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# Dopamine Influences Primary Motor Cortex Plasticity and Dorsal Premotor-to-Motor Connectivity in Parkinson's Disease

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**We investigated abnormal premotor to motor (PMd-to-M1) connectivity in Parkinson's disease (PD) with repetitive transcranial magnetic stimulation (rTMS). We studied 28 patients off and on dopaminergic therapy and 28 healthy subjects. We delivered 5 Hz rTMS over M1 before and after conditioning PMd with 5 Hz rTMS. In healthy subjects, motor-evoked potentials (MEPs) elicited by M1-rTMS were facilitated and PMd-rTMS left MEPs unchanged. In patients, before PMd-rTMS, M1-rTMS induced no MEP facilitation, whereas after PMd-rTMS, it significantly facilitated MEPs only when patients were on therapy. In the second experiment, we delivered M1-rTMS under 3 different attention-demanding tasks: eyes closed, attention directed to the stimulated hand, and attention directed to the nonstimulated hand. In healthy subjects, a more pronounced MEP facilitation was present when subjects directed attention to the stimulated hand. In patients, the MEP facilitation was present when attention was directed to the stimulated hand only when patients were on therapy. Finally, we delivered M1-rTMS in patients on therapy while they were looking at the stimulated hand, before and after 1 Hz PMd-rTMS. PMd-rTMS reduced the attention-induced MEP facilitation. We conclude that in addition to abnormal M1 plasticity, the reduced MEP facilitation in PD also reflects altered PMd-to-M1 connectivity.**

**Keywords:** Parkinson's disease, premotor cortex, primary motor cortex, synaptic plasticity, TMS

## Introduction

Supporting the idea that connectivity between dorsal premotor cortex (PMd) and primary motor cortex (M1) is involved in human motor planning and motor execution, neurophysiological studies in primates have detected strong facilitatory and inhibitory connections between PMd and M1. Inputs from the PMd enable M1 to select appropriate movements from a set of prepared possible responses and to suppress activity in other muscles thus preventing inappropriate release of other responses (Tokuno and Nambu 2000; Picard and Strick 2001; Dum and Strick 2002, 2005; Cisek and Kalaska 2005; Chouinard and Paus 2006).

A useful technique for investigating the connections between PMd and M1 in humans is repetitive transcranial magnetic stimulation (rTMS). In healthy subjects, rTMS delivered over the PMd influences the amplitude of motor-evoked potentials (MEPs) in response to single and paired pulses over M1 (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). One Hertz rTMS conditioning over the PMd reduces, whereas 5 Hz rTMS enhances the amplitude of MEPs elicited by single pulses over the ipsilateral M1. A number of experiments have shown that spread of nonsynaptic current is an unlikely mechanism for explaining how PMd-rTMS influences M1 excitability (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). Current knowledge therefore implies that the PMd-rTMS–induced changes in M1 excitability reflect the activation of PMd-to-M1 connections (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). In healthy subjects, we have already shown that conditioning PMd with 1 Hz rTMS also reduces mechanisms of short-term plasticity (STP) in ipsilateral M1 tested by delivering 5 Hz rTMS in short trains (Suppa et al. 2008). In healthy subjects, when suprathreshold 5 Hz rTMS is delivered over M1, MEPs elicited by each stimulus progressively increase in amplitude during the train (Pascual-Leone et al. 1994; Jennum et al. 1995; Berardelli et al. 1998). This normal MEP facilitation reflects STP resembling N-methyl-D-aspartate (NMDA)–dependent short-term potentiation of synaptic connections described in animal experiments (Pascual-Leone et al. 1994; Jennum et al. 1995; Berardelli et al. 1998; Di Lazzaro et al. 2002; Inghilleri et al. 2004, 2005; Cooke and Bliss 2006).

Conversely, in patients with Parkinson's disease (PD), during 5 Hz rTMS over M1, MEP size remains unchanged (Gilio et al. 2002), showing that STP is abnormal in PD. Studies using the paired associative stimulation (PAS) technique in PD have nevertheless shown abnormalities also in M1 long-term plasticity (Bagnato et al. 2006; Morgante et al. 2006; Ueki et al. 2006). In PD patients off therapy, MEPs after PAS were unchanged (Morgante et al. 2006; Ueki et al. 2006) or abnormally increased in amplitude (Bagnato et al. 2006). In patients with PD, in addition to altered STP and long-term M1 plasticity, PMd-to-M1 connectivity is also abnormal. In de novo PD, conditioning 1 Hz rTMS to the PMd reduced in the ipsilateral M1 the abnormal baseline intracortical excitability tested with the paired-pulse technique at interstimulus intervals of 5 ms (Buhmann et al. 2004). In addition, 5 Hz rTMS over the PMd failed to facilitate MEPs elicited by single pulses over M1 (Mir et al. 2005). Dopaminergic treatment partly restored normal PMd-to-M1 modulatory patterns (Buhmann et al. 2004; Mir et al. 2005).

