bilateral effects were observed despite unilateral stimulation.

Discussion

In the present study, the effects of STN lesioning and chronic STN-DBS on expression levels of various NFs within STN target regions were analysed in a pharmacological rodent model of PD, using 2 different analytical methods: qRT-PCR and ELISA. To our knowledge, this is the first study investigating the effects of STN-DBS on VEGF expression in an animal model of PD, and it is the first study investigating the effect of STN lesioning on expression levels of NFs. The main finding of the study was that STN lesioning and STN-DBS were in fact able to modify expression levels of all NFs within the STN target regions analysed. The direction of alteration was antidromic, dependent on the model used. The unpulsed electrode led to a moderate reduction in NFs, while STN-

DBS produced elevated levels of NFs. The extent of the modification was variable depending on the brain region and the respective NF analysed. The most distinctive finding was the upregulation of BDNF in the ipsilateral striatum through STN-DBS, reproduced both by mRNA and protein analysis.

The Effects of STN Lesioning

The INACTIVE cohort in the presented study may serve as an STN lesion model based on the tissue damage/mechanical irritation within the STN caused by the electrode. After all, the INACTIVE group consists of animals, in which an electrode was implanted into the STN, yet the current was not switched on. Interestingly, a reduction in NF was observed after STN lesioning for almost all NFs and regions analysed. In the analysis of protein levels, this reduction was significant in the ipsilateral striatum for all NFs, and in both motor cortices for BDNF and GDNF. How this protein suppression is mediated on a molecular level is unclear. It may most likely be explained through the established activity-controlled translational regulation of NF-synthesis: Regional NF translation is (at least partly) independent from transcriptional regulation. However, not all translational regulators involved have been fully identified as of yet. So far, they have been investigated most extensively for BDNF: for example, Baj et al. [44] have demonstrated that dendritic BDNF mRNA was translated to BDNF protein by KCl induction after the dendrite was spacially disconnected from the cell soma (i.e., the place of mRNA transcription), furnishing evidence for translational regulation mechanisms independent of those modulating transcription. Later, it had been shown that BDNF translation into protein was triggered by the activation of glutamate and tyrosine kinase receptors conditional to electrical activation of the neuron [45]. Since it is assumed that STN lesioning reduces excitatory transmission to the striatum [46], and since NF translation is stimulation dependent, it is well possible that the reduced STN output within the INACTIVE group may reduce translation of NF protein in STN target regions.

PCR also showed a slight downregulation at least for VEGF and GDNF after lesioning, however, not statistically relevant. It bears mentioning that the transcription of NFs is also dependent on electrical activity. Generally, there are Ca^{2+} -responsive elements in the NF promoter regions. Ca^{2+} -mediated transcription is controlled by activity-dependent L-type voltage-gated calcium channels or NMDA receptors [47, 48]. Thus, the moderate reduction of NF mRNA in the INACTIVE group may to a cer-

tain extent also be attributed to a reduction in STN output to the striatum.

Increases in NF levels, however, could only be observed after high-frequency stimulation (HFS) of the STN. Therefore, at least inferred from the animal model used in this study, STN lesioning alone was not sufficient to produce elevated NF levels in the 2 STN target structures examined. Electrical stimulation appears to be prerequisite for this. If it is hypothesized that STN lesioning leads to a reduced glutamatergic stimulation of the striatum through “silencing” of the STN, just as it is the case in DBS [49], it turns out to be rather unlikely that NF upregulation is directly mediated by the reduction of glutamatergic input. Taken together, a theory where DBS disrupts pathological input signals and dissociates output signals, as suggested, for example, by Chiken and Nambu [50], still providing controlled electrical stimulation to the target regions, appears more likely.

The Effects of STN-DBS

Generally, the effects of STN-DBS on NF expression were upregulatory. On the protein level, statistically significant increases in all NFs were registered in the ipsilateral striatum with the statistics we used (ANOVA + Tukey's post hoc test). However, these levels increased by maximum 100% and thus barely reached the benchmark value of the NAIVE animals. While DBS produced non-significant and rather similar increases of mRNA transcription for VEGF and GDNF (respective RQ values about 2-fold), BDNF was upregulated significantly within the ipsilateral striatum (RQ values of up to almost 17-fold). BDNF was also the only NF that produced significant upregulation in the motor cortex, both on the mRNA and the protein levels.

