Differential effects of deep brain stimulation and levodopa on brain activity in Parkinson's disease

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Abstract

Levodopa is the first-line treatment for Parkinson's disease, although the precise mechanisms mediating its efficacy remain elusive. We aimed to elucidate treatment effects of levodopa on brain activity during execution of fine movements and to compare them with deep brain stimulation of the subthalamic nuclei.

We studied 32 patients with Parkinson's disease using functional magnetic resonance imaging during execution of finger-tapping task, alternating epochs of movement and rest. The task was performed after withdrawal and administration of a single levodopa dose. A subgroup of patients (n=18) repeated the experiment after electrode implantation with stimulator on and off.

Investigating levodopa treatment, we found a significant interaction between both factors of treatment state (off, on) and experimental task (finger tapping, rest) in bilateral putamen, but not in other motor regions. Specifically, during the off state of levodopa medication, activity in the putamen at rest was higher than during tapping. This represents an aberrant activity pattern probably indicating derangement of basal ganglia network activity due to lack of dopaminergic input. Levodopa medication reverted this pattern, so that putaminal activity during finger tapping was higher than during rest, as previously described in healthy controls. Within-group comparison with deep brain stimulation underlines the specificity of our findings with levodopa treatment. Indeed, a significant interaction was observed between treatment approach (levodopa, deep brain stimulation) and treatment state (off, on) in in bilateral putamen.

Our functional MRI study compared for the first time the differential effects of levodopa treatment and deep brain stimulation on brain motor activity. We showed modulatory effects of levodopa on brain activity of the putamen during finger movement execution which were not

observed with deep brain stimulation.

Keywords

Parkinson's disease, deep brain stimulation, functional magnetic resonance imaging, levodopa, dopaminergic treatment

List of abbreviations

CDT	Cluster-defining threshold
DBS	Deep brain stimulation
DBS-OFF	Deep brain stimulation, off state
DBS-ON	Deep brain stimulation, on state
EPI	Echo planar imaging
FIR	Finite impulse response
fMRI	Functional magnetic resonance imaging
FWE	Family-wise error
GLM	General linear model
GPi	Globus pallidus internus
HRF	Hemodynamic response function
LEFT	Left hand finger tapping

LDOPA	Levodopa
LDOPA-OFF	Levodopa, medication off state
LDOPA-ON	Levodopa, medication on state
MDS	International Parkinson and Movement Disorder Society
MNI	Montreal neurological institute
MP-RAGE	Magnetization prepared rapid acquisition gradient echo
NIfTI	Neuroimaging informatics technology initiative
OFF	Treatment off state
ON	Treatment on state
PD	Parkinson's disease
REST	Resting, experimental condition
RIGHT	Right hand finger tapping
SPM	Statistical parametric mapping
STN	Subthalamic nucleus
TAP	Finger tapping, experimental condition
TE	Echo time
TIME	Time factor
TR	Repetition time
UCL	University College London
UPDRS	Parkinson's Disease Rating Scale
UTHSCSA	University of Texas Health Science Center at San Antonio

Introduction

Parkinson's disease (PD) is a frequent neurodegenerative disease characterized by progressive and relentless loss of motor functions (Fahn, 2003; Poewe *et al.*, 2017). Cardinal motor symptoms with diagnostic validity for PD are bradykinesia combined with rigidity and/or tremor at rest (Postuma *et al.*, 2015). These symptoms are often accompanied by loss of postural reflexes, forward bended posture and freezing, but also by non-motor manifestations, such as sleep disturbances, constipation, mild cognitive impairment and hallucinations (Fahn *et al.*, 2011). The symptomatology is burdensome for patients and limits everyday activities. No therapeutic option is currently available to stop or revert the neurodegenerative process, but symptomatic treatments are effective and widely used. The most common pharmacological treatments for PD target the dopaminergic system, exploiting either levodopa (LDOPA), a precursor of dopamine in the brain, or dopamine agonists (Connolly and Lang, 2014). Indeed, a marked loss of dopaminergic nigro-striatal neurons is the most evident brain abnormality in PD, and the pharmacological restoration of dopamine transmission leads to considerable symptom attenuation (Poewe *et al.*, 2017). Therefore, LDOPA and dopamine agonists represent the foundation of PD therapy for decades and are the first-choice interventions in early disease stages. Although LDOPA treatment was already introduced in 1961, its precise mechanisms of action in the brain were largely unknown (Hornykiewicz, 2010). Several studies tried to clarify how LDOPA modulates brain functions. In the last two decades functional magnetic resonance imaging (fMRI) studies have investigated the brain activity in patients during the execution of simple movements while unmedicated (LDOPA-OFF) and/or after taking the medication (LDOPA-ON) (Haslinger *et al.*, 2001; Rowe *et al.*, 2008; Kraft *et al.*, 2009; Maillet *et al.*, 2012; Herz *et al.*,

2014; Michely *et al.*, 2015). Herz *et al.* (2014) performed a meta-analysis showing that, during movements, PD patients OFF medication compared to controls presented a reduced activity in the posterior putamen and a mixed pattern of increased and decreased functional activations in cortical regions, encompassing supplementary and primary motor areas and inferior and superior parietal lobes. Of note, dopaminergic medication attenuated the deficits in the posterior putamen and reduced the hyperactivation of the primary motor cortex. However, studies included in the meta-analysis presented heterogeneous movement paradigms and experimental protocols, thus limiting the generalizability of the findings. Previous results (Holiga *et al.*, 2012; Holiga *et al.*, 2013) indicated that LDOPA increases putamen activation during finger tapping and underlined the importance to control for motor performance and severity of clinical symptoms for improved sensitivity of fMRI. To date, a clear and extensive evaluation of the effects of LDOPA on brain functions during movement execution is still lacking.

Although LDOPA leads to excellent clinical improvements during the first years of treatment, it is also associated with the emergence of severe side effects, affecting both motor and cognitive/behavioral domains, and its efficacy degrades after years of treatment (Fahn, 1989; Obeso *et al.*, 2000). Deep-brain stimulation (DBS) was introduced as an additional treatment option for PD, especially when the pharmacological therapy is less beneficial and its side effects are intolerable (Moro and Lang, 2006; Bronstein *et al.*, 2011). DBS is based on the electrical stimulation of deep brain nuclei with high frequencies, typically focused on either the globus pallidus internus (GPi) or the subthalamic nucleus (STN) in PD (Benabid *et al.*, 2009; Bronstein *et al.*, 2011). Indeed, PD induces brain abnormalities in the firing patterns of the basal ganglia, in particular generating hyperactivity of the GPi and the STN that in turn lead to suppression of

thalamo-cortical activity (Galvan *et al.*, 2015). The specific mechanisms of DBS are not completely understood, and likely comprehend a variety of mechanisms related to the stimulation (e.g. desynchronization of aberrant oscillations, inhibition of abnormal firing) and not only to the lesion effect (Benabid *et al.*, 2009). It is thus expected that the therapeutic effects of LDOPA and DBS should be mediated by different mechanisms. Previous studies investigated differential effects of DBS and LDOPA on movement performance (Rocchi *et al.*, 2002; Vingerhoets *et al.*, 2002; Timmermann *et al.*, 2008; Bäumer *et al.*, 2009) but did not focus on the modulation of brain activations during movement execution.

In this study, we aim to characterize the modulatory effect of LDOPA on brain activations during movement execution. To this aim we collected fMRI data from 32 PD patients in both LDOPA-OFF and LDOPA-ON states employing a sequential finger tapping task. Sequential finger-thumb opposition is a useful diagnostic tool to assess motor impairment in PD, and specifically bradykinesia. It is included in the unified Parkinson's Disease Rating Scale (UPDRS-III) and has been shown to be more sensitive compared to gross movements (e.g. forearm pronation-supination) because it requires fine motor control (Agostino *et al.*, 1998; Agostino *et al.*, 2003). We hypothesized that LDOPA modulates basal ganglia activity during fine movement execution and expected that fMRI investigations shed light on treatment-induced modulation of the interplay between basal ganglia and cortical regions. The basal ganglia have been shown to influence cortical activity via two mechanisms: (i) facilitation of motor activity via the thalamo-cortical projections, and (ii) inhibition of competing motor patterns from unwanted movements (Mink, 1996; Rubchinsky *et al.*, 2003). To further test the specificity of our results, a subgroup of patients, who underwent DBS surgery, performed the same experimental protocol for LDOPA-

OFF and LDOPA-ON, and also after DBS implantation with the electrodes switched on (DBS-ON) or off (DBS-OFF). Comparing ON and OFF treatment effects between LDOPA and DBS provides a rare perspective into treatment-related brain activity changes.

Methods

LDOPA cohort

Functional MRI was performed in 32 PD patients (Hoehn-Yahr stages II-III, 26 males, age 56.1±7.7 years, mean±standard deviation), disease duration 12.2±2.5 years, levodopa treatment duration 9.0±3.0 years. The selection of relatively young patients was based on the rationale that this group was planned to undergo the DBS procedure. Clinical assessment and MRI were performed in two sessions, without dopaminergic medication and after acute levodopa challenge: LDOPA-OFF and LDOPA-ON. Four days before all measurements, dopamine agonists were substituted by equivalent doses of levodopa (Tomlinson *et al.*, 2010). Other anti-PD medications (selegiline, amantadine, anticholinergics) were suspended. After an overnight withdrawal of levodopa (at least 12 hours), clinical and first fMRI data were obtained in the LDOPA-OFF session. Clinical and second fMRI assessment with medication was performed in the LDOPA-ON session approximately one hour after administration of 250/50mg of levodopa/carbidopa after the patient's clinical improvement. PD symptoms were assessed with the UPDRS motor score (part III) in both sessions. All patients gave informed written consent. All procedures conformed to the Declaration of Helsinki. The study protocol had been approved by the Ethics Committee of the General University Hospital in Prague.

LDOPA-DBS cohort

For a subgroup of 18 patients (Hoehn-Yahr stages II-III, 15 males, age 54.6±7.1 years, disease duration 12.2±2.7 years, levodopa treatment duration 9.5±3.1 years), implantation of the DBS system was performed separately in two surgeries following previously described procedures (Jech *et al.*, 2001). Within 15.5±12.5 days after the LDOPA-OFF and LDOPA-ON sessions, the first DBS surgery was carried out in awake state, during which the patient with attached Leksell stereotactic frame and motor microdriver underwent electrophysiology mapping of the subthalamic area with five parallel microelectrodes. Then, the intraoperative stimulation by macroelectrode was performed in a region with a neuronal signal typical for STN to confirm clinical benefit and to monitor potential adverse effects of DBS. The macroelectrode was eventually replaced by the permanent electrode (type 3389, Medtronic, MN) connected to external leads.

