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Cerebellar Sensory Processing Alterations Impact Motor Cortical Plasticity in Parkinson's Disease: Clues from Dyskinetic Patients

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The plasticity of primary motor cortex (M1) in patients with Parkinson's disease (PD) and levodopa-induced dyskinesias (LIDs) is severely impaired. We recently reported in young healthy subjects that inhibitory cerebellar stimulation enhanced the sensorimotor plasticity of M1 that was induced by paired associative stimulation (PAS). This study demonstrates that the deficient sensorimotor M1 plasticity in 16 patients with LIDs could be reinstated by a single session of real inhibitory cerebellar stimulation but not sham stimulation. This was evident only when a sensory component was involved in the induction of plasticity, indicating that cerebellar sensory processing function is involved in the resurgence of M1 plasticity. The benefit of inhibitory cerebellar stimulation on LIDs is known. To explore whether this benefit is linked to the restoration of sensorimotor plasticity of M1, we conducted an additional study looking at changes in LIDs and PAS-induced plasticity after 10 sessions of either bilateral, real inhibitory cerebellar stimulation or sham stimulation. Only real and not sham stimulation had an antidyskinetic effect and it was paralleled by a resurgence in the sensorimotor plasticity of M1. These results suggest that alterations in cerebellar sensory processing function, occurring secondary to abnormal basal ganglia signals reaching it, may be an important element contributing to the maladaptive sensorimotor plasticity of M1 and the emergence of abnormal involuntary movements.

Keywords: cerebellum, dyskinesias, L-DOPA, motor cortex plasticity, Parkinson's disease

Introduction

The production of a normal, smooth movement requires the timely involvement of numerous structures that issue concerted commands adapted to a given context and the integration of the information from within and outside the body (Shadmehr et al. 2010). Impairment in the functioning of any element in the chain of command will result in movements unsuited for the desired aim or undesired movements. Levodopa-induced dyskinesias (LIDs) exemplify such involuntary movements and are related to abnormal signaling in the striato-thalamo-cortical system in Parkinson's disease (PD). Non-invasive cortical stimulations studies in PD patients with LIDs have shown an impaired ability of the primary motor cortex (M1) to develop LTP and LTD-like plasticity (Morgante et al. 2006; Kishore et al. 2012) as well as to depotentiate LTP-like plasticity (Huang et al. 2011). These cortical abnormalities are viewed as a manifestation of the maladaptive cortico-striatal plasticity that is transmitted to M1 through the striato-thalamo-cortical network (Picconi et al. 2008). Human studies in PD indicate that the cerebello-thalamo-cortical network may also influence LIDs (Nimura et al. 2004; Koch et al. 2009).

We recently reported that in healthy young adults (Popa et al. 2013), the plasticity induced in M1 by paired associative stimulation (PAS) was enhanced when PAS was preceded by inhibitory stimulation of cerebellar cortex. Such a plasticity-enhancing effect of cerebellar stimulation was not seen when theta-burst stimulation (TBS), which does not involve peripheral sensory input, was used instead of PAS to stimulate M1. This suggested that the cerebellum or related structures (thalamus, dentato-olivary nuclear complex) have an important role in controlling the plastic changes occurring within M1 by modulating the sensory afferents upstream of M1. Here, we took advantage of the same experimental paradigm to explore 1) if cerebellar inhibitory stimulation is able to enhance the deficient and L-DOPA-unresponsive plasticity of M1 associated with LIDs in PD (Morgante et al. 2006; Kishore et al. 2012) and 2) whether this could be the functional mechanism underlying the reduction of LIDs after multiple sessions of cerebellar stimulation reported earlier (Koch et al. 2009). As the effects of L-DOPA on motor signs in PD are primarily mediated through the striato-thalamo-cortical pathway, any demonstrable influence of cerebellar modulation on both dyskinesias and the sensorimotor plasticity of M1 would provide an evidence for the functional link between the striato-thalamo-cortical network and the cerebello-thalamo-cortical network in human PD.

Materials and Methods

Subjects

Sixteen patients with PD (mean age: 55 ± 2.2 years) who experienced peak-dose LIDs participated in the single-session study and 20 patients in the multiple-sessions study (included 10 patients from the single-session study and 10 new dyskinetic patients). All patients satisfied the UK Parkinson's Disease Society brain bank clinical criteria (Hughes et al. 1992) and were selected by a movement disorders specialist from the Movement Disorders clinic of a university hospital (SCTIMST). All patients were right handed according to the Edinburgh handedness inventory (Oldfield 1971). None of the patients had any signs of dementia or were treated with antidepressants. All patients agreed to maintain stable doses of the antiparkinsonian medications for at least 1 month prior to the study and till the end of the last follow-up session. The Unified Parkinson's Disease Rating Scale (UPDRS) III scores in OFF and ON (Fahn et al. 1987), as well as

the severity of LIDs, were measured at the screening visit using the CAPSIT protocol (Defer et al. 1999). The study was approved by the local Ethics Committee and performed according to the ethical standards laid down in the Declaration of Helsinki. All subjects gave their written informed consent before the experiments.

Experimental Protocol

Single-Session Study

All 16 patients recruited in the study underwent 3 independent transcranial magnetic stimulation (TMS) sessions on 3 different days (Fig. 1*A*):

1. One session was performed in OFF (after overnight withdrawal of dopaminergic drugs). It consisted of PAS delivered on M1 (PAS_{OFF}) contralateral to the more affected side of body, without any preceding cerebellar stimulation.
2. A second session was performed in ON (1 h after the usual morning dose of levodopa equivalent of drugs given as L-DOPA/C-DOPA, and after clinical confirmation of the ON state). It consisted of a continuous theta-burst stimulation (cTBS) of the cerebellar hemisphere ipsilateral to the more parkinsonian side of the body, followed immediately by PAS delivered on the contralateral M1 ($cTBS_{CB\text{-}ON} \rightarrow PAS$).
3. A third session was performed in ON and consisted of a sham cTBS of the ipsilateral cerebellar hemisphere, followed immediately by PAS delivered on the contralateral M1 ($Sham_{CB\text{-}ON} \rightarrow PAS$).

The order of the 3 sessions was randomized.

Single-Session Study: Control Experiments

1. In order to test whether the effects observed in the single-session study were specific to PAS (i.e., dependent on the peripheral input and on the heterosynaptic plasticity), some of the patients ($n = 7$) were tested further in ON, on 2 different days. In this experiment, PAS was replaced with intermittent theta-burst stimulation (iTBS) of M1 (i.e., a stimulation that induces LTP-like plasticity through a homosynaptic mechanism, independent of sensory inputs). These additional sessions were coded similar to the sessions in the single-session study as $cTBS_{CB\text{-}ON} \rightarrow iTBS$ and $Sham_{CB\text{-}ON} \rightarrow iTBS$. In our previous study in young healthy volunteers (HV), the effects of cerebellar modulation were specific to the PAS-induced plasticity, without any influence on the iTBS-induced plasticity within M1 (Popa et al. 2013).
2. The effects of cerebellar modulation on PAS-induced plasticity were also tested in 16 gender- and age-matched (54.5 ± 2.5 years) HV. Cerebellar cTBS was followed immediately by PAS delivered on the contralateral M1 ($cTBS_{CB} \rightarrow PAS$), and sham cerebellar stimulation was followed immediately by PAS on the contralateral M1 ($Sham_{CB} \rightarrow PAS$). The HV were not tested after L-DOPA.

Multiple-Sessions Study

A beneficial effect of multiple sessions of bilateral cerebellar inhibitory stimulation on LIDs has already been demonstrated (Koch et al. 2009). In order to confirm such an effect and to find out whether the antidyskinetic effect of cerebellar stimulation is linked to the improvement of sensorimotor plasticity of M1, we conducted an additional study with ten sessions spread over 10 days. There was a minimum of a 1-month gap between the single-session and the multiple-sessions studies. Twenty PD patients with severe LIDs (i.e., duration of LIDs for >25% of the awake time; UPDRS IV A, item $32 \geq 2$) that were at least moderately disabling (UPDRS IV A, item $33 \geq 2$) were randomized in 2 groups undergoing either real or sham, multiple cerebellar stimulation (Fig. 1*B*). Ten of these patients had already participated in the single-session study.

Each group received 10 daily sessions of bilateral, real inhibitory cerebellar, or sham cerebellar stimulation in ON (bilateral-$cTBS_{CB\text{-}ON}$ or bilateral-$Sham_{CB\text{-}ON}$). The cerebellar stimulation was performed only in ON to demonstrate the effect of cerebellar modulation on cortical plasticity, after potentially normalizing the dysfunction in the striato-thalamo-cortical circuit by dopamine replacement. The severity of LIDs was evaluated before treatment and 2 weeks after the end of the last stimulation session in both groups. Patients who were randomized into the sham arm for the first session, crossed over to real stimulation arm 2 weeks after the end of the sham stimulation and after completing the second week clinical assessment (Fig. 1*B*). Those in the real stimulation arm did not cross over to sham stimulation, as the duration of benefit of real stimulation was also an outcome measure of the study. Patients were told that they could receive either sham or real stimulation in the first session, but they were not told that those receiving real stimulation would not cross over to sham till the end point of the second week assessment was completed. Those who crossed over to the second session were not aware of the type of stimulation they received in each session. Thus, it was ensured that both groups remained blinded to the type of stimulation received till the end of the second week assessment.