Compared with current knowledge on how PMd-to-M1 connectivity affects M1 cortical excitability in PD, how the PMd influences STP in M1 as tested by the MEP facilitation and through which underlying mechanisms remain less clear. Given that the absent MEP facilitation in PD might depend not only on altered M1 plasticity but also on abnormal functional PMd-to-M1 connectivity, the altered STP in PD could in theory be restored by delivering 5 Hz rTMS to the PMd.

Besides its role in motor planning and motor execution, the PMd also intervenes in mechanisms of motor attention. Neurophysiological studies conducted in monkeys and humans and designed to dissociate neuronal activity reflecting motor preparation from activity related to attention have shown that the PMd has a role in orienting attention and maintaining

visuospatial information relevant for a goal-directed action (Caminiti et al. 1998; Corbetta et al. 1998; Coull and Nobre 1998; Courtney et al. 1998; Boussaoud 2001; Lebedev and Wise 2001; Simon et al. 2002). In healthy subjects, M1 cortical plasticity as tested with transcranial magnetic stimulation (TMS) techniques is strongly modulated by attention (Stefan et al. 2004; Conte et al. 2007, 2008). Research conducted in our laboratory showed that increasing subjects' attention levels enhances the M1 MEP facilitation elicited by 5 Hz rTMS (Conte et al. 2007). In healthy subjects, conditioning 1 Hz rTMS over PMd also reduces the attention-related effects on the MEP facilitation (Conte et al. 2007), suggesting that the PMd intervenes in motor attention as part of the frontoparietal network involved in the self-recognition process (Van den Bos and Jeannerod 2002; Chouinard et al. 2003; MacDonald and Paus 2003). Given that the lack of MEP facilitation in PD might depend on abnormal activation of PMd-to-M1 connectivity by attention, the altered STP in PD could in theory be restored by increasing levels of motor attention. The possible link between the PMd and motor attention in PD could be investigated by finding out whether 1 Hz rTMS over the PMd reduces the attention-induced changes in the MEP facilitation in patients with PD as it does in healthy subjects (Conte et al. 2007).

Current data leave considerable gaps in the knowledge of the PMd-to-M1 connections in PD, especially on mechanisms underlying altered M1 cortical plasticity, nor do they fully explain their relationship with different attention levels. More information is especially needed on how dopaminergic therapy in PD restores PMd-to-M1 connectivity and possibly M1 STP before developing new therapeutic neurostimulation approaches for patients with PD.

We designed this study in patients with mild-to-moderate PD first to investigate whether conditioning PMd with 5 Hz rTMS enhances STP as tested by the MEP facilitation during 5 Hz rTMS. Finally, to gain further insight into the PMd-to-M1 connections in PD, we tested whether attention restores STP and whether real or sham 1 Hz rTMS over the PMd reduces these attention-induced changes. To see whether dopaminergic therapy restores STP and whether it does so by modulating functional PMd-to-M1 connectivity, we studied PD patients on and off therapy.

## Materials and Methods

### *Subjects*

The study group comprised 28 PD patients (20 men and 8 women; mean age ± standard deviation [SD]: 62 ± 7.6 years, range 45–77 years) and 28 age-matched healthy subjects (13 men and 15 women; mean age ± SD: 65 ± 7.5 years, range 50–76 years). All participants were right-handed. The diagnosis of idiopathic PD was made using the UK Brain Bank Criteria (Gibb and Lees 1988). PD patients with a predominantly akinetic-rigid manifestation were recruited from the movement disorder outpatient clinic of the Department of Neurological Sciences, Sapienza University of Rome. PD patients enrolled in the study had mild-to-moderate PD and had neither dyskinesias nor additional neuropsychiatric disorders. Patients were studied off and on dopaminergic therapy. Patients were considered on when they had dopaminergic therapy, whereas they were considered off after drug withdrawal for at least 12 h. None of the PD patients involved in this study was taking long-acting dopaminergic drugs. The patients' clinical features are summarized in Table 1. PD patients were clinically evaluated before starting each experimental session. Motor signs were scored using the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS) and the Hoehn and Yahr scale. All subjects gave their informed consent, and the study was approved by the local ethical committee and conformed with the Declaration of Helsinki.

