Acute dopamine boost has a negative effect on plasticity of the primary motor cortex in advanced Parkinson’s disease

Asha Kishore, Traian Popa, Balu Velayudhan, Thomas Joseph, Ammu Balachandran and Sabine Meunier

Plasticity of primary motor cortex is severely impaired in Parkinson’s disease and chronic dopaminergic treatment is reported not to rescue it. The effect of an acute dose of levodopa on cortical plasticity reported so far is variable. In this study, it was hypothesized that cortical plasticity would be restored in Parkinson’s disease as a long duration response to treatment in stable responders while those with motor complications would have a reduction or loss of plasticity similar to the decay of long duration response of motor signs. Patients were carefully stratified based on their motor response to levodopa into stable responders (n = 17), fluctuating non-dyskinetics (n = 18) and fluctuating dyskinetics (n = 20). Theta burst stimulation was applied to the motor cortex to induce long-term potentiation and long-term depression-like plasticity in both OFF and ON conditions. In OFF, stable responders could express both types of plasticity, fluctuating non-dyskinetics had long-term potentiation, but no long-term depression and both types of plasticity were lost in fluctuating dyskinetics. This suggests the presence of a long duration response in early stages of levodopa treatment and a gradual loss of chronic treatment benefit on plasticity, particularly for long-term depression, when motor complications develop. An acute dose of levodopa led to a worsening of long-term potentiation in fluctuating non-dyskinetic patients, and it did not have any effect on the plasticity that was absent in OFF in the fluctuating dyskinetic patients. Acute dosing led to a worsening of long-term depression in all the groups. In the fluctuating dyskinetic patients, there was a paradoxical potentiation instead of depression. Our results suggest that an acute non-physiological dopamine boost has a negative effect on cortical plasticity as disease advances. We propose that the loss of long duration response and the negative effect of acute doses on cortical plasticity with progression of disease may contribute to the pathophysiology of motor complications. Repeated non-physiological surges in synaptic dopamine during acute levodopa dosing could potentially lead to persistent dysfunction of key enzymes of the intracellular signalling cascade that are involved in the induction and maintenance of both forms of plasticity.
Levodopa (L-DOPA) remains the cornerstone for the medical treatment of motor symptoms of Parkinson’s disease but long-term treatment with L-DOPA is associated with motor fluctuations and dyskinesias (Cotzias et al., 1969; Ahlskog et al., 2001). The clinical response of motor signs of Parkinson’s disease to L-DOPA has two components: the short and long duration responses (Anderson and Nutt, 2011). Short duration response is evident with the first dose of L-DOPA and provides, within minutes, a clinically apparent improvement in motor disability which lasts up to a few hours (Meunter et al., 1971). In contrast, long duration response is a sustained anti-parkinsonian effect derived from prolonged administration of L-DOPA and may last for up to 2 weeks after cessation of drug treatment (Fahn et al., 2004). Both short and long duration responses are present from the initiation of therapy but long duration response masks the detection of short duration response in the early stages and patients are unable to perceive the benefits of individual doses of L-DOPA (Nutt et al., 1992, 1996). The exact mechanisms of these phenomena are unknown. Short duration response corresponds to plasma levels of L-DOPA and long duration response is attributed to postsynaptic mechanisms and recently proposed to be related to restoration of synaptic plasticity in the corticostriatal pathways (Beeler et al., 2010).

The roles of short and long duration responses in the development of the ‘wearing off’ type of motor fluctuation during chronic dopaminergic therapy are not fully established. Wearing off fluctuation was previously considered a consequence of shortening of short duration response during the course of L-DOPA treatment (Contin et al., 1994; Hughes et al., 1994). However, there is a current view that a gradual loss of long duration response and an increased magnitude of short duration response may play a critical role in the genesis of wearing off (Stocchi et al., 2010). There are no previous studies that have explored whether the dynamics of short and long duration responses extend to the phenomena of long-term potentiation (LTP) or long-term depression (LTD) at corticostriatal synapses during chronic dopaminergic treatment. This is pertinent as there is a great deal of interest in the hypothesis that defective corticostriatal synaptic plasticity may be responsible for the motor complications of L-DOPA therapy (Calabresi, 2009).

In animal models of Parkinson’s disease, striatal neurons were unable to generate LTP and LTD (Calabresi et al., 2007) but chronic L-DOPA treatment restored it (Picconi et al., 2003). In human Parkinson’s disease, primary motor cortex (M1) plasticity is severely impaired in de novo patients (Kishore et al., 2012), as is the corticostriatal plasticity in parkinsonian rodents (Calabresi et al., 2007). However, in contrast with the beneficial effect of L-DOPA in restoring LTP and LTD in chronically treated animal models of Parkinson’s disease, several studies dealing with treated patients with Parkinson’s disease have found persisting impairment of M1 plasticity (Gilio et al., 2002; Morgante et al., 2006; Ueki et al., 2006; Suppa et al., 2011). In these studies, patients were not stratified according to the type of their motor response to L-DOPA (stable responders, fluctuators with and without dyskinesias) and had wide ranges of disease and treatment durations. We hypothesized that M1 plasticity would be restored as a long duration response to chronic dopaminergic treatment in stable responders. We further proposed that patients in the more advanced stages of disease with both fluctuations and dyskinesias may have a reduced or absent long duration response of M1 plasticity similar to the reduced long duration response of motor signs in these stages. Such a loss of chronic treatment effect could allow the detection of acute L-DOPA-induced changes in M1 plasticity.