Within 1-3 days after the first surgery, DBS-OFF and DBS-ON with clinical assessment and fMRI were scheduled when the electrodes were externalized and connected to an external stimulator working in bipolar mode (Dual Screen 3628, Medtronic, Minneapolis, MN). Note that clinical assessment in the DBS-ON session used bilateral STN DBS while fMRI was performed using unilateral STN DBS contralateral to finger tapping. The DBS parameters were kept below threshold for dyskinesias and above the threshold for rigidity and akinesa in all patients (dyskinesias were not observed with STN DBS during MRI). Since the therapeutic effect of STN DBS might last even after switching off the neurostimulator, the DBS-OFF and DBS-ON conditions were randomized across the group to avoid order effects. Implantation of the internal pulse generator in the subclavial region was done under general anesthesia one day after fMRI.

MRI data acquisition

Functional MRI data were obtained in two sessions (LDOPA-OFF and LDOPA-ON) for the LDOPA cohort, and in four sessions (LDOPA-OFF, LDOPA-ON, DBS-OFF, and DBS-ON) for the LDOPA-DBS subgroup. In each session, the patients performed a simple tapping task for each hand separately while lying supine with both hands in a resting position, resulting in four and eight data sets for the LDOPA and the LDOPA-DBS cohorts, respectively. The finger tapping experiment consisted of 25 consecutive movement and rest epochs (TAP and REST), each lasting 10 s, resulting in a total session duration of 500s. During rest epochs, a visual 'rest signal' (centered static red fixation cross on a black background) was presented on a projection screen, whereas during movement epochs, 10 pacing 'movement cues' (yellow square behind the fixation cross displayed for 100 ms) were presented with a frequency of 1 Hz. While viewing the 'rest signal', patients were instructed to remain motionless. They had to perform a unilateral index finger-thumb opposition whenever the 'movement signal' appeared.

All data were acquired with a 1.5-T Siemens Symphony scanner (Siemens, Erlangen, Germany) using a gradient-echo echo planar imaging (EPI) sequence (TR=1000 ms, TE=54 ms, nominal in-plane resolution 3×3 mm^2, 3 mm slice thickness, 1 mm inter-slice gap). Ten oblique slices were acquired, oriented along the central sulcus and covering the rolandic cortex, basal ganglia and thalamus in a region between the anterior border of the caudate nuclei and the posterior border of the red nuclei. For image registration and further morphological analysis, axial T1-weighted magnetization prepared rapid acquisition gradient echo (MP-RAGE; TR=2140 ms, TE=3.93 ms) and T2-weighted turbo spin echo (TR=5520 ms, TE=86 ms, 4 mm slice thickness)

images were acquired.

Data pre-processing and first-level analysis

Functional MRI data were processed using SPM12 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK) and Matlab (The MathWorks Inc., Natick, MA). Standard pre-processing included realignment, normalization to the Montreal Neurological Institute (MNI) space based on the unified segmentation approach (Ashburner and Friston, 2005), and spatial filtering using a Gaussian kernel with 10-mm full width at half maximum. The quality of pre-processing was carefully assessed by visual inspection to exclude misalignment and segmentation faults.

Each data set was further processed by least-squares parameter estimation using the general linear model (GLM) with serially correlated observations (first-level analysis) (Friston *et al.*, 2002a; Friston *et al.*, 2002b). A high-pass filter was used for baseline correction with a cutoff frequency of 1/96 Hz. The design matrix was generated with the onsets of all 50 TAP and REST blocks. Using the standard model in SPM12, the stimulus function (generated from the onsets) was convolved with a canonical hemodynamic response function (HRF) (Friston *et al.*, 1998) including its first derivative resulting in two columns for each condition. Finally, parameter maps (beta images) were estimated for both conditions, and a contrast image was generated by subtracting the beta images (TAP–REST).

To investigate dynamic changes during finger tapping and rest in relationship to medication, another first-level analysis was performed using a design matrix generated with a finite impulse response (FIR) model for an entire 20-s cycle of TAP and REST. Thus, instead of 4 basis functions in the model described above (2 conditions and 2 temporal derivatives), the FIR

model was implemented with 20 basis functions (i.e., 1 basis function for each functional volume of the cycle). Parameter estimation was performed for each individual data set resulting in 20 parameter maps. Note that this FIR model did not include any assumption about the shape of the HRF.

Statistical analysis (second-level analysis)

For all types of analyses described below, significant differences were obtained with $P<0.05$ using family-wise error (FWE) correction at the voxel level as well as a minimum cluster size of 25 voxels. This combination of voxel-level FWE correction plus a minimum cluster size substantially reduces the appearance of false positive clusters (see voxel inference displayed in Figure 1 in Eklund *et al.*, 2016, right column). However, in order to reduce false negative findings, we additionally show all results using an uncorrected voxel-threshold of $P<0.001$ in combination with FWE correction at the cluster-level at $P<0.05$ in the supplementary material. Note that this "cluster-defining threshold" (CDT) procedure is prone to produce false positive findings and should be used carefully (see SPM results Figure 1 in Eklund *et al.*, 2016, middle column, using a CDT of $P<0.001$). Four types of second-level analyses were performed for the LDOPA cohort:

(1.1) The first analysis was performed with all TAP–REST contrast images using a flexible factorial design with factors LEFT/RIGHT (finger tapping) and OFF/ON (LDOPA state) for 32 PD patients (i.e., 32×2×2=128 contrast images). The model was implemented with both factors as main effects for investigating both OFF/ON- and LEFT/RIGHT-differences in a paired fashion. After parameter estimation, contrast images and *t*-statistics were computed for OFF/ON- and LEFT/RIGHT-differences.

(1.2) Two additional group analyses were performed using the individual beta images to find OFF/ON- and LEFT/RIGHT-differences for TAP and REST conditions separately (identical design as above with 128 beta images for both TAP and REST). Contrast images and *t*-statistics were computed for OFF/ON- and LEFT/RIGHT-differences separately for the TAP and REST condition.

(1.3) To investigate interactions between TAP/REST- and OFF/ON-differences, a model was generated with factors TAP/REST, LEFT/RIGHT and OFF/ON. The design matrix was created implementing an interaction of factors TAP/REST and OFF/ON (factor LEFT/RIGHT as main effect; 32×2×2×2=256 beta images). Two contrasts for testing both directions of interaction between factors TAP/REST and OFF/ON were computed and processed with *t*-statistics. An *F*-contrast was computed to investigate the amount of variance explained by both factors TAP/REST and OFF/ON within the model.

(1.4) In addition to the above analyses based on the canonical HRF, another second-level analysis was based on the beta images obtained with the FIR model. Here, we generated a model with the factors LEFT/RIGHT, OFF/ON and TIME (represented by the 20 basis functions for a full TAP-REST-cycle). Note that instead of two levels of each factor in the other analyses, the factor TIME included 20 levels. The design matrix was created implementing an interaction of factors TIME and OFF/ON (factor LEFT/RIGHT as main effect; 32×20×2×2=2560 beta images). Two contrasts for testing both directions of interaction between the factors TIME and OFF/ON were computed and further processed with *t*-statistics. An *F*-contrast was computed to investigate the amount of variance explained by both factors.

Five types of second-level analyses were additionally performed for the LDOPA-DBS cohort:

(2.1) The first analysis aimed at detecting activity differences between OFF and ON states of both treatment approaches using the TAP–REST contrast images. The OFF-ON comparison for LDOPA treatment was identical as in analysis (1.1) in the LDOPA cohort. The same OFF-ON analysis was also performed for the DBS-OFF and DBS-ON sessions (each 18×2×2=72 contrast images). The model was implemented with both factors OFF/ON and LEFT/RIGHT to investigate differences in a paired fashion. After parameter estimation, contrast images and *t*-statistics were computed for OFF/ON- and LEFT/RIGHT-differences.

(2.2) After investigating OFF-ON differences for LDOPA and DBS separately, a three-factorial model was generated with the factors LDOPA/DBS, OFF/ON and LEFT/RIGHT (interaction between factors LDOPA/DBS and OFF/ON, factor LEFT/RIGHT implemented as main effect; 18×2×2×2=144 TAP–REST contrast images). Two contrasts for testing both interaction directions were computed and further processed with t-statistics. An *F*-contrast was computed to investigate the amount of variance explained by both factors LDOPA/DBS and OFF/ON.

(2.3) The third analysis aimed at detecting activity differences upon the treatment change using all TAP–REST contrast images in both ON-states (two-factorial model with factors LDOPA/DBS and LEFT/RIGHT; 18×2×2=72 contrast images). Subsequently, the same analysis was repeated to compare the TAP–REST contrast images from the LDOPA-OFF and DBS-OFF session. Both models were implemented with factors LDOPA/DBS and LEFT/RIGHT as main effects, and contrast images and *t*-statistics were computed for LDOPA/DBS- and LEFT/RIGHT-differences. Note that the difference between both ON-ON- and OFF-OFF-effects can be expressed by the same interaction analysis as in (2.2).

(2.4) Further analyses included the beta images to investigate both conditions TAP and

REST separately. To study OFF-ON differences for LDOPA and DBS, beta images of the TAP and the REST condition were used in a flexible factorial model implementing an interaction between factors OFF/ON and TAP/REST (factor LEFT/RIGHT as main effect; 18×2×2×2=144 beta images). In addition to their interaction, we also studied the main effect of both factors OFF/ON and TAP/REST and computed the *F*-contrasts to look at contrast estimates.

(2.5) Finally, an analysis was performed including beta images (TAP and REST) from all sessions (LDOPA-OFF, LDOPA-ON, DBS-OFF, DBS-ON). A flexible factorial model was created using factors LDOPA/DBS, OFF/ON, and TAP/REST as an interaction, and LEFT/RIGHT as main effect (18×2×2×2×2=288 beta images). Potential interaction between factors OFF/ON and TAP/REST were first tested separately for LDOPA treatment and DBS. Thereafter, a statistical analysis was performed including all three factors. In addition, we computed an *F*-contrast containing all columns of the design matrix associated with the three factors. This contrast was used to plot contrast estimates within regions of interest.

