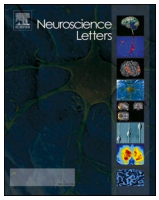
Neuroscience Letters 814 (2023) 137459

Contents lists available at ScienceDirect

Neuroscience Letters

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



Subthalamic nucleus deep brain stimulation does not alter growth factor expression in a rat model of stable dopaminergic deficiency

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ARTICLE INFO

Keywords:
Deep brain stimulation
Subthalamic nucleus
Parkinson's disease
6-OHDA
6-hydroxydopamine
Stable dopaminergic deficiency
BDNF
Brain-derived neurotrophic factor
GDNF
Glial cell line-derived neurotrophic factor

ABSTRACT

Background: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been a highly effective treatment option for mid-to-late-stage Parkinson's disease (PD) for decades. Besides direct effects on brain networks, neuroprotective effects of STN-DBS – potentially via alterations of growth factor expression levels – have been proposed as additional mechanisms of action.
Objective: In the context of clarifying DBS mechanisms, we analyzed brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) levels in the basal ganglia, motor and parietal cortices, and dentate gyrus in an animal model of stable, severe dopaminergic deficiency.
Methods: We applied one week of continuous unilateral STN-DBS in a group of stable 6-hydroxydopamine (6-OHDA) hemiparkinsonian rats (6-$OHDA_{STIM}$) in comparison to a 6-OHDA control group (6-$OHDA_{SHAM}$) as well as healthy controls ($CTRL_{STIM}$ and $CTRL_{SHAM}$). BDNF and GDNF levels were determined via ELISAs.
Results: The 6-OHDA lesion did not result in a persistent alteration in either BDNF or GDNF levels in a model of severe dopaminergic deficiency after completion of the dopaminergic degeneration. STN-DBS modestly increased BDNF levels in the entopeduncular nucleus, but even impaired BDNF and GDNF expression in cortical areas.
Conclusions: STN-DBS does not increase growth factor expression when applied to a model of completed, severe dopaminergic deficiency in contrast to other studies in models of modest and ongoing dopaminergic degeneration. In healthy controls, STN-DBS does not influence BDNF or GDNF expression. We consider these findings relevant for clinical purposes since DBS in PD is usually applied late in the course of the disease.

1. Introduction

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a highly effective treatment option in mid-to-late-stage Parkinson's disease (PD), offering long-lasting motor symptom control for PD patients suffering from motor fluctuations and dyskinesia [1]. In contrast to various animal studies [2–5], there is currently no convincing evidence for disease modification through DBS in PD on the clinical level [6,7].

The mechanisms of action of STN-DBS have been investigated for several decades, though they have not been entirely clarified so far: while initially, a local inhibitory effect on STN neurons was assumed, more recent studies discussed a correction of abnormal motor network activity as indicated by disruption of pathological beta oscillations [8,9]. Additional attention has been focused on STN-DBS effects on a molecular level, e.g., regarding alterations in neurotrophin expression. Brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) have been identified as critical trophic factors in the survival of dopaminergic neurons in the substantia nigra (SN), and decreased levels of these neurotrophins have been found in dopaminergic brain regions in PD patients [10–12]. In animal models, reduced neurotrophin levels have also been repeatedly reported [13–15]. Conveniently, experimental growth factor addition resulted in neuroprotective effects in the dopaminergic system in these models [16–19], though human studies on GDNF enhancement yielded so far disappointing results [20–23]. A previous study by Spieles-Engemann and colleagues on STN-DBS and growth factors demonstrated an increase in BDNF levels, but unchanged GDNF expression in the basal ganglia and primary motor cortex in the 6-hydroxydopamine (6-OHDA)

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https://doi.org/10.1016/j.neulet.2023.137459
Received 29 June 2023; Received in revised form 11 August 2023; Accepted 21 August 2023
Available online 23 August 2023

animal model after 14 days of continuous STN-DBS [24]. In a more recent study by Faust and co-workers, STN-DBS over two weeks resulted in increased levels of BDNF, GDNF, and vascular endothelial growth factor (VEGF) in the striatum, but only elevated BDNF levels in cortical regions [25]. These effects were far less pronounced in a PD rat model of synucleinopathy with a modest rescue of STN-DBS on striatal BDNF levels [26]. The molecular mechanisms behind these findings have only been assessed in animal models of psychiatric diseases, e.g., for depression and dementia, in which DBS altered gene expression profiles, RNA splicing or acted via mi-RNA suppression, respectively [27–29].