Materials and methods

Subjects

Patients with Parkinson’s disease attending the Movement Disorders Clinic in a University Hospital setting participated in this study. A movement disorder specialist selected and recruited all patients focusing on the response of the clinical signs to dopaminergic therapy and the occurrence of dyskinesias. The diagnosis of Parkinson’s disease was based on UK Parkinson’s Disease Society Brain bank clinical criteria (Hughes et al., 1992). At the initial screening visit, all patients were examined in the OFF condition (after overnight withdrawal of drugs) and again in the ON condition (after the morning dose of drugs) to confirm the presence of wearing off motor fluctuation (recurrence of motor symptoms and signs of Parkinson’s disease within 4 h of turning ON) and peak-dose dyskinesias. Patients were assigned to three clinical groups: (i) stable responders: patients on chronic dopaminergic treatment (for >6 months) and not experiencing any motor fluctuations or dyskinesias; (ii) fluctuating non-dyskinetics (FND): patients had only wearing off fluctuation but no dyskinesias; and (iii) fluctuating dyskinetics (FD): patients with both wearing off fluctuation and peak dose dyskinesias. The severity of dyskinesias was scored using the dyskinesias rating scale proposed by Goetz et al. (1994) and severity of fluctuations using the Unified Parkinson’s Disease Rating Scale (UPDRS) Part IV-B (sum of scores 36–39). Only patients with mild dyskinesias in ON (score of 1 on the more affected limb in Goetz scale) and mild tremor in OFF that would not interfere with transcranial magnetic stimulation testing were included. Patients were also selected to have a duration of the ON of at least 2.5 h (to complete the transcranial magnetic stimulation session in ON). In the final analysis, we also included 10 healthy volunteers, whose results were previously reported (Kishore et al., 2012). All subjects were right-handed according to the Edinburgh handedness inventory (Oldfield, 1971). All treated recruited patients were on stable dose of drugs for at least 3 months prior to the study and until all transcranial magnetic stimulation sessions were over. None of the subjects were clinically depressed.
Electrophysiological studies

Transcranial magnetic stimulation

Transcranial magnetic stimulation was delivered with an air-cooled 7 cm inner diameter, figure-of-eight coil connected to a Magstim Rapid² stimulator (The Magstim Company). The coil was held with the handle pointing backwards and laterally at 45° (Brasil Neto et al., 1992) over the optimal position for eliciting motor evoked responses in the first dorsal interosseous muscle. The current pulses were biphasic. EMG responses were amplified (×1000) and filtered (100–3000 Hz) with a Digitimer D360 amplifier (Digitimer Ltd.), then digitally transformed at a sampling rate of 10,000 Hz (CED Power 1401 MkII, CED Ltd.) and stored for offline analysis (Signal 4.02, CED Ltd.).

Theta-burst stimulation

TBS was given as bursts of three pulses at 50 Hz delivered every 200 ms. In the excitatory intermittent TBS mode, 10 bursts were given for 2 s and repeated every 10 s for 20 cycles (600 pulses; Huang et al., 2005). In the inhibitory continuous TBS mode, 200 bursts were delivered continuously, for 40 s (600 pulses; Huang et al., 2005). The intensity of transcranial magnetic stimulation was set at 80% of the active motor threshold for both TBS protocols.

Experimental design

Each session started with the assessment of parkinsonism using the UPDRS (Fahn and Elton, 1987). Patients were scored in both OFF and ON condition using UPDRS Part III (total). UPDRS Part III subscores (sum of items 20–26) for the more affected side were then calculated. The total score and subscore for the more affected side were used in the correlation analysis. The mean percentage of clinical improvement in UPDRS Part III total score and subscore after acute dose of L-DOPA was calculated at the end of all sessions using the formula ([UPDRS in OFF – UPDRS in ON]/UPDRS in OFF × 100). L-DOPA equivalent dose of anti-parkinsonian drugs were calculated using conversion factors from a recent recommendation (Tomlinson et al., 2010).

At baseline, the resting and active motor thresholds were measured. The resting motor threshold was the minimum stimulus intensity that produced motor evoked potentials with an amplitude of at least 50 μV in 5 out of 10 consecutive trials. The active motor threshold was the minimum stimulus intensity that produced motor evoked potentials with an amplitude of at least 200 μV in 5 out of 10 consecutive trials while subjects performed an isotonic contraction of the first dorsal interosseus muscle of 20–30% of maximum contraction. Just before TBS, 15 individual motor evoked potentials at baseline (S1) were averaged, adjusting the intensity of the magnetic stimulus to 120% of resting motor threshold. Patients then received the intermittent TBS or continuous TBS over the M1 representation of first dorsal interosseus of the more affected hemisphere. Fifteen individual motor evoked potentials were averaged immediately after the TBS (T0) and 10 min (T1), 20 min (T2) and 30 min (T3) later. For the post-TBS motor evoked potentials, stimulus intensity was kept the same as at S1 (baseline). During transcranial magnetic stimulation, subjects were asked to look straight ahead and be in a relaxed position. Alertness level was monitored throughout the experiment. EMG activity was continuously monitored and audiovisual feedback from EMG signal was also provided to subjects to ensure muscle relaxation. Trials contaminated by EMG activity were discarded from offline analysis.

Statistical analysis

Approximately 50% of patients underwent OFF and ON sessions in both intermittent TBS and continuous TBS protocols (Table 1). Matched patients completed the second protocol when the originally selected patients could not attend. Therefore, we first statistically verified that patients in both the intermittent and continuous TBS protocols in each of the clinical groups (stable responders, FND and FD) were similar. This was done by comparing the clinical features (age, duration of illness, UPDRS Part III scores OFF and ON and percentage of improvement in UPDRS Part III scores after an acute dose of L-DOPA) and treatment aspects (duration of treatment, morning dose of L-DOPA and total L-DOPA equivalent dose per day) of patients in each clinical group by a factorial ANOVA with the ‘type of TBS’ (interrupted and continuous) and the patient group (stable responders, FND and FD) as variables. Subsequent comparison of groups, 2 × 2, was done using the post hoc Fisher’s test. Effect of L-DOPA on the resting and active motor thresholds was tested by a repeated ANOVA with ‘l-DOPA’ as the repeated variable (OFF and ON) and ‘GROUP’ as the independent variable. For the patients who had attended the intermittent and continuous TBS sessions, the mean values of the two measures of resting motor threshold, active motor threshold and motor evoked potential amplitudes at baseline (S1) in the intermittent and continuous TBS sessions were used in the final analysis.

