Excitability of the supplementary motor area in Parkinson's disease depends on subcortical damage

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ABSTRACT

Background: Cortical dysfunctioning significantly contributes to the pathogenesis of motor symptoms in Parkinson's disease (PD).
Objective: We aimed at testing whether an acute levodopa administration has measurable and specific cortical effects possibly related to striatal dopaminergic deficit.
Methods: In thirteen PD patients, we measured the electroencephalographic responses to transcranial magnetic stimulation (TMS/EEG) of the supplementary motor area and superior parietal lobule (n = 8) before and after an acute intake of levodopa. We also performed a single-photon emission computed tomography and [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane to identify the more affected and the less affected brain side in each patient, according to the dopaminergic innervation loss of the putamen. Cortical excitability changes before and after an acute intake of levodopa were computed and compared between the more and the less affected brain side at the single-patient as well as at the group level.
Results: We found that levodopa intake induces a significant increase (P < 0.01) of cortical excitability nearby the supplementary motor area in the more affected brain side, greater (P < 0.025) than in the less affected brain side. Notably, cortical excitability changes nearby the superior parietal lobule were not statistically significant.
Conclusions: These results strengthen the idea that dysfunction of specific cortico-subcortical circuits may contribute to pathophysiology of PD symptoms. Most important, they support the use of navigated TMS/EEG as a non-invasive tool to better understand the pathophysiology of PD.

Introduction

Functional neuroimaging studies using positron emission tomography (PET) [1], single-photon emission computed tomography (SPECT) [2] and functional magnetic resonance imaging (fMRI) [3] have shown that cortical dysfunctioning contributes to the pathogenesis of symptoms in Parkinson's disease (PD). Specifically, the cortical regions that have been mostly studied are the Supplementary Motor Area (SMA), the Dorsolateral Prefrontal Cortex (DLPFC) and the Primary Motor Cortex (M1). While for M1 findings are more controversial [4], an abnormal reduction of SMA excitability has been consistently reported and also confirmed by neurophysiological studies of the electrophysiological signature of motor preparation, such as movement-related potentials [5,6]. Overall, several experimental evidences foster the hypothesis that SMA dysfunctioning should be taken into account to explain the pathogenesis of bradykinesia, which is the cardinal symptom of PD [7].

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Abbreviations			
AI	Asymmetry index	LMFP	Local mean field power
DAT	Dopamine reuptake transporter	M1	Primary motor cortex
DLPFC	Dorsolateral prefrontal cortex	MRI	Magnetic resonance imaging
EEG	Electroencephalography	PBR	Putamen-specific binding ratio
EF	Electric field	PET	Positron emission tomography
fMRI	Functional magnetic resonance imaging	rTMS	Repetitive transcranial magnetic stimulation
ICA	Independent Component Analysis	SMA	Supplementary motor area
H+	Less affected hemisphere	SPECT	Single-photon emission computed tomography
H-	More affected hemisphere	SPL	Superior parietal lobule
H&Y	Hoehn and Yahr scale	SS	Symptom sub-score
IRA	Immediate response area	TEPs	TMS-evoked potentials
		TMS	Transcranial magnetic stimulation
		UPDRS-III	Unified Parkinson Disease Rating Scale motor part

A reduction of SMA activity follows the typical dopaminergic impairment within the basal ganglia that characterizes PD: this cortical area is a crucial component of the basal ganglia-thalamo-cortical circuit model, which is composed of parallel reentrant cortico-subcortical circuits spreading from specific cortical areas, passing through the basal ganglia and thalamus, and projecting back to their respective areas of origin [8,9]. In particular, the basal ganglia [10,11] and especially the putamen [12,13] are densely interconnected with the motor and premotor cortex as well as the SMA. A pathological hyper-connectivity between SMA and putamen, but not with caudate, has also been shown in PD patients using a resting state fMRI approach [14].

Therefore, several functional and anatomical characteristics of the SMA provide an empirical rationale for using this cortical area as a potential therapeutic target of neuromodulatory approaches. Indeed, repetitive TMS (rTMS) applied over the SMA in randomized, double-blind studies has been reported to modestly improve motor symptoms in patients with PD [15,16], but the clinical benefit of cortical stimulation at the single patient level is still debated [17].

Besides its possible therapeutic use, single-pulse TMS is also suitable to investigate motor cortex dysfunctioning through motor potentials evoked by direct stimulation of M1 [7]. This conventional neurophysiologic approach has revealed an excessive corticospinal motor output at rest and a reduced facilitation of muscular activation during voluntary muscle contraction in PD patients [7,18—20]. In this context, the combination of navigated TMS with simultaneous high-density electroencephalography (TMS/EEG) allows to non-invasively probe both local and global changes of brain excitability and connectivity [21] through the recording of TMS-evoked potentials (TEPs), which represent genuine cortical responses to a direct perturbation [22]. In addition, the neuro-navigated TMS/EEG system offers the unique opportunity to target associative cortical areas, and to obtain a direct readout of cortical function that does not involve either the cortico-spinal tract or peripheral muscle activation.