### *Stimulation of M1 and PMd*

rTMS was delivered over the left M1 through a high-frequency magnetic stimulator (Magstim Super Rapid; The Magstim Company Ltd, Whitland, UK) connected to a figure-of-eight coil with mean loop diameter of 9 cm. The magnetic stimulus had a biphasic waveform with a pulse width of ~300 μs. During the first phase of the stimulus, the current in the center of the coil flowed toward the handle. The coil was held tangentially to the scalp with the handle pointing back and away from the midline at 45°, inducing posteroanterior followed by anteroposterior (PA–AP) current in the brain. The coil was placed over the optimum scalp position (hot spot) to elicit motor responses (MEPs) in the contralateral first dorsal interosseous (FDI) muscle.

Motor threshold at rest (RMT) was determined as the lowest intensity able to evoke an MEP of greater than 50 μV in at least 5 of 10 consecutive trials in the FDI muscle. Active motor threshold (AMT) was determined as the lowest intensity able to evoke an MEP of 200 μV during slight contraction of FDI muscle. For RMT and AMT determination, we used a step width 1% of the maximum stimulator output. rTMS over the left PMd was delivered through a high-frequency magnetic stimulator connected to a figure-of-eight coil with mean loop diameter of 9 cm. The left PMd was considered as being located at a site 2.5 cm anterior to the M1 hot spot (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004; Suppa et al. 2008). The coil was held tangentially to the scalp with the handle pointing anteromedially from the midline at 45°, inducing anteroposterior followed by posteroanterior (AP–PA) current in the brain (Gerschlager et al. 2001; Kammer et al. 2001; Rizzo et al. 2004; Suppa et al. 2008).

### *Recording Techniques and Measurements*

The electromyographic (EMG) activity was recorded through pairs of surface electrodes (Ag/AgCl) placed over the right FDI muscle using a belly–tendon montage. EMG signals were recorded, amplified, and filtered with a Digitimer D360 (Digitimer Ltd, Welwyn Garden City, UK) (bandwidth 5 Hz to 1 kHz); acquired at a sampling rate of 5 kHz through a 1401 plus AD laboratory interface (Cambridge Electronic Design, Cambridge, UK); and stored on a personal computer for off-line analysis (Signal software; Cambridge Electronic Design). The level of baseline EMG activity was controlled by visual feedback through an oscilloscope screen and by auditory feedback through a loudspeaker. Trials with background EMG activity (with involuntary EMG activity greater than 50 μV in a time window of 500 ms preceding MEPs) were rejected to exclude possible confounding effects of involuntary muscular contraction. The amplitude of MEPs evoked by each of the 10 stimuli in the M1-rTMS train was measured peak to peak (millivolt), and MEPs in the same range (from 1 to 10) were then averaged. MEP amplitude is expressed in figures as a percentage of the first MEP amplitude evoked by M1-rTMS.

### *Experiment 1—MEP Facilitation in Healthy Subjects and in Patients with PD: The Effect of PMd-rTMS*

A group of 14 PD patients off and on therapy (10 men and 4 women; mean age ± SD: 64 ± 7.9 years, range 52–77 years) and 19 healthy subjects (8 men and 11 women; mean age ± SD: 66 ± 6.4 years, range 56–79 years) participated in this experiment (Fig. 1). PD patients were studied in 2 separate sessions randomly assigned and at least 5 days apart. Subjects were asked to relax fully and keep their eyes open without a fixation point. A conditioning-test rTMS paradigm was used (Fig. 1). Conditioning PMd-rTMS was delivered in all sessions at 90% of AMT (Rizzo et al. 2004; Mir et al. 2005; Suppa et al. 2008). Conditioning PMd-rTMS was delivered at 5 Hz and consisted of a total of 1500 stimuli delivered in 5 blocks, each of 300 pulses separated by intertrain intervals of 1 min (10 min in total) (Rizzo et al. 2004; Mir et al. 2005; Suppa et al. 2008). Test M1-rTMS consisted of trains of 10 stimuli at 5 Hz (2 s of stimulation). To avoid cumulative aftereffects (Gilio et al.