In the next step, statistical methods were designed to answer the following questions: (i) Is motor cortex plasticity impaired to a different extent in Parkinson’s disease groups with different motor response patterns to L-DOPA compared with healthy volunteers? (ii) Does long-term dopaminergic replacement therapy restore the severe impairment of M1 plasticity in Parkinson’s disease?; and (iii) Does an acute dose of L-DOPA induce a change of M1 plasticity that would differ among the Parkinson’s disease groups?

To answer the first question, we performed a repeated ANOVA with the normalized values of mean motor evoked potentials at T0, T1 and T2 in the OFF condition forming the repeated values and GROUP (i.e. healthy volunteers, stable responders, FND and FD) being the inter-subject variable. To answer the second question, we examined whether TBS was able to induce LTP/LTD-like plasticity in each group separately (stable responders, FND, FD and healthy volunteers). For this, repeated ANOVA
Table 1 Clinical characteristics of all enrolled subjects

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<th>Subjects enrolled</th>
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<th>Age</th>
<th>Gender</th>
<th>Duration of disease in years</th>
<th>Duration of treatment in years</th>
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<th>Total LED mg/day</th>
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cTBS = continuous theta burst stimulation; DA = dopamine agonist; iTBS = intermittent theta burst stimulation; LD = L-DOPA; LED = L-DOPA equivalent dose of drugs; MAOI = monoamine oxidase inhibitor.
was performed on the raw values of the mean motor evoked potentials at S1, T0, T1 and T2 of each group. As the TBS-evoked response is over at 30 min post-intervention (T3) in healthy volunteers, we did not include this measure in the analyses.

To answer the third question, we compared the additional effects of acute L-DOPA dosing (OFF versus ON) among the patient groups (i.e. stable responders, FND and FD) by performing repeated ANOVA with the six normalized values of motor evoked potentials (T0, T1, T2 in OFF and ON) forming the repeats and GROUP as inter-subject variable.

We looked for correlations between the characteristics of the disease (disease duration and severity of motor symptoms assessed by UPDRS Part III in OFF) or treatment (duration of treatment, total L-DOPA equivalent dose/day and morning dose of L-DOPA) on the one hand and physiological parameters on the other. The physiological parameters were the baseline excitability of the motor cortex (motor evoked potential size at S1 in OFF and ON) and the responsiveness of M1 to TBS protocol as assessed by mean motor evoked potential sizes at T0, T1, T2 or their grand average (i.e. mean of motor evoked potentials at T0, T1, T2 or motor evoked potentials T0 + T1 + T2) in OFF and ON. We also examined whether DOPA responsiveness of M1 plasticity, as assessed by the values of the normalized motor evoked potentials at T0, T1, T2 or their average (motor evoked potentials T0 + T1 + T2) in ON or by their variation between OFF and ON (motor evoked potential in ON – motor evoked potential in OFF/motor evoked potential in OFF), correlated with the clinical improvement after acute L-DOPA dose [(UPDRS in OFF – UPDRS in ON)/UPDRS in OFF]. Correlations were tested in the whole patient cohort (i.e. all three groups taken together) and in each group separately.

Non-parametric Spearman rank correlation test was used for these correlations. When a positive correlation was found, it was further explored by looking for a linear regression between the two variables. Values were considered significant at $P < 0.05$. Fisher’s test was used for the post hoc analysis. All results are presented as mean ± SEM.

**Results**

Fifty-five patients were enrolled in this study. Their clinical characteristics are provided in Table 1. In the stable group ($n = 17$, range of disease duration 1.5–7 years), seven patients completed both protocols while an additional five patients completed intermittent TBS alone and another five patients completed continuous TBS alone (in all, 12 subjects had intermittent TBS in ON and OFF and 12 had continuous TBS in ON and OFF). In the FND group ($n = 18$, range of disease duration 2–15 years), six patients underwent both protocols in ON and OFF while an additional six had intermittent TBS alone and six had continuous TBS alone (total of 12 patients had intermittent TBS in ON and OFF and 12 had continuous TBS in ON and OFF). In the FD group ($n = 20$, range of disease duration 5–12 years), 12 completed both protocols, while an additional four had either continuous TBS alone or intermittent TBS alone (total of 16 patients had intermittent TBS and continuous TBS in ON and OFF). It was statistically verified that patients in the final set of intermittent and continuous TBS experiments in each clinical group, shared similar clinical and treatment characteristics.

**Clinical characteristics of the patient groups**

The mean age at study was not different among the groups (GROUP $F = 0.1$, $P = 0.8$). The duration of motor symptoms, showed an increase from stable responders to FND, but with no significant difference between FND and FD (GROUP $F = 9.1$, $P < 0.004$; Fisher’s test: stable responders versus FND $P < 0.007$, stable responders versus FD $P < 0.0001$, FD versus FND $P = 0.2$). The UPDRS Part III total score in OFF was higher in the FD group than in the other two groups (GROUP $F = 10.1$, $P < 0.0002$; Fisher’s test: stable responders versus FND $P = 0.2$, stable responders versus FD $P < 0.0001$, FND versus FD $P < 0.002$). This was less marked in the ON condition (GROUP $F = 5.1$, $P < 0.009$; Fisher’s test: stable responders versus FND $P = 0.09$, stable responders versus FD $P < 0.002$, FD versus FND $P = 0.1$), with almost similar percentage of clinical improvement in UPDRS Part III total score after the acute L-DOPA dose (GROUP $F = 2.3$, $P = 0.1$). Severity of motor fluctuations was, as expected, higher in the FD group than in the FND group (GROUP $F = 21.8$, $P < 0.001$).