Therefore, following previous approaches aimed at detecting cortical dysfunctions in PD, TMS/EEG may be used to disclose functional properties of secondary cortical regions involved in the basal ganglia-thalamo-cortical loop and to obtain complementary information related to the complex pathophysiology of PD motor signs and symptoms.

With this aim, we measured TEPs recorded during stimulation of SMA before and after levodopa intake in PD patients. Specifically, in the present study we tested i) whether an acute levodopa intake has specific and measurable effects on cortical excitability (here referred to as the strength of the cortical response to a direct perturbation) and ii) whether these neurophysiological effects parallel the asymmetry of akinetic-rigid symptoms and the corresponding dopaminergic striatal innervation loss.

Materials and methods

Patient population. We enrolled thirteen patients with idiopathic PD (Table 1), diagnosed according to the UK Parkinson Disease Brain Bank criteria [23]. All patients were on stable dopaminergic treatment for at least three months prior to this study and were showing a significant benefit from levodopa administration. Clinical assessment was performed with the Unified Parkinson Disease Rating Scale motor part (UPDRS-III) [24]: a left and a right symptom sub-score (SS) was computed by summing the items between 22 and 26, in which a lateralized score is available [25] (Table 1). The following clinical inclusion criteria were applied: (i) UPDRS part I score of 0, (ii) Hoehn and Yahr (H&Y) scale [26] stage 2; (iii) no psychiatric disorders or other neurological diseases other than PD; and (iv) absence of any sign indicative for atypical parkinsonism (e.g., gaze abnormalities, autonomic dysfunction, psychiatric disturbances, etc.). All patients had no cognitive decline as assessed by the Mini-Mental State examination, Clock Drawing Test and Frontal Assessment Battery. Patients with medical history of seizures, loss of consciousness and traumatic brain injury, intracranial metallic devices and/or of cardiac pacemakers were excluded to prevent potential adverse effects of TMS. Anatomical T1-weighted magnetic resonance images (MRI; 1.5 T scanner, Achieva, Philips Medical Systems, Amsterdam, The Netherlands; $0.94 \times 0.94 \times 1$ mm spatial resolution) were collected within a week prior to TMS/EEG assessment; only patients without major structural abnormalities (e.g, white matter lesions or cortical atrophy) were enrolled in the study. The local ethical committee approved the study design and all patients signed an informed consent form.

SPECT imaging. All patients were evaluated with single-photon emission computerized tomography (SPECT) in order to objectively ascertain the asymmetry of dopaminergic degeneration. The striatal dopamine reuptake transporter (DAT) density was measured with SPECT and [^{123}I]N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (FP-CIT) (for more details, refer to [27]). Scanning was performed with a triple detector gamma-camera (Prism 3000, Philips, Eindhoven, The Netherlands) equipped with low-energy ultra-high resolution fan beam collimators (4 subsets of acquisitions, matrix size 128×128, radius of rotation 12.9—13.9 cm, continuous rotation, angular sampling: 3°, duration: 28 min) about 3—4 h after intravenous administration of 110—185 MBq of FP-CIT (DaTSCAN™, GE Healthcare, Arlington Heights, IL, USA) preceded by thyroid blockade (10—15 mg of Lugol solution per os). Brain sections were reconstructed with an iterative algorithm (OSEM, 4 iterations and 15 subsets), followed by 3D

Table 1
Demographic and clinical characteristics of Parkinson's disease patients. y = years; f = female; m = male; LEDD = levodopa equivalent daily dose [54]; UPDRS-III = Unified Parkinson Disease Rating Scale motor part; SS = symptom sub-score; PBR = putamen-specific binding ratio; AI = asymmetry index; H- is the brain side with lower PBR (i.e. the more affected side); N/A = not applicable.

Patient	age (y)/gender	disease duration (y)	LEDD (mg/day)	UPDRS-III		SS meds-off/ meds-on		PBR		AI	H-
				meds-off	meds-on	left	right	left	right		
1	73/f	1	100	10	8	6/5	3/3	0.88	0.73	−18.63	right
2	57/m	4	505	13	7	3/1	9/5	1.20	1.41	16.09	left
3	66/m	18	1150.45	21	17	4/3	8/6	0.62	0.65	4.72	left
4	53/f	2	505	13	7	3/1	7/4	0.97	0.80	−19.21	right
5	70/m	6	715	9	2	7/2	0/0	0.49	0.40	−20.22	right
6	58/m	14	738.15	16	4	4/0	5/1	0.25	0.31	21.43	left
7	51/f	3	505	9	5	2/0	2/1	1.07	1.09	1.85	N/A
8	69/m	5	600	9	3	5/2	1/0	0.67	0.40	−50.47	right
9	63/m	4	610	19	8	7/3	8/3	0.77	0.89	14.46	left
10	55/m	7	718.2	7	0	5/0	2/0	1.00	0.61	−48.45	right
11	73/m	9	480	16	8	5/3	6/4	1.15	1.50	26.42	left
12	61/m	4	405	14	4	2/0	5/1	0.60	0.82	30.99	left
13	53/f	5	460	19	6	9/2	4/1	1.27	0.76	−50.25	right