**Table 1**
Clinical features of the patients with PD who participated in the 3 experiments

| | | Age (years) | Sex | UPDRS | | Hoehn and Yahr | Disease duration (years) | Treatment (L-Dopa equivalent dose) (mg) | Neuropsychology | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | On | Off | | | | MMSE | AM | WCST |
| Exp. 1 | 1 | 63 | M | 13 | 19 | II | 3 | 140 | | | |
| | 2 | 52 | F | 15 | 20 | II | 5 | 550 | | | |
| | 3 | 68 | M | 17 | 25 | III | 10 | 200 | | | |
| | 4 | 55 | F | 13 | 16 | II | 5 | 140 | | | |
| | 5 | 68 | M | 15 | 22 | II | 4 | 740 | | | |
| | 6 | 56 | M | 16 | 20 | II | 7 | 333 | | | |
| | 7 | 72 | M | 20 | 25 | II | 5 | 400 | | | |
| | 8 | 77 | F | 20 | 28 | III | 10 | 300 | | | |
| | 9 | 72 | F | 16 | 21 | III | 10 | 440 | | | |
| | 10 | 67 | M | 14 | 21 | II | 6 | 240 | | | |
| | 11 | 72 | M | 16 | 24 | II | 4 | 740 | | | |
| | 12 | 55 | M | 16 | 28 | III | 15 | 240 | | | |
| | 13 | 62 | M | 21 | 27 | III | 10 | 400 | | | |
| | 14 | 58 | M | 18 | 24 | III | 11 | 600 | | | |
| | AV | 64 | | 16 | 23 | | 8 | 390 | | | |
| | SD | 7.9 | | 2.5 | 4.2 | | 3.5 | 203 | | | |
| Exp. 2 | 1 | 57 | F | 19 | 39 | III | 6 | 440 | 25 | 50 | 54 |
| | 2 | 63 | F | 10 | 25 | III | 8 | 540 | 30 | 55 | 51 |
| | 3 | 60 | M | 13 | 35 | III | 17 | 740 | 30 | 54 | 54 |
| | 4 | 60 | F | 5 | 15 | II | 6 | 540 | 29 | 50 | 54 |
| | 5 | 58 | F | 5 | 13 | II | 5 | 540 | 29 | 54 | 52 |
| | 6 | 45 | M | 5 | 14 | II | 3 | 140 | 30 | 60 | 55 |
| | 7 | 54 | M | 9 | 21 | II | 6 | 240 | 26 | 54 | 54 |
| | 8 | 55 | M | 13 | 26 | III | 2 | 440 | 30 | 55 | 51 |
| | 9 | 61 | M | 5 | 15 | II | 2 | 140 | 28 | 51 | 53 |
| | AV | 57 | | 9 | 22 | | 6 | 418 | 29 | 54 | 53 |
| | SD | 5.3 | | 4.9 | 9.5 | | 4.6 | 205 | 2 | 3 | 1 |
| Exp. 3 | 1 | 69 | M | 21 | | III | 6 | 440 | 30 | 55 | 51 |
| | 2 | 65 | M | 14 | | II | 6 | 340 | 28 | 50 | 51 |
| | 3 | 54 | M | 14 | | III | 13 | 470 | 28 | 54 | 54 |
| | 4 | 73 | M | 18 | | II | 3 | 400 | 29 | 51 | 52 |
| | 5 | 59 | M | 12 | | II | 3 | 140 | 30 | 55 | 53 |
| | AV | 64 | | 16 | | | 6 | 358 | 29 | 53 | 51 |
| | SD | 7.6 | | 3.6 | | | 4.1 | 131 | 1 | 2 | 1 |

Note: L-Dopa equivalent dose (mg) was calculated for each patient according to the criteria of Hobson et al. (2002). AV, average.

2007; Suppa et al. 2008), 15 trains were delivered with an intertrain interval of 1–2 min. The stimulation intensity was set at 120% RMT. Test M1-rTMS was delivered before and immediately after conditioning PMd-rTMS ended.

### *Experiments 2 and 3—Attention Levels and MEP Facilitation in Healthy Subjects and in Patients with PD*

A group of 9 PD patients off and on therapy (5 men and 4 women; mean age ± SD: 57 ± 5.3 years, range 45–63 years) and 9 healthy subjects (3 men and 6 women; mean age ± SD: 62 ± 9.4 years, range 54–76 years) participated in experiment 2 (Fig. 1). To exclude cognitive impairment, executive dysfunction, and attention deficits, all patients did the Mini-Mental State Examination (MMSE), Wisconsin Card Sorting Test (WCST), and Attentive Matrices (AM) (Halligan et al. 2003; Koerts et al. 2009). In this experiment, subjects were asked to relax fully. The MEP facilitation evoked by 5 Hz rTMS over the left M1 was studied in 3 different attention-demanding tasks tested in a single experimental session with an "intertask" interval of 1–2 min: Before each M1-rTMS train, subjects were randomly asked to keep the eyes closed ("eyes closed" condition), to open the eyes and look at the right hand ("target hand" condition), and to open the eyes and look at the left hand ("nontarget hand" condition) (Conte et al. 2007, 2008) (Fig. 1). To avoid cumulative aftereffects (Gilio et al. 2007; Suppa et al. 2008), 15 rTMS trains with an intertrain interval of 1–2 min were collected in each attention-demanding task (total of 45 rTMS trains).