EMG Recordings

The subjects were seated comfortably in an armchair, with both hands resting symmetrically on a pillow in their lap. MEPs were recorded from the Abductor pollicis brevis (APB) muscle. Responses were amplified (1000×) and filtered (100–3000 Hz) with a Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, UK), then digitally transformed at a sampling rate of 10 000 Hz (CEDPower 1401 MkII, CED Ltd, Cambridge, UK), and stored offline for analysis (Signal 4.02, CED Ltd, Cambridge, UK). The EMG activity was continuously monitored to ensure muscle relaxation. Patient's alertness was monitored and verified. Trials contaminated by EMG activity anywhere within 500 ms around each MEP were discarded from the offline analysis.

TMS Sessions

Evaluation of Cortico-Spinal Excitability

The TMS pulses were applied over the M1 of the more affected hemisphere (contralateral to the limbs with more LIDs) in PD patients and over the M1 of the dominant (left) hemisphere in HV, with a 70-mm figure-of-eight coil connected to a Bistim magnetic stimulator (The Magstim Company, Whitland, UK). The "hot spot" for the APB muscle was identified and marked on a default brain reconstruction with the help of an MRI-based neuronavigation system (Brainsight2, Rogue Resolutions, Cardiff, UK). It allowed us to maintain the ideal stimulation position throughout each session and from one session to the next, in the same patient. The resting (RMT) and active (AMT) motor thresholds were calculated according to the standard procedures (Rossi et al. 2009).

The cortico-spinal excitability was assessed with single-pulse TMS delivered at 0.2 Hz and with an intensity of 130% RMT. Fifteen MEPs were averaged prior to the intervention (T0), and at 5 min (T5), 15 min (T15), and 30 min (T30) after the end of the PAS.

Intracortical Inhibitions

Intracortical inhibitions were evaluated with paired-pulse TMS, where the intensity of the test TMS pulse was set to evoke an MEP ~1 mV. Fifteen trials were recorded for each condition.

For the short-interval intracortical inhibition (SICI), the intensity for the conditioning TMS pulse was set at 70% RMT and the interstimulus interval (ISI) was 2.5 ms (Fisher et al. 2002). For the long-interval intracortical inhibition (LICI), the intensity of the conditioning stimulus was set at 110% RMT; the ISI was 100 ms. For the short-latency afferent inhibition (SAI) and the long-latency afferent inhibition (LAI), the conditioning stimulation was an electrical stimulus (200-μs square pulse) delivered to the median nerve with intensity 2 × perceptual threshold using a Digitimer DS7A Constant Current Stimulator (Digitimer, Welwyn Garden City, Herts, UK). The ISI was 20 ms for SAI, and 100 ms for LAI.

Inhibition was expressed as a percentage of the mean peak-to-peak amplitude of the conditioned MEP referred to the unconditioned MEP in the respective block. The inhibitions were measured pre- and post-intervention, with the test TMS pulse intensity adjusted post-intervention, if necessary, to get a test MEP ~1 mV; the

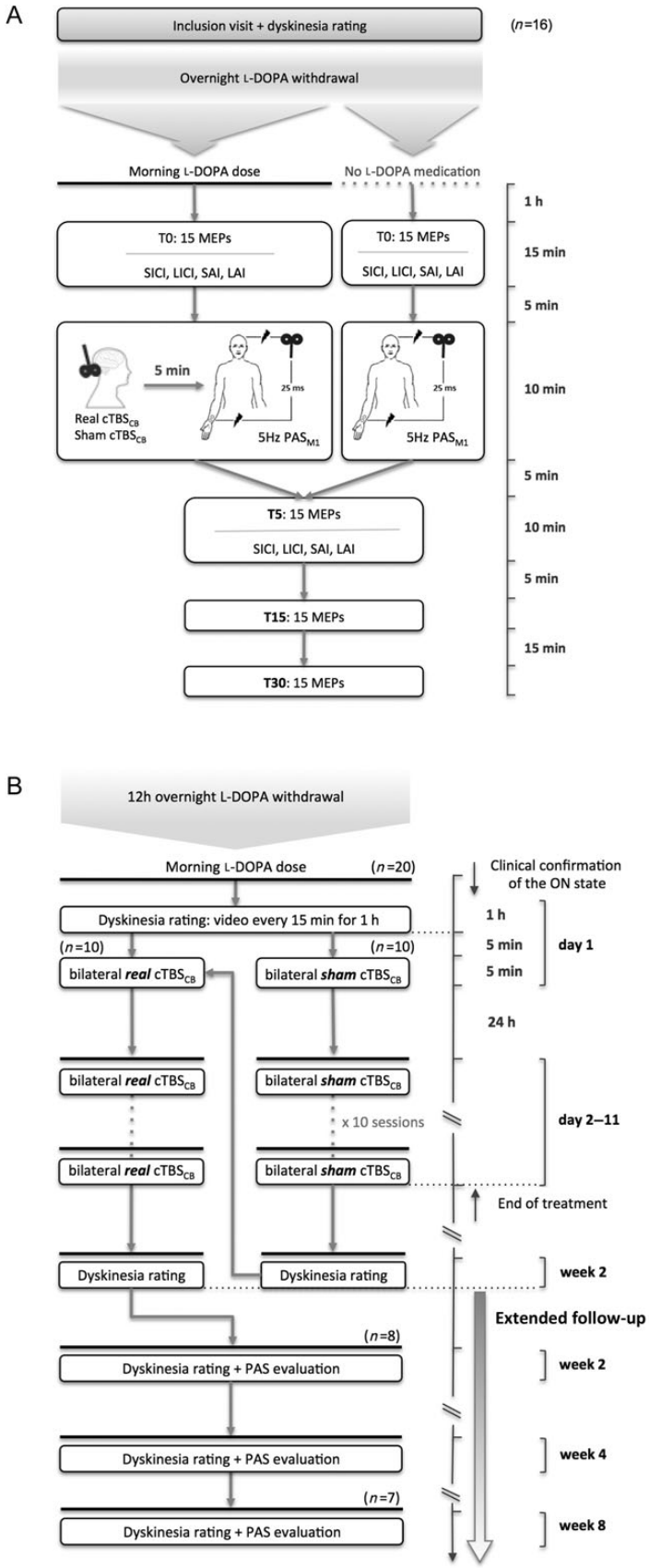

Figure 1. Experimental design. Flow diagram of patients enrolled in the (*A*) single-session study and (*B*) multiple sessions study.

conditioning pulse intensity was kept at the same level pre- and post-intervention.

Paired Associative Stimulation of M1

For PAS, electric stimulation pulses were delivered over the median nerve at the wrist at 2.5× the perceptual threshold, or just below the electromyographically measured motor threshold, whichever was lower. Each pulse was followed 25 ms later by a magnetic pulse delivered over the hotspot of APB at 90% AMT. Six hundred pairs of stimuli were delivered at 5 Hz. This stimulation increases the excitability of M1 when delivered alone (Quartarone et al. 2006). All experiments using PAS were performed at the same hour in the morning in order to reduce variability (Sale et al. 2007).

Cerebellar Stimulation Target

Cerebellar stimulation targeted the lateral part of lobule VIII (Popa et al. 2010), since this is the most superficial part of the sensorimotor cerebellum (Stoodley and Schmahmann 2009) and thus easily accessible to low-intensity rTMS. The landmark of 2 cm lower and 4 cm lateral to the inion was used for identifying this area (Popa et al. 2010) and it was confirmed with MRI-guidance in patients having their own MRI.

Single-Session Cerebellar Inhibitory Stimulation

The stimulation protocol was detailed in a previous paper (Popa et al. 2013) and will only be summarized here. A figure-of-eight cooled coil (inner diameter of each loop 70 mm) connected to a SuperRapid^2 magnetic stimulator (Magstim Company, Whitland, Wales, UK) was used to deliver classical cTBS stimulation (Huang et al. 2005) to the cerebellum ipsilateral to the more affected side of the body. In young healthy subjects, such stimulation potentiates for at least 20–30 min the facilitatory effect of a subsequent PAS delivered over the M1 (Popa et al. 2013). It is unknown whether such a potentiation occurs following cerebellar stimulation in older subjects. This was tested in this study in a group of HV who were age-matched with the patient group.