The present study investigates the effects of continuous STN-DBS over one week on the expression of BDNF and GDNF in the basal ganglia circuitry, certain cortical areas, and the dentate gyrus. In contrast to previous studies in the field, we investigated growth factor levels not during the ongoing dopaminergic degeneration, i.e., within 5 to 14 days post (striatal) lesioning [24,25], but in a state of stable dopaminergic deficiency six weeks after initial 6-OHDA application, which is – in our opinion – more relatable to the clinical situation. PD patients usually receive DBS treatment after suffering from the disease for 5 to 15 years [30,31], while at the same time, there is already a degeneration of at least 50–70% of dopaminergic neurons in the substantia nigra at symptom onset [32] and a loss of ~ 90% of striatal dopaminergic innervation within four years post-diagnosis [33]. We hypothesize that a fully degenerated dopaminergic system similar to the situation in human DBS patients might respond differently to external stimuli, such as DBS, compared to animal models of ongoing dopaminergic degeneration.

2. Methods

Animals. All procedures were permitted by responsible authorities (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany; TVV 7331.3–1.075/18) and carried out in line with ARRIVE guidelines and the EU Directive 2010/63/EU. We used 30 male Wistar-Han rats (260–280 g at the time of arrival (Charles River Laboratories, Germany)) of which 27 were included into final analyses (three drop-outs due to implant failures or insufficient lesioning; Fig. 1**a**). A subset of rats (n = 17) underwent right-sided unilateral 6-OHDA lesioning to generate a reliable dopaminergic degeneration in the ventral midbrain (see Supplementary Methods for details). Successful 6-OHDA lesioning was quantified by apomorphine-induced rotational behavior two and four weeks after lesioning. We used 0.25 mg/kg body weight apomorphine (0.2 mg/ml in 0.9% NaCl; Teclapharm, Germany) and quantified rotational behaviour for a total of 40 min; then, lesioned animals were divided into two closely matched groups (6-OHDA$_{STIM}$ (mean ± S.E.M.): 8.2 ± 0.9 rotations/min; 6-OHDA$_{SHAM}$: 6.5 ± 1.2 rotations/min; $P = 0.328$ from Welch's test, n = 6–8; see **Suppl.** Fig. S1**a** for details).

Surgery. Electrode implantations were performed unilaterally into the right subthalamic nucleus (STN) of both healthy and 6-OHDA-lesioned animals the day after the second rotational testing as described in detail in ([2]; see Fig. 1**a** for experimental setups). We used an external high-frequency stimulator carried in a rodent backpack (as detailed in [34]). After one week of stimulation, all animals were anesthetized and transcardially perfused with heparinized saline, followed by ice-cold saline. Brains were harvested, snap-frozen, and stored at −80 °C. Dissections of the brain regions of interest were carried out as adapted from [24] (see Supplementary Methods for details).

Protein assay. For BDNF, GDNF and dopamine quantifications, we used commercially available ELISA kits with streptavidin–biotin detection (RayBiotech Life, GA, US) according to the manufacturer's protocol (see Supplementary Methods for details).

Statistics. All calculations were carried out with SPSS (version 27, IBM, NY, US), while data plots and figures were created with GraphPadPrism 9.4.1 (GraphPad Software, CA, US) and BioRender.com (BioRender, Canada). The statistical methods are described in detail in the Supplementary Methods section.

3. Results

To determine the possible effects of seven days of continuous STN-DBS or respective sham stimulation in both healthy and dopamine-deficient animals on BDNF and GDNF levels in the basal ganglia, motor and parietal cortices, and dentate gyrus, we used quantitative protein analyses in hemiparkinsonian rats (6-OHDA$_{SHAM}$; 6-OHDA$_{STIM}$) and healthy controls (CTRL$_{SHAM}$; CTRL$_{STIM}$; study design in Fig. 1**a**). The effects of 6-OHDA lesioning were estimated by apomorphine-induced rotational testing and measuring striatal dopamine using the ELISA technology content as well as dopaminergic immunohistochemistry analyses of the substantia nigra pars compacta in additional cohorts (refer to **Suppl. Methods and Suppl. Fig. 1,2**). All data are presented as percentage of growth factor levels relative to the contralateral, non-lesioned side without electrode placement.