Finally, for experiment 3, we enrolled 5 further PD patients on therapy (5 men; mean age ± SD: 64 ± 7.6 years, range 54–73 years) (Fig. 1). Patients participated in 2 sessions that took place at least 1 week apart and were randomly assigned to receive real or sham 1 Hz PMd-rTMS as the first interventional procedure. One Hertz PMd-rTMS consisted of 1500 stimuli delivered in 2 blocks, each of 750 pulses separated by intertrain intervals of 1 min (25 min in total), at 90% AMT intensity (Rizzo et al. 2004). Conditioning sham 1 Hz rTMS stimulation was applied at 90% AMT intensity over the left PMd with the coil held anteromedially and angled at 90° (Suppa et al. 2008). The MEP facilitation evoked by 5 Hz rTMS over the left M1 was studied in the target hand condition.

### *Statistical Analysis*

Data collected in all experimental sessions were analyzed as absolute values (millivolt).

Student's *t*-test was used to compare in healthy subjects and in patients with PD on and off therapy RMT and AMT values, the amplitude of the first MEP evoked by M1-rTMS, and the intensity of M1-rTMS and PMd-rTMS in experiments 1–3.

In experiment 1, to test the effect of conditioning PMd-rTMS on the amplitude of MEPs evoked by each stimulus of the test M1-rTMS in healthy subjects and in PD patients on and off therapy, we used a between-group analysis of variance (ANOVA) with factors "Group" (healthy subjects vs. PD patients on and healthy subjects vs. PD patients off), "Time" (before vs. after conditioning PMd-rTMS), and "Number of Stimuli" (1 vs. 2, 3, 4, 5, 6, 7, 8, 9, and 10). A 3-way repeated measures ANOVA with factors "Therapy" (on vs. off), Time (before vs. after conditioning PMd-rTMS), and Number of Stimuli (1 vs. 2, 3, 4, 5, 6, 7, 8, 9, and 10) was also used to test the effect of conditioning PMd-rTMS on the amplitude of MEPs evoked by each stimulus of the test M1-rTMS in PD patients on and off state of therapy.

In experiment 2, the effect of attention on the amplitude of MEPs evoked by each stimulus of the test M1-rTMS in healthy subjects and in PD patients on and off therapy was tested in a between-group ANOVA with factors Group (healthy subjects vs. PD patients on and healthy subjects vs. PD patients off), "Condition" (target hand vs. nontarget hand vs. eyes closed), and Number of Stimuli (1 vs. 2, 3, 4, 5, 6, 7, 8, 9, and 10). A 3-way repeated measures ANOVA with factors Therapy (on vs. off), Condition (target hand vs. nontarget hand vs. eyes closed), and