Multiple Sessions of Cerebellar Inhibitory Stimulation

Twenty dyskinetic patients were randomized to receive a 10-day course of bilateral cerebellar stimulation (bilateral-cTBS$_{CB\text{-}ON}$) or bilateral sham stimulation of cerebellum in ON (bilateral-Sham$_{cB\text{-}ON}$) (Fig. 1*B*). The severity of LIDs was evaluated at 2 weeks after the end of the last stimulation session. The cerebellum was stimulated bilaterally to have a clinical effect on both sides of the body. Sessions were conducted daily at the same hour in the morning (9 AM) for each patient, 1h after the morning dose of drugs and after confirming the clinical ON state. There were 10 consecutive days of stimulation (with a break only on the seventh day, a Sunday). Two trains of cTBS (each of 600 pulses and lasting 40 s) were applied at 80% AMT over the left and right lateral cerebellum, with a pause of 2 min between the 2 trains. The order of the stimulated side was pseudo-randomized in each subject in every session. Sham stimulation was performed by maintaining the stimulation intensity at 80% AMT, but moving the coil down by 5 cm. This type of stimulation has been shown not to influence the cerebellar output, but only induce the same twitch in the neck muscles (Popa et al. 2010).

Eight patients (either from real cerebellar stimulation alone arm or from sham and real stimulation arm) after the end of their real treatment sessions were monitored beyond the second week follow-up from the end of treatment sessions, up to 4 and 8 weeks (Fig. 1*B*). In this group, PAS-induced plasticity was tested in ON at each follow-up session to find out whether clinical benefits on LIDs and restoration of cortical plasticity after multiple bilateral-cTBS$_{CB\text{-}ON}$ had similar time courses.

Dyskinesia Rating

LIDs were measured with the CAPSIT dyskinesias scale (Defer et al. 1999) at baseline before the single-session study and then at the beginning and the specific time points of the follow-up in the multiple sessions study (Fig. 1). The LED of the usual morning dose of drugs was administered as L-DOPA/C-DOPA after 12 h of overnight medication withdrawal. LIDs were videotaped every 15 min for 1 h, starting from the clinically confirmed beginning of ON. At the end of the study, LIDs from both pre-and post-treatment assessments were rated on the videos presented in a random order to 2 blinded movement disorder specialists. Total dyskinesia scores over the 4 time points and the worst score among the 4 time points (both pre- and post-treatment) were used for analyses.

Patients also completed an hourly "ON–dyskinesia–OFF "diary for 5 days before the start of the treatment sessions, and 5 days prior to the 2 weeks follow-up from the end of each treatment session (real or sham). Prior to the study, all patients were trained in the identification of 4 motor states: no mobility or worst mobility (complete OFF), moderate mobility (partial OFF), good mobility without troublesome dyskinesia (ON without troublesome dyskinesias), and mobility with troublesome dyskinesia (ON with troublesome dyskinesias).

Data Analysis

Single-Session Study

1. The effects of PAS were compared "within" each group (dyskinetic PD patients in OFF, in ON and HV) using repeated-measures ANOVA (rANOVA) with the raw values of the MEPs forming the repeats (at T0, T5, T15, T30, factor "TIME"). The effects of PAS were compared "among" the 3 groups using rANOVA with the normalized values of the MEPs (MEP_{T5}/MEP_{T0}, MEP_{T15}/MEP_{T0}, MEP_{T30}/MEP_{T0}) forming the repeats (TIME) and "GROUP" (PD ON, PD OFF and HV) being the intersubject variable.
2. The effects of PAS or iTBS delivered to M1 were compared between the 2 groups and between the different interventions using rANOVA with the 6 normalized values of the averaged MEPs forming the repeats (MEP_{T5}/MEP_{T0}, MEP_{T15}/MEP_{T0}, MEP_{T30}/MEP_{T0}, factor "TIME"), after $Sham_{CB}$ and $cTBS_{CB}$ (factor "INTERVENTION") and the factor "GROUP" (HV, PD patients in ON) being the intersubject variable. Bonferroni correction in post-hoc test was applied to characterize the time course of the parameters after each type of intervention.
3. The effect of L-DOPA on RMT, AMT, SICI, LICI, SAI and LAI were evaluated by comparing their respective values at baseline in OFF (i.e., prior to PAS_{OFF}) and in ON (i.e., prior to $Sham_{CB\text{-}ON}$) using paired *t*-tests.
4. The effect of cerebellar stimulation on PAS-induced changes of cortical excitability was evaluated by comparing the normalized values of RMT, AMT, SICI, LICI, SAI and LAI recorded after the 2 interventions in ON ($Sham_{CB\text{-}ON} \rightarrow PAS$ and $cTBS_{CB\text{-}ON} \rightarrow PAS$) with paired *t*-tests. The normalization was done using the formula: [(value postcerebellar stimulation) − (value at baseline)]/(value at baseline).
5. The possible placebo effect of the sham cerebellar stimulation was tested by comparing the effects of $Sham_{CB\text{-}ON} \rightarrow PAS$ and PAS_{ON} alone on M1 plasticity in 6 of the subjects who underwent both interventions, using rANOVA.
6. Linear regression analysis was used to correlate the effect of PAS_{OFF}, $Sham_{CB\text{-}ON} \rightarrow PAS$, and $cTBS_{CB\text{-}ON} \rightarrow PAS$ with the clinical parameters (in Table 1). The effect of PAS was assessed at its peak, that is, mean MEP amplitude at 15 min after the end of the PAS (MEP_{T15}).

Multiple-Sessions Study

1. Variations of the clinical scores [$(score_{baseline} - score_{2weeks})/score_{baseline}$] after 10 sessions of real ($n = 20$) or sham ($n = 10$) stimulation were compared using unpaired *t*-test.
2. In order to find out whether one session of bilateral cerebellar stimulation alone is able to improve LIDs, the worst dyskinesias score was recorded in a subgroup of 10 patients receiving the real stimulation, on the second day of the treatment, just before the due stimulation. This second day score was compared with the scores of the first day and at 2 weeks after the end of all ten sessions.

Table 1
Clinical characteristics of the 16 subjects in the single-session cerebellar stimulation study

Subjects enrolled	Age (years)	Sex	Disease duration (years)	Duration of treatment (years)	UPDRS III OFF	UPDRS III ON	Worst dyskinesia score	Total dyskinesia score	Total LED (mg/day)	Morning dose of DOPA given
Patient 1[a]	65	M	4	4	37	23	9	16	500	100
Patient 2	53	M	11	11	48	10	10	18	654	250
Patient 3	65	M	10	3	47	8	7	6	890	200
Patient 4[a]	63	M	10	8	23	5	13	31	665	150
Patient 5[a]	50	M	10	7	60	11	8	22	750	150
Patient 6	57	M	8	6	34	5	7	14	665	200
Patient 7[a]	50	M	6	6	24	7	7	26	2225	350
Patient 8	67	F	9	9	46	14	10	20	532	150
Patient 9	45	M	6	6	69	15	7	16	1025	275
Patient 10[a]	38	M	12	10	39	14	13	52	482	150
Patient 11[a]	52	M	11	10	49	15	7	30	330	100
Patient 12[a]	50	M	11	10	51	18	9	33	1350	375
Patient 13	45	M	8	7	22	6	8	12	800	200
Patient 14[a]	62	M	15	15	22	10	8	35	375	100
Patient 15[a]	50	F	6	6	39	3	12	36	680	150
Patient 16[a]	63	F	6	6	24	9	14	57	1100	250
Mean ± SEM	**54.5 ± 2.2**	***M* = 13 *F* = 3**	**8.9 ± 0.71**	**7.8 ± 0.74**	**39.6 ± 3.59**	**10.8 ± 1.34**	**9.3 ± 0.61**	**26.5 ± 3.51**	**688.9 ± 74.26**	**196.9 ± 21.02**

Note: SEM, standard error of mean.
[a]Patients who also participated in the multiple sessions study.

Table 2
Clinical characteristics of subjects in the multiple-sessions cerebellar stimulation study

Subjects enrolled	Age (years)	Gender	Disease duration (years)	UPDRS III OFF	UPDRS III ON	Worst dyskinesia score	Total dyskinesia score	Total LED (mg/day)	Morning dose L-DOPA (mg/day)
Active only									
Patient 1[a]	65	M	4	37	23	9	16	500	100
Patient 2[a]	63	M	10	23	5	13	31	665	150
Patient 3[a]	50	M	10	60	11	8	22	750	150
Patient4[a]	38	M	12	39	14	13	52	482	150
Patient 5[a]	52	M	11	49	15	7	30	330	100
Patient6[a]	62	M	15	22	10	8	35	375	100
Patient7[a]	63	F	6	24	9	9	57	1100	250
Patient8	60	F	10	70	26	9	36	1085	250
Patient 9	70	F	10	51	20	15	60	625	150
Patient 10	56	F	10	41	11	13	56	400	100
Mean ± SEM	**57.9 ± 2.91**	M = **6** F = **4**	**9.8 ± 0.95**	**41.6 ± 5.11**	**14.4 ± 2.10**	**10.4 ± 0.69**	**39.5 ± 4.95**	**631.2 ± 87.51**	**150 ± 18.22**
Sham and active									
Patient 11[a]	50	M	11	51	18	9	33	1350	375
Patient12[a]	50	F	6	39	3	12	36	680	150
Patient13[a]	57	M	12	26	1	7	26	2225	350
Patient14	67	F	18	38	17	10	27	675	75
Patient 15	52	F	12	62	13	18	69	685	175
Patient16	48	F	12	24	11	6	22	880	250
Patient17	44	M	8	18	4	23	84	700	100
Patient18	60	M	7	35	15	8	37	200	50
Patient19	64	M	14	54	18	11	14	1700	150
Patient 20	62	M	13	35	15	15	52	600	100
Mean ± SEM	**55.4 ± 1.91**	M = **6** F = **4**	**11.3 ± 0.89**	**38.2 ± 3.49**	**12.1 ± 1.78**	**11.9 ± 1.3**	**40.1 ± 6.96**	**969.5 ± 151.73**	**177.5 ± 28.17**

Note: SEM, standard error of mean.
[a]Patients who also participated in the single-session study.