Initially, we analyzed the effects of the 6-OHDA lesion on growth factor expression levels. In our model of stable dopaminergic deficiency, i.e., six weeks after initial lesioning and therefore after completion of the degenerative process [3], we found no significant differences in BDNF and GDNF levels between healthy and 6-OHDA-lesioned groups (for details and statistics, see **Suppl. Table S1**).

Since we were mainly interested in DBS effects, we next evaluated alterations in growth factor levels in both CTRL$_{SHAM}$ vs. CTRL$_{STIM}$ and 6-OHDA$_{SHAM}$ vs. 6-OHDA$_{STIM}$ animals. We found no significant alterations in BDNF or GDNF levels in healthy control animals (CTRL) that had been submitted to one week of unilateral STN-DBS in all examined regions of the basal ganglia, cortex, and dentate gyrus (Fig. 1**,2**, for numeric data and statistics, refer to **Suppl. Table S2**).

In 6-OHDA-lesioned animals, relative amounts of BDNF levels in the subthalamic nucleus were 102.6 ± 11.4% and 92.2 ± 9.3% in 6-OHDA$_{SHAM}$ and 6-OHDA$_{STIM}$ groups ($P = 0.49$; Fig. 1**b**), while in the substantia nigra, we detected 111 ± 6.5% and 106.5 ± 8.4% of BDNF, respectively ($P = 0.40$; Fig. 1**c**). In the entopeduncular nucleus, BDNF levels were significantly increased by STN-DBS with 108.3 ± 9.0% compared to 79.3 ± 2.0% in the 6-OHDA$_{SHAM}$ group ($P = 0.05$; Fig. 1**d**). In the external globus pallidus, BDNF levels remained unchanged with 94.8 ± 6.6% and 94.0 ± 10.4% in 6-OHDA$_{SHAM}$ and 6-OHDA$_{STIM}$ groups ($P = 0.96$; Fig. 1**e**).

In the dentate gyrus of the hippocampus, we found 117.2 ± 20.1% and 91.1 ± 5.3% in 6-OHDA$_{SHAM}$ vs. 6-OHDA$_{STIM}$ groups ($P = 0.26$; Fig. 1**f**), while in the M2 cortex, STN-DBS led to a significant decrease in BDNF levels with 116.1 ± 9.3% vs. 90.0 ± 1.7% ($P = 0.04$; Fig. 1**g**). Similarly, we detected a decrease of relative BDNF levels with 118.3 ± 6.1% vs. 92.1 ± 6.9% in 6-OHDA$_{SHAM}$ vs. 6-OHDA$_{STIM}$ groups ($P = 0.02$) in the parietal cortex **(**Fig. 1**h).**

Regarding GDNF levels, we found again no alterations after one week of STN-DBS with 96.3 ± 8.2% and 95.8 ± 13.0% of GDNF levels in 6-OHDA$_{SHAM}$ and 6-OHDA$_{STIM}$ groups in the subthalamic nucleus ($P = 0.98$; Fig. 2**a**) and 128.9 ± 14.4% and 125.3 ± 20.7% in the substantia nigra ($P = 0.57$; Fig. 2**b**). Similar to stimulation-induced BDNF alterations, GDNF levels in the entopeduncular nucleus were significantly increased in 6-OHDA$_{STIM}$ animals with 107.8 ± 3.1% compared to 94.0 ± 3.4% in the 6-OHDA$_{SHAM}$ group ($P = 0.01$; Fig. 2**c**). In the external globus pallidus, GDNF levels remained unchanged with 88.0 ± 6.8% and 109.8 ± 7.7% in 6-OHDA$_{SHAM}$ and 6-OHDA$_{STIM}$ groups ($P = 0.06$; Fig. 2**d**).

In the dentate gyrus, GDNF levels were 85.6 ± 6.0% and 101.1 ± 12.0% in 6-OHDA$_{SHAM}$ vs. 6-OHDA$_{STIM}$ groups ($P = 0.14$; Fig. 2**e**). No significant alterations were found in the M2 cortex, with 95.7 ± 7.3% vs. 100.8 ± 12.2% in 6-OHDA$_{SHAM}$ vs. 6-OHDA$_{STIM}$ animals ($P = 0.77$; Fig. 2**f**), though there was again a significant decrease of GDNF levels in the parietal cortex after STN-DBS with 81.2 ± 9.2% compared to 110.9 ± 5.7% ($P = 0.03$; Fig. 2**g**).