filtering of sections obtained (Butterworth, order 5, cut-off 0.31 Ny) and attenuation correction (Chang method, factor 0.12). The putamen-specific binding ratio (PBR) for the left and right hemisphere was estimated by semi-quantitative image analysis with the two dimensional Crescent ROI algorithm (12 mm-thickness) implemented in QuantiSPECT (GE Healthcare, Arlington Heights, IL, USA) software package [28]. The asymmetry index (AI) was computed as [PBR(right) − PBR(left)]/[PBR(right) + PBR(left)] x 200 (as reported by Ref. [29]). The brain side with lower PBR was labeled as H-, whereas the one with higher PBR was labeled as H+ (Table 1). Within the patient population, PBR was lower on the left brain side in 7 patients (AI > 0) and on the right brain side (AI < 0) in the remaining 6 patients (Table 1). However, in two patients (n. 3 and n. 7) AI was lower than 10%, indicating that dopaminergic denervation was almost symmetric according to SPECT imaging. We decided to exclude patient n. 7 from group analysis because the lateralized symptom sub-score in meds-off session was not asymmetric as well (SS(left) = SS(right) = 2, Table 1). Conversely, we decided to include patient n. 3 into group analysis considering the left hemisphere as H-, because behavioral motor deficits were definitely prevalent on the right body side, consistent with the asymmetry direction (AI = 4.72) provided by SPECT imaging. In the remaining 11 patients, clinical assessment was congruent with SPECT imaging asymmetry (i.e. worse motor impairments contralateral to H-), except for patient n. 4 who showed higher SS score on the body side ipsilateral to H-.

Experimental protocol. The experimental protocol (Fig. 1A) consisted in two experimental sessions: the first one (meds-off) was performed after that levodopa had been withdrawn overnight for at least 12 h, dopamine-agonists had been suspended for three days and all other dopaminergic drugs (e.g. MAO-B inhibitors) for one week prior to the experiment. The second session (meds-on) started 60 min after oral intake of 200/50 mg of fast-released soluble levodopa/benserazide. Each session involved a clinical assessment with the UPDRS-III (about 10 min duration) and a neurophysiological assessment by means of TMS/EEG measurements (between 25 and 45 min duration).

TMS/EEG measurement. Brain responses to TMS were recorded with a 60-channel TMS-compatible EEG amplifier (Nexstim Ltd., Helsinki, Finland). Impedance at all electrodes was kept below 5 kΩ. EEG was referenced to an additional electrode on the forehead, band-pass filtered between 0.1 and 350 Hz and sampled at 1450 Hz with 16 bit resolution. Vertical electrooculogram was recorded with two extra sensors in order to monitor ocular movements and blinks. TMS was delivered with a Focal Bipulse 8-Coil (mean/outer winding diameter ca. 50/70 mm, biphasic pulse shape, pulse length ca. 280 μs, focal area of the stimulation hot spot 0.68 cm^2; eXimia TMS Stimulator, Nexstim Ltd., Helsinki, Finland). The coil was always placed tangentially to the scalp, in order to optimize transmission of the magnetic field to the cortical surface. Stimulation parameters were carefully controlled by means of a Navigated Brain Stimulation (NBS) system (Nexstim Ltd., Helsinki, Finland), that employs a 3D infrared tracking position sensor unit (Polaris, Northern Digital Inc., Waterloo, Canada) and integrates T1-weighted MRIs recorded from all patients. Stimulation intensity was set to induce an estimated maximum electric field (EF) in the target area of about 120 V/m, in order to elicit robust and reproducible EEG responses [30,31]. Since supra-threshold stimulation of the primary motor cortex produces TEPs that are also affected by a peripheral sensory feedback [32], we always checked that stimulation intensity did not evoke muscle twitches to prevent a confounding factor. Single TMS pulses were delivered with an inter-stimulus interval randomly jittering between 1500 and 1800 ms (equivalent to ca. 0.56−0.67 Hz), which does not induce any reorganization/plasticity processes possibly interfering with longitudinal measurements [31]. During TMS stimulation, patients wore in-ear headphones continuously playing a customized masking noise [33] to maximally reduce the contribution of auditory potentials elicited by TMS-associated clicks to genuine brain responses to TMS [34].