The effect of STN DBS on BDNF has been investigated previously [40]. Even though Spieles-Engemann et al. [40] used an externalized neurostimulation system, they also performed long-term DBS in the STN of the rat. Our data are in part conformable with theirs, in that we also observed a marked upregulation of BDNF, particularly both in the frontal motor cortex and in the striatum. Spieles-Engemann et al. [40] also looked at GDNF, however only in intact, non-lesioned rats.

Our results here suggest a rather generalized, less-specific effect of STN DBS on many growth and trophic factors (GDNF and VEGF), at least within the regions investigated here. However, our data allocate the strongest effect to BDNF. From this, 2 things may be concluded: first, DBS may have the potential to influence or regulate the transcription of NFs. Second, the increase in BDNF with-

in the striatum after stimulation of the STN might be a more relevant trophic influence of DBS in PD.

It is well known that electrical stimulation can modify gene expression [51], as well as synaptic plasticity and transmission [49]. It is assumed that DBS works through altering the patterns of neuronal activity [52], including discharge rates. Altered neuronal discharge rates can influence transmitter release, as well [53]. Clearly, changes in neuronal firing patterns and transmitter homeostasis will also have effect on many other parameters within the DBS target structures, including receptor expression as well as synthesis and release of metabolic and trophic factors. We hypothesize that altered expression levels of several neuroprotective factors might contribute to the neuroprotective effect of DBS therapy.

Taking into account previous animal studies [39], it may be important to emphasize that the upregulatory (and potentially neuroprotective) effects of DBS on NF expression appear not to be limited to a certain brain target, nor to a specific disease model. Since HFS of the fornix also produces NF upregulation in a remote target region, as demonstrated by Gondard et al. [39], it is more than likely that the upregulation of NFs by HFS-DBS in upstream brain regions constitutes a rather general physiological phenomenon of the technique that may thus hold the potential to be applied to a multitude of further neurodegenerative diseases.

What bears mentioning is that the considerable increase in BDNF mRNA in the ipsilateral striatum was not paralleled proportionately to the protein level. After all, proteins are the final effectors of neuroprotection and not mRNA. Several possible reasons for an imbalance between mRNA and protein expression after gene upregulation are well documented in the literature. The most common ones involve around protein transport, protein stability, post-translational modifications, and translation efficiency. As is the case with NFs, the NF protein is always translated and synthesized in the cytosoma or in the dendrites and then packed into vesicles to be transported via axonal transport to the respective synapses the neuron is projecting to [48]. If the invagination and transport of the new protein away from the soma are effective, it is well possible that mRNA levels are higher than protein levels at the site of synthesis (i.e., in the brain region where the soma lies). Also, it may be hypothesized that part of the transcribed mRNA could be degraded (e.g., based on potential nonproductive splice isoforms) or transcripts may be retained. Especially for BDNF, it is known that not all mRNAs are translated into proteins automatically, but that the initiation of translation is part-

ly dependent on cell environment and activation triggered messengers [46]. A further possible explanation could be that post-translational modifications of the protein (e.g., ubiquitinylation) may lead to a fast cleaving or degrading, resulting in different half-lives of mRNA and protein. Post-translational modifications at the site of the epitope may result in the protein not being detected by the antibody, producing false low protein measurements. Last, experimental limitations of the method, involving the sample preparation technique and failure of antibody binding, can never be fully excluded. While all these mechanisms are conceivable, the authors assume that the first possible explanation, in which a portion of the synthesized NF proteins leaves the striatum and is transported via axonal vesicular transport to the respective target tissue, will account for these differences most relevantly.