Visualization

Figures showing orthogonal brain slices were generated using the Mango software v4.1 (Research Imaging Institute, UTHSCSA) with the 'Build Surface' option and the 'Cut Plane' feature. Finally, SPM *T*-maps were imported using the 'Add Overlay' function. The bar plots for the contrast estimates were directly obtained from SPM and plotted with Matlab.

Data availability

Datasets analyzed during the current study are available on reasonable request. All data will be

anonymized. Functional MRI data will be available in pre-processed fashion in the NIfTI format without any personal meta-data.

Results

LDOPA cohort

After the overnight withdrawal of dopaminergic treatment, the patients showed moderate PD symptoms in the LDOPA-OFF session with a UPDRS-III score of 33.0±8.5. One hour after the single dose of 250/50 mg of levodopa/carbidopa, all 32 patients improved in the LDOPA-ON session showing fewer PD symptoms resulting in a decreased UPDRS-III score (11.2±5.3). A paired *t*-test showed a significant decrease with $P<10^{-17}$. The analysis of the fMRI data revealed significant results in the motor system, particularly in the primary left and right motor cortex as well as in the left and right putamen. Note that all results described below were obtained with $P<0.05$ using FWE correction at the voxel-level in order to prevent false-positive findings (Eklund *et al.*, 2016). However, all results were re-checked using the more liberal CDT approach in order to reduce false negative findings (see tables in the supplementary material). All reported non-significant results remained non-significant with the CDT approach.

(1.1) The pairwise comparison of the TAP–REST contrast images between the LDOPA-OFF and LDOPA-ON states (i.e., the OFF/ON factor in the GLM) revealed a significant increase in the left and right putamen (see *O* in Table 1; Supplementary Table 1; Figure 1). We did not find any significant OFF/ON-decrease of the TAP-REST contrast. As a verification of the experimental design and plausibility of the data analysis, we also performed a pairwise comparison of the TAP–REST contrast images between finger tapping with the left and right hand (i.e. the LEFT/RIGHT

factor in the GLM). As expected, we obtained significant LEFT–RIGHT and RIGHT–LEFT differences in the contralateral primary motor cortex, i.e., in the right and left primary motor cortex, respectively.

(1.2) The second analysis aimed at investigating LDOPA OFF/ON-differences for the TAP and the REST condition separately using the beta images. For the TAP condition, we obtained a significant increase of brain activity difference with levodopa medication in the left putamen (see A in Table 1 and A in Figure 2; see also Supplementary Table 1). (In the right putamen, we observed an activity increase with an uncorrected threshold of $P<0.001$, see values in Table 1 plotted in grey.) Using the beta images from the REST condition, we obtained an inverse pattern showing significant activity decrease with levodopa medication in both left and right putamen (see B in Table 1 and B in Figure 2). We also looked at inverse contrasts for brain activity decrease with TAP, and for brain activity increase with REST, however, we did not find any significant results. For both analyses with TAP and REST, we also looked at the LEFT/RIGHT factor that was included in both models looking at LEFT–RIGHT and RIGHT–LEFT differences. With TAP, we obtained similar results as described under (1.1), however, when using the REST beta images, we obtained an inverse pattern showing LEFT–RIGHT and RIGHT–LEFT differences in the ipsilateral primary motor cortex, i.e., in the left and right primary motor cortex, respectively.

(1.3) The third analysis aimed at investigating the differential results on levodopa-induced brain activity change with the TAP and the REST condition. Using a full model including all beta files, we obtained a significant interaction between the factors TAP/REST and OFF/ON showing a differential pattern of putamen activity change with levodopa treatment in the TAP (increase) and the REST (decrease) condition (see A-B in Table 1 and A-B in Figure 2; see also Supplementary

Table 1). The inverse interaction contrast did not show significance, i.e. we did not observe any decrease of putamen activity during TAP or any increase of putamen activity during REST with levodopa treatment. Finally, an F contrast including all experimental conditions related to the interaction between the factors TAP/REST and OFF/ON yielded a differential pattern of brain activity change in the left and right putamen with dopaminergic treatment during finger tapping and rest. Figure 3 shows the contrast estimates using the local maxima of the TAP/REST-OFF/ON interaction. The putamen activity decrease in the OFF vs. ON state during REST appeared much more prominent than the increase during TAP, which is in line with our other analyses, particularly with analysis (1.1) using the TAP–REST contrast images. To understand the TAP–REST increase in the ON state in Figure 1, REST bars shown in Figure 3 would need to be flipped because of the subtraction of REST in the contrast TAP–REST.

(1.4) In the fourth analysis, employing the FIR model instead of an HRF, we obtained a significant interaction between TIME and OFF/ON in the left and right putamen. Contrast estimates for each basis function allowed investigating the temporal dynamics of the fMRI signal in the OFF and in the ON state of levodopa medication without prior assumptions about form and shape of the HRF. Looking at contrast estimates for the left and right putamen, we obtained different response patterns in the two different medication states. Most interestingly, we obtained an HRF-shaped response with the FIR model that supports the usefulness of our HRF models. In addition to the interaction analysis between the factors TIME and OFF/ON, the main effect of the LEFT/RIGHT factor yielded a significant LEFT–RIGHT and RIGHT–LEFT differences in the contralateral primary motor cortex, i.e., in the right and the left primary motor cortex, respectively. Note that we did not find a levodopa-related differential response in the motor

cortex. Here, we obtained the same response pattern of brain activity in both medication states showing an increase with finger tapping.

LDOPA-DBS cohort

The subgroup of 18 patients with additional DBS showed a similar improvement of PD symptoms with both treatment approaches. After withdrawal of dopaminergic treatment, patients showed PD symptoms in the LDOPA-OFF session with a UPDRS-III score of 31.7±8.8. One hour after the single dose of 250/50 mg of levodopa/carbidopa, all patients improved in the LDOPA-ON session resulting in a decreased UPDRS-III score (9.3±4.4; $P<10^{-9}$). After implanting the electrodes, patients showed PD symptoms in the DBS-OFF session with a UPDRS-III score of 23.0±6.0. The observed PD symptoms reduction in DBS-OFF was significantly larger compared to LDOPA-OFF (the so-called microlesion effect, $P<0.0003$). Finally, we obtained a significant UPDRS-III decrease in the DBS-ON session (10.0±4.6) compared to both LDOPA-OFF ($P<10^{-7}$) and DBS-OFF ($P<10^{-9}$).

Although LDOPA-ON and DBS-ON led to similar improvement of UPDRS-III scores, analysis of the fMRI data revealed different patterns of brain activity change for LDOPA treatment and DBS. We observed a differential pattern of putamen activity with LDOPA, however, not with DBS. Moreover, we found a significant difference between the LDOPA- and DBS-related putamen response patterns that reflect different mechanisms of both treatment approaches.

(2.1) The comparison between LDOPA-ON and LDOPA-OFF states yielded increased TAP–REST contrast under treatment in the left and right putamen (see A in Table 2 and A in Figure 4; see also Supplementary Table 2). Thus, the main finding from the entire LDOPA cohort (see O in Table 1 and Figure 1) was replicated with the LDOPA-DBS subgroup. The comparison between the DBS-ON and the DBS-OFF states showed no significant differences, neither positive nor

negative, even when using the CDT approach (see B in Table 2 and B in Figure 4; see Supplementary Table 2).

(2.2) In order to disentangle the LDOPA- from the DBS-effects obtained in the analyses described in (2.1) above, we investigated the interaction between the factors LDOPA/DBS and OFF/ON including all eight TAP–REST contrast images for each subject within a single model. Here, we found a significant interaction between the factors LDOPA/DBS and OFF/ON in both left and right putamen (see A-B in Table 2 and A-B in Figure 4). No further interaction was observed in other brain regions. The inverse interaction contrast (activity decrease with levodopa treatment) did not yield significant results.

(2.3) Although differences between the UPDRS-III scores in the LDOPA-ON and DBS-ON sessions were insignificant (p=0.58), the fMRI data revealed significant brain activity differences between both ON states. Using the TAP–REST contrast images, we obtained a significant decrease of the TAP–REST contrast with the treatment switch (from LDOPA-ON to DBS-ON) in the left and right putamen (see C in Table 2 and C in Figure 4; see also Supplementary Table 2). The opposite contrast of an increase of the TAP–REST difference with the treatment switch did not reveal significant results. Comparing the DBS-OFF and LDOPA-OFF sessions, we found a significant improvement of PD symptoms, presumably due to microlesion effects. Such decreased UPDRS-III values were accompanied by a decrease of the TAP–REST contrast. Using the same flexible factorial design but including both OFF states instead of the ON states, we observed significant activity decrease in various subcortical regions in the vicinity of the anterior thalamus and the internal globus pallidus (see D in Table 2, and D in Figure 4). The inverse contrast of an increase of the TAP–REST difference upon switching from LDOPA-OFF to DBS-OFF did not reveal significant

results. Using the same analysis as in (2.2), we found a significant interaction between the factors LDOPA/DBS and OFF/ON in both left and right putamen (see C-D in Table 2 and C-D in Figure 4), which allows to separate treatment effects from microlesion effects. The interaction was observed in the left and right putamen but not in other subcortical regions that is in line with both ON-ON- and OFF-OFF-analyses.

(2.4) Investigating only DBS using the DBS-ON and the DBS-OFF session with the beta images from both the TAP and the REST condition, we were looking for a potential interaction between the factors OFF/ON and TAP/REST. However, in contrast to our findings with the LDOPA cohort, we did not find a significant interaction between both factors (both directions). Moreover, we did not find significant OFF-ON-differences, neither for the TAP nor for the REST condition (Figure 5). However, independent of the OFF or ON state of DBS, we found a reversed pattern of putamen and motor cortex activity. As expected, activity increased with tapping in the primary motor cortex, but decreased in left and right putamen (Figure 5). Note that a similar decrease in the putamen was observed for LDOPA-OFF (see Figure 3, left column, bars in red color) but not LDOPA-ON.

(2.5) The final analysis was performed using all beta images of the TAP and the REST condition for all sessions LDOPA-OFF, LDOPA-ON, DBS-OFF, and DBS-ON. The comparison between LDOPA-OFF and LDOPA-ON revealed a significant interaction between the factors OFF/ON and TAP/REST that is a replication of the result of analysis (1.3) for the subgroup of 18 PD patients of the LDOPA-DBS cohort. There was no significant interaction between the factors DBS-OFF/DBS-ON and TAP/REST, however, similar to analysis (2.4), we found significant brain activity decrease with finger tapping in the left and right putamen. The analysis including all three

factors LDOPA/DBS, OFF/ON, and TAP/REST did not reveal a significant interaction, however, using a merged contrast containing the interaction between LDOPA-OFF/LDOPA-ON and TAP/REST, and REST>TAP for both DBS-OFF and DBS-ON, we obtained significant clusters in the left and right putamen (Table 3; see also Supplementary Table 3).