TMS targeting. The caudal portion of the middle-superior frontal gyrus (Supplementary Motor Area - SMA, Brodmann area - BA6) was stimulated with TMS in all patients on both brain sides. In 8 out of 12 patients (n. 1-2-3-4-6-8-11-12), the superior parietal lobule (SPL, Brodmann area - BA7) was additionally targeted bilaterally as a control region not specifically involved in the dopamine-dependent cortical-basal ganglia-thalamo-cortical circuitry [9]. Supplementary Table 1 reports the MNI-transformed anatomical coordinates of the TMS hotspots in each patient (see also Fig. 1B and Supplementary Fig. 1). In each cortical target, the location of the maximum EF was always kept on the convexity of the gyrus with the induced current perpendicular to its main axis, about 1 cm lateral to the midline in order to prevent unwanted direct activation of scalp or facial muscles [35]. Homologous cortical areas of the left and right hemisphere were stimulated with the same estimated EF intensity and direction in a counterbalanced order across patients and sessions. Therefore, either two (SMA left and right) or four (SMA left and right; SPL left and right) TMS/EEG measurements were collected in each patient during each experimental session.
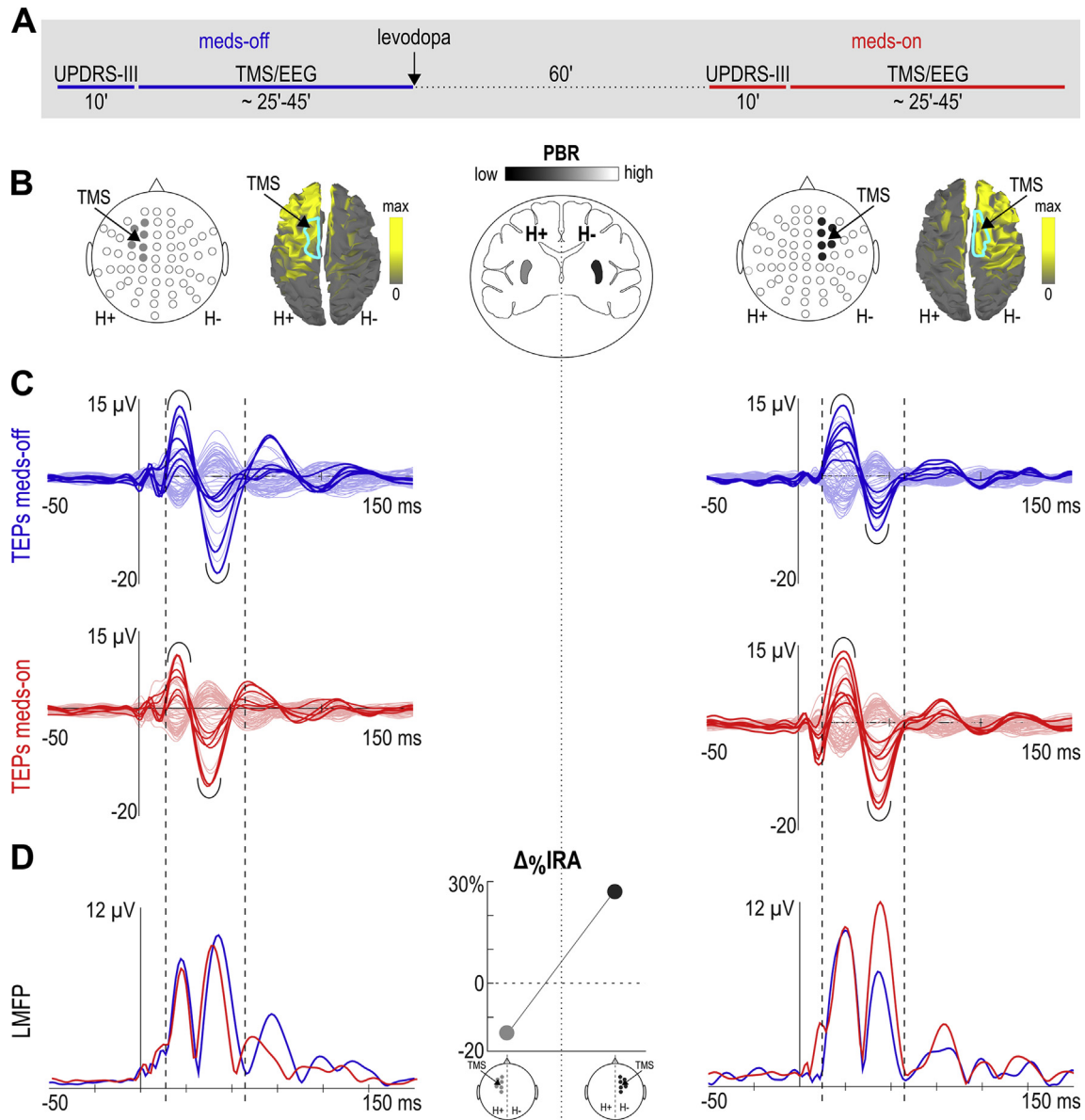
Fig. 1. Outline of the experimental protocol and data analysis procedures. A. Experimental protocol timeline. B. The central plot shows that the dopaminergic impairment of the putamen (as measured by the putamen-specific binding ratio - PBR) is asymmetric: specifically, the left part of panels B, C and D refers to the less affected brain side (H+) while the right part to the more affected brain side (H-). The topographical scalp maps show the overall EEG channels layout: the selected clusters of channels nearby the SMA (Supplementary Motor Area) target are highlighted. The location of the SMA (cyan closed path) and the spatial extent of the cortical sources that project to the corresponding cluster of selected channels (yellow) are depicted on brain maps (see Supplementary Fig. 1 for methodological details). C. Average TMS-evoked potentials (TEPs) recorded from a representative patient (n. 1) during meds-off (blue) and meds-on (red) sessions. The large and early positive and negative TEP components are respectively highlighted by a reversed U-shaped black line and a U-shaped black line. Vertical dashed lines mark the time intervals for computing the immediate response area (IRA). D. Local mean field power (LMFP) computed on the cluster of channels nearby the SMA target during meds-off (blue) and meds-on (red) sessions. The central plot shows the percentage change of IRA ($\Delta_\%IRA$) between meds-off and meds-on sessions computed nearby the stimulated SMA target on the less affected brain side (H+) and on the more affected brain side (H-).