**Treatment characteristics across the groups**

The duration of treatment was significantly longer in the FND and FD in comparison with the stable responders group (GROUP $F = 10.4$, $P < 0.0002$; Fisher’s test: stable responders versus FND $P < 0.003$, stable responders versus FD $P < 0.0001$, FD versus FND $P = 0.2$). L-DOPA equivalent dose and morning dose of DOPA were larger when motor complications were present (L-DOPA equivalent dose: GROUP $F = 9.1$, $P < 0.004$; Fisher’s test: stable responders versus FND $P = 0.2$, stable responders versus FD $P < 0.0001$, FD versus FND $P < 0.008$; morning dose of L-DOPA: GROUP $F = 5.6$, $P < 0.006$; Fisher’s test: stable responders versus FND $P = 0.05$, stable responders versus FD $P < 0.002$, FD versus FND $P = 0.2$).

**Motor thresholds and mean motor evoked potentials at baseline in OFF and ON conditions**

In OFF at baseline, resting motor threshold and active motor threshold (Table 2) were not different between healthy volunteers and other groups of patients (resting motor threshold: GROUP $F = 1.4$, $P = 0.2$; active motor threshold: GROUP $F = 2.1$, $P = 0.08$). Acute challenge with L-DOPA did not induce significant changes in resting motor threshold (DOPA $F = 1.0$, $P = 0.3$; GROUP $F = 0.6$, $P = 0.6$, no interaction). In contrast, it modified the active motor threshold according to the GROUP (DOPA $F = 1.8$, $P = 0.2$; GROUP $F = 0.8$, $P = 0.4$; DOPA $\times$ GROUP $F = 2.9$, $P < 0.04$). Only in the stable responders group, an acute challenge with L-DOPA induced a decrease in active motor threshold (stable responders group: ON versus OFF $F = 20.1$, $P < 0.0004$, $P = 0.007$ for other groups). The mean size of motor evoked potentials at baseline (S1) elicited at
A trend to be different (GROUP P = 0.06; TIME P = 0.6). In the FD group, there was neither any LTD-like plasticity to continuous TBS in OFF (TIME P = 0.9) nor continuous TBS-induced LTD-like plasticity in OFF (TIME P = 0.1, P = 0.9).

The analysis concerning the long-term effect of the dopaminergic replacement therapy on the TBS-induced effects in each group revealed different responses to intermittent and continuous TBS according to the clinical status (Table 3). In the healthy volunteers group, intermittent TBS induced an LTP-like plasticity (TIME F = 10.8, P < 0.0001) and continuous TBS induced LTD-like plasticity (TIME F = 11.7, P < 0.0001). In the stable responders group, intermittent TBS-induced LTD-like plasticity (TIME F = 13.6, P < 0.0001) and continuous TBS-induced LTD-like plasticity in OFF (TIME F = 8.3, P < 0.0003). In the FND group, intermittent TBS-induced LTD-like plasticity (TIME F = 3.0, P < 0.04) but there was no LTD-like plasticity to continuous TBS in OFF (TIME P = 0.1, P = 0.6). In the FD group, there was neither any significant intermittent TBS-induced LTD-like plasticity (F = 1.1, P = 0.3) nor continuous TBS-induced LTD-like plasticity in OFF (F = 0.1, P = 0.9).

### Additional effect of an acute L-DOPA dose on primary motor cortex plasticity

#### Long-term potentiation-like plasticity

The additional effect of an acute L-DOPA dose (OFF versus ON) on intermittent TBS-induced plasticity (Fig. 2) differed among the patient groups (GROUP F = 0.9, P = 0.4; DOPA F = 0.6, P = 0.4; TIME F = 5.0, P < 0.0009; GROUP × DOPA interaction F = 4.4, P = 0.009).

---

### Table 2 Baseline motor thresholds in the different patient groups

<table>
<thead>
<tr>
<th>Group</th>
<th>OFF</th>
<th>ON</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>T0</td>
<td>T1</td>
</tr>
<tr>
<td>Stable responders</td>
<td>58.5 ± 1.7</td>
<td>59.1 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FND</td>
<td>58.1 ± 2.1</td>
<td>56.6 ± 1.9</td>
<td>0.05</td>
</tr>
<tr>
<td>FD</td>
<td>59.1 ± 2.8</td>
<td>59.1 ± 1.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>60.4 ± 2.8</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Healthy volunteers did not receive L-DOPA.

### Table 3 Effect of intermittent and continuous TBS in healthy volunteers and patients in different stages of Parkinson’s disease in OFF and ON conditions

The motor evoked potential amplitudes are given as raw values (in mV) at baseline (S1), immediately after TBS (T0), after 10 min (T1), and after 20 min (T2). Motor evoked potential at S1 was obtained with a calibrated stimulation 1.2 × resting motor threshold.

SR = stable responders; HV = healthy volunteers; NA = not applicable as healthy volunteers were not given L-DOPA.

120% of resting motor threshold (Table 3) was not different among the patient groups or between the patients and the healthy volunteers (GROUP: F = 0.8, P = 0.5).

### Theta burst stimulation-induced plasticity of primary motor cortex

To find out whether LTD-like plasticity was impaired with respect to healthy volunteers, the groups were compared using the normalized values of the motor evoked potentials in the OFF condition (Fig. 1). The plasticity induced in the four groups showed only a trend to be different (GROUP F = 2.6, P = 0.06; TIME F = 2.7, P = 0.06, no interaction). This trend is explained by the lower level of plasticity in all patient groups compared with healthy volunteers (Fig. 1).