Discussion

Using fMRI, we investigated brain connectivity alterations in PD patients related to acute treatment effects. Overall, we showed that, during finger tapping, LDOPA specifically modulates activity in the basal ganglia, but not in the motor cortex. We validated the robustness of our findings in two independent analyses and assessed their specificity for LDOPA through the comparison against DBS. To the best of our knowledge, our fMRI study is the first to present the comparison between therapeutic effects of LDOPA and DBS on brain motor activity.

Brain activity changes with LDOPA during finger-tapping

Concerning the LDOPA effects, the motor cortex in both LDOPA-ON and LDOPA-OFF conditions showed an activity pattern related to finger tapping consistent with the previous literature (Witt *et al.*, 2008; Gountouna *et al.*, 2010) and expectations from basic brain physiology (Kandel *et al.*, 2000). The REST condition was characterized by a low cortical activity, while tapping execution was associated with significant activity increases in the contralateral primary motor cortex and, in particular, in the hand areas. On the contrary, during the LDOPA-OFF state, the basal ganglia, and more specifically the bilateral putamen, showed an elevated activity during REST condition and a lower activity during the TAP execution. The LDOPA-ON state reverted this activity pattern

in the putamen, so that REST and TAP were associated, respectively, with lower and higher activities, thus resembling the pattern observed in cortical motor regions. Of note, we found a significant interaction in the putamen between levodopa medication (OFF/ON) and task (TAP/REST). We propose that the increased activity in the putamen during REST in LDOPA-OFF state might have a pathological meaning, reflecting the derangement of the basal ganglia network in PD as shown with electrophysiological recordings (Galvan and Wichmann, 2008; Galvan *et al.*, 2015). For example, Singh *et al.* (2016) reported with in vivo electrophysiology increased firing rates in striatal projections neurons (both from putamen and, to a lower extent, caudate nucleus) in PD patients. Nevertheless, a conclusive statement concerning the aberrant nature of the increased putaminal activity in LDOPA-OFF would require the comparison with a group of healthy controls, which was not available for the present study. Here, the previous literature on finger-tapping in healthy subjects might provide valuable information to clarify this point. Several studies, recently summarized in a quantitative meta-analysis, reported the brain functional correlates of finger tapping (Witt *et al.*, 2008). Gountouna *et al.* (2010) assessed their consistency across centers and the robustness against confounds such as scanner variability. Specifically, the TAP-REST comparison has been mostly associated with activations focused in the primary motor, premotor and supplementary motor areas, and also in the basal ganglia, thalamus and cerebellum. This means that the activation in the putamen in normal controls is higher in the TAP as compared to the REST condition. We observed this same pattern (TAP>REST) in PD patients for LDOPA-ON state, but not for LDOPA-OFF, thus supporting the idea that the LDOPA-OFF state represents a difference from a normal healthy cohort. Moreover, PD patients in our study showed reduced activations in the putamen during the TAP condition that was reversed by

the levodopa intake. A similar activity pattern in the putamen has been previously reported by Holiga et al. (2012; 2013) comparing LDOPA-OFF and LDOPA-ON in a subset of our cohort, and also in a meta-analysis of fMRI studies including 283 patients with PD compared to healthy controls (Herz *et al.*, 2014). The meta-analysis showed consistent reductions in the activity of the posterior putamen comparing LDOPA-OFF PD patients and controls during the execution of movement. Interestingly, consistently with our observations, this deficit was attenuated by levodopa medication. This finding supports the idea that neural activity in striatal regions is impaired due to the lack of nigrostriatal dopaminergic input in PD. Finally, at difference with our findings, previous investigations reported that the basal ganglia hypoactivity also associates with presumably compensatory hyperactivity in cortical and cerebellar regions in PD (Thobois *et al.*, 2000; Yu *et al.*, 2007). However, Haslinger *et al.* (2001) reported that only cerebellar hyperactivity has a compensatory meaning, while motor cortex hyperactivity relates to specific motor symptoms (i.e. upper limb rigidity).

As aforementioned, we report here the interaction between finger tapping task and levodopa medication in two independent subgroups of subjects, thus providing an internal validation of our results. However, for external validation, our results need to be replicated with different cohorts of PD patients. Note that validation is of particular importance as recent studies pointed at poor reproducibility as one of the major pitfalls of neuroimaging studies (Pernet and Poline, 2015). We further suggest using a particularly stringent correction for multiple comparisons (P<0.05 FWE at the voxel-level), thus minimizing the likelihood of false positives (Nichols and Hayasaka, 2003).

Differences between LDOPA and DBS

The comparison between LDOPA and DBS revealed that the modulation of basal ganglia activity during finger tapping is specific for LDOPA, and it is not a general feature of treatment effects in PD. Indeed, we found an interaction between LDOPA/DBS and OFF/ON factors in the bilateral putamen. This finding is in line with previous knowledge concerning the therapeutic activity of LDOPA and DBS. The former aims at restoring the impaired dopaminergic transmission in the nigro-striatal system (Connolly and Lang, 2014; LeWitt and Fahn, 2016), while the latter specifically interferes with the electrical signaling of the hyperactive subthalamic nuclei (Benabid *et al.*, 2009). More specifically, in ON treatment conditions, the activity associated with finger tapping was higher in the bilateral putamen with LDOPA than with DBS (i.e. LDOPA-ON>DBS-ON). Note that the DBS-ON condition comprises both the effect of active DBS itself and the mircolesion effect due to the electrode placement during the surgery that is known to modulate brain network organization and activity (Singh *et al.*, 2012; Holiga *et al.*, 2015). In the OFF condition, instead, we found an increased activity in various brain regions in the LDOPA-OFF state as compared to the DBS-OFF (i.e. as compared to the microlesion effect alone, LDOPA-OFF>DBS-OFF). This finding might be a result of the microlesion effect that attenuates the basal ganglia hyperactivity during motor execution (Jech *et al.*, 2012).

However, we were not able to show a specific difference between DBS-ON and DBS-OFF during finger tapping, even using the more liberal CDT approach in the correction for multiple comparisons. The relatively small sample size of the LDOPA-DBS cohort might be a factor that limits our ability to capture subtle changes due to DBS. Additionally, we recognize at least three

more reasons that might explain this negative finding. First, our analysis was focused on the core motor regions, including the motor and premotor cortex, basal ganglia and thalamus, but excluding several other areas whose activity might be modulated by the treatment. Indeed, previous fMRI studies during both motor activity and rest showed that the dopaminergic system has a broad influence on the brain, encompassing both motor and non-motor regions (Postuma and Dagher, 2005; Wu *et al.*, 2012; Ballarini *et al.*, 2018). For example, a previous resting-state fMRI study from our group showed that DBS, as compared to LDOPA, is associated with increases in functional interconnectedness of the motor cortical regions that are in turn more connected to the thalamus and the cerebellum (Mueller *et al.*, 2018). Second, the presence of electrodes in the brain after DBS surgery requires that implantation foci and neighboring regions are masked out from the analysis. This hinders the detection of treatment-related activity changes in deep brain regions in the proximity of the electrodes. It has been indeed proposed that DBS, through the electrical stimulation of the subthalamic nuclei, influences cortical activity either through reducing activity in the indirect pathway (Bergman *et al.*, 1990) or directly via the hyperdirect pathway (Nambu *et al.*, 2002; Akram *et al.*, 2017) of the basal ganglia. Third, the still present microlesion effect in the DBS-OFF state modifies brain activity related to finger tapping compared to the LDOPA-OFF state. This effect is known to be transitory and might hide from our analysis the 'true', long-term effect of DBS-ON (Jech *et al.*, 2012).

Limitations and Strengths

A first limitation of our study is the lack of a healthy control cohort that could provide additional information regarding the specificity of our finding for PD. However, the implemented study

design provided an unprecedented framework to investigate the core question of our research, namely the differential effects of LDOPA and DBS within PD patients. Indeed, since PD patients did not receive levodopa during the DBS part of the study, the pre-surgery LDOPA sessions can be used as a baseline for the post-surgery DBS one and vice versa. Of note, the distance between LDOPA and DBS sessions was on average about two-weeks, thus minimizing the impact of long-term disease-related changes. A second limitation of our study is the fixed order (OFF before ON) of clinical and fMRI assessments in the LDOPA condition. As the study was performed in a clinical setting typical for conventional LDOPA test, this experimental design was necessary to document responsiveness of dominant clinical symptoms to dopaminergic treatment routinely required before DBS implantation procedure. On the other hand, DBS-ON and DBS-OFF conditions were randomized, thus mitigating in the interaction analyses the fault of the fixed LDOPA order.

As an additional cautionary note for the interpretation of our results, one has to consider that fMRI sessions with DBS were run one to three days after surgery. Therefore, our findings likely reflect short-term effects of DBS, and we cannot exclude that further brain functional changes would come into play in a later – chronic – DBS stage. In addition, due to the randomized order of the DBS conditions DBS-ON and DBS-OFF, we were able to take the microlesion effect into account (Jech *et al.*, 2012). Finally, despite most of the current fMRI research is acquiring MRI data at 3T or even stronger magnetic field strength, we performed our measurements on a 1.5T device. This choice was imposed by safety concerns regarding the application of higher magnetic fields in patients with DBS and by recommendations of manufacturers producing implantable DBS devices (Tagliati *et al.*, 2009).

Conclusions

Our investigation provides an in-depth perspective on the effects of LDOPA therapy for PD on activity in the basal ganglia during finger movement execution. We showed a strong interaction between LDOPA effects and finger-tapping in the bilateral putamen, but not in the motor cortex. The LDOPA-OFF state was associated with an abnormal pattern of activity in the putamen, where the activity during REST exceeded that during TAP. The medication (LDOPA-ON) normalized this pattern, so that the activity in the TAP phase was larger than in the REST one, as reported for healthy controls (Witt *et al.*, 2008; Gountouna *et al.*, 2010). Moreover, the within-group comparison with DBS treatment highlighted the specificity of our findings for the LDOPA medication. Here, we found a significant interaction between LDOPA/DBS and OFF/ON in in the bilateral putamen, showing that LDOPA medication, but not DBS, has a modulatory effect on basal ganglia activity.