Data preprocessing. Data analysis was carried out using MATLAB® (2014b, The MathWorks Inc.). Single-trial TMS-evoked potentials (TEPs) were visually inspected and artifact-contaminated trials (e.g. sporadic movement artifacts) were rejected from further analysis. EEG was band-pass filtered between 1 and 80 Hz, down-sampled to 725 Hz, and re-referenced to the common average reference after removal and interpolation of bad channels (always less than six and located on the scalp periphery) with the spherical method implemented in the EEGLAB toolbox [36]. The channels nearby the stimulated sites were never rejected because signal quality was always acceptable. Typical artifacts of muscular and ocular origin were reduced by subtracting visually-selected

components estimated by Independent Component Analysis (ICA) (*runica* function, EEGLAB toolbox). Average TMS-evoked potentials were computed from a minimum number of 90 artifact-free single trials (mean ± standard error across all TMS/EEG measurements: 233 ± 8 trials; range 97—380 trials) for each stimulation site and session.

Computation of the Immediate Response Area. TEPs were specifically analyzed to measure cortical excitability (here referred to as the strength of the cortical response to a direct perturbation) and its modulation by acute levodopa intake; therefore, we focused data analysis on the early components of the EEG responses evoked by TMS nearby the stimulated site, similarly to [37]. Four clusters of

six neighboring channels each were defined nearby the TMS coil positions during stimulation, according to the Nexstim manufacturer labelling (which does not rigorously correspond to the International 10−20 nomenclature): {AF1, F5, F1, FC3, FC1, C1} and {AF2, F2, F6, FC2, FC4, C2} corresponding to the left and right SMA target respectively and {C1, CP3, CP1, P3, P1, PO3} and {C2, CP2, CP4, P2, P4, PO4} corresponding to the left and right SPL target respectively (Fig. 1B and Supplementary Fig. 1 show the spatial extent of the cortical regions that project to each cluster). For each cluster, the local mean field power (LMFP) was computed as square root of squared TEPs averaged across the neighboring channels. The immediate response area (IRA) was computed as the area subtended by the LMFP over a specific time interval, which was individually defined according to the same procedure described in Ref. [37]. Specifically, we considered the early and large TEP components locally triggered by TMS, consisting in a positive wave (Fig. 1B, black reversed U-shaped line) followed by a negative wave (Fig. 1B, black U-shaped line). The time intervals were defined in correspondence to the local minima of the LMFP encompassing the early positive and negative evoked waves. For each stimulated site and patient, we defined a specific time interval based on the EEG responses obtained during the meds-off session and we used the same interval also for the corresponding meds-on session. At the group level, the following time intervals (mean ± standard deviation) were defined for the LMFP computed nearby the SMA and the SPL targets respectively: between 15.2 ± 2.76 ms and 63.4 ± 9.65 ms and between 13.8 ± 1.38 ms and 57.9 ± 11.3 ms.

In order to measure the effect of acute levodopa intake on cortical excitability, the percentage change of IRA between meds-off and meds-on sessions was computed as follows:

$$\Delta_\% IRA = \left(IRA_{on} - IRA_{off}\right)\big/ IRA_{off} \times 100$$

For each stimulated cortical region, $\Delta_\% IRA$ was computed from the cluster of channels nearby the TMS target as well as from the corresponding contralateral cluster using the same time interval.

Non-parametric permutation-based statistical analysis, as described in Ref. [37], was applied to compare IRA values between meds-off and meds-on sessions at the single-patient level. Specifically, under the null hypothesis of equivalence between meds-off and meds-on session, 1000 surrogate TEPs were computed by averaging single trials randomly selected from the two sessions; then, a null distribution of IRA values was obtained from the LMFPs

of surrogate TEPs. $\Delta_\% IRA$ was considered significant at $P < 0.05$ when either IRA_{off} or IRA_{on} values laid beyond the 2.5-th and the 97.5-th percentile tails of the null distribution.

After the identification of the H+ and H- brain sides in each patient using the PBR, group analysis was performed by pooling together the EEG responses to stimulation of either the H+ or the H- brain side, irrespective of specific anatomic side. At the group level, non-parametric Wilcoxon signed-rank test was applied to identify whether $\Delta_\% IRA$ was significantly different from 0 across patients as well as to compare between the $\Delta_\% IRA$ values obtained from stimulation of H+ and H- brain sides. The same statistical test was also used to compare clinical scores between meds-off and med-on sessions at the group level.

Results

From a clinical perspective, as expected acute levodopa intake resulted in a significant improvement of motor symptoms in all patients (Table 1). In fact, at the group level we found a significant decrease of the UPDRS-III score ($W(13) = -91$, $P = 0.0002$) as well as of the SS ($W(13) = -91$, $P = 0.0002$ on the left and $W(13) = -66$, $P = 0.001$ on the right body side) in meds-on as compared to meds-off sessions.