Comparison of the normalized values of motor evoked potentials in OFF showed that LTD-like plasticity was different among the four groups (GROUP F = 4.2, P < 0.02; TIME F = 0.4, P = 0.7, no interaction). LTD-like plasticity in the stable responders group was restored to normal (stable responders versus healthy volunteers P = 0.3) while it was less than in healthy volunteers in the others groups (FND versus healthy volunteers P < 0.05, FD versus healthy volunteers P < 0.001, stable responders versus FND P = 0.3, stable responders versus FD P < 0.02, FD versus FND P = 0.1).
Figure 1 Comparison of LTP and LTD-like plasticity levels among healthy volunteers and patients in different stages of Parkinson's disease in the OFF condition. Normalized values of the motor evoked potentials (MEP post-TBS/MEP pre-TBS) immediately (T0), 10 min (T1) and 20 min (T2) after the end of the TBS intervention in OFF are compared among healthy controls (HV), patients with stable response to L-DOPA (SR), patients with wearing-off fluctuations but no dyskinesia (FND) and patients with fluctuations and dyskinesias (FD). Values >1 indicate an LTD-like effect; values <1 indicate an LTP-like effect. S1 is the pre-TBS motor evoked potential amplitude of 1 mV. LTP-like plasticity was present, though at a lower level compared with healthy volunteers in all patient groups. LTD-like plasticity in the stable response group was restored to normal (stable response versus healthy volunteers P = 0.3) while it was less than in healthy volunteers in the FD group and zero in the FD group. cTBS = continuous TBS; iTBS = intermittent TBS.

P < 0.02; TIME × GROUP F = 1.0, P = 0.4; DOPA × TIME F = 0.2, P = 0.8; DOPA × TIME × GROUP F = 1.9, P = 0.8; post hoc Fisher’s test: T0 versus T1 P = 0.2, T0 versus T2 P < 0.002, T1 versus T2 P = 0.06). In stable responders, L-DOPA increased, though not significantly, the intermittent TBS-induced effect on motor evoked potentials (DOPA F = 2.2, P = 0.1; TIME F = 1.6, P = 0.2; DOPA × TIME F = 0.6, P = 0.5). In the FND group, L-DOPA dosing led to a decline in plasticity when compared with OFF (DOPA F = 5.1, P < 0.04; TIME F = 5.3, P < 0.01; DOPA × TIME F = 2.8, P = 0.08). At T2, plasticity was lower in ON than OFF (T0 versus T1 P = 0.1, T0 versus T2 P < 0.004, T1 versus T2 P = 0.1). In the FD group, there was no effect of L-DOPA (DOPA F = 0.8, P = 0.4; TIME F = 0.2, P = 0.8; DOPA × TIME F = 0.2, P = 0.8).

Long-term depression-like plasticity

Acute L-DOPA had an adverse effect on continuous TBS-induced plasticity. Plasticity declined in all groups from OFF to ON (Fig. 2) but to a different extent according to the group (GROUP F = 5.2, P < 0.01; DOPA F = 37.1, P < 0.0001; GROUP × DOPA interaction F = 1.4, P = 0.3; TIME F = 3.8, P < 0.02; TIME × GROUP F = 0.9, P = 0.4; DOPA × TIME F = 5.4, P < 0.007; DOPA × TIME × GROUP F = 10.5, P = 0.7; Fisher’s test for TIME: T0 versus T1 P < 0.007, T0 versus T2 P = 0.06, T1 versus T2 P = 0.03. In the stable responders group, LTD-like response was present at the same level in OFF and ON at T0 but was decreased thereafter in ON (DOPA F = 22.6, P < 0.0006; TIME F = 2.7, P = 0.09; DOPA × TIME interaction F = 6.5, P < 0.006). In the FND group, LTD-like response almost disappeared after T0 following acute L-DOPA dose (DOPA F = 5.7, P < 0.03; TIME F = 1.7, P = 0.2, no interaction). The most striking result was observed in the FD group in which acute L-DOPA induced a paradoxical LTP-like facilitatory response instead of the expected inhibition after continuous TBS (DOPA F = 13.0, P < 0.004; TIME F = 1.6, P = 0.2; DOPA × TIME F = 0.7, P = 0.5).

In order to get an overview of the effect of an acute dose of L-DOPA on LTP and LTD-like plasticity in each of the patient groups, we compared the TBS-induced plastic responses in OFF and ON at the peak effect after the stimulation (T1, i.e. 10 min post-TBS). Figure 3 clearly shows that the reported enhancing effect of acute L-DOPA on plasticity (Morgante et al., 2006; Ueki et al., 2006) is preserved, though to a very low level, only in the stable responders group and only for the LTD-like plasticity. In the FND group, acute DOPA was detrimental to the plastic response and decreases pre-existing plasticity. In the FD group, acute L-DOPA was unable to restore the absent plasticity and even reversed the expected inhibitory effect of continuous TBS to a facilitation (LTP: stable responders P = 0.08, FND P < 0.01, FD P = 0.77; LTD: stable responders P < 0.001, FND P < 0.06, FD P < 0.03).

Correlations between clinical and treatment features and excitability of primary motor cortex or propensity of primary motor cortex to develop plasticity

Severity of illness

When considering the whole patient cohort (all intermittent TBS/all continuous TBS), there was no correlation between the duration
The additional effect of acute L-DOPA dose (OFF versus ON) in patients in different stages of Parkinson’s disease. Normalized values of the motor evoked potentials (MEP post-TBS/MEP pre-TBS) immediately (T0), 10 min (T1) and 20 min (T2) after the end of the TBS intervention in OFF and ON are compared among stable responders (SR), patients with ‘wearing off’ fluctuation but no dyskinesia (FND) and patients with both wearing off fluctuation and dyskinesias (FD). In OFF, plasticity was present in all groups except the FD group. Only in the stable response group for intermittent TBS, acute L-DOPA induced an additional positive effect (but not significant) of the plasticity from OFF. In the stable response group for continuous TBS and in the FND group for both intermittent and continuous TBS, acute L-DOPA had a negative effect on plasticity when compared with OFF. In the FD group, TBS-induced plasticity was zero in OFF and not restored by L-DOPA. Note that for continuous TBS, acute L-DOPA induced a paradoxical LTP-like facilitatory response instead of inhibition. OFF = after overnight withdrawal of all dopaminergic drugs; ON = 1 h after intake of the regular morning dose of dopaminergic drugs. cTBS = continuous TBS; iTBS = intermittent TBS.
or the severity of illness (UPDRS Part III total score or subscore in OFF) and the baseline excitability of the motor cortex (motor evoked potential at S1 in OFF) or the propensity of M1 to develop LTP or LTD-like plasticity in OFF (motor evoked potential T0 + T1 + T2). In contrast, the less severe the motor status of the patients (UPDRS Part III subscore in OFF), the better was the inhibitory response or LTD-like plasticity during ON (Fig. 4A) as revealed by the Spearman test on UPDRS Part III subscore versus motor evoked potential T0 + T1 + T2 (P < 0.05, r = 0.3) and on UPDRS Part III subscore versus motor evoked potential T1 (P < 0.03, r = 0.4). Those with more severe disease tended to show less inhibition (or more paradoxical facilitation). This did not hold true for the UPDRS Part III total score or the LTD-like plasticity.