Bilateral Stimulation Effects

The fact that unilateral stimulation of the left STN produced bilateral, relatively similar, effects on mRNA and protein levels may be surprising. However, a relatively recent study on the projections of the STN in the rat by Cavdar et al. [54], where tracers (Fluoro Gold and biotinylated dextran) were injected into the STN unilaterally to visualize STN connects after 1 week, has demonstrated strong direct interhemispheric connections between left and right STN. Furthermore, bilateral connections of the STN were also found to the SN, as well as to several further thalamic and brainstem targets [54]. It is worth mentioning that Spieles-Engemann et al. [40], who employed a similar experimental set-up to ours, also observed bilateral effects in STN target regions after unilateral stimulation. Taken together, at least in the rodent model, we have to assume a robust interhemispheric connectivity between both STNs, making bilateral effects after unilateral stimulation rather likely. What may be interesting is that even in humans, there is certain clinical evidence of bi-hemispheric effects after unilateral STN stimulation to some extent. Clinical studies have shown that unilateral STN stimulation for PD has produced a bilateral improvement in rigidity, bradykinesia, and other gait parameters, similar to that in bilateral stimulation [55–57] making a direct or indirect bihemispheric connectivity of the STN appear conceivable even in humans.

Limitations to the Study

There are a few shortcomings in this study that bear mentioning: we did not include a healthy control, that is, a 6-OHDA-non-lesioned control group. Therefore, we cannot give evidence whether the observed effects on NF

were specific to the PD model used, or rather unspecific stimulation effects, also seen in otherwise healthy rats. Second, we did not include the SNc into our biochemical analysis (the SNc was isolated and then used for TH staining, data not shown here). However, the SNc also constitutes a target structure of the STN, and since degeneration of dopaminergic neurons takes place here, the effect of DBS on SNc-NF levels is of interest and should certainly be considered in future studies. Third, group sizes were relatively small and not uniform (group sizes were ranging from 5 to 9). Last, we used a pharmacological animal model of PD. Even though the 6-OHDA model still is one of the traditionally and widely used animal models for PD, it associates with all the well-known weaknesses of pharmacological animal models. It is therefore often suggested that genetic animal models (such as the α -synuclein model) may constitute a closer representative for the human PD condition.

Conclusion

Our results suggest an upregulatory effect of STN-DBS on trophic factor expression in STN target regions and are thus consistent with the hypothesis that DBS might exert neuroprotective and neurorestorative effects to some extent within the complex network of the motor system.

References

- Perlmutter JS, Mink JW. Deep brain stimulation. *Annu Rev Neurosci*. 2006;29:229–57.