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Competing Interests

There are no competing interests.

Table captions

Table 1. List of significant clusters of brain activity change with levodopa treatment for the LDOPA cohort including 32 PD patients.

The upper rows of the table show significant clusters of a pairwise comparison of the TAP–REST contrast images between the LDOPA-ON and the LDOPA-OFF state using a flexible factorial design (O, see also clusters in Figure 1). Middle rows show LDOPA OFF-ON differences for the TAP and the REST condition separately (A and B, respectively). During the TAP condition, we obtained a significant brain activity increase with levodopa treatment in the left putamen (A, below significance for the right putamen shown in grey, see also A in Figure 2). During the REST condition, we found a reversed pattern of major brain activity decrease with levodopa treatment during (B, see also B in Figure 2). The lower rows of the table shows clusters of a significant interaction between both factors OFF/ON and TAP/REST within a flexible factorial design (A-B, see also A-B in Figure 2). Height threshold $P<0.05$ family-wise error (FWE) corrected at the voxel-level.

Table 2. List of significant clusters of brain activity change with levodopa treatment and DBS using the TAP–REST contrast images of the LDOPA-DBS cohort of 18 PD patients.

The upper part of the table (A, B) shows clusters of a pairwise comparison of the TAP–REST contrast images between the ON and the OFF state of LDOPA treatment (A) and DBS (B) in the subgroup of 18 PD patients of the LDOPA-DBS cohort. Here we obtained a significant brain activity increase with levodopa treatment in the putamen (A, see A in Figure 4, see also Figure 1

and Table 1 for the full LDOPA cohort). In contrast to the levodopa case, we did not find any significant differences between the TAP–REST contrast images of the DBS-OFF and the DBS-ON state (B, see also B in Figure 4) even when using a more liberal cluster-defining threshold approach (see Supplementary Table 2). We obtained a significant interaction between both factors LDOPA/DBS and OFF/ON within a flexible factorial design (A-B, see also A-B in Figure 4). The lower part of the table (C, D) shows a direct comparison of the TAP–REST contrast images between both treatment approaches, LDOPA and DBS, in ON and OFF states separately (ON-ON and OFF-OFF). Comparing both ON-states of LDOPA and DBS, we obtained significant brain activity differences in the putamen (C, see also C in Figure 4). The middle part of the table shows the pairwise comparison of the TAP–REST contrast images between the LDOPA-OFF and the DBS-OFF state (the so-called microlesion effect) showing a subcortical region in the vicinity of the anterior thalamus and the internal globus pallidus (D, see also D in Figure 4). In order to investigate a pure effect of LDOPA-DBS treatment change, the microlesion effect (OFF-OFF) must be subtracted from the treatment change (ON-ON) (C-D) which can be performed by the same interaction analysis between the factors LDOPA/DBS and OFF/ON already shown in the upper part of the table (A-B). Height threshold $P<0.05$ family-wise error (FWE) corrected at the voxel-level. n.s. not significant.

Table 3. List of significant clusters obtained by a three-factorial model containing (1) both experimental conditions of finger tapping and rest (TAP/REST), (2) both treatment approaches with levodopa and deep brain stimulation (LDOPA/DBS), and (3) both treatment states (OFF/ON), for the LDOPA-DBS cohort of 18 PD patients.

The table shows the result of a three-factorial model containing the factors 'condition' (TAP/REST), 'treatment approach' (LDOPA/DBS), and 'treatment state' (OFF/ON) using a merged contrast containing the interaction between LDOPA-OFF/LDOPA-ON and TAP/REST, and REST>TAP for both DBS-OFF and DBS-ON. Two significant clusters were found in the left and right putamen. Height threshold $P<0.05$ family-wise error (FWE) corrected at the voxel-level.

Figure Captions

Figure 1. Brain activity increase with levodopa treatment in the LDOPA cohort of 32 PD patients.

Using an experiment of consecutive blocks of finger tapping (TAP) and rest (REST), contrast images of TAP-REST were created for each participant. With a pairwise comparison of these contrast images (between the ON and the OFF session with and without levodopa treatment, respectively), a significant increase of the TAP-REST contrast was obtained after levodopa treatment in the left and right putamen (P<0.05 family-wise error (FWE) corrected at the voxel-level, see Table 1 for details).

Figure 2. Differential pattern of brain activity change with levodopa treatment during finger tapping and rest within the LDOPA cohort of 32 PD patients.

Using an experimental design with consecutive blocks of finger tapping and rest in both treatment states with (ON) and without (OFF) levodopa medication, we observed a differential pattern of brain activity change in the putamen. During phases of finger tapping (TAP), an increased brain activity was obtained with levodopa medication (left column, A, color-coded in red). In contrast, during resting periods (REST), putamen activity was decreased with levodopa (middle column, B, color-coded in blue). A significant interaction between both factors of experimental condition (TAP/REST) and levodopa treatment (OFF/ON) was observed in the left and right putamen (right column, A-B). All results were obtained with P<0.05 with family-wise error (FWE) correction at the voxel-level (see Table 1 for details).

Figure 3. Contrast estimates of a factorial model containing both experimental conditions of finger tapping and rest in both treatment states without and with levodopa medication for the LDOPA cohort of 32 PD patients.

Contrast estimates of the putamen showed a differential pattern of brain activity change after levodopa treatment (ON vs. OFF) during finger tapping (TAP) and rest (REST). In particular, during REST periods, we found a significant activity decrease (see A and C on the left, see also B in Figure 2). In contrast to the differential pattern of brain activity in the left and right putamen, we did not observe any brain activity differences between the OFF and the ON state in the left or right motor cortex M1, neither in the TAP nor in the REST condition (see B and D on the right).

Figure 4. Differential pattern of brain activity change with finger tapping during levodopa treatment and deep brain stimulation in the LDOPA-DBS cohort of 18 PD patients.

Using the subcohort of patients who underwent deep brain stimulation (DBS), the pairwise ON-OFF comparison revealed a brain activity increase with levodopa (LDOPA) treatment with finger tapping in the left and the right putamen (top row, A, color-coded in red, $P<0.05$ family-wise error (FWE) corrected at the voxel-level, see also Figure 1 for the full cohort). In contrast, we did not observe any significant brain activity change when comparing the ON and OFF state of DBS even when using the more liberal cluster-defining threshold approach (top row, B, see also Table 2 and Supplementary Table 2). The interaction model using a flexible factorial design with both factors LDOPA/DBS and OFF/ON revealed a significant result in the left and right putamen showing a significant difference between the ON-OFF-differences of LDOPA and DBS (top row, A-B). The pairwise comparison between both ON states of levodopa (LDOPA) treatment and deep brain

stimulation (DBS) revealed a significant brain activity decrease with finger tapping when changing the treatment from LDOPA to DBS (bottom row, C). Comparing both OFF states between LDOPA and DBS (the so-called microlesion effect), we did not find any significant brain activity differences in the left and right putamen but in the vicinity of the anterior thalamus and the internal globus pallidus (bottom row, D). Note that the interaction C-D is exactly the same as A-B sown in the top row.

Figure 5. Contrast estimates of a factorial model containing both experimental conditions of finger tapping and rest in both treatment states without and with deep brain stimulation within the subgroup of 18 PD patients.

In contrast to a differential pattern of brain activity change with levodopa treatment (see Figure 3), we did not find any significant brain activity differences with deep brain stimulation (ON vs. OFF), neither for finger tapping (TAP), nor for the rest (REST) condition. Independent of the ON or OFF state of DBS, we found a reversed pattern of brain activity in the putamen and in the primary motor cortex M1.

References

Agostino R, Berardelli A, Currà A, Accornero N, Manfredi M. Clinical impairment of sequential finger movements in Parkinson's disease. Movement disorders 1998; 13(3): 418-21.

Agostino R, Currà A, Giovannelli M, Modugno N, Manfredi M, Berardelli A. Impairment of individual finger movements in Parkinson's disease. Movement disorders 2003; 18(5): 560-5.

Akram H, Sotiropoulos SN, Jbabdi S, Georgiev D, Mahlknecht P, Hyam J*, et al.* Subthalamic deep brain stimulation sweet spots and hyperdirect cortical connectivity in Parkinson's disease. Neuroimage 2017; 158: 332-45.

Ashburner J, Friston KJ. Unified segmentation. Neuroimage 2005; 26(3): 839-51.

Ballarini T, Růžička F, Bezdicek O, Růžička E, Roth J, Villringer A*, et al.* Unraveling connectivity changes due to dopaminergic therapy in chronically treated Parkinson's disease patients. Scientific reports 2018; 8(1): 14328.

Bäumer T, Hidding U, Hamel W, Buhmann C, Moll CK, Gerloff C*, et al.* Effects of DBS, premotor rTMS, and levodopa on motor function and silent period in advanced Parkinson's disease. Movement disorders: official journal of the Movement Disorder Society 2009; 24(5): 672-6.

Benabid AL, Chabardes S, Mitrofanis J, Pollak P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. The Lancet Neurology 2009; 8(1): 67-81.

Bergman H, Wichmann T, DeLong MR. Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 1990; 249(4975): 1436-8.

Bronstein JM, Tagliati M, Alterman RL, Lozano AM, Volkmann J, Stefani A*, et al.* Deep brain stimulation for Parkinson disease: an expert consensus and review of key issues. Archives of neurology 2011; 68(2): 165.

Connolly BS, Lang AE. Pharmacological treatment of Parkinson disease: a review. Jama 2014; 311(16): 1670-83.

Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci U S A 2016; 113(28): 7900-5.

Fahn S. Adverse effects of levodopa in Parkinson's disease. Drugs for the Treatment of Parkinson's Disease: Springer; 1989. p. 385-409.

Fahn S. Description of Parkinson's disease as a clinical syndrome. Annals of the New York Academy of Sciences 2003; 991(1): 1-14.

Fahn S, Jankovic J, Hallett M. Principles and Practice of Movement Disorders E-Book: Expert Consult: Elsevier Health Sciences; 2011.

Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg M, Turner R. Event-related fMRI: characterizing differential responses. Neuroimage 1998; 7(1): 30-40.

Friston KJ, Glaser DE, Henson RN, Kiebel S, Phillips C, Ashburner J. Classical and Bayesian inference in neuroimaging: applications. Neuroimage 2002a; 16(2): 484-512.

Friston KJ, Penny W, Phillips C, Kiebel S, Hinton G, Ashburner J. Classical and Bayesian inference in neuroimaging: theory. Neuroimage 2002b; 16(2): 465-83.