**Duration of disease and treatment**

In the whole group, we found significant correlations between disease and treatment durations and the normalized motor evoked potentials in ON after intermittent TBS (motor evoked potential T0 + T1 + T2) indicating that shorter the duration of motor symptoms or treatment, the better was the responsiveness of M1 plasticity to L-DOPA (Fig. 4B) as revealed by the Spearman test on disease duration versus motor evoked potential T0 + T1 + T2 (P < 0.006, r = 0.5), or on treatment duration versus motor evoked potential T0 + T1 + T2 (P < 0.02, r = 0.4).

**Responsiveness to acute L-DOPA**

In the whole cohort of patients, the responsiveness of the motor status (UPDRS Part III in ON) to an acute L-DOPA dose did not correlate with the additional effect of acute L-DOPA on LTD-like plasticity. This was not an unexpected result because of the different responses of M1 plasticity to acute L-DOPA in the three groups. However, in the stable responders group, the greater the clinical improvement of motor signs with L-DOPA, the larger was the additional effect of acute L-DOPA on LTP-like plasticity (Fig. 4C), as revealed by the Spearman test: on UPDRS Part III subscore improvement [(subscore in OFF – subscore in ON)/subscore in OFF] versus motor evoked potential T0 in ON (P < 0.04, r = 0.4) and on UPDRS Part III subscore improvement versus motor evoked potential improvement [(motor evoked potential in ON – motor evoked potential in OFF)/motor evoked potential in OFF] at T0 (P < 0.02, r = 0.7). This did not hold true for the UPDRS Part III total score, for later time points, for LTD-like plasticity or for the other two patient groups (i.e. FND or FD).

In the whole cohort of patients, the additional effect of L-DOPA on LTD-like plasticity and on motor status (Fig. 4D) showed that the best motor response in ON (i.e. lower UPDRS Part III subscore) was accompanied by a reversal of the continuous TBS effect at T0 (P < 0.002, r = 0.5) leading to facilitation instead of inhibition. This negative correlation was observed in the FND (P < 0.02, r = 0.6) and FD groups (Fig. 4D) but not in the stable responders group (P = 0.2).
**Discussion**

The key finding of this study was that the propensity of the motor cortex in parkinsonian patients to develop plasticity was tightly linked to the pattern of the motor response to dopaminergic therapy. When patients were stratified on this basis, we found important differences among them: (i) stable responders to L-DOPA showed LTP and LTD-like plasticity in OFF; (ii) fluctuating non-dyskinetic patients in OFF expressed LTP but not LTD-like plasticity; (iii) fluctuating dyskinetic patients did not manifest either LTP- or LTD-like plasticity when tested in OFF and (iv) acute L-DOPA dosing revealed a negative effect on M1 plasticity in those with motor complications.
In short, if the motor response to l-DOPA was stable, the motor cortex was responsive to plasticity-induction protocols but if the motor response was complicated, the motor cortex was less responsive or unresponsive to plasticity-induction protocols and even developed paradoxical responses in the most advanced stages of motor complications. Whether the impairment in plastic response of motor cortex is a cause or a consequence of the motor complications remains an open question. Some of the results presented here may favour the former hypothesis.

**Primary motor cortex plasticity in patients with Parkinson’s disease never exposed to l-DOPA is severely reduced**

In a previous study (Kishore et al., 2012), we showed that the motor cortex of de novo patients (n = 21) did not express either LTP- or LTD-like plasticity when compared with healthy volunteers. These patients reported only unilateral symptoms, yet M1 of both hemispheres were unresponsive to TBS. These changes in M1 plasticity resembled the deficient plasticity of striatal neurons in parkinsonian rats (Centonze et al., 1999; Calabresi et al., 2000, 2007; Picconi et al., 2003). Additionally, the impairment of M1 plasticity in de novo Parkinson’s disease did not correlate with the severity of motor signs of Parkinson’s disease and failed to improve with an acute dose of l-DOPA. This was in stark contrast with the motor signs that showed a marked short duration response to an acute l-DOPA dose (Kishore et al., 2012). Based on these observations in de novo Parkinson’s disease, we proposed, in our earlier study, that M1 plasticity may be restored only as a long duration response to l-DOPA therapy.

**Primary motor cortex plasticity may be restored as a long duration response to l-DOPA therapy in stable responders**

The response of M1 plasticity to chronic l-DOPA treatment demonstrated in this study has features in common with the long duration response of motor signs of Parkinson’s disease. Patients with a stable response to l-DOPA expressed both LTP- and LTD-like plasticity 12–18 h after discontinuation of dopaminergic medications (OFF), indicating a chronic treatment benefit resembling the long duration response of motor signs (Anderson and Nutt, 2011). This response is in agreement with slice studies in which chronic l-DOPA treatment rescued the defective LTP (Picconi et al., 2003) and LTD (Picconi et al., 2011) of the cortico-striatal synapses in parkinsonian rats. In the stable responders, an acute dose of l-DOPA (ON) induced a brief and small, though not statistically significant, additional enhancement of LTP-like plastic response (short duration response) when compared with OFF (Fig. 2). This again resembles the characteristic of a strong long duration response of motor signs that masks the detection of an additional, possibly substantial, improvement in motor status (short duration response) in response to an individual dose of l-DOPA (Nutt et al., 1992, 1996). In the more advanced stages of the disease with motor complications, there is a decline of long duration response of motor signs (Nutt et al., 2000; Zapia and Nicoletti, 2010). We found the same tendency for the long duration response of M1 plasticity to decline as the disease progressed and motor complications developed (schematically shown in Fig. 5); this decline occurring earlier in LTD than LTP (Fig. 2). However, we did not find a negative correlation between the responsiveness of M1 to plasticity induction protocols in OFF and the disease or treatment durations or the UPDRS score in OFF that one would expect. A possible explanation for the failure to establish this relation could be the large overlap of disease duration and UPDRS scores in OFF among the three groups of patients, especially for the low values (Table 1). An alternative possibility is that the loss of plasticity in advanced Parkinson’s disease is not due to loss of long duration response of M1 plastic response but an effect of duration of disease. Such a view fits with the correlation between disease (and treatment) duration and responsiveness of M1 to intermittent TBS in ON (Fig. 4B), but it is not supported by the lack of correlation between any physiological parameter in OFF (resting motor threshold, active motor threshold, motor evoked potential size at baseline, responsiveness of M1 to TBS) and duration of disease.