3. Linear regression analysis was used to correlate the change in the dyskinesia scores with the additional effect elicited by one session of $cTBS_{CB\text{-}ON} \rightarrow PAS$ on M1 plasticity beyond the L-DOPA effect, that is, [(MEP after $cTBS_{CB\text{-}ON} \rightarrow PAS$) − (MEP after $Sham_{CB\text{-}ON} \rightarrow PAS$)]/(MEP after $Sham_{CB\text{-}ON} \rightarrow PAS$), in the 10 patients who participated in both the single-session and multiple-sessions studies. M1 plasticity was measured at its peak effect (MEP_{T15}). We also looked for a correlation between the baseline severity of LIDs and the additional effect elicited by one session of $cTBS_{CB\text{-}ON} \rightarrow PAS$.
4. Because of the small size of the sample ($n = 8$), long-term effects (at second, fourth, and eighth week follow-up from end of treatment) of 10 sessions of bilateral-$cTBS_{CB\text{-}ON}$ on clinical scores were assessed by nonparametric Wilcoxon test (*W*-test). The Bonferroni correction for multiple comparisons set the level of significance to $P < 0.017$, since 3 comparisons were performed. The long-term effects of the treatment were also tested on the PAS_{ON}-induced plasticity using rANOVA with the normalized values of the MEPs forming the repeats, and "FOLLOW-UP" (weeks 2, 4, and 8) being the intrasubject variable.

For all statistical analyses, a $P < 0.05$ was assumed to denote significance, unless adjusted for multiple comparisons. Stat View software (SAS Institute, Inc., Cary, NC, USA) was used for all statistical analyses.

Results

The clinical characteristics of the subjects who participated in the 2 studies are provided in Tables 1 and 2. The subjects did

not report any adverse effects after any of the interventions. The intraclass correlation for the video-based dyskinesia ratings by the 2 movement disorder specialists was 0.92, and a consensus score was derived when there was a difference between individual scores.

Single-Session Study

Plastic Response of M1 to PAS Is Weak in Dyskinetic PD Patients in OFF and ON and Similar to Age-Matched HV

We found that M1 of dyskinetic PD patients in OFF was only weakly responsive to the 5 Hz PAS protocol (raw MEP values: rANOVA: TIME $F = 0.8$, $P = 0.5$). Even in ON, patients remained poorly responsive to 5 Hz PAS (TIME $F = 0.4$, $P = 0.7$). The effect of PAS in older age-matched HV was similarly low ($F = 1.6$, $P = 0.2$) (Fig. 2*A*). This was confirmed by comparing the normalized values of MEPs (T5/T0, T15/T0, T30/T0) between HV and patients in OFF and ON (rANOVA: "GROUP" $F = 0.1$, $P = 0.7$, TIME $F = 0.8$, $P = 0.5$, no interaction $F = 1.4$, $P = 0.2$).

There was an effect of age on plasticity in both groups HV and PD subjects. Indeed, when the overall effect of PAS was compared between PD in OFF and HV with age as a covariant, there was a significant effect of age ($P < 0.05$) and no effect of "GROUP" ($P = 0.7$) or interaction ("GROUP" * "AGE" $P = 0.8$). As shown in Figure 2*B*, the older was the subject, the lower was the PAS-induced effect.

Cerebellar Inhibitory Stimulation Modifies PAS-Induced M1 Plasticity in PD Patients in ON and not in Age-Matched Healthy Volunteers

When preceded by $Sham_{CB}$, PAS induced a similar, small increase of the test MEP size, both in PD patients in ON and in HV. In contrast, when preceded by $cTBS_{CB}$, PAS induced a large and significant facilitation of the test MEP size in patients in ON but not in the HV (Fig. 3). This was confirmed by comparing the normalized values of the MEPs (T5/T0, T15/T0, T30/T0) between the 2 groups and the 2 sessions. The analysis found a significant interaction between "GROUP" and "INTERVENTION" due to the additional effect of $cTBS_{CB\text{-}ON}$ on PAS-induced plasticity in the patient and not in the HV group (see Table 3).

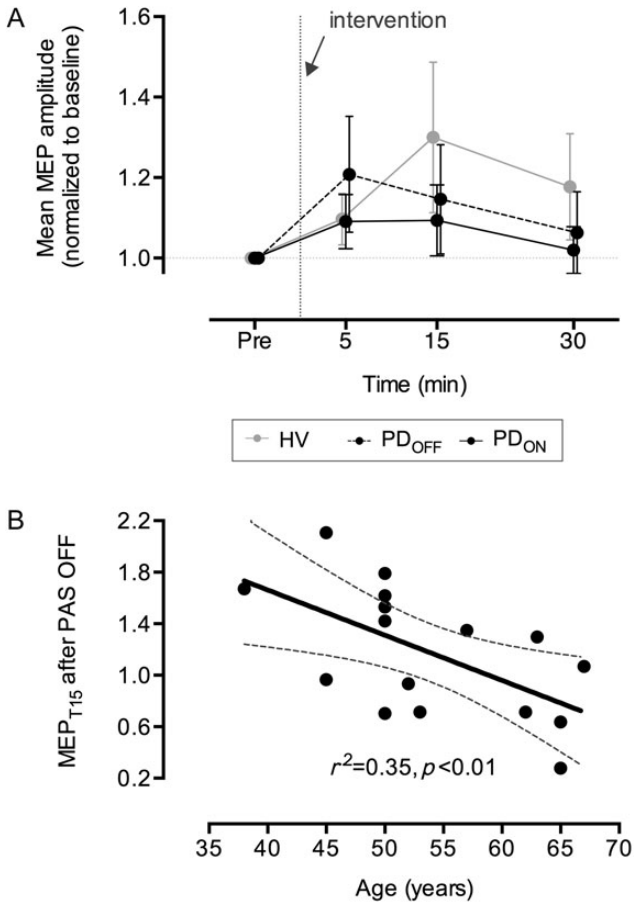

Figure 2. Comparison of PAS-induced effects in PD patients in OFF and ON and age-matched HV. (*A*) The mean amplitudes of the motor-evoked potentials (MEP) normalized to their mean pre-PAS amplitude are plotted against the elapsed time from the end of the PAS. There was no significant difference in the effect of PAS among the 3 groups. PD patients were tested OFF and ON. HV were not tested with L-DOPA. (*B*) Linear regression analysis shows that the older the patient, the lower was the peak effect of PAS-induced plasticity (MEP_{T15}). Dotted lines represent the respective 95% confidence intervals of the regression.

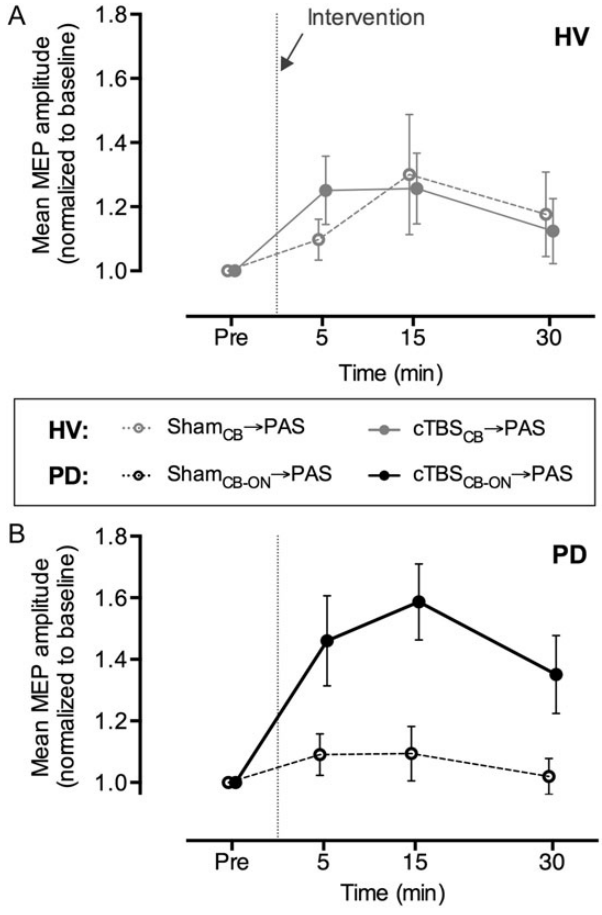

Figure 3. Comparison of PAS-induced effects when preceded by a single session of sham or real inhibitory stimulation of cerebellum. The mean amplitude of the MEPs normalized to their mean pre-PAS amplitude is plotted against the time elapsed from the end of PAS. PAS was preceded by a sham or real cTBS cerebellar stimulation. Upper panel: In HV, who were not given L-DOPA, real cerebellar inhibitory stimulation did not induce any additional effect on PAS when compared with sham cerebellar stimulation. Lower panel: In PD patients tested in ON, the real cerebellar inhibitory stimulation enhanced the PAS-induced effect at all tested time points when compared with the sham cerebellar stimulation.