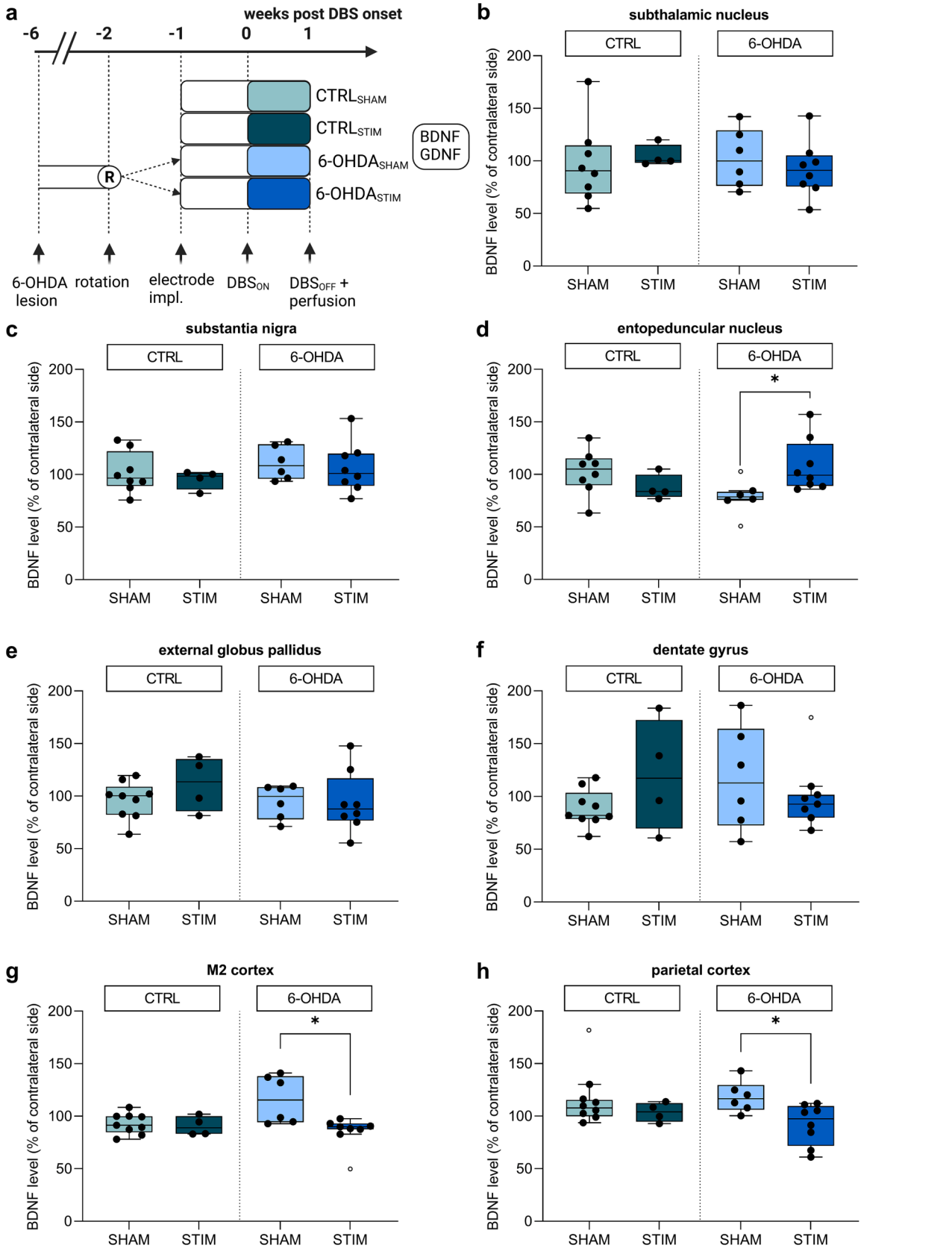

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Fig. 1. BDNF expression levels after one week of sham stimulation (SHAM) or STN-DBS (STIM) in healthy control animals (CTRL) or in 6-OHDA-lesioned hemiparkinsonian rats (6-OHDA) normalized to contralateral non-lesioned, non-stimulated hemispheres, respectively. (a) Experimental design (Created with BioRender.com). **(b-h)** Expression levels of BDNF normalized to contralateral, non-lesioned hemisphere without electrode placement as detected by ELISA in the following brain regions: subthalamic nucleus **(b),** substantia nigra **(c),** entopeduncular nucleus **(d),** external globus pallidus **(e),** dentate gyrus **(f),** supplementary motor cortex (M2 cortex; **g**) and parietal cortex **(h).** There was no significant effect of the 6-OHDA-induced dopaminergic deficiency on BDNF levels in our model. However, STN-DBS significantly decreased BDNF levels in cortical areas, i.e., the M2 cortex and the parietal cortex. Abbreviations: DBS – deep brain stimulation; 6-OHDA – 6-hydroxydopamine; SHAM – sham stimulation; STIM – verum stimulation; CTRL – control; 6-OHDA – 6-hydroxydopamine-lesioned animals; STN – subthalamic nucleus; BDNF – brain-derived neurotrophic factor; GDNF – glial cell line-derived neurotrophic factor. Data are presented as mean with all individual values; outliers were defined as all values ± 1.5x S.D.; $P < 0.05$ were deemed significant after unpaired two-sided Student's *t*-test **(b-e,h)** or Welchs *t*-test **(f,g)**, as appropriate.

4. Discussion

In the present study, we analyzed the influence of continuous unilateral STN-DBS for seven days on BDNF and GDNF expression levels in various brain regions in 6-OHDA-lesioned hemiparkinsonian rats. To our knowledge, this is the first study to investigate the effects of STN-DBS on growth factor levels after completing a quite extensive degenerative process in the dopaminergic system, a model that closely resembles the condition of human PD patients at the time of STN-DBS. Interestingly, we found no significant alterations in either BDNF or GDNF expression levels in 6-OHDA-lesioned animals compared to healthy controls. These findings contrast the literature, where a persistent decrease in BDNF and/or GDNF levels, e.g., in the striatum or hippocampus, is reported after 6-OHDA-induced lesioning, though usually in models of incomplete dopaminergic deficiency. This discrepancy might also be attributable to the time interval between 6-OHDA injections and STN-DBS onset, resulting in DBS onset during the ongoing toxic degeneration [24,25]. The actual time course of dopaminergic degeneration