- Schuepbach WM, Rau J, Knudsen K, Volkmann J, Krack P, Timmermann L, et al. Neurostimulation for Parkinson's disease with early motor complications. *N Engl J Med*. 2013; 368(7):610–22.
- Harnack D, Meissner W, Jira JA, Winter C, Morgenstern R, Kupsch A. Placebo-controlled chronic high-frequency stimulation of the subthalamic nucleus preserves dopaminergic nigral neurons in a rat model of progressive Parkinsonism. *Exp Neurol*. 2008;210(1):257–60.
- Maesawa S, Kaneoke Y, Kajita Y, Usui N, Misawa N, Nakayama A, et al. Long-term stimulation of the subthalamic nucleus in hemiparkinsonian rats: neuroprotection of dopaminergic neurons. *J Neurosurg*. 2004;100(4):679–87.
- Spieles-Engemann AL, Behbehani MM, Collier TJ, Wohlgenant SL, Steece-Collier K, Paumier K, et al. Stimulation of the rat subthalamic nucleus is neuroprotective following significant nigral dopamine neuron loss. *Neurobiol Dis*. 2010;39(1):105–15.
- Temel Y, Visser-Vandewalle V, Kaplan S, Kozan R, Daemen MA, Blokland A, et al. Protection of nigral cell death by bilateral subthalamic nucleus stimulation. *Brain Res*. 2006; 1120(1):100–5.
- Wallace BA, Ashkan K, Heise CE, Foote KD, Torres N, Mitrofanis J, et al. Survival of mid-brain dopaminergic cells after lesion or deep brain stimulation of the subthalamic nucleus in MPTP-treated monkeys. *Brain*. 2007 Aug; 130(Pt 8):2129–45.
- Winkler C, Sauer H, Lee CS, Björklund A. Short-term GDNF treatment provides long-term rescue of lesioned nigral dopaminergic neurons in a rat model of Parkinson's disease. *J Neurosci*. 1996;16(22):7206–15.
- Musacchio T, Rebenstorff M, Fluri F, Brothie JM, Volkmann J, Koprich JB, et al. Subthalamic nucleus deep brain stimulation is neuroprotective in the A53T α -synuclein Parkinson's disease rat model. *Ann Neurol*. 2017;81(6): 825–36.
- Faucheux BA, Bonnet AM, Agid Y, Hirsch EC. Blood vessels change in the mesencephalon of patients with Parkinson's disease. *Lancet*. 1999;353(9157):981–2.
- Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. *Pharmacol Ther*. 2013 May;138(2):155–75.
- Sullivan AM, Toulouse A. Neurotrophic factors for the treatment of Parkinson's disease. *Cytokine Growth Factor Rev*. 2011 Jun;22(3): 157–65.
- Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling – in control of vascular function. *Nat Rev Mol Cell Biol*. 2006;7(5):359–71.
- Sun Y, Jin K, Childs JT, Xie L, Mao XO, Greenberg DA. Vascular endothelial growth factor-B (VEGFB) stimulates neurogenesis: evidence from knockout mice and growth factor administration. *Dev Biol*. 2006;289(2):329–35.
- Guan J, Pavlovic D, Dalkie N, Waldvogel HJ, O'Carroll SJ, Green CR, et al. Vascular degeneration in Parkinson's disease. *Brain Pathol*. 2013 Mar;23(2):154–64.
- Falk T, Zhang S, Sherman SJ. Vascular endothelial growth factor B (VEGF-B) is up-regulated and exogenous VEGF-B is neuroprotective in a culture model of Parkinson's disease. *Mol Neurodegener*. 2009;10(4):49.
- Falk T, Yue X, Zhang S, McCourt AD, Yee BJ, Gonzalez RT, et al. Vascular endothelial growth factor-B is neuroprotective in an in vivo rat model of Parkinson's disease. *Neurosci Lett*. 2011;496(1):43–7.