Galvan A, Devergnas A, Wichmann T. Alterations in neuronal activity in basal ganglia-thalamocortical circuits in the parkinsonian state. Frontiers in neuroanatomy 2015; 9: 5.

Galvan A, Wichmann T. Pathophysiology of parkinsonism. Clinical neurophysiology 2008; 119(7): 1459-74.

Gountouna V-E, Job DE, McIntosh AM, Moorhead TWJ, Lymer GKL, Whalley HC*, et al.* Functional Magnetic Resonance Imaging (fMRI) reproducibility and variance components across visits and scanning sites with a finger tapping task. Neuroimage 2010; 49(1): 552-60.

Haslinger B, Erhard P, Kämpfe N, Boecker H, Rummeny E, Schwaiger M*, et al.* Event-related functional magnetic resonance imaging in Parkinson's disease before and after levodopa. Brain 2001; 124(3): 558-70.

Herz DM, Eickhoff SB, Løkkegaard A, Siebner HR. Functional neuroimaging of motor control in parkinson's disease: A meta-analysis. Human brain mapping 2014; 35(7): 3227-37.

Holiga Š, Möller HE, Sieger T, Schroeter ML, Jech R, Mueller K. Accounting for movement increases sensitivity in detecting brain activity in Parkinson's disease. PLoS One 2012; 7(5): e36271.

Holiga Š, Mueller K, Möller HE, Sieger T, Schroeter ML, Vymazal J*, et al.* Motor matters: tackling heterogeneity of Parkinson's disease in functional MRI studies. PloS one 2013; 8(2): e56133.

Holiga Š, Mueller K, Möller HE, Urgošík D, Růžička E, Schroeter ML*, et al.* Resting-state functional magnetic resonance imaging of the subthalamic microlesion and stimulation effects in Parkinson's disease: indications of a principal role of the brainstem. NeuroImage: Clinical 2015; 9: 264-74.

Hornykiewicz O. A brief history of levodopa. Journal of neurology 2010; 257(2): 249-52.

Jech R, Mueller K, Urgosik D, Sieger T, Holiga S, Ruzicka F*, et al.* The subthalamic microlesion story in Parkinson's disease: electrode insertion-related motor improvement with relative cortico-subcortical hypoactivation in fMRI. PLoS One 2012; 7(11): e49056.

Jech R, Urgošík D, Tintěra J, Nebuželský A, Krásenský J, Liščák R*, et al.* Functional magnetic resonance imaging during deep brain stimulation: a pilot study in four patients with Parkinson's disease. Movement disorders: official journal of the Movement Disorder Society 2001; 16(6): 1126-32.

Kandel ER, Schwartz JH, Jessell TM, Biochemistry Do, Jessell MBT, Siegelbaum S*, et al.* Principles of neural science: McGraw-hill New York; 2000.

Kraft E, Loichinger W, Diepers M, Lule D, Schwarz J, Ludolph AC*, et al.* Levodopa-induced striatal activation in Parkinson's disease: a functional MRI study. Parkinsonism & related disorders 2009; 15(8): 558-63.

LeWitt PA, Fahn S. Levodopa therapy for Parkinson disease: a look backward and forward. Neurology 2016; 86(14 Supplement 1): S3-S12.

Maillet A, Krainik A, Debû B, Troprès I, Lagrange C, Thobois S*, et al.* Levodopa effects on hand and speech movements in patients with Parkinson's disease: a FMRI study. PLoS One 2012; 7(10): e46541.

Michely J, Volz LJ, Barbe MT, Hoffstaedter F, Viswanathan S, Timmermann L*, et al.* Dopaminergic modulation of motor network dynamics in Parkinson's disease. Brain 2015; 138(3): 664-78.

Mink JW. The basal ganglia: focused selection and inhibition of competing motor programs. Progress in neurobiology 1996; 50(4): 381-425.

Moro E, Lang AE. Criteria for deep-brain stimulation in Parkinson's disease: review and analysis. Expert Review of Neurotherapeutics 2006; 6(11): 1695-705.

Mueller K, Jech R, Růžička F, Holiga Š, Ballarini T, Bezdicek O*, et al.* Brain connectivity changes when comparing effects of subthalamic deep brain stimulation with levodopa treatment in Parkinson's disease. NeuroImage: Clinical 2018; 19: 1025-35.

Nambu A, Tokuno H, Takada M. Functional significance of the cortico–subthalamo–pallidal 'hyperdirect'pathway. Neuroscience research 2002; 43(2): 111-7.

Nichols T, Hayasaka S. Controlling the familywise error rate in functional neuroimaging: a comparative review. Statistical methods in medical research 2003; 12(5): 419-46.

Obeso JA, Olanow CW, Nutt JG. Levodopa motor complications in Parkinson's disease. Elsevier; 2000.

Pernet C, Poline J-B. Improving functional magnetic resonance imaging reproducibility. Gigascience 2015; 4(1): 15.

Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J*, et al.* Parkinson disease. Nature reviews Disease primers 2017; 3: 17013.

Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W*, et al.* MDS clinical diagnostic criteria for Parkinson's disease. Movement Disorders 2015; 30(12): 1591-601.

Postuma RB, Dagher A. Basal ganglia functional connectivity based on a meta-analysis of 126 positron emission tomography and functional magnetic resonance imaging publications. Cerebral cortex 2005; 16(10): 1508-21.

Rocchi L, Chiari L, Horak F. Effects of deep brain stimulation and levodopa on postural sway in Parkinson's disease. Journal of Neurology, Neurosurgery & Psychiatry 2002; 73(3): 267-74.

Rowe J, Hughes L, Ghosh B, Eckstein D, Williams-Gray C, Fallon S*, et al.* Parkinson's disease and dopaminergic therapy—differential effects on movement, reward and cognition. Brain 2008; 131(8): 2094-105.

Rubchinsky LL, Kopell N, Sigvardt KA. Modeling facilitation and inhibition of competing motor programs in basal ganglia subthalamic nucleus–pallidal circuits. Proceedings of the National Academy of Sciences 2003; 100(24): 14427-32.

Singh A, Kammermeier S, Mehrkens JH, Bötzel K. Movement kinematic after deep brain stimulation associated microlesions. J Neurol Neurosurg Psychiatry 2012; 83(10): 1022-6.

Singh A, Mewes K, Gross RE, DeLong MR, Obeso JA, Papa SM. Human striatal recordings reveal abnormal discharge of projection neurons in Parkinson's disease. Proceedings of the National Academy of Sciences 2016; 113(34): 9629-34.

Tagliati M, Jankovic J, Pagan F, Susatia F, Isaias IU, Okun MS*, et al.* Safety of MRI in patients with implanted deep brain stimulation devices. Neuroimage 2009; 47 Suppl 2: T53-7.

Thobois S, Dominey P, Decety J, Pollak P, Gregoire MC, Broussolle E. Overactivation of primary motor cortex is asymmetrical in hemiparkinsonian patients. Neuroreport 2000; 11(4): 785-9.

Timmermann L, Braun M, Groiss S, Wojtecki L, Ostrowski S, Krause H*, et al.* Differential effects of levodopa and subthalamic nucleus deep brain stimulation on bradykinesia in Parkinson's disease.

Movement Disorders 2008; 23(2): 218-27.

Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010; 25(15): 2649-53.

Vingerhoets FJ, Villemure J-G, Temperli P, Pollo C, Pralong E, Ghika J. Subthalamic DBS replaces levodopa in Parkinson's disease: two-year follow-up. Neurology 2002; 58(3): 396-401.

Witt ST, Laird AR, Meyerand ME. Functional neuroimaging correlates of finger-tapping task variations: an ALE meta-analysis. Neuroimage 2008; 42(1): 343-56.

Wu T, Wang J, Wang C, Hallett M, Zang Y, Wu X*, et al.* Basal ganglia circuits changes in Parkinson's disease patients. Neuroscience letters 2012; 524(1): 55-9.

Yu H, Sternad D, Corcos DM, Vaillancourt DE. Role of hyperactive cerebellum and motor cortex in Parkinson's disease. Neuroimage 2007; 35(1): 222-33.

Table 1. List of significant clusters of brain activity change with levodopa treatment for the LDOPA cohort including 32 PD patients*

	cluster-level		voxel-level					
	pFWE	k_E	*pFWE*	*T*	*Z*	*x*	*y*	*z*
O:TAP-REST ON>OFF	<0.001	590	<0.001	7.60	6.69	-26	-2	10
	<0.001	543	<0.001	6.86	6.16	24	6	10
A: TAP ON>OFF	0.016	25	0.016	4.42	4.20	-24	-2	10
			0.172	3.60	3.48	24	4	12
B: REST ON<OFF	<0.001	644	<0.001	7.58	6.68	-26	-2	12
	<0.001	476	<0.001	6.84	6.14	24	2	12
A – B	0.003	160	<0.001	5.73	5.52	-24	0	12
	0.003	170	<0.001	5.30	5.14	24	4	12

*The upper rows of the table show significant clusters of a pairwise comparison of the TAP–REST contrast images between the LDOPA-ON and the LDOPA-OFF state using a flexible factorial design (O, see also clusters in Figure 1). Middle rows show LDOPA OFF-ON differences for the TAP and the REST condition separately (A and B, respectively). During the TAP condition, we obtained a significant brain activity increase with levodopa treatment in the left putamen (A, below significance for the right putamen shown in grey, see also A in Figure 2). During the REST condition, we found a reversed pattern of major brain activity decrease with levodopa treatment during (B, see also B in Figure 2). The lower rows of the table shows clusters of a significant interaction between both factors OFF/ON and TAP/REST within a flexible factorial design (A-B,

see also A-B in Figure 2). Height threshold $P<0.05$ family-wise error (FWE) corrected at the voxel-level.