after 6-OHDA administration in rats has already been described in the literature in detail and is completed 4 weeks after the procedure [3]. In addition, we already demonstrated the stability of the functional deficit over up to 18 weeks [2]. In the present study, we measured the extent of striatal dopamine depletion with a decrease in dopamine levels of ≈98% in the dorsomedial striatum close to the ventricular surface (**Suppl.** Fig. S1**b**). In a previous, unpublished cohort with a similar experimental design and similar apomorphine-induced rotational behaviour results (mean 7.3 ± 0.5 turns/min compared to 6.5 turns/min (6-$OHDA_{SHAM}$) and 8.2 turns/min (6-$OHDA_{STIM}$) in the present study; **Suppl.** Fig. S1**a**), we performed immunohistological quantifications of midbrain dopaminergic neurons in the substantia nigra pars compacta and found an excessive depletion of > 97% of nigral dopaminergic neurons in the lesioned compared to the non-lesioned hemisphere (**Suppl.** Fig. S1**c,d**).

Regarding stimulation effects, we only detected minor alterations in growth factor levels in the basal ganglia with an increase in BDNF and GDNF levels after one week of STN-DBS exclusively in the entopeduncular nucleus, a region that receives substantial input from the subthalamic nucleus, the actual site of stimulation [35,36]. In addition, we even found detrimental effects of STN-DBS on neurotrophin expression in cortical areas, such as the M2 and parietal cortices. These findings are in contrast to previous studies in the field, in which STN-DBS induced an increase in BDNF levels and – more inconsistently – also in GDNF levels in different brain areas, e.g., the basal ganglia and certain cortical areas [24,25]. In contrast to our study, Spieles-Engemann and colleagues normalized their protein concentrations in DBS-treated animals to SHAM cohorts, making comparison of results almost impossible. As mentioned above, and besides a shorter duration of STN-DBS of only seven days, the main difference in our study compared to previous publications was the type of 6-OHDA model. While the other studies used a model of striatal lesioning, which induces a partial dopaminergic degeneration resembling early- or mid-stage PD [37,38], our MFB lesion induces a severe dopaminergic deficit comparable to a later PD stage [39]. In addition, stimulation onset in our study was postponed to the completion of the degenerative process six weeks after the initial 6-OHDA application in contrast to 5 to 14 days in previous studies [39,40]. Therefore, such a severely degenerated system might no longer be able to respond to external stimuli, such as high-frequency stimulation.