Table 3
Statistical results for the single-session study

Time	Group	Time × group	Intervention	Group × intervention	Time × intervention
PAS-induced plasticity in PD OFF (raw data)					
$F = 0.8$, $P = 0.5$					
PAS-induced plasticity in PD ON (raw data)					
$F = 0.8$, $P = 0.5$					
PAS-induced plasticity in HV (raw data)					
$F = 0.8$, $P = 0.5$					
PAS-induced plasticity in PD OFF vs. PD ON vs. HV (normalized data)					
$F = 0.8$, $P = 0.5$	$F = 0.1$, $P = 0.7$	$F = 1.4$, $P = 0.2$			
Effect of $cTBS_{CB}$ vs. $Sham_{CB}$ on PAS-induced plasticity in PD and HV (normalized data)					
$F = 2.8$, $P = 0.07$	$F = 0.5$, $P = 0.5$	$F = 0.1$, $P = 0.8$	$F = 6.4$, $P = 0.02$	$F = 5.4$, $P = 0.03$	$F = 0.6$, $P = 0.9$
Post hoc: effect of $cTBS_{CB}$ vs. $Sham_{CB}$ in PD ON (normalized data)					
$F = 1.8$, $P = 0.2$			$F = 16.5$, $P = 0.001$		$F = 0.4$, $P = 0.6$
Post hoc: effect of $cTBS_{CB}$ vs. $Sham_{CB}$ in HV (normalized data)					
$F = 1.7$, $P = 0.2$			$F = 0.3$, $P = 0.6$		$F = 0.9$, $P = 0.4$
Effect of $cTBS_{CB}$ vs. $Sham_{CB}$ on $iTBS_{M1}$-induced plasticity in PD (normalized data)					
$F = 0.6$, $P = 0.6$			$F = 0.006$, $P = 0.9$		$F = 0.8$, $P = 0.5$
No placebo effect of $Sham_{CB}$ in PD ON: $Sham_{CB} \rightarrow$ PAS vs. PAS alone (normalized data)					
$F = 6.5$, $P = 0.05$			$F = 0.06$, $P = 0.8$		$F = 0.005$, $P = 0.9$

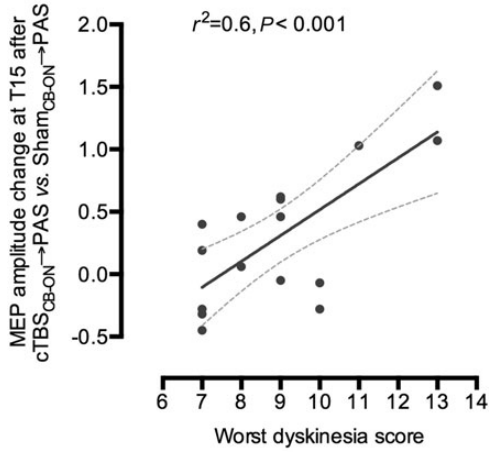

Figure 4. The severity of dyskinesia at baseline predicts the efficacy of one session of real inhibitory stimulation of cerebellum in enhancing PAS-induced plasticity. Linear regression shows that the more severe the worst dyskinesia scores at baseline, the larger was the additional effect of one session of real cerebellar inhibitory stimulation on PAS over that of sham stimulation.

In PD patients, the additional effect of $cTBS_{CB\text{-}ON} \rightarrow$ PAS versus $Sham_{CB\text{-}ON} \rightarrow$ PAS on the peak effect of plasticity (MEP_{T15}) correlated with the worst dyskinesia score at baseline: in patients with more severe LIDs, this additional effect of cerebellar stimulation on PAS-induced plasticity was larger ($R^2 = 0.6$, $P < 0.001$) (Fig. 4).

There was no difference between the effect of $cTBS_{CB\text{-}ON} \rightarrow$ PAS and $Sham_{CB\text{-}ON} \rightarrow$ PAS on RMT, AMT, SICI, LICI, SAI or LAI (Table 4).

Cerebellar Inhibitory Stimulation Does Not Enhance iTBS-Induced Plasticity of M1

In dyskinetic patients, iTBS of M1 did not induce any significant plastic response when preceded by $Sham_{CB\text{-}ON}$ or $cTBS_{CB\text{-}ON}$ (Fig. 5). This was confirmed by comparing the normalized values of the MEPs (T5/T0, T15/T0, T30/T0) after $Sham_{CB\text{-}ON} \rightarrow iTBS_{M1}$ and $cTBS_{CB\text{-}ON} \rightarrow iTBS_{M1}$ (see Table 3).

Table 4
Measures of cortical excitability following $SHAM_{CB\text{-}ON} \rightarrow$ PAS and $cTBS_{CB\text{-}ON} \rightarrow$ PAS

	Preintervention	Post-$SHAM_{CB\text{-}ON} \rightarrow$ PAS	Post-$cTBS_{CB\text{-}ON} \rightarrow$ PAS	*P*
RMT	46.3 ± 1.7	45.9 ± 1.9	45.6 ± 1.3	0.9
AMT	32.2 ± 1.4	31.7 ± 1.4	31.8 ± 1.2	0.7
SICI	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4
LICI	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.3
SAI	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.2
LAI	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.5

RMT, resting motor threshold; AMT, active motor threshold; SICI, short-interval intracortical inhibition; LICI, long-interval intracortical inhibition; SAI, short-latency afferent inhibition; LAI, long-latency afferent inhibition. $P < 0.05$ is considered significant. All values are mean ± SEM.

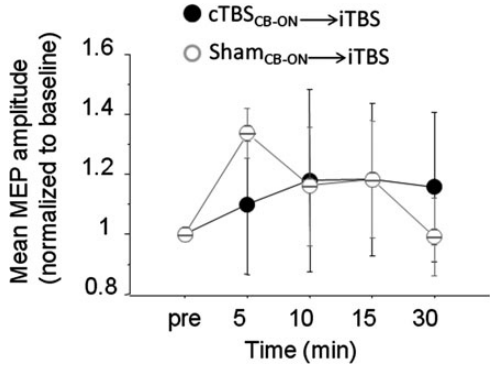

Figure 5. Comparison of iTBS-induced effects when preceded by a sham or real cerebellar inhibitory stimulation. The mean amplitude of the MEPs normalized to their mean pre-iTBS amplitude is plotted against the time elapsed from the end of the iTBS. iTBS was preceded by a single session of sham or real inhibitory stimulation of cerebellum. iTBS-induced plasticity was weak after sham cerebellar stimulation and was not modified after real inhibitory stimulation of cerebellum in a subgroup of PD patients ($n = 7$).

L-DOPA Responsiveness of PAS-Induced Plasticity, Cortical Excitability, and Cortical Inhibitions in Dyskinetic PD

After L-DOPA, the responsiveness of the M1 of dyskinetic PD patients to PAS protocol was not enhanced compared with OFF (see result 1). The RMT, AMT, SICI, LICI, LAI were not significantly modified in ON (RMT = 46.3 ± 1.7% and AMT = 32.2 ± 1.4% of the maximum stimulator output, SICI = 0.70 ± 0.11 of the unconditioned MEP amplitude,

LICI = 0.23 ± 0.09, LAI = 0.79 ± 0.08) compared with OFF (RMT = 45.1 ± 2%, AMT = 32.1 ± 1.9%, SICI = 0.72 ± 0.12, LICI = 0.38 ± 0.16, LAI = 0.74 ± 0.10). In contrast, SAI was significantly decreased ($P < 0.01$) in ON (0.97 ± 0.09 of the unconditioned MEP amplitude) when compared with OFF (0.69 ± 0.07).

No Placebo Effect of Sham Cerebellar Stimulation

MEP were minimally changed after $SHAM_{CB\text{-}ON} \rightarrow PAS$ and PAS_{ON} alone in the 6 tested subjects: MEP_{T15}/MEP_{T0} 1.12 ± 0.0 after PAS_{ON} alone and 1.08 ± 0.15 after $SHAM_{CB\text{-}ON} \rightarrow PAS$; MEP_{T30}/MEP_{T0} 0.95 ± 0.07 after PAS_{ON} alone and 0.90 ± 0.15 after $SHAM_{CB\text{-}ON} \rightarrow PAS$. rANOVA confirmed that $SHAM_{CB\text{-}ON}$ had no placebo effect as PAS-induced plasticity was not significantly changed by $SHAM_{CB\text{-}ON} \rightarrow PAS$ compared with PAS_{ON} alone (Table 3).