From a neurophysiological perspective, Fig. 2A shows the grandaverage TEPs and LMFPs obtained from the clusters of channels nearby the stimulated SMA in meds-off (blue) and meds-on (red) sessions (grandaverage TEPs obtained from all stimulation sites and sessions are shown in Supplementary Fig. 2). At the group level, $\Delta_\% IRA$ between meds-off and meds-on sessions was significantly different from 0 only on the more affected brain side H- (Table 2; $P < 0.01$). Specifically, $\Delta_\% IRA$ nearby the stimulated SMA was significantly higher ($P = 0.021$) in H- than H+ (Fig. 2B). At the single-patient level, $\Delta_\% IRA$ was always significantly different from 0 nearby the stimulated SMA on the H- brain side (Table 2). Of note, $\Delta_\% IRA$ was negative in H- and higher in H+ than H- only in one patient (n. 4), who also showed contrasting evaluations between clinical and SPECT assessments (Table 1).

As a control for the spatial extent of this effect, we applied the same analysis to the clusters of channels contralateral to the stimulated SMA and we did not find any significant difference ($P = 0.79$) of $\Delta_\% IRA$ between H- and H+ sides (Fig. 3A and Table 2). This result suggests that levodopa intake has mainly a local effect on cortical excitability nearby SMA rather than producing global
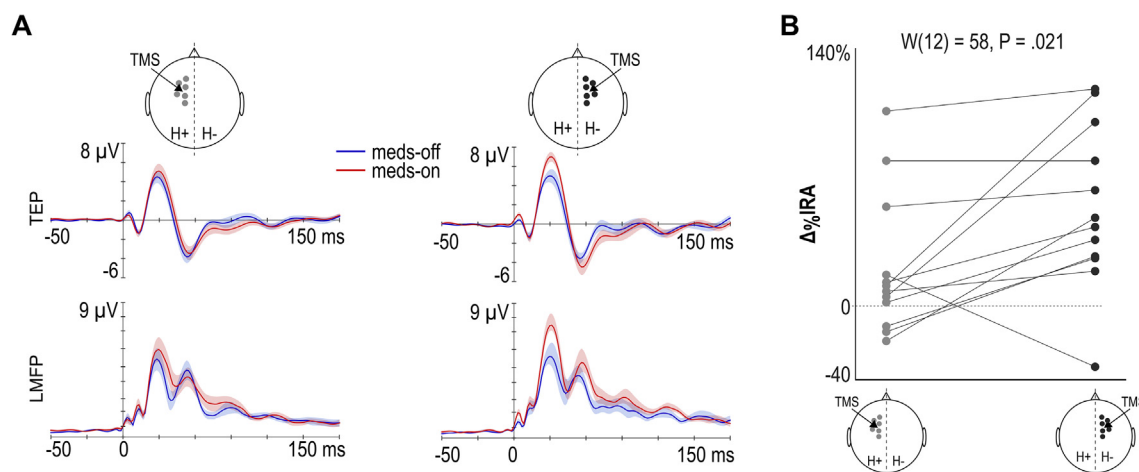

Fig. 2. Cortical excitability changes in the supplementary motor area. Grandaverage of TEPs averaged across the channels nearby the SMA target and corresponding grandaverage LMFP (shading represents the standard error). B. Percentage change of the immediate response area ($\Delta_\% IRA$) between meds-off and meds-on sessions nearby the stimulated SMA in each patient.

Table 2
Percentage change of the immediate response area ($\Delta_{\%}$IRA) between meds-off and meds-on sessions nearby and contralateral to the stimulated supplementary motor area (SMA) and superior parietal lobule (SPL) on H + and H- brain sides. H+ and H- refer to the brain side with higher and lower putamen-specific binding ratio respectively. *P < 0.05,**P < 0.01 when testing whether $\Delta_{\%}$IRA was significantly different from 0 (either assessed by non-parametric permutation-based statistical analysis at the single-patient level or by non-parametric Wilcoxon signed-rank at the group level). μ = group average; SE = standard error.

patient	SMA				SPL			
	nearby		contralateral		nearby		contralateral	
	H+	H-	H+	H-	H+	H-	H+	H-
1	−0.14*	0.27*	−0.27*	−0.05	−0.21*	−0.14	0.01	0.26
2	−0.19*	0.48*	−0.09	0.21	0.55*	0.12*	0.65*	−0.23*
3	0.11	1.16*	0.11*	0.33*	1.02	0.11	0.62	−0.03
4	0.17	−0.33*	0.15	−0.49	−0.02	−0.02	−0.09	−0.21
5	0.13	0.43*	0.05	0.62*				
6	0.08	0.19*	−0.08	0.29*	−0.03	0.24	0.17*	0.22
7								
8	0.02	0.36*	0.02	0.46*				
9	0.79*	1.00*	2.02*	0.44*				
10	1.06*	1.18*	1.09*	0.72*	0.4*	0.45*	0.53	0.36
11	−0.11	0.26*	−0.12*	−0.55*	0.07	1.59*	0.77*	0.69*
12	0.05	0.10*	0.18*	0.29*	−0.14*	1.5*	−0.01	0.84*
13	0.54*	0.63*	0.09	1.21*				
μ±SE	0.21 ± 0.11	0.48** ± 0.13	0.26 ± 0.19	0.29 ± 0.14	0.21 ± 0.15	0.48 ± 0.24	0.33 ± 0.12	0.24 ± 0.14

changes in brain responses to TMS. In addition, in order to control for the effect of levodopa on other brain regions, we analyzed the EEG responses to TMS of SPL: $\Delta_{\%}$IRA was not significantly different between H- and H+ neither nearby (Fig. 3B; P = 0.38) nor contralateral (Fig. 3C; P = 0.55) to the stimulated SPL (Table 2).