Statement of Ethics

All experiments were carried out with permission from local authorities and in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC) for the care of laboratory animals. The study is in compliance with the ARRIVE guidelines.

Conflict of Interest Statement

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Funding Sources

The study received no funding, except for the University Clinic research funds.

Author Contributions

D.H. conceived the study. D.H. and K.F. carried out the animal experiments. D.H. performed the intrastriatal lesions and the histological staining. D.H. and K.F. performed the electrode/stimulation system implantations. K.F. carried out the tissue preparation, PCR, and ELISA; analysed the data; and drafted the manuscript. B.X. helped with the PCR and ELISA. P.V., K.F., and D.H. contributed to the study design, coordination and execution, and manuscript writing. All authors read and approved the final manuscript.

- 18 Yasuhara T, Shingo T, Muraoka K, Wen Ji Y, Kameda M, Takeuchi A, et al. The differences between high and low-dose administration of VEGF to dopaminergic neurons of in vitro and in vivo Parkinson's disease model. *Brain Res*. 2005 Mar 15;1038(1):1–10.
- 19 Yasuhara T, Shingo T, Muraoka K, Kameda M, Agari T, Wen Ji Y, et al. Neurorescue effects of VEGF on a rat model of Parkinson's disease. *Brain Res*. 2005 Aug 16;1053(1–2):10–8.
- 20 Tian YY, Tang CJ, Wang JN, Feng Y, Chen XW, Wang L, et al. Favorable effects of VEGF gene transfer on a rat model of Parkinson disease using adeno-associated viral vectors. *Neurosci Lett*. 2007 Jun 29;421(3):239–44.
- 21 Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science*. 1993;260(5111):1130–2.
- 22 Yamamoto M, Sobue G, Yamamoto K, Terao S, Mitsuma T. Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, trkA, trkB, and trkC) in the adult human peripheral nervous system and nonneural tissues. *Neurochem Res*. 1996;21(8):929–38.
- 23 Bowenkamp KE, Lapchak PA, Hoffer BJ, Miller PJ, Bickford PC. Intracerebroventricular glial cell line-derived neurotrophic factor improves motor function and supports nigrostriatal dopamine neurons in bilaterally 6-hydroxydopamine lesioned rats. *Exp Neurol*. 1997;145(1):104–17.
- 24 Lapchak PA, Miller PJ, Collins F, Jiao S. Glial cell line-derived neurotrophic factor attenuates behavioural deficits and regulates nigrostriatal dopaminergic and peptidergic markers in 6-hydroxydopamine-lesioned adult rats: comparison of intraventricular and intranigral delivery. *Neuroscience*. 1997;78(1):61–72.
- 25 Lapchak PA, Miller PJ, Jiao S. Glial cell line-derived neurotrophic factor induces the dopaminergic and cholinergic phenotype and increases locomotor activity in aged Fischer 344 rats. *Neuroscience*. 1997;77(3):745–52.
- 26 Sauer H, Rosenblad C, Björklund A. Glial cell line-derived neurotrophic factor but not transforming growth factor beta 3 prevents delayed degeneration of nigral dopaminergic neurons following striatal 6-hydroxydopamine lesion. *Proc Natl Acad Sci U S A*. 1995;92(19):8935–9.
- 27 Gill SS, Patel NK, Hutton GR, O'Sullivan K, McCarter R, Bunnage M, et al. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med*. 2003; 9(5):589–95.
- 28 Lang AE, Gill S, Patel NK, Lozano A, Nutt JG, Penn R, et al. Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann Neurol*. 2006;59(3):459–66.
- 29 He YY, Zhang XY, Yung WH, Zhu JN, Wang JJ. Role of BDNF in central motor structures and motor diseases. *Mol Neurobiol*. 2013 Dec; 48(3):783–93.
- 30 Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos GD, Squinto SP, et al. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature*. 1991;350(6315): 230–2.
- 31 DeLong MR. Primate models of movement disorders of basal ganglia origin. *Trends Neurosci*. 1990 Jul;13(7):281–5.
- 32 Lui NP, Chen LW, Yung WH, Chan YS, Yung KK. Endogenous repair by the activation of cell survival signalling cascades during the early stages of rat Parkinsonism. *PLoS One*. 2012; 7(12):e51294.
- 33 Stahl K, Mylonakou MN, Skare O, Amiry-Moghaddam M, Torp R. Cytoprotective effects of growth factors: BDNF more potent than GDNF in an organotypic culture model of Parkinson's disease. *Brain Res*. 2011;1378:105.
- 34 Howells DW, Porritt MJ, Wong JY, Batchelor PE, Kalnins R, Hughes AJ, et al. Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp Neurol*. 2000; 166(1):127–35.