Multiple-Sessions Study

Multiple Sessions of Bilateral Cerebellar Inhibitory Stimulation in ON Have Antidyskinetic Effects

Treatment-induced changes of the dyskinesia scores (total and worst scores from the blinded video ratings) and in the scores extracted from the patient-diaries were compared between the real ($n = 20$) and sham cerebellar stimulation ($n = 10$) sessions at the end of the second week after the termination of each type of treatment session. Real cerebellar inhibitory and not sham stimulation, led to a decrease of the total ($P < 0.04$) and worst ($P < 0.007$) dyskinesia scores and a reduction in the self-rated "time spent in ON with troublesome dyskinesias" ($P < 0.05$). Self-rated "time spent in ON" ($P = 0.3$) and "in OFF" ($P = 0.8$) remained unchanged.

Effects of 1 Versus 10 Sessions

There was no durable change in the worst dyskinesia score lasting until the second day, after just one session of cerebellar stimulation, while this score in these patients was improved 2 weeks after the end of the 10 sessions of stimulations (rANOVA: TIME $F = 9.6$, $P < 0.001$, Fisher's test: pretreatment vs. day 2, $P = 0.4$; pretreatment vs. day 28, $P < 0.0006$; day 2 vs. day 28, $P < 0.004$).

The Enhancement of PAS-Induced Plasticity After a Single-Session of Cerebellar Inhibitory Stimulation in ON Predicts Improvement in LIDs After Multiple Bilateral Sessions in ON

In the subgroup of the 10 patients from the single-session study who participated in the multiple sessions study, bilateral cerebellar stimulation in ON induced a significant improvement of LIDs, just as in the whole group of 20 patients. There was a substantial decrease in the blinded video rated total ($P < 0.008$) and worst dyskinesia scores ($P < 0.003$), shortening of the time spent in ON with troublesome dyskinesias ($P < 0.02$) and lengthening of the "time spent in ON without troublesome dyskinesias" ($P < 0.04$).

The larger the additional plasticity generated by a single session of $cTBS_{CB\text{-}ON} \rightarrow PAS$, that is, [($MEP_{T15}$ after $cTBS_{CB\text{-}ON} \rightarrow PAS$) − ($MEP_{T15}$ after $Sham_{CB\text{-}ON} \rightarrow PAS$)]/($MEP_{T15}$ after $Sham_{CB\text{-}ON} \rightarrow PAS$), the greater was the decrease in the worst dyskinesia score after 10 days of bilateral-$cTBS_{CB\text{-}ON}$ ($P < 0.007$, $R^2 = 0.6$) (Fig. 6).

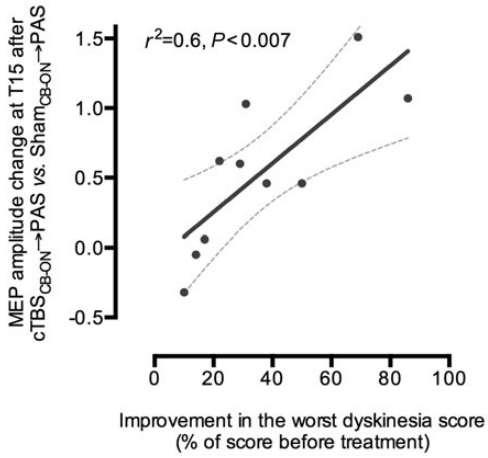

Figure 6. Efficacy of one session of real cerebellar inhibitory stimulation in enhancing PAS-induced plasticity is a good predictor of the antidyskinetic effect of 10 sessions of real, bilateral, inhibitory stimulation of the cerebellum. Linear regression shows that the larger the additional effect on PAS after one session of real cerebellar inhibitory stimulation over that of sham stimulation, the greater was the improvement in the worst dyskinesias scores after 10 sessions of real bilateral cerebellar inhibitory stimulation.

Enhancement of PAS-Induced Plasticity Paralleled the Antidyskinetic Effect of 10 Sessions of Bilateral cTBSCB-ON

The results obtained in the 8 patients who had an extended follow-up for 8 weeks from the end of the multiple sessions study are presented in Figure 7. PAS-induced plasticity that was weak at baseline became strongly enhanced at the end of the second week follow-up and again dropped by the end of the fourth week follow-up (rANOVA: FOLLOW-UP $F = 5.2$, $P < 0.008$; TIME $F = 0.04$, $P = 0.9$; no interaction $F = 0.8$, $P = 0.5$; Bonferroni: baseline vs. second week $P < 0.005$). A similar time course was observed for the antidyskinetic effect. Indeed, both total and worst dyskinetic scores were significantly decreased at the end of the second week follow-up but were back to the baseline values by the end of the fourth week (Fig. 7*C*,*D*). The self-rated scores (time spent in ON with and without troublesome dyskinesia) were also improved at the end of the second week and remained so till the end of the fourth week (Fig. 7*E*,*F*). The "time spent in OFF" did not change (Fig. 7*B*). There was a trend for the improvement of the total dyskinesia score to correlate with the additional effect of PAS-induced plasticity at the second week follow-up compared with the baseline ($P = 0.07$, $R^2 = 0.4$).

Discussion

The M1 of PD patients with LIDs in the present study had a poor or absent responsiveness to a plasticity-induction protocols, be it PAS or iTBS, as in previous reports (Morgante et al. 2006; Kishore et al. 2012). In striking contrast with the similar impairment of the plasticity after PAS and iTBS, a single session of real cerebellar stimulation combined with L-DOPA enhanced the PAS-induced plasticity, while it did not induce any change in the iTBS-induced plasticity. The main difference between the 2 protocols is the involvement of a sensory component in the PAS but not in the iTBS protocol. The differential effect of cerebellar modulation on PAS-induced plasticity points to a change in cerebellar sensory processing underlying the effect of cerebellar stimulation. When repeated