Our study is limited regarding its small animal numbers per group due to the very high experimental effort in such cohorts. Nevertheless, the final group sizes are in the range of similar experimental DBS studies in small animal models [4,24,41]. We tried to minimize this limitation by using closely matched groups according to their apomorphine-induced rotational behavior, which exhibits modest correlations between dopaminergic cell loss and test results [42]. In addition, since the experiments were designed as a pilot study, we included only male animals, which might have influenced the generalizability of our results. However, the literature is quite controversial about the actual influences of sex and sex hormones on neurotrophin levels [43–46]. In contrast to Faust and colleagues [25], we performed unilateral electrode implantations also in healthy cohorts. This might underestimate the influences of inflammatory responses and local scar formation due to the implantation procedure on growth factor levels [47,48], though STN-DBS has already been shown to reduce neuroinflammation [49]. On the other hand, electrode implantations and associated foreign body reactions are a prerequisite for targeted electrical stimulation in DBS and do not necessarily need to be controlled for. Furthermore, the 6-OHDA model – though it has been characterized extensively over several decades – does not adequately reflect the neuropathological and clinical hallmarks of human PD, such as α-synuclein accumulation and chronic progressive course of the disease [50]. To date, there is only a single study on the interplay of BDNF and STN-DBS in a preclinical model of synucleinopathy, which showed increased striatal BDNF levels, though no impact on α-synuclein accumulation and neuroinflammation [26].

In line with clinical findings, the lack of adaptivity in a fully degenerated dopaminergic system might explain the absence of convincing evidence of neuroprotection or disease-modification of STN-DBS in human PD. As recently reviewed by Mahlknecht and colleagues [51], STN-DBS induces long-term improvement of cardinal motor symptoms and disability over more than ten years [52,53]; however, both neuropathological studies and functional imaging data argue against a relevant disease modification [6,7]. Regarding the neuroprotective effect of BDNF and GDNF, most preclinical studies in animal models of PD applied neurotrophins before the toxic damage to the dopaminergic system, resulting in a much more pronounced neuroprotective effect than vice versa [19,54,55]. To date, clinical studies on neurotrophins in human PD have only been conducted for GDNF enhancements, with so far disappointing results [20,22,23].

Our study indicates that putative neuroprotective effects of STN-DBS mediated via growth factor expression might not be evident in a condition of completed dopaminergic deficiency similar to late-stage Parkinsońs disease. Future studies might assess these findings in models with neuropathological findings similar to the human disease, e.g., intraneuronal α-synuclein accumulations.

CRediT authorship contribution statement

Meike Statz: Formal analysis, Writing – original draft. **Frederike Schleuter:** Investigation. **Hanna Weber:** Investigation, Formal analysis. **Maria Kober:** Methodology, Investigation. **Franz Plocksties:** Resources. **Dirk Timmermann:** Resources, Supervision. **Alexander**