Since only 2/3 of the patients have been stimulated on SPL, we have verified whether the significance of the difference of $\Delta_{\%}$IRA between H- and H+ brain sides obtained from SMA stimulation was confirmed on 100 random samples of 7 out of 11 patients (patient n. 4 has been excluded from this analysis because of its peculiar characteristics with respect to SPECT and clinical assessment). Non-parametric Wilcoxon signed-rank test confirmed that $\Delta_{\%}$IRA nearby the stimulated SMA was significantly higher (P = 0.016) in H- than H+ in all 100 random samples.

When disregarding SPECT imaging, the comparison of $\Delta_{\%}$IRA between the left and right brain side did not reveal any significant difference (nearby the stimulated SMA: W(12) = −22, P = 0.42; contralateral to the stimulated SMA: W(12) = 20, P = 0.47; nearby the stimulated SPL: W(8) = −2, P = 0.95; contralateral to the stimulated SPL: W(8) = 6, P = 0.74). This result further highlights that the observed modulation of cortical excitability nearby SMA is

specifically driven by the asymmetric impairment of dopaminergic neurons in the putamen.

Discussion

This study is the first to demonstrate that TMS/EEG allows non-invasively measuring the neurophysiological effects of an acute levodopa intake in cortical areas other than the primary motor cortex.

We observed that cortical excitability, as measured by the early EEG responses to TMS elicited nearby the stimulated site, significantly changes following levodopa administration. More specifically, we found that cortical excitability nearby the SMA was significantly increased ipsilateral to the more affected brain side and that this increase was higher than in the less affected hemisphere. Notably, in accordance with the very unique role of SMA in the cortico-basal-ganglia-thalamo-cortical circuitry, the EEG responses to TMS of the parietal cortex (SPL) were not significantly affected by levodopa intake on both sides.

Previous fMRI studies [3,38] have shown that the hemodynamic SMA activity is abnormally reduced during meds-off condition and
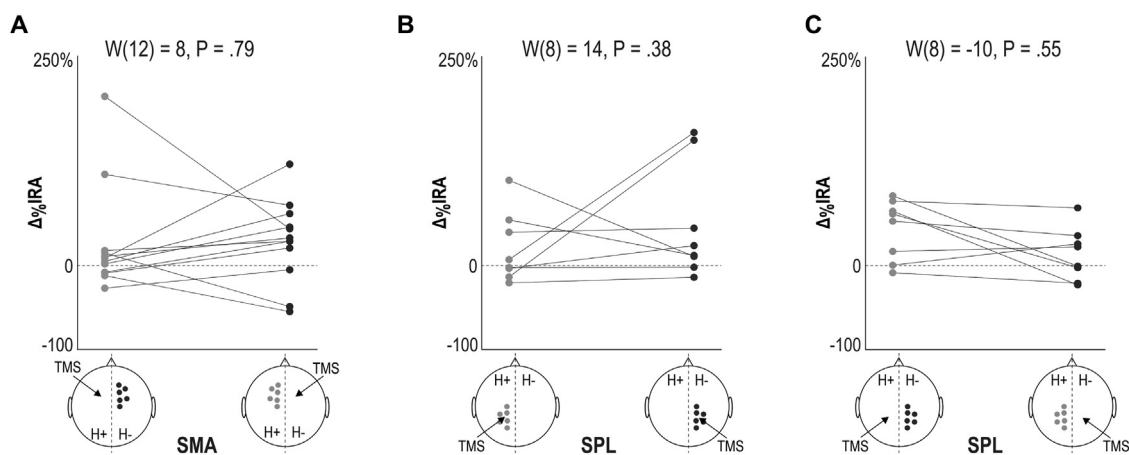

Fig. 3. **Group results concerning control sessions.** Percentage change of the immediate response area ($\Delta_{\%}$IRA) between meds-off and meds-on sessions (A) contralateral to the stimulated SMA, (B) nearby the stimulated SPL and (C) contralateral to the stimulated SPL in each patient.

is relatively normalized after levodopa intake. In line with these previous imaging findings, our TMS/EEG based neurophysiological approach further confirms that levodopa replacement therapy affects the cortical activity nearby the SMA by producing an increase of the local neural response to a direct stimulation.

On the more affected brain side, the levodopa-related modulation of cortical excitability nearby the SMA was significant at the single-patient level (Table 2): notably, the direction of this change was the same (an increase) in all but one patient, who showed a significant decrease on the more affected and increase on the less affected brain side. This pattern of "reversed excitability" was particularly interesting because only in this patient worse motor clinical signs were ipsilateral to the most affected putamen. Therefore, in this case, the modulation of cortical excitability nearby the SMA induced by acute levodopa intake was more in agreement with the clinical assessment. Since the degree of motor asymmetry can be more pronounced than the one measured by FP-CIT and SPECT [39], other factors possibly involving non-dopaminergic derangements [40,41] might contribute to this peculiar presentation.