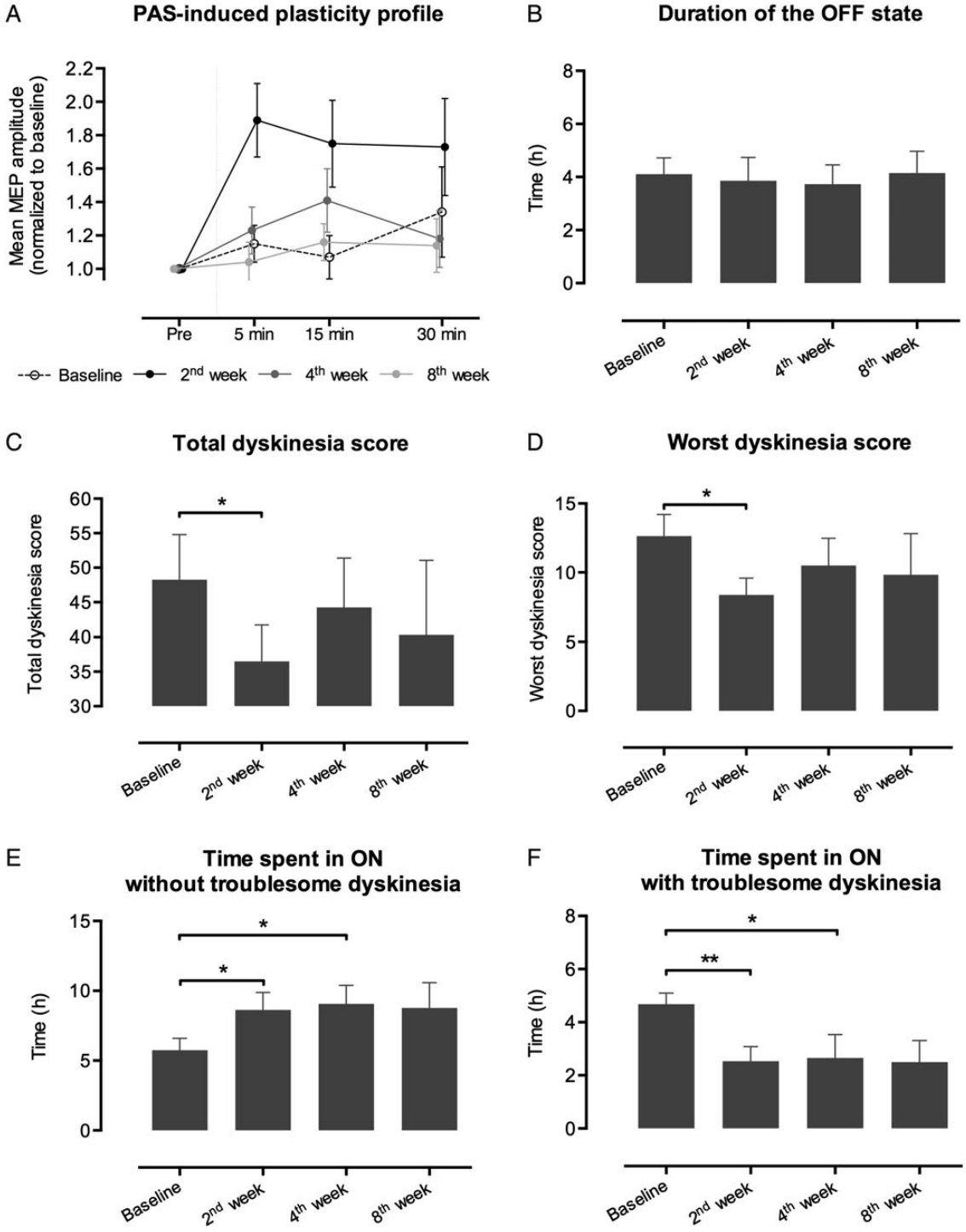

Figure 7. Extended follow-up after 10 sessions of real and bilateral cerebellar inhibitory stimulation. A: Extended follow-up of the M1 plasticity changes. The mean MEP amplitude normalized to the mean pre-PAS value is plotted against the time elapsed from the end of the PAS. PAS-induced effects are compared among baseline, 2, 4, and 8 weeks after completion of the 10 days of bilateral real cTBS stimulation of cerebellum. A prominent PAS effect was observed at the end of the second week follow-up, lesser at the end of the fourth week, and back to the baseline level by the end of the eighth week. (*B–F*): Extended follow-up of dyskinesias and motor fluctuations. Black bars represent the mean (±SEM) of the clinical scores at baseline and 2, 4, and 8 weeks after the completion of 10 days of bilateral real cTBS of cerebellum. The total dyskinesia score (*C*) and the worst dyskinesia score (*D*) were rated on videos by a blinded investigator. The time spent in ON without (*E*) or with (*F*) troublesome dyskinesias as well as the duration of the OFF state (*B*) were self-rated by the patients and recorded in their diaries. Total and worst dyskinesia scores were decreased at the end of the second week follow-up. "Time spent in ON without and with troublesome dyskinesias" were increased/decreased at the second and fourth week follow-up. Duration of the "Time spent in OFF" was not changed at these time points.

over 10 days, real inhibitory stimulation of cerebellum had an antidyskinetic effect that was clearly superior to sham stimulation. This antidyskinetic effect was accompanied by a sustained restoration of the responsiveness of M1 to PAS; both lasting for more than 2 weeks after the completion of the sessions, but lost by the end of the fourth week.

Inhibitory Stimulation of Cerebellum Reinstates Sensorimotor Plasticity of M1 in Dyskinetic PD

Inhibitory cerebellar stimulation in ON (applied either in a single session or repeatedly) reinstates the responsiveness of M1 to PAS in dyskinetic PD patients. Cerebellar stimulation could act by priming the M1 cortical excitability directly through the cerebello-thalamo-cortical circuit (Koch et al. 2008) before the plasticity-induction protocol to M1 was delivered. If it were true, then PAS- and iTBS-induced plasticity of M1 should be similarly modulated after cerebellar stimulation. This was not the case, as iTBS-induced plastic response of M1 was not modulated by cerebellar stimulation. It is unlikely that the difference between the group sizes ($n = 16$ for PAS, $n = 7$ for iTBS) could bias the difference observed; such a differential effect of cerebellar stimulation on PAS versus iTBS-induced plasticity has been already reported in healthy young subjects and the implications discussed in detail (Popa et al. 2013). The control experiments using iTBS of M1 were only aimed at verifying whether this differential effect of cerebellar stimulation was influenced by the presence of PD or by the age-related differences in baseline responsiveness of M1 to a plasticity-induction protocol (i.e., high in young healthy subjects, low in PD patients who are older). Taken together, the results of the 2 previous studies in young healthy subjects (Hamada et al. 2012; Popa et al. 2013) and the present study in dyskinetic PD patients, suggest that cerebellar stimulation does not influence M1 plasticity through a change in the tonic output of the cerebello-thalamo-cortical pathway directly to M1. We propose that the effect of cerebellar stimulation in re-establishing M1 responsiveness to PAS is mediated by a change of gain of the sensory afferent volley reaching the motor cortex. This change could intervene either at the level of the dentato-olivary complex, the cerebellar cortex or the thalamic nuclei (Popa et al. 2013). This argument is in keeping with the adaptive filtering role of the cerebellum on sensory afferents (Dean et al. 2010). The possibility that cerebellar stimulation may influence sensory processing in the primary somatosensory cortex was ruled out in previous studies by the lack of change in cortical somatosensory-evoked potentials after cerebellar stimulation in HV (Hamada et al. 2012; Popa et al. 2013). The lack of change in $SAI_{20\,ms}$ after $cTBS_{CB\text{-}ON} \rightarrow PAS$ compared with $Sham_{CB\text{-}ON} \rightarrow PAS$ in the present study means that cerebellar stimulation is unlikely to modulate the afferent volley in the pathway activated in SAI_{20ms}, which includes, most probably, the thalamic VP nucleus and M1 (Asanuma et al. 1980; Hirai and Jones 1988; Tokimura et al. 2000).

Cerebellar Involvement in LIDs

Koch et al. (2009) have already shown that multiple sessions of bilateral cerebellar inhibitory stimulation in ON led to a sustained reduction of LIDs. The authors discussed several potential mechanisms for this effect, but had not examined whether the clinical benefit was accompanied by any change in the deficient M1 plasticity in ON that is associated with LIDs and which contrasts with the preserved plasticity in those without dyskinesias (Morgante et al. 2006; Kishore et al. 2012).

Two evidences from the current study support the view that the reduction of LIDs is linked to the resurgence of M1 responsiveness to PAS after $cTBS_{CB\text{-}ON}$: 1) the larger the facilitation of M1 plasticity after a single session of cerebellar inhibitory stimulation in ON in dyskinetic patients, the greater was the antidyskinetic effect of 10 days of repeated stimulation in the same subjects at the end of second week (Fig. 7); and 2) the time course of the improvement of LIDs and that of the resurgence of M1 responsiveness to PAS after 10 $cTBS_{CB\text{-}ON}$ sessions was similar (Fig. 7). We also found that patients with more severe pretreatment LIDs showed a larger responsiveness of M1 to $cTBS_{CB\text{-}ON} \rightarrow PAS$ (Fig. 4). This could be interpreted as an increasing involvement of the cerebellum in the pathophysiology of dyskinesias as the severity of LIDs increases, making cerebellar inhibitory stimulation more effective in reversing the cerebellar dysfunction. The critical involvement of the cerebellum in LIDs, but not in parkinsonian signs (akinesia, rigidity), is supported by our finding that 10 days of cerebellar inhibitory stimulation improved the severity of LIDs and the "time spent in ON without troublesome dyskinesias," but not the duration of the OFF periods. Koch et al. (2009) also observed that multiple sessions of cerebellar stimulation did not induce any change in the UPDRS motor scores.

It is worth noting that there was a temporal dissociation between the responses of the physiological parameter (resurgence of responsiveness of M1 to PAS) and the clinical measure (severity of LIDs) after cerebellar stimulation. While a single session of $cTBS_{CB\text{-}ON}$ was able to immediately influence the responsiveness of M1 to PAS, multiple sessions were necessary for the antidyskinetic effects to occur. This does not contradict the study by Koch et al. (2009) in which a beneficial effect of one session of bilateral $cTBS_{CB}$ on the dyskinesia score was found at only 30 and 45 min after the stimulation, but not at 60 min.

This is in line with the accepted view that cumulative effects of multiple sessions of rTMS are necessary to influence clinical symptoms (Khedr et al. 2006; Lomarev et al. 2006; Huang et al. 2012; Popa et al. 2012). This might indicate that different mechanisms support the physiological effects seen after one session and the clinical effects seen after multiple sessions. While the effects of one session of rTMS are linked to LTP/LTD mechanisms, how multiple sessions work is still not fully understood. Putative mechanisms include postsynaptic changes of GABA receptors, metaplasticity that induces a slide of the threshold for inducing LTP/LTD and structural changes of the synapses.

Is the Effect of Cerebellar Inhibitory Stimulation on the Responsiveness of M1 to PAS, Dependent on Dopamine?

The results of the study raise the question whether dopamine is necessary for the effect of cerebellar inhibitory stimulation to develop. In this study, it was noted that plasticity induced by 5 Hz PAS is smaller in the older HV than the effect reported in the young HV in our earlier study (Popa et al. 2013). This is similar to and reinforces the observations of studies using the classical PAS, that the responsiveness of M1 to PAS is critically dependent on age (Muller-Dahlhaus et al. 2008; Fathi et al. 2010). It also highlights the need for an appropriately age-matched control group when cortical plasticity is measured. The loss of ability of cerebellar inhibitory stimulation to modulate M1 plasticity in the older HV was unexpected and contrasted with the dramatic effect of cerebellar stimulation on dyskinetic PD patients who were tested in ON. It is plausible that the decline in striatal dopamine levels with aging (Fearnley and Lees 1991; Kish et al. 1992; Cordes et al. 1994; Darbin

2012) and the low responsiveness of M1 to plasticity induction with aging (whatever its origin, including a possible role of the low dopamine levels) are responsible for the lack of effects of cerebellar stimulation in older HV. Dyskinetic PD patients in ON also had a similar low responsiveness of M1 to plasticity induction, but they are known to have abnormally high striatal synaptic dopamine release after exogenous L-DOPA intake (de la Fuente-Fernandez et al. 2004). This replenishment of dopamine could be a key factor that enabled the effect of cerebellar stimulation to occur in PD patients when compared with HV who were tested without exogenous dopamine.