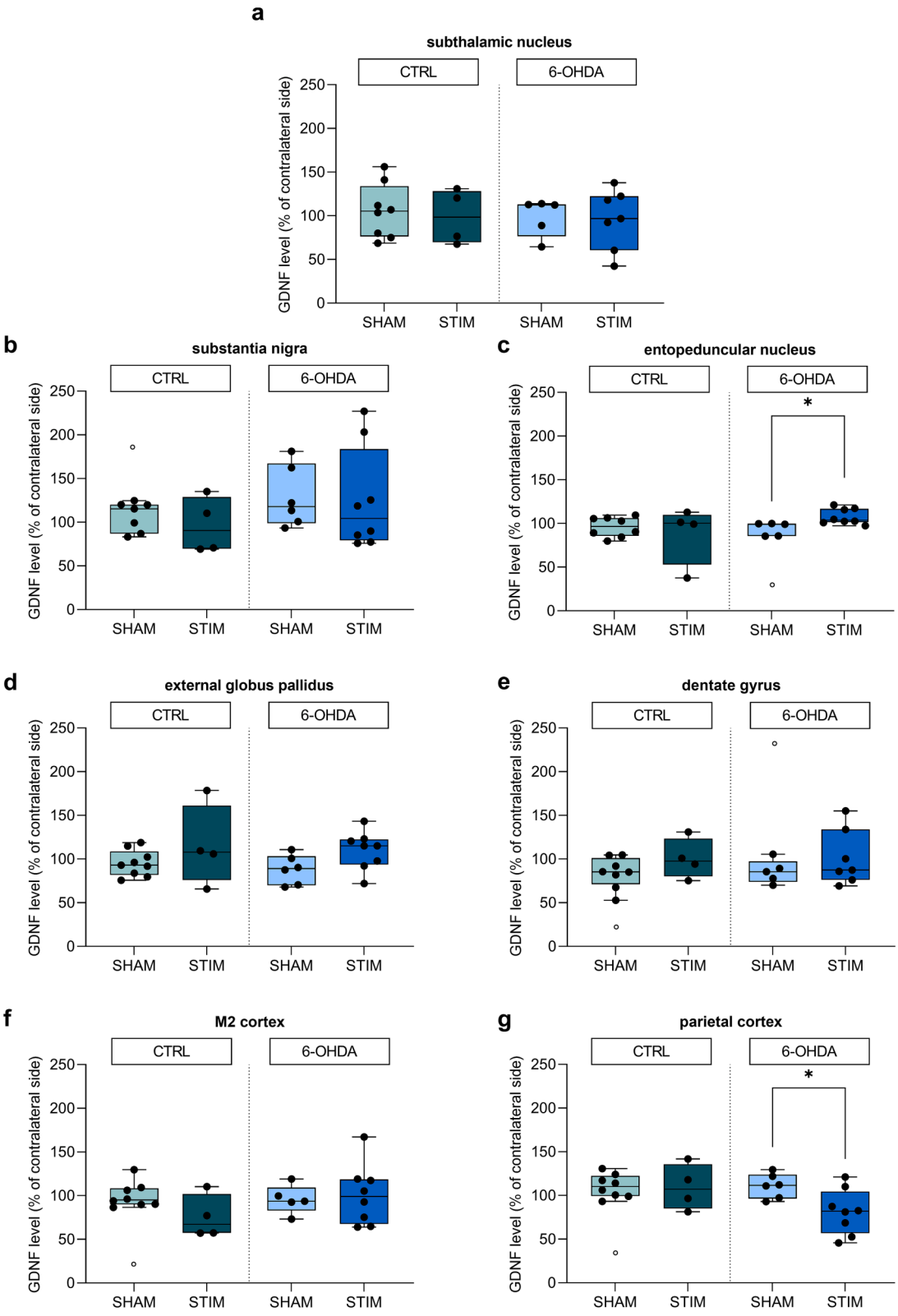

Fig. 2. GDNF expression levels after one week of sham stimulation (SHAM) or STN-DBS (STIM) in healthy control animals (CTRL) or in 6-OHDA-lesioned hemiparkinsonian rats (6-OHDA) normalized to contralateral non-lesioned, non-stimulated hemispheres, respectively. (a-h) Expression levels of GDNF normalized to contralateral, non-lesioned hemisphere without electrode placement as detected by ELISA in the following brain regions: subthalamic nucleus **(a),** substantia nigra **(b),** entopeduncular nucleus **(c),** external globus pallidus **(d),** dentate gyrus **(e),** supplementary motor cortex (M2 cortex; **f**) and parietal cortex **(g).** Abbreviations: SHAM – sham stimulation; STIM – verum stimulation; CTRL – control; 6-OHDA – 6-hydroxydopamine; GDNF – glial cell line-derived neurotrophic factor. Data are presented as mean with all individual values; outliers were defined as all values ± 1.5x S.D.; $P < 0.05$ were deemed significant after unpaired two-sided Student's *t*-test **(a,d,f,g)**, Welch's *t*-test **(e)** or Mann Whitney *U* test **(b,c)**, as appropriate.

Storch: Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Mareike Fauser:** Visualization, Supervision, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

F.S. included these data in her masteŕs thesis. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) through the Collaborative Research Centre CRC 1270 “Electrically Active Implants” (DFG; SFB 1270/1,2 – 299150580) to A.S., M.S. and H.W. M.F. was supported by the CRC 1270 “Electrically Active Implants” (DFG; SFB 1270/1 – 299150580) through the rotation position program for clinician scientists and by the Else Hirschberg Womeńs Advancement Program of the University Medical Center Rostock. F.S. was supported by the CRC 1270 “Electrically Active Implants” (DFG; SFB 1270/2 – 299150580) through an Integrated Research Training Program fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neulet.2023.137459.

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