Another possibility is that plasticity of M1 was restored by chronic l-DOPA therapy not as a long duration response but as a direct effect of l-DOPA on cortical excitability since acute l-DOPA dosing decreased the active motor threshold, a marker of enhanced cortical excitability, only in stable responders. In this study, we did not explore whether increased cortical excitability is linked to a decrease of intracortical inhibition under the dual effects of TBS and l-DOPA, either acute or chronic. This argument is not supported by previous studies that have raised the question whether intracortical inhibitions (short and long intracortical inhibitions) tested with paired-pulse transcranial magnetic stimulation paradigms are really abnormal (Ridding et al., 1995;
Parkinson’s disease (Zamir et al., 2012) in healthy volunteers (Huang et al., 2005) failed to produce such changes in patients with Parkinson’s disease (Zamir et al., 2012).

Primary motor cortex plasticity declines in the stage of motor complications

We have discussed above how the long duration response to M1 plasticity becomes less effective as motor complications worsen and disease progresses. Besides this, we observed that an acute dose of L-DOPA added a modest benefit to the M1 plasticity in stable responders (Fig. 2), suggesting a short duration response. The improvement in the plastic response of M1 correlated with the acute improvement of motor signs from OFF to ON (Fig. 4C); suggesting that the short duration response to L-DOPA of M1 plasticity, might be directly involved in the stable recovery of motor signs of Parkinson’s disease. From this perspective, it seems that both long and short duration response to L-DOPA are necessary to support an optimal plastic response. We assumed that the size of the motor evoked potential in ON is likely to reflect the summation of the long duration response and short duration response, while the size of motor evoked potential in OFF selectively reflects the long duration response of M1 plasticity. Correlations revealed that the higher the UPDRS scores in OFF or the longer the disease duration (i.e. more severe the dopaminergic denervation), the smaller was the effect of the plasticity-induction protocols in ON (Fig. 4A and B). In contrast, the plastic response in ON did not correlate with total daily L-DOPA equivalent dose or the morning dose of L-DOPA. This shows that as the disease progresses, the plastic response of M1 in ON depends more on the residual endogenous dopamine availability than on the dose of exogenous dopamine.

Correlations represented in Fig. 4A and B reveal that LTP- and LTD-like plasticity of M1 are susceptible to different extents with disease progression: facilitation of motor evoked potentials after intermittent TBS in ON that is initially robust, shows a very progressive decline to zero (Fig. 4B), while the inhibition of motor evoked potentials after continuous TBS in ON is present only at the lowest UPDRS Part III scores, is lost soon after and then turns into a paradoxical facilitation at UPDRS Part III scores >15 (Fig. 4A). This was further explored in each group of patients with motor complications. In the fluctuating non-dyskinetic patients, long duration response of both forms of plasticity was preserved. There was no additional effect of acute dopamine boost at T0 and even a detrimental effect at later time points (Fig. 2). Considering the positive relation between the acute L-DOPA-induced boost in LTP-like plasticity and the clinical improvement in stable responders, the negative effect of L-DOPA on plasticity seen in fluctuating patients might underlie their inability to sustain clinical improvement at this stage. In dyskinetic patients, both forms of long duration response of M1 plasticity were severely impaired with a complete loss of LTD-like plasticity (Fig. 2).

Moreover, the lower the scores in ON (due to improvement in motor signs), the higher was the paradoxical facilitation after continuous TBS in ON (Fig. 4D). The fact that paradoxical facilitation was largest in those patients with highest L-DOPA response to motor signs (lowest residual scores in ON), particularly in those with motor complications (Fig. 4D), fits with the view that both phenomena are related to the high levels of synaptic dopamine.

Since the severity of motor signs is an established risk factor for L-DOPA-induced dyskinesias (Benbir et al., 2006), it is reasonable to assume that the loss of LTD and the paradoxical LTP in those with more severe disease (Fig. 4A), may play a role in the genesis of dyskinesias.

A possible confounding aspect in the interpretation of results in the dyskinetic group is the occurrence of dyskinetic movements during or immediately after the induction of plasticity. Indeed, it was previously shown that in healthy subjects, muscle contraction during TBS abolishes both LTP- and LTD-like effects, while contraction immediately after TBS enhances an LTP-like effect and reverses an LTD-like effect to an LTP-like effect (Huang et al., 2008). Although not completely ruling out a possible role of levodopa-induced dyskinesias in the reversal of plastic response to an inhibitory protocol in dyskinetic patients, the progressive build-up from stable responders to fluctuating non-dyskinetic to dyskinetic patients argue against it (Figs 2 and 3).

In addition, the paradoxical facilitation after continuous TBS correlated only with disease severity (Fig. 4A) and not with the total L-DOPA equivalent dose, even if dyskinesias are strongly linked to both (Nutt et al., 2000). Since only patients with mild dyskinesias were selected for this study, a relation between the paradoxical facilitation and severity of dyskinesias could not be tested.