Previous studies have speculated whether the worsening of proprioception (O'Suilleabhain et al. 2001; Zia et al. 2000) and/or of SAI (Sailer et al. 2003, 2007) caused by dopaminergic drugs may contribute to dyskinesias. While LAI was found to be lost or even replaced by a small facilitation in PD patients OFF drugs in a previous report (Sailer et al. 2003, 2007), LAI was present in the dyskinetic patients OFF drugs in the present study. The discrepancy between the results might be explained by the differences in the intensities used by us for median nerve stimulation which were always below the motor twitch and therefore lower than in the previous studies (Sailer et al. 2003, 2007). As in their study, LAI was not influenced by dopamine intake, suggesting that LAI may be related to nondopaminergic features of PD (Sailer et al. 2007). Circuits supporting LAI are not precisely known, but may involve cortical areas other than M1 and S1 or even subcortical areas. However, as LAI was not modified by cerebellar stimulation in HV or PD patients, it is unlikely that cerebellum is part of the LAI circuit.

In this study, $SAI_{20\ ms}$ was reduced in ON in dyskinetic patients when compared with OFF. This effect of L-DOPA is congruent with an earlier report (Sailer et al. 2003) in which it was tentatively explained by an inhibitory effect of dopamine on the response of pyramidal and nonpyramidal neurons in M1 to inputs from the ventrolateral thalamus, as observed in cats. The decrease of $SAI_{20\ ms}$ in ON could be the confirmation that dopamine enhances M1 excitability at the cost of impairing the normal inhibitory response to peripheral stimuli in the lemniscal pathway. This decreased inhibition of M1 in ON when combined with the increase in the gain of the peripheral afferent input triggered by the cerebellar cortex inhibition may lead to the enhancement of the responsiveness of M1 to PAS after cerebellar inhibitory stimulation in PD patients in ON. However, the fact that SAI is impaired irrespective of whether the patients had LIDs (the present study) or not (Sailer et al. 2003) does not support a direct role of altered SAI in LIDs.

It is still debatable to what extent the level of striatal dopamine influences the effects of cerebellar stimulation. We cannot rule out the possibility that cerebellar inhibitory stimulation by itself, in the absence of L-DOPA, may have some effect on PAS-induced plasticity in dyskinetic PD, as the patients in the present study were not tested with cerebellar inhibitory stimulation in OFF. Even so, the influence of the chronic dopaminergic treatment in PD could be a confounding factor in a comparison with HV. Future studies will therefore have to test the effect of cerebellar inhibitory stimulation on PAS-induced plasticity, both alone and when combined with dopamine replacement therapy in untreated PD.

Cerebellum Versus Striatal Involvement in Dyskinesia

The view that cerebellum is involved in LIDs might appear to be in contradiction to the existing view that LIDs are due to increased activity in the motor areas, secondary to the abnormal output in the striato-thalamo-cortical circuit. Neuroimaging studies have found increased activity of motor and premotor areas in dyskinetic PD patients when compared with nondyskinetic patients (Rascol et al. 1998; Brooks et al. 2000). This led to the view that hyperactivity in cortical motor areas might be responsible for LIDs (Bezard et al. 2001; Koch et al. 2005). However, the results of studies using 1 Hz rTMS of SMA (Koch et al. 2005; Brusa et al. 2006) or of M1 (Wagle-Shukla et al. 2007; Filipović et al. 2009) to reduce dyskinesias were conflicting. Even multiple sessions of rTMS failed to show more than a transient or mild improvement in dyskinesias. In comparison to these results, the antidyskinetic effect of cerebellar inhibition demonstrated in an earlier study (Koch et al. 2009) and confirmed by the present study, were more robust and lasted up to 4 weeks. In a recent study, a global reduction in metabolism in bilateral cerebellar hemispheres and dentate nuclei was found after multiple sessions of cerebellar cTBS that led to reduction in LIDs in PD patients (Brusa et al. 2012). These evidences indicate that the cerebellum is excessively active in dyskinesias and that this activity might play an important role in the pathophysiology of dyskinesias. Cerebellar cTBS may act by reversing the overactivity. SMA, premotor cortex and M1 are all targets of cerebellar output (Akkal et al. 2007). Therefore, altered cerebellar outputs in dyskinetic PD might indeed trigger abnormal fMRI activations in such areas, yet make them less suitable targets for a direct stimulation for the treatment of LIDs.

We propose that the abnormal signaling within the striato-thalamo-cortical circuit, possibly due to nonphysiological and excessive release of striatal synaptic dopamine in dyskinetic patients (de la Fuente-Fernandez et al. 2004), could impinge on the cerebello-thalamo-cortical circuit either at the cortical level or at a subcortical level through topographically specific connections (Hoshi et al. 2005; Bostan et al. 2010). The lack of cerebellar stimulation effects on intrinsic M1 plasticity (as evoked by $iTBS_{M1}$) and on M1 excitability (measured by RMT, AMT, SICI, LICI) argue against an interaction at the cortical level. Anatomical data have shown that the subthalamic nucleus (STN) is connected to the cerebellar cortex through the pontine nuclei via an excitatory glutamatergic pathway (Bostan et al. 2010). This pathway could be a good candidate for the transmission of signals between the 2 circuits (schematically shown in Fig. 8). An interaction between the 2 circuits has been suggested to drive the tremor in PD (Helmich et al. 2011). Such a propagation of abnormal signals from basal ganglia circuits could affect the normal modulatory filtering/processing of the sensory afferents by the cerebellum, resulting in relevant sensory inputs to the cerebellum not getting transformed into motor-relevant output signals to M1, thus leading to dyskinetic movements. Cerebellar inhibitory stimulation, by increasing the final gain of the sensory volley before projecting to M1, may attenuate this defect and reduce the severity of dyskinesias.

Conclusion

The results of the present study bring a new insight into the functional connectivity between the basal ganglia and the cerebellum in human PD. Exploiting the pathological model offered by the dyskinetic state of advanced PD, this study strengthens the existing view of an involvement of the

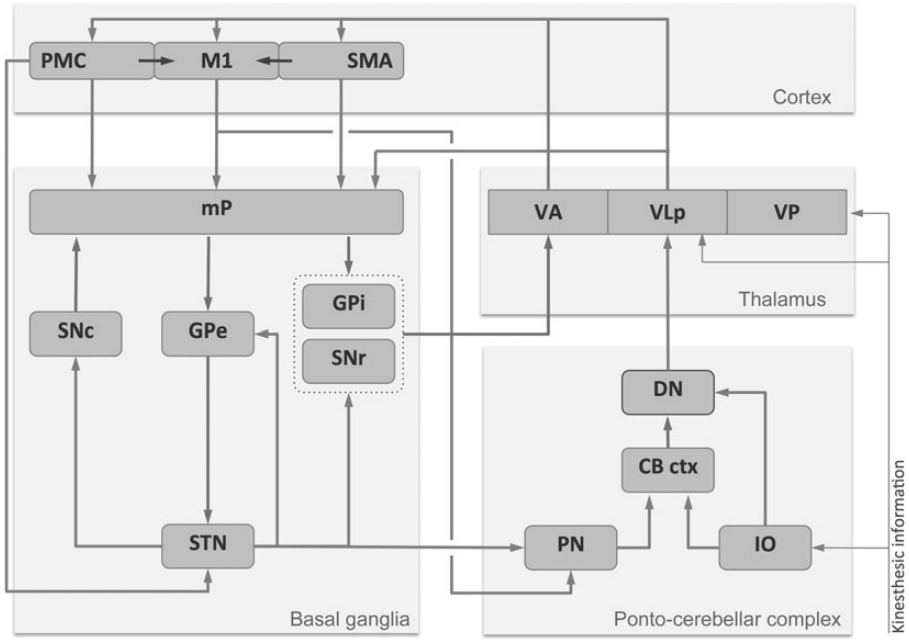

Figure 8. Schematic diagram showing the interconnections of the basal ganglia and cerebellar circuits. PMC, premotor cortex; M1, primary motor cortex; SMA, supplementary motor area; GPe, globus pallidus externum; GPi, globus pallidus internum; STN, subthalamic nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VA, ventral anterior thalamic nucleus; VLp, posterior part of the ventrolateral thalamic nucleus; VP, ventral posterior thalamic nucleus; DN, dentate nucleus; CBctx, cerebellar cortex.

cerebellum in LIDs and raise a new hypothesis on how changes in the striatal levels of dopamine may negatively impact the activity of the cerebello-thalamo-cortical network. We propose that abnormal signaling in the basal ganglia circuits causes alterations in the sensory processing function of the cerebellum. This leads to an inappropriate filtering of the relevant sensory volley, which is responsible for a maladaptive state of cortical plasticity. This, in turn, predisposes to the selection of abnormal motor programs and emergence of undesired, abnormal movements. Cerebellar inhibition, by increasing the gain of the sensory afferent volley to M1, permits better sensorimotor integration, thereby reducing involuntary movements.

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