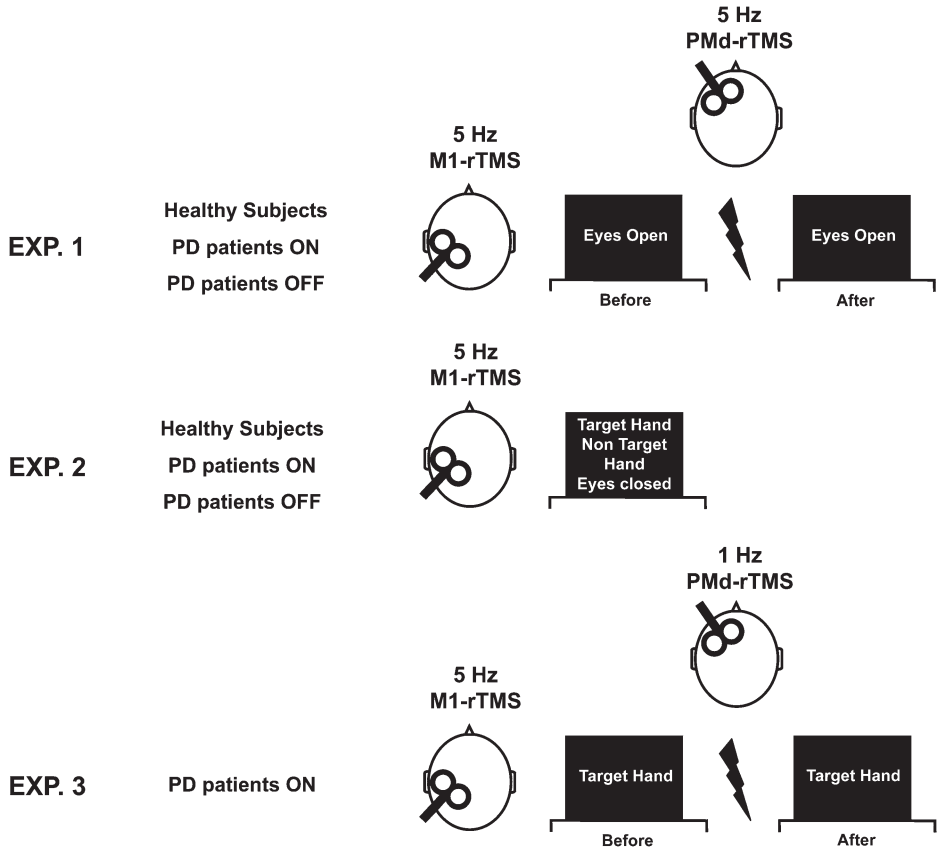


**Figure 1.** Experimental protocol used in the study. In experiment 1, we tested the MEP facilitation in response to 5 Hz rTMS over the primary motor cortex (M1) before and after conditioning 5 Hz rTMS over the dorsal premotor cortex (PMd) in healthy subjects and in PD patients off and on therapy. In experiment 2, we tested the MEP facilitation in response to 5 Hz rTMS in the 3 attention-demanding tasks in healthy subjects and in PD patients off and on therapy. Finally, in experiment 3, we tested the MEP facilitation in the "target hand" condition in PD patients on therapy.

Number of Stimuli (1 vs. 2, 3, 4, 5, 6, 7, 8, 9, and 10) was also used to test the effect of attention on the amplitude of MEPs evoked by each stimulus of the test M1-rTMS in PD patients on and off state of therapy.

In experiment 3, the effect of real and sham 1 Hz PMd-rTMS on the amplitude of MEPs evoked by each stimulus of the test M1-rTMS in PD patients on therapy in the target hand condition was tested in a 3-way repeated measures ANOVA with factors "Stimulation" (real vs. sham 1 Hz PMd-rTMS), Time (before vs. after 1 Hz PMd-rTMS), and Number of Stimuli (1 vs. 2, 3, 4, 5, 6, 7, 8, 9, and 10).

Finally, a 1-way repeated measures ANOVA with factor "Number of Train" (1 vs. 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15) was also used to test separately in healthy subjects and in PD patients off and on therapy whether the first MEP elicited by M1-rTMS progressively increased or decreased in amplitude from the 1st to the 15th train throughout the experiments because M1 plasticity mechanisms built up.

Tukey Honest Significant Difference test was used for all post hoc analyses. The Greenhouse–Geisser correction was used when necessary to correct for nonsphericity.

The Wilcoxon matched pairs test was used to compare UPDRS values in PD patients on and off therapy participating in experiments 1 and 2.

Spearman rank correlation test was used to assess correlations between changes in UPDRS values and changes in the amplitude of MEPs evoked by M1-rTMS in the experiment testing the effect of PMd-rTMS and attention on the MEP facilitation.

$P$ values $<0.05$ were considered to indicate statistical significance.

## Results

None of the subjects experienced any adverse effects during or after rTMS, and none of the patients had side effects when drugs were withdrawn.

According to the criteria used for rejecting trials with involuntary EMG activity, only a few trials were rejected (less than 5% on average) in healthy subjects and in PD patients off and on therapy. A similar number of trials were rejected in experiments 1–3.

Student's $t$-test showed that RMT and AMT values, the amplitude of the first MEP evoked by M1-rTMS, and the intensities used for M1-rTMS and PMd-rTMS in experiments 1–3 were all comparable in healthy subjects and in PD patients on and off therapy (Tables 2–4).

### *Experiment 1—MEP Facilitation in Healthy Subjects and in Patients with PD: The Effect of PMd-rTMS*

In this experiment, ANOVA showed that in healthy subjects, M1-rTMS induced a significant MEP facilitation and PMd-rTMS left the facilitation unchanged (Fig. 2*A*). Conversely, in PD patients on and off therapy, M1-rTMS induced no MEP facilitation and PMd-rTMS induced an MEP facilitation only in patients on therapy (Fig. 2*B*,*C*).

PMd-rTMS–induced changes in STP differed in healthy subjects and in PD patients off and on therapy. When we compared healthy subjects and PD patients off therapy, between-group ANOVA showed a significant 2-way interaction between factors Group and Number of Stimuli ($F_{9,279} = 4.99$, $P < 0.01$) and a nonsignificant effect of factor Time ($F_{1,31} = 1.18$, $P = 0.29$). Conversely, when we compared healthy subjects and PD patients on therapy, between-group ANOVA showed