Table 2. List of significant clusters of brain activity change with levodopa treatment and DBS using the TAP–REST contrast images of the LDOPA-DBS cohort of 18 PD patients*

		cluster-level		voxel-level					
		pFWE	k_E	*pFWE*	*T*	*Z*	*x*	*y*	*z*
A: LDOPA ON>OFF		<0.001	627	<0.001	8.54	6.72	-28	-2	8
		<0.001	584	<0.001	7.23	5.99	26	0	8
B: DBS ON<>OFF		n.s.		n.s.					
A – B		0.001	183	<0.001	6.30	5.85	-28	0	8
		0.001	270	<0.001	5.33	5.05	26	4	6
C: ON LDOPA>DBS		0.004	154	<0.001	5.70	5.00	-26	2	4
		0.012	65	0.001	5.21	4.65	22	0	0
		<0.001	522	<0.001	5.77	5.05	10	-2	20
D: OFF LDOPA>DBS		<0.001	597	<0.001	7.30	6.03	10	-4	8
C – D		0.001	183	<0.001	6.30	5.85	-28	0	8
		0.001	270	<0.001	5.33	5.05	26	4	6

*The upper part of the table (A, B) shows clusters of a pairwise comparison of the TAP–REST contrast images between the ON and the OFF state of LDOPA treatment (A) and DBS (B) in the subgroup of 18 PD patients of the LDOPA-DBS cohort. Here we obtained a significant brain

activity increase with levodopa treatment in the putamen (A, see A in Figure 4, see also Figure 1 and Table 1 for the full LDOPA cohort). In contrast to the levodopa case, we did not find any significant differences between the TAP–REST contrast images of the DBS-OFF and the DBS-ON state (B, see also B in Figure 4) even when using a more liberal cluster-defining threshold approach (see Supplementary Table 2). We obtained a significant interaction between both factors LDOPA/DBS and OFF/ON within a flexible factorial design (A-B, see also A-B in Figure 4). The lower part of the table (C, D) shows a direct comparison of the TAP–REST contrast images between both treatment approaches, LDOPA and DBS, in ON and OFF states separately (ON-ON and OFF-OFF). Comparing both ON-states of LDOPA and DBS, we obtained significant brain activity differences in the putamen (C, see also C in Figure 4). The middle part of the table shows the pairwise comparison of the TAP–REST contrast images between the LDOPA-OFF and the DBS-OFF state (the so-called microlesion effect) showing a subcortical region in the vicinity of the anterior thalamus and the internal globus pallidus (D, see also D in Figure 4). In order to investigate a pure effect of LDOPA-DBS treatment change, the microlesion effect (OFF-OFF) must be subtracted from the treatment change (ON-ON) (C-D) which can be performed by the same interaction analysis between the factors LDOPA/DBS and OFF/ON already shown in the upper part of the table (A-B). Height threshold $P<0.05$ family-wise error (FWE) corrected at the voxel-level. n.s. not significant.

Table 3. List of significant clusters obtained by a three-factorial model containing (1) both experimental conditions of finger tapping and rest (TAP/REST), (2) both treatment approaches with levodopa and deep brain stimulation (LDOPA/DBS), and (3) both treatment states (OFF/ON), for the LDOPA-DBS cohort of 18 PD patients*

cluster-level		**voxel-level**					
pFWE	k_E	*pFWE*	*T*	*Z*	*x*	*y*	*z*
0.001	195	<0.001	5.52	5.36	-28	-2	8
<0.001	461	<0.001	6.54	6.29	28	0	8

*The table shows the result of a three-factorial model containing the factors 'condition' (TAP/REST), 'treatment approach' (LDOPA/DBS), and 'treatment state' (OFF/ON) using a merged contrast containing the interaction between LDOPA-OFF/LDOPA-ON and TAP/REST, and REST>TAP for both DBS-OFF and DBS-ON. Two significant clusters were found in the left and right putamen. Height threshold $P<0.05$ family-wise error (FWE) corrected at the voxel-level.

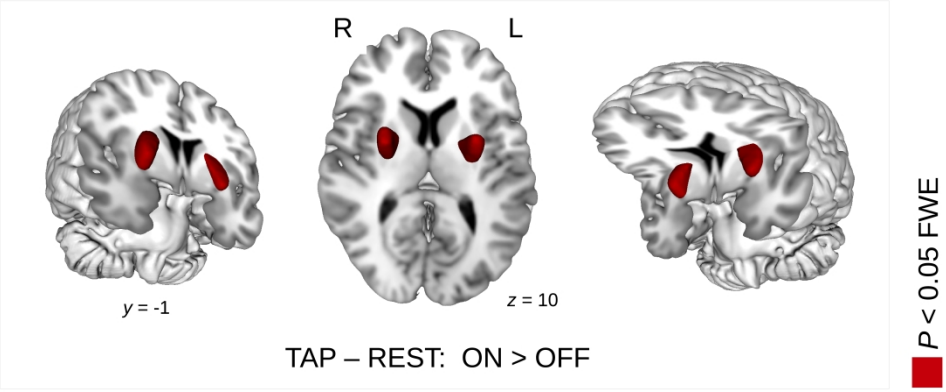

Figure 1. Brain activity increase with levodopa treatment in the LDOPA cohort of 32 PD patients. Using an experiment of consecutive blocks of finger tapping (TAP) and rest (REST), contrast images of TAP-REST were created for each participant. With a pairwise comparison of these contrast images (between the ON and the OFF session with and without levodopa treatment, respectively), a significant increase of the TAP-REST contrast was obtained after levodopa treatment in the left and right putamen (P<0.05 family-wise error (FWE) corrected at the voxel-level, see Table 1 for details).

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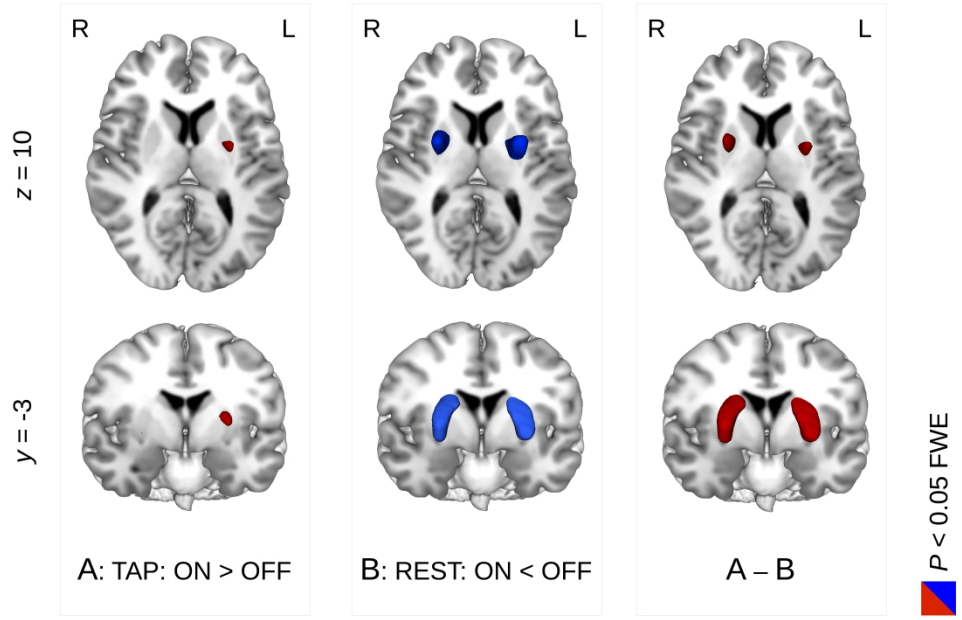

Figure 2. Differential pattern of brain activity change with levodopa treatment during finger tapping and rest within the LDOPA cohort of 32 PD patients. Using an experimental design with consecutive blocks of finger tapping and rest in both treatment states with (ON) and without (OFF) levodopa medication, we observed a differential pattern of brain activity change in the putamen. During phases of finger tapping (TAP), an increased brain activity was obtained with levodopa medication (left column, A, color-coded in red). In contrast, during resting periods (REST), putamen activity was decreased with levodopa (middle column, B, color-coded in blue). A significant interaction between both factors of experimental condition (TAP/REST) and levodopa treatment (OFF/ON) was observed in the left and right putamen (right column, A-B). All results were obtained with P<0.05 with family-wise error (FWE) correction at the voxel-level (see Table 1 for details).

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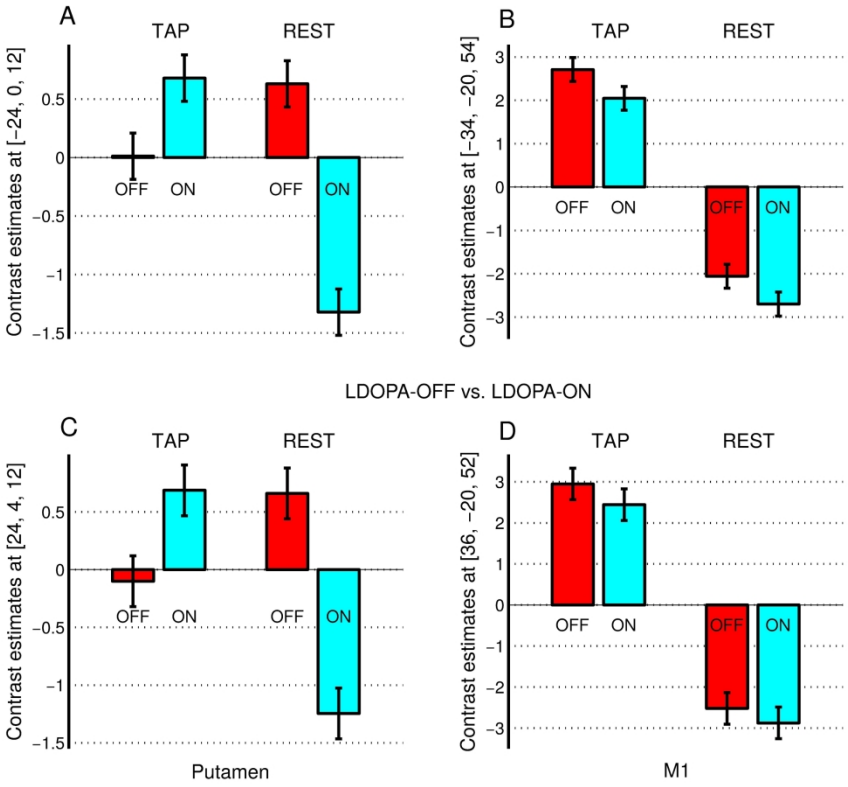

Figure 3. Contrast estimates of a factorial model containing both experimental conditions of finger tapping and rest in both treatment states without and with levodopa medication for the LDOPA cohort of 32 PD patients. Contrast estimates of the putamen showed a differential pattern of brain activity change after levodopa treatment (ON vs. OFF) during finger tapping (TAP) and rest (REST). In particular, during REST periods, we found a significant activity decrease (see bars on the left, see also B in Figure 2). In contrast to the differential pattern of brain activity in the left and right putamen, we did not observe any brain activity differences between the OFF and the ON state in the left or right motor cortex M1, neither in the TAP nor in the REST condition (see bars on the right).