Although the entire basal ganglia system is widely altered in PD, DAT measurements of the putamen returned better predictors of motor disability in comparison with equivalent measurements of the whole striatum [42,43]. Interestingly, specific anatomical and functional connections have been reported between the putamen and the SMA in both animal [10,44] and human studies by means of resting-state functional magnetic resonance imaging [13,14] and diffusion tensor imaging [12]. Our study provides further evidence for this preferential relationship by showing that the levodopa-related asymmetric increase of cortical excitability nearby the SMA parallels the clinical presentation and the dopaminergic impairment of the putamen.

So far, measurements of cortical excitability in PD have been conducted by applying TMS on the primary motor cortex to measure motor threshold, motor-evoked potentials, electromyographic silent period to cortical stimulation and intracortical facilitation/inhibition [7]. The most consistent finding is an excessive cortico-spinal output at rest and a reduced intracortical inhibition, especially on the more affected brain side [18]: these results has been mainly interpreted as compensatory for deficiency of movement facilitation, which characterizes PD [19,20,45]. Our results cannot be directly compared to these previous studies because we targeted different cortical sites (i.e. SMA and SPL) and directly measured the evoked brain responses to TMS instead of the evoked peripheral activity. However, functional neuroimaging studies have associated the increase of motor cortex excitability with a concurrent hypo-activation of the SMA and have revealed that these abnormal patterns can be relatively normalized by therapy [3]. Therefore, the analysis we carried out at the single-subject level further supports the existence of pathological as well as compensatory mechanisms that involve an abnormal flow of information, reaching the SMA.

While SMA hyperactivation has been shown to predict the development and severity of levodopa-induced dyskinesia [46,47], SMA hypoactivation has been frequently linked to the pathogenesis of bradykinesia [48]. In agreement with these hypotheses, while low-frequency inhibitory rTMS on the SMA has been shown to transiently improve involuntary movements [49], high-frequency excitatory rTMS has been successfully applied to reduce hypo-kinetic symptoms in some patients, possibly by facilitating SMA activity [15,48].

Levodopa disposition is highly variable both within and between patients because of several peripheral and central pharma-cokinetic features, which also account for its marked plasmatic concentration fluctuations over time and eventually affect the actual drug amount reaching the neural targets [38,50,51]. In this study, we stimulated the different cortical targets in a counter-balanced order across patients and sessions, starting about 60' after intake of the same amount of levodopa. Although the pharmaco-logical effects on neurophysiological parameters is time-dependent, we chose to assess excitability of different cortical regions in a counterbalanced order across patients and sessions in order to prevent any bias towards a specific brain region.

The present study is the first to show an asymmetric effect of levodopa intake on cortical excitability associated with the corresponding degree of dopaminergic impairment in the putamen. We did not include a control group to evaluate the performance of a physiological dopaminergic system and the placebo effect since normative values for cortical excitability are not available: nonetheless, the less affected hemisphere could be used as a reliable intra-individual control [38]. It would be certainly interesting to replicate and further confirm these experimental results in a selected and larger population with a more controlled and homogeneous difference between the left and right basal ganglia system.

The results reported in this study refer to the signals recorded at the scalp level: this approach allows to pool together the EEG responses to stimulation of either the H+ or the H- brain side, irrespective of specific anatomic side. Clearly, the interpretation of these results in terms of source activity locally generated at specific cortical areas is challenging because of several reasons. First, TMS pulses actually excite a patch of cortical surface as large as several cm^2 [52,53], thus preventing to constrain direct perturbation within a small anatomical region. Second, the electrical activity of a point-like source actually projects over several EEG electrodes as well as the signal recorded at a single electrode is affected by the activity of a spatially large cortical area. Third, different EEG electrodes display partially correlated signals because of a certain overlap among the cortical regions projecting to them. The application of rigorous source modelling methods would be useful to increase the spatial accuracy of the results and could possibly reveal local effects of levodopa within specific cortical regions. However, in this case, patients with the H- brain side on the left should be analyzed separately from patients with the brain side H- on the right, in order to properly account for inter-hemispheric anatomical differences.

In conclusion, our findings support the use of navigated TMS/EEG as a non-invasive tool to better understand the role of different cortical regions in the pathophysiology of PD. From a neuro-modulatory perspective, the pattern of cortical excitability nearby the SMA revealed by TMS/EEG should be further exploited to identify and candidate PD patients to specific inhibitory (low-frequency) or excitatory (high-frequency) rTMS protocols. In this respect, TMS/EEG approach could also be used to monitor cortical excitability over time and possibly predict the onset of levodopa related adverse events (e.g. dyskinesia) along with disease progression.

Conflicts of interest disclosure

The authors declare no competing financial interests.

Authors' contributions

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Francesco Turco: 1BC.
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Mario Rosanova: 1AC, 2C, 3AB
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1. Research project: A. Conception, B. Organization, C. Execution;
2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique;
3. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique;

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2018.10.011.

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