**Table 2**
TMS data in the experiment testing the effect of conditioning rTMS over the dorsal premotor cortex (PMd) on MEP facilitation elicited by 5 Hz rTMS over the primary motor cortex (M1)

| Subjects | Before PMd-rTMS | | | AMT (%) | PMd-rTMS (%) | After PMd-rTMS | | |
|---|---|---|---|---|---|---|---|---|
| | RMT (%) | M1-rTMS (%) | First MEP amp. (mV) | | | RMT (%) | M1-rTMS (%) | First MEP amp. (mV) |
| Healthy subjects | | | | | | | | |
| AV | 53 | 66 | 0.29 | 37 | 34 | 53 | 66 | 0.28 |
| SD | 6.6 | 7.9 | 0.15 | 5.6 | 5.1 | 6.6 | 7.8 | 0.14 |
| PD patients off therapy | | | | | | | | |
| AV | 51 | 61 | 0.34 | 37 | 32 | 51 | 61 | 0.28 |
| SD | 9.5 | 11 | 0.12 | 5.6 | 4.3 | 9.3 | 11 | 0.08 |
| PD patients on therapy | | | | | | | | |
| AV | 51 | 61 | 0.26 | 37 | 32 | 51 | 61 | 0.23 |
| SD | 10.2 | 11.9 | 0.1 | 5.5 | 4.2 | 9.8 | 11.8 | 0.12 |

Note: M1-rTMS, intensity of M1-rTMS; first MEP amp., amplitude of the first MEP evoked by M1-rTMS; AV, average.

**Table 3**
TMS data in the experiment testing the effect of attention on MEP facilitation elicited by 5 Hz rTMS over the primary motor cortex (M1)

| Subjects | RMT (%) | M1-rTMS (%) | First MEP amp. | | |
|---|---|---|---|---|---|
| | | | Target hand (mV) | Nontarget hand (mV) | Eyes closed (mV) |
| Healthy subjects | | | | | |
| AV | 54 | 64 | 0.27 | 0.29 | 0.28 |
| SD | 10.1 | 11.5 | 0.1 | 0.1 | 0.1 |
| PD patients off therapy | | | | | |
| AV | 55 | 65 | 0.36 | 0.41 | 0.38 |
| SD | 5.7 | 7 | 0.12 | 0.18 | 0.14 |
| PD patients on therapy | | | | | |
| AV | 56 | 66 | 0.34 | 0.38 | 0.36 |
| SD | 5.7 | 7.1 | 0.21 | 0.13 | 0.1 |

Note: M1-rTMS, intensity of M1-rTMS; first MEP amp., amplitude of the first MEP evoked by M1-rTMS; AV, average.

a significant 3-way interaction between factors Group, Time, and Number of Stimuli ($F_{9.279} = 3.37$, $P < 0.01$). The PMd-rTMS–induced changes in STP also differed in PD patients off and on therapy. The 3-way repeated measures ANOVA disclosed a significant 3-way interaction between factors Therapy, Time, and Number of Stimuli ($F_{9.117} = 1.89$, $P = 0.05$).

In healthy subjects (Fig. 2*A*), post hoc 2-way ANOVA showed a significant effect of factor Number of Stimuli ($F_{9.162} = 9.94$, $P < 0.01$) but no significant effect of factor Time ($F_{1.18} = 0.68$, $P = 0.42$). Five Hz rTMS over M1 induced a significant MEP facilitation before ($P < 0.01$) and after ($P < 0.01$) PMd-rTMS, and PMd-rTMS left the MEP facilitation unchanged ($P < 0.01$ before and after PMd-rTMS). Conversely, in PD patients off therapy (Fig. 2*B*), post hoc 2-way ANOVA showed that factors Time and Number of Stimuli were nonsignificant ($F_{1.13} = 0.56$, $P = 0.47$ and $F_{9.117} = 0.56$, $P = 0.83$). Five Hertz rTMS over M1 failed to evoke a significant MEP facilitation before and after PMd-rTMS, and PMd-rTMS left MEPs statistically unchanged (before, $P = 0.44$ and after, $P = 0.97$). Finally, in PD patients on therapy (Fig. 2*C*), post hoc 2-way ANOVA showed a significant interaction between factors Time and Number of Stimuli ($F_{9.117} = 2.23$, $P = 0.02$). Although 5 Hz rTMS over M1 left MEPs statistically unchanged before PMd-rTMS ($F_{9.117} = 0.71$, $P = 0.7$), MEPs significantly increased in amplitude after PMd-rTMS ($F_{9.117} = 5.68$, $P < 0.01$).

### *Experiments 2 and 3—Attention Levels and MEP Facilitation in Healthy Subjects and in Patients with PD*

In these experiments, in healthy subjects, ANOVA showed a more pronounced MEP facilitation when subjects directed attention to the stimulated hand (Fig. 3*A*). When PD patients directed attention to the stimulated hand, MEPs were significantly facilitated only when patients were on therapy (Fig. 3*B,C*). One Hertz real but not sham PMd-rTMS significantly reduced the attention-induced changes in STP in PD patients on therapy (Fig. 4).