Aberrant plasticity may participate in generating dyskinesias

In healthy volunteers, high dose L-DOPA converts LTP-like plasticity of M1 (induced by paired associative stimulation) to LTD. It was proposed that such a reversal may be secondary to the abnormal levels of NMDA receptor activation caused by high levels of D1 receptor stimulation (Thirugnanasambandam et al., 2011). A similar negative effect of high levels of dopamine and excessive D1 signalling might exist in patients with advanced Parkinson’s disease with motor complications and could be involved in the paradoxical response observed in our dyskinetic group in response to an inhibitory plastic intervention. Indeed, PET studies revealed a dramatic and prolonged increase in synaptic dopamine levels in dyskinetic Parkinson’s disease and fluctuating patients when compared with stable responders, following the same acute L-DOPA dose (de la Fuente-Fernández et al., 2001, 2004). Such non-physiological synaptic dopamine boosts could interfere with the function of key enzymes (Greengard et al., 1999; Santini et al., 2007; Calabresi et al., 2010) that are necessary for the induction of normal plasticity (Barria et al., 1997; Lee et al., 1998, 2000). This could potentially lead to an inability to sustain good motor response to L-DOPA or selection of unwanted motor programmes and thereby involuntary movements.
Dyskinesias are linked to the loss of inhibitory synaptic regulation such as LTD and depotentiation (i.e. ability to inhibit an already facilitated synapse) in corticostriatal neurons of parkinsonian rats in which chronic L-DOPA treatment failed to restore LTD or depotentiation (Picconi et al., 2011). Recently, loss of depotentiation was also demonstrated in the M1 of dyskinetic patients with Parkinson’s disease that could not be rescued by L-DOPA (Huang et al., 2012). There are no previous reports of the changes in LTD of M1 in dyskinetic human Parkinson’s disease or the effect of L-DOPA on it. The severe impairment of LTD-like plasticity demonstrated in this study provides additional evidence that there is dysregulation of inhibitory synapses in dyskinetic patients. We also propose that besides the loss of LTD and depotentiation, the paradoxical LTP-like response to an inhibitory stimulation protocol after acute dopamine boost may additionally hamper the inhibitory synaptic regulation and contribute to abnormal selection of motor programmes and dyskinesias.

Implications of the current study

In most of the previous studies, plasticity induction protocols, both paired associative stimulation (Morgante et al., 2006; Ueki et al., 2006) and TBS (Suppa et al., 2011; Eggers et al., 2010) failed to induce plasticity in treated patients with Parkinson’s disease. A single recent study reported that TBS-induced M1 plastic response in chronically treated patients was similar to healthy subjects (Zamir et al., 2012). It was also controversial whether M1 of dyskinetic patients responded like non-dyskinetic patients (Suppa et al., 2011) or not (Morgante et al., 2006) and whether chronic L-DOPA therapy restored plasticity of M1 (Morgante et al., 2006; Ueki et al., 2006) or not (Suppa et al., 2011). Previous studies that compared M1 plasticity and its response to L-DOPA in dyskinetic versus non-dyskinetic patients included patients with wide ranges of disease duration and thus of dopaminergic treatment duration (2–12 years disease duration in non-dyskinetic and 5–19 years in dyskinetic group in Suppa et al., 2011; 6–13 years and 7–20 years in Morgante et al., 2006; 2–11 years in Zamir et al., 2012). Our results concur with the reports of loss of plasticity demonstrated in dyskinetic Parkinson’s disease, be it with paired associative stimulation (Morgante et al., 2006) or intermittent TBS (Suppa et al., 2011). The beneficial effect of L-DOPA on paired associative stimulation-induced plasticity in Parkinson’s disease (Ueki et al., 2006) and similar levels of TBS-induced plasticity reported in treated patients with Parkinson’s disease and in normal controls (Zamir et al., 2012) are compatible with our observations in the stable responders with shorter disease and treatment duration.

Our study shows that brain plasticity is strongly influenced by the persistent treatment effects and the degree of dopaminergic denervation. This needs to be factored in while comparing patients with variable durations of disease and treatment and also when planning future studies that examine brain plasticity or modulate brain plasticity for therapeutic purposes. We assume that changes in M1 plasticity and motor signs reflect improved corticostriatal and striato-thalamo-cortical efferent signalling secondary to dopamine replacement (both chronic and acute-on-chronic treatment). However, a direct effect of dopamine on the M1 dopamine receptors (Molina-Luna et al., 2009) cannot be excluded. A long duration response of dopaminergic treatment can extend to physiological functions like brain plasticity supports the observation of Beeler et al. (2010) that long duration response could influence motor learning in animals with dorsal striatal dopamine deficiency that were chronically treated with L-DOPA.

Conclusions

Our previous and the current studies bring to the fore a spectrum of changes in the plasticity of M1 in Parkinson’s disease. As the disease advances and motor complications appear in response to L-DOPA, there is a clear loss of the long duration response of motor cortex plasticity and development of abnormal plastic responses to acute L-DOPA dosing. The strong correlation between the improvement in M1 plasticity and the clinical signs of Parkinson’s disease after an acute L-DOPA dosing in stable responders and between the decline in LTD-like plasticity of M1 in dyskinetic patients and the severity of motor signs in them argue in favour of a relation between the clinical motor response to L-DOPA and plasticity of M1. The fact that the variation in plastic response of M1 from OFF to ON correlated only with clinical improvement of parkinsonian signs on the contralateral side of body (UPDRS Part III subscore) and not with the improvement in the total motor scores (that includes, in addition, the assessment of gait, balance, posture, speech and facial expression) reinforces the view of a close link between M1 plasticity and motor signs of Parkinson’s disease. Further investigations in animals are needed to explore the molecular changes induced by acute pulsatile dosing of L-DOPA that may ultimately interfere with the expression of synaptic plasticity and the development of dyskinesias and fluctuations. A greater understanding of the pathophysiological role of changes in M1 plasticity may open the field of more specific therapeutic approaches aimed at modulating the plasticity of the motor cortex.

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