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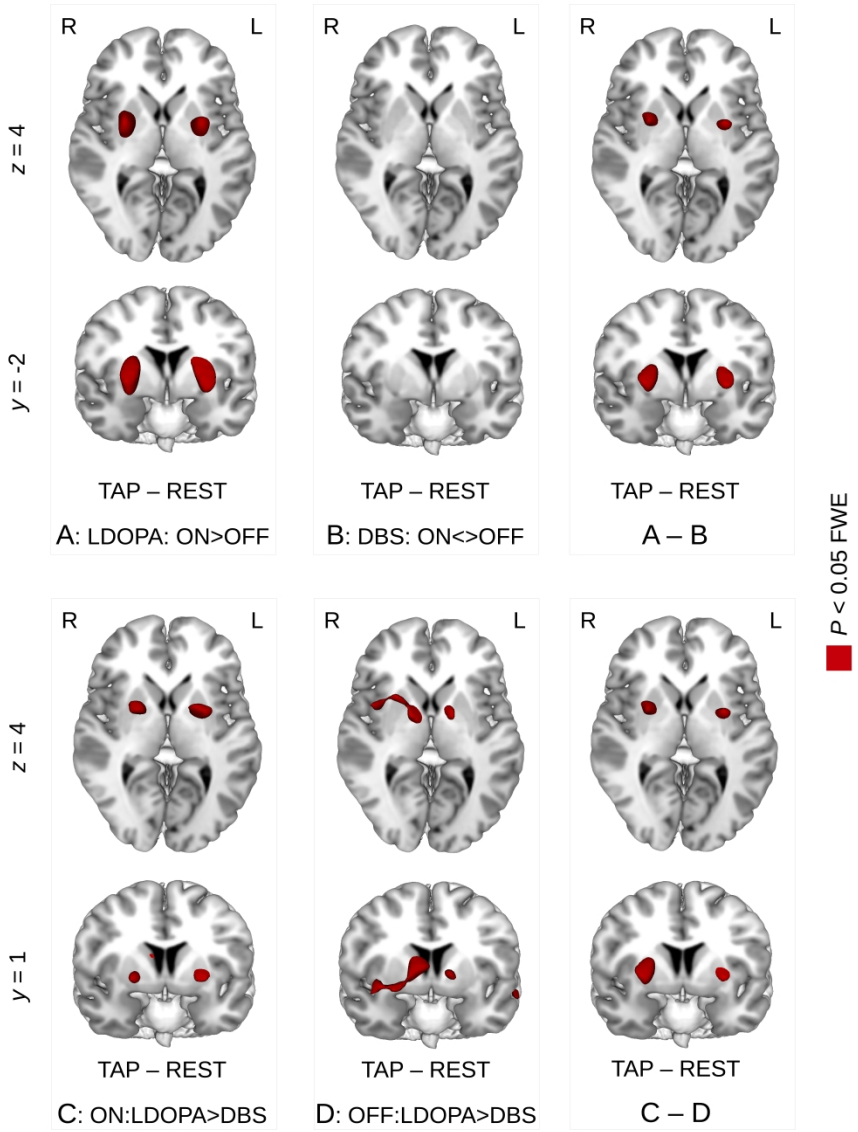

Figure 4. Differential pattern of brain activity change with finger tapping during levodopa treatment and deep brain stimulation in the LDOPA-DBS cohort of 18 PD patients. Using the subcohort of patients who underwent deep brain stimulation (DBS), the pairwise ON-OFF comparison revealed a brain activity increase with levodopa (LDOPA) treatment with finger tapping in the left and the right putamen (top row, A, color-coded in red, P<0.05 family-wise error (FWE) corrected at the voxel-level, see also Figure 1 for the full cohort). In contrast, we did not observe any significant brain activity change when comparing the ON and OFF state of DBS even when using the more liberal cluster-defining threshold approach (top row, B, see also Table 2 and Supplementary Table 2). The interaction model using a flexible factorial design with both factors LDOPA/DBS and OFF/ON revealed a significant result in the left and right putamen showing a significant difference between the ON-OFF-differences of LDOPA and DBS (top row, A-B). The pairwise comparison between both ON states of levodopa (LDOPA) treatment and deep brain stimulation (DBS) revealed a significant brain activity decrease with finger tapping when changing the treatment from LDOPA to DBS (bottom row, C). Comparing both OFF states between LDOPA and DBS (the so-called microlesion effect), we did not find any significant brain activity differences in the left and right putamen but in the vicinity of the

anterior thalamus and the internal globus pallidus (bottom row, D). Note that the interaction C-D is exactly the same as A-B sown in the top row.

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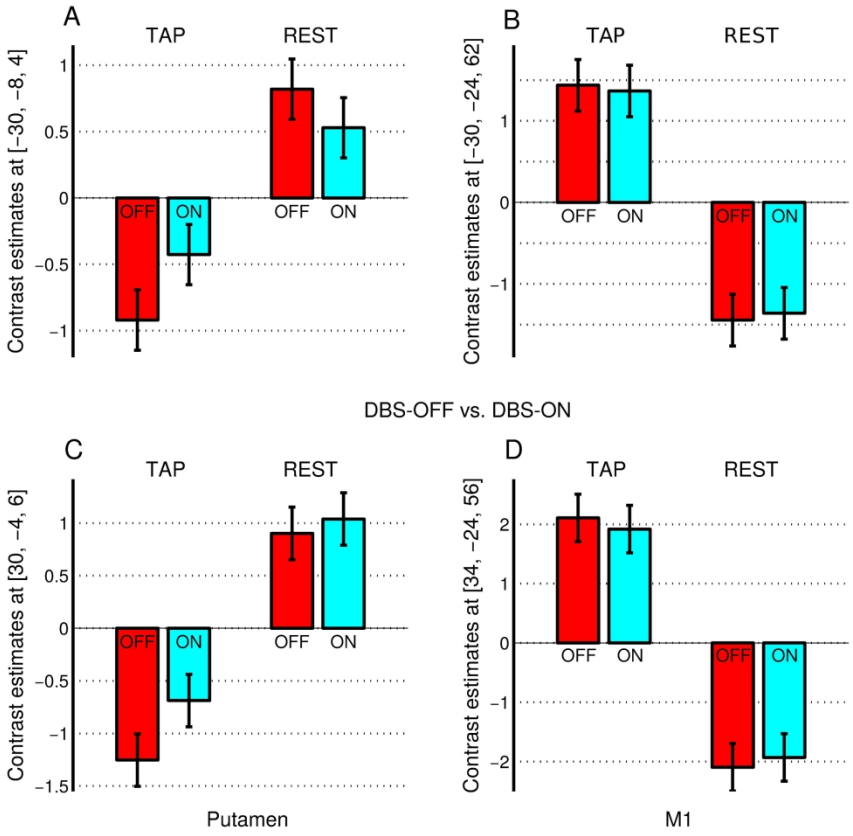

Figure 5. Contrast estimates of a factorial model containing both experimental conditions of finger tapping and rest in both treatment states without and with deep brain stimulation within the subgroup of 18 PD patients. In contrast to a differential pattern of brain activity change with levodopa treatment (see Figure 3), we did not find any significant brain activity differences with deep brain stimulation (ON vs. OFF), neither for finger tapping (TAP), nor for the rest (REST) condition. Independent of the ON or OFF state of DBS, we found a reversed pattern of brain activity in the putamen and in the primary motor cortex M1.

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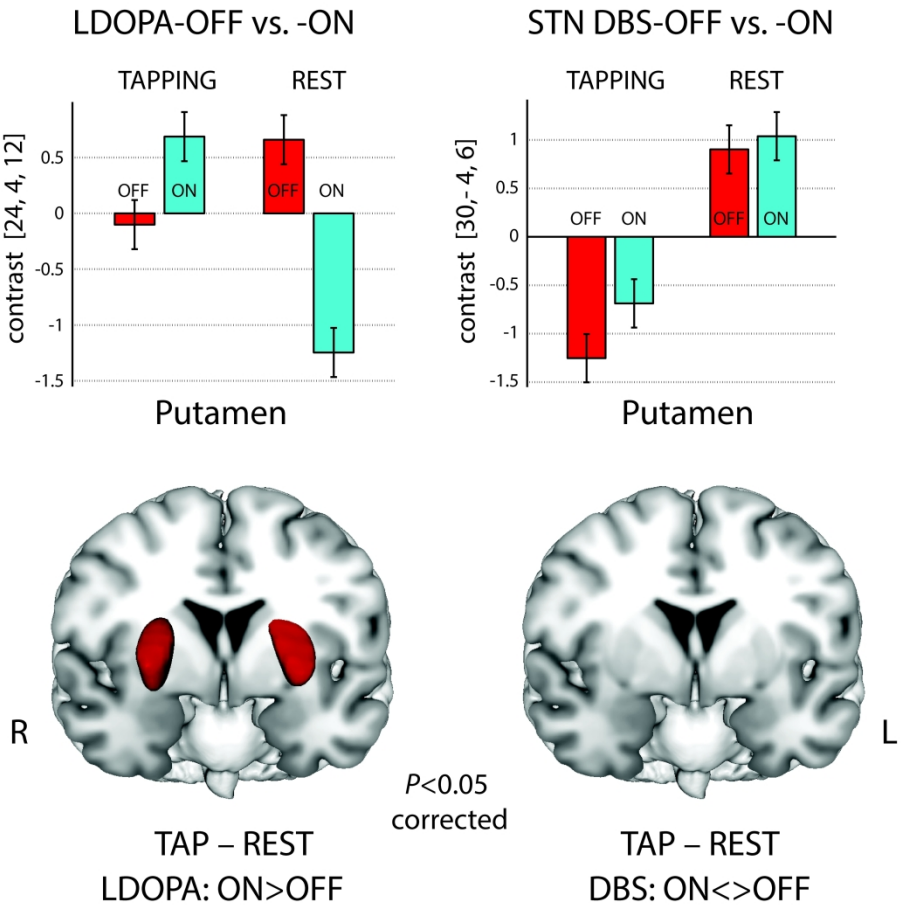
Short abstract:

The study shows fundamentally different effects of symptomatic treatment in Parkinson's disease on activity of motor network during motion and rest. The decreased motion-related activity in the putamen after medication withdrawal was reversed by levodopa but not with subthalamic deep brain stimulation.



Graphical abstract

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