- 35 Ziebell M, Khalid U, Klein AB, Aznar S, Thomsen G, Jensen P, et al. Striatal dopamine transporter binding correlates with serum BDNF levels in patients with striatal dopaminergic neurodegeneration. *Neurobiol Aging*. 2012; 33(2):428–5.
- 36 McIntyre CC, Anderson RW. Glutamate and neurotrophic factors in neuronal plasticity and disease. *Ann N Y Acad Sci*. 2008 Nov;1144:97–112.
- 37 Lee KH, Chang SY, Roberts DW, Kim U. Neurotransmitter release from high-frequency stimulation of the subthalamic nucleus. *J Neurosurg*. 2004 Sep;101(3):511–7.
- 38 Hagg T. From neurotransmitters to neurotrophic factors to neurogenesis. *Neuroscientist*. 2009 Feb;15(1):20–7.
- 39 Gondard E, Chau HN, Mann A, Tierney TS, Hamani C, Kalia SK, et al. Rapid modulation of protein expression in the rat hippocampus following deep brain stimulation of the fornix. *Brain Stimul*. 2015 Nov–Dec;8(6):1058–64.
- 40 Spieles-Engemann AL, Steece-Collier K, Behbehani MM, Collier TJ, Wohlgenant SL, Kemp CJ, et al. Subthalamic nucleus stimulation increases brain derived neurotrophic factor in the nigrostriatal system and primary motor cortex. *J Parkinsons Dis*. 2011;1(1):123–36.
- 41 Harnack D, Meissner W, Paulat R, Hilgenfeld H, Müller WD, Winter C, et al. Continuous high-frequency stimulation in freely moving rats: development of an implantable micro-stimulation system. *J Neurosci Methods*. 2008 Jan 30;167(2):278–91.
- 42 Oiwa Y, Sanchez-Pernaute R, Harvey-White J, Bankiewicz KS. Progressive and extensive dopaminergic degeneration induced by convection-enhanced delivery of 6-hydroxydopamine into the rat striatum: a novel rodent model of Parkinson disease. *J Neurosurg*. 2003 Jan; 98(1):136–44.
- 43 Paxinos G, Watson C, Pennisi M, Topple A. Bregma, lambda and the interaural midpoint in stereotaxic surgery with rats of different sex, strain and weight. *J Neurosci Methods*. 1985; 13(2):139–43.
- 44 Baj G, Leone E, Chao MV, Tongiorgi E. Spatial segregation of BDNF transcripts enables BDNF to differentially shape distinct dendritic compartments. *Proc Natl Acad Sci U S A*. 2011 Oct 4;108(40):16813–8.
- 45 Baj G, Pinheiro V, Vaghi V, Tongiorgi E. Signaling pathways controlling activity-dependent local translation of BDNF and their localization in dendritic arbors. *J Cell Sci*. 2016 Jul 15;129(14):2852–64.
- 46 Walker RH, Moore C, Davies G, Dirling LB, Koch RJ, Meshul CK. Effects of subthalamic nucleus lesions and stimulation upon corticostriatal afferents in the 6-hydroxydopamine-lesioned rat. *PLoS One*. 2012;7(3):e32919.
- 47 Zheng F, Zhou X, Moon C, Wang H. Regulation of brain-derived neurotrophic factor expression in neurons. *Int J Physiol Pathophysiol Pharmacol*. 2012;4(4):188–200.
- 48 Levy YS, Gilgun-Sherki Y, Melamed E, Offen D. Therapeutic potential of neurotrophic factors in neurodegenerative diseases. *BioDrugs*. 2005;19(2):97–127.
- 49 Agnesi F, Johnson MD, Vitek JL. Deep brain stimulation: how does it work? *Handb Clin Neurol*. 2013;116:39–54.
- 50 Chiken S, Nambu A. Mechanism of deep brain stimulation: inhibition, excitation, or disruption? *Neuroscientist*. 2016 Jun;22(3):313–22.
- 51 Ho DX, Tan YC, Tan J, Too HP, Ng WH. High-frequency stimulation of the globus pallidus interna nucleus modulates GFRα1 gene expression in the basal ganglia. *J Clin Neurosci*. 2014;21(4):657–60.
- 52 Mattson MP. Deep brain stimulation mechanisms: the control of network activity via neurochemistry modulation. *J Neurochem*. 2016 Oct;139(Suppl 1):338–45.
- 53 Kopell BH, Rezai AR, Chang JW, Vitek JL. Anatomy and physiology of the basal ganglia: implications for deep brain stimulation for Parkinson's disease. *Mov Disord*. 2006; 21(Suppl 14):S238–46.
- 54 Cavdar S, Özgür M, Çakmak YÖ, Kuvvet Y, Kunt SK, Sağlam G. Afferent projections of the subthalamic nucleus in the rat: emphasis on bilateral and interhemispheric connections. *Acta Neurobiol Exp*. 2018;78(3):251–63.
- 55 Walker HC, Watts RL, Guthrie S, Wang D, Guthrie BL. Bilateral effects of unilateral subthalamic deep brain stimulation on Parkinson's disease at 1 year. *Neurosurgery*. 2009; 65(2):302–10.
- 56 Tabbal SD, Ushe M, Mink JW, Revilla FJ, Wernle AR, Hong M, et al. Unilateral subthalamic nucleus stimulation has a measurable ipsilateral effect on rigidity and bradykinesia in Parkinson disease. *Exp Neurol*. 2008 May;211(1): 234–42.
- 57 Rizzone MG, Ferrarin M, Lanotte MM, Lopian L, Carpinella I. The dominant-subthalamic nucleus phenomenon in bilateral deep brain stimulation for Parkinson's disease: evidence from a gait analysis study. *Front Neurol*. 2017 Oct 30;8:575.