In experiment 2, attention-related changes in STP differed in healthy subjects and in PD patients off and on therapy. The between-group ANOVA comparing healthy subjects and PD patients off therapy showed a significant 3-way interaction between factors Group, Condition, and Number of Stimuli ($F_{18.288} = 3.47$, $P < 0.01$). Conversely, the between-group ANOVA comparing healthy subjects and PD patients on therapy showed a significant 2-way interaction between factors Group and Number of Stimuli ($F_{9.144} = 6.13$, $P < 0.01$) and between factors Condition and Number of Stimuli ($F_{18.288} = 8.71$, $P < 0.01$).

The attention-induced changes in STP also differed in PD patients off and on therapy. The 3-way repeated measures ANOVA showed a significant 3-way interaction between factors Therapy, Condition, and Number of Stimuli ($F_{18.144} = 3.03$, $P < 0.01$).

In healthy subjects (Fig. 3*A*), the post hoc 2-way ANOVA showed a significant 2-way interaction between factors Condition and Number of Stimuli ($F_{18.144} = 5.3$, $P < 0.01$). Although in healthy subjects MEP amplitude significantly increased during 5 Hz rTMS under all conditions ($\text{Condition}_{\text{Target hand}}$: $P < 0.01$, $\text{Condition}_{\text{Nontarget hand}}$: $P < 0.01$, $\text{Condition}_{\text{Eyes closed}}$: $P < 0.01$), the degree of MEP facilitation was higher when healthy subjects looked at the target hand than at the nontarget hand and eyes closed conditions ($\text{Condition}_{\text{Target hand}}$ vs. $\text{Condition}_{\text{Nontarget hand}}$: $P < 0.05$, $\text{Condition}_{\text{Target hand}}$ vs. $\text{Condition}_{\text{Eyes closed}}$: $P = 0.02$, $\text{Condition}_{\text{Nontarget hand}}$ vs. $\text{Condition}_{\text{Eyes closed}}$: $P = 0.9$). Conversely, in PD patients off therapy (Fig. 3*B*), the post hoc 2-way ANOVA showed that factors Condition and Number of Stimuli were nonsignificant ($F_{2.16} = 0.68$, $P = 0.52$ and $F_{9.72} = 1.84$, $P = 0.08$, respectively). Five Hertz rTMS elicited no MEP facilitation ($\text{Condition}_{\text{Target hand}}$: $P = 0.15$, $\text{Condition}_{\text{Nontarget hand}}$: $P = 0.21$, $\text{Condition}_{\text{Eyes closed}}$: $P = 0.11$), and MEPs remained unchanged under all experimental conditions ($\text{Condition}_{\text{Target hand}}$ vs. $\text{Condition}_{\text{Nontarget hand}}$: $P = 0.98$, $\text{Condition}_{\text{Target hand}}$ vs. $\text{Condition}_{\text{Eyes closed}}$: $P = 0.96$, $\text{Condition}_{\text{Nontarget hand}}$ vs. $\text{Condition}_{\text{Eyes closed}}$: $P = 0.88$). Finally, in PD patients on therapy (Fig. 3*C*), the post hoc 2-way ANOVA showed a significant 2-way interaction of factors Condition and Number of Stimuli ($F_{18.144} = 4.55$, $P < 0.01$). MEPs differed significantly in the target hand and in the nontarget hand and eyes closed conditions ($\text{Condition}_{\text{Target hand}}$ vs. $\text{Condition}_{\text{Nontarget hand}}$: $P < 0.05$, $\text{Condition}_{\text{Target hand}}$ vs.

**Table 4**

TMS data in the experiment testing the effect of conditioning real and sham 1 Hz rTMS over the dorsal premotor cortex (PMd) on the attention-induced MEP facilitation elicited by 5 Hz rTMS over the primary motor cortex (M1) in PD patients on therapy

| | RMT (%) | M1-rTMS (%) | First MEP amp. (mV) | AMT (%) | real PMd-rTMS (%) | RMT (%) | M1-rTMS (%) | First MEP amp. (mV) |
|---|---|---|---|---|---|---|---|---|
| | Before real PMd-rTMS | | | | | After real PMd-rTMS | | |
| PD patients on therapy | | | | | | | | |
| AV | 55 | 66 | 0.28 | 41 | 36 | 55 | 66 | 0.33 |
| SD | 2.8 | 7.5 | 0.1 | 4.1 | 3.8 | 3.5 | 6.3 | 0.16 |
| | Before sham PMd-rTMS | | | | | After sham PMd-rTMS | | |
| PD patients on therapy | | | | | | | | |
| AV | 55 | 66 | 0.27 | 40 | 36 | 55 | 66 | 0.31 |
| SD | 3.4 | 8.2 | 0.12 | 4 | 4.1 | 3.6 | 8.2 | 0.18 |

Note: M1-rTMS, intensity of M1-rTMS; first MEP amp., amplitude of the first MEP evoked by M1-rTMS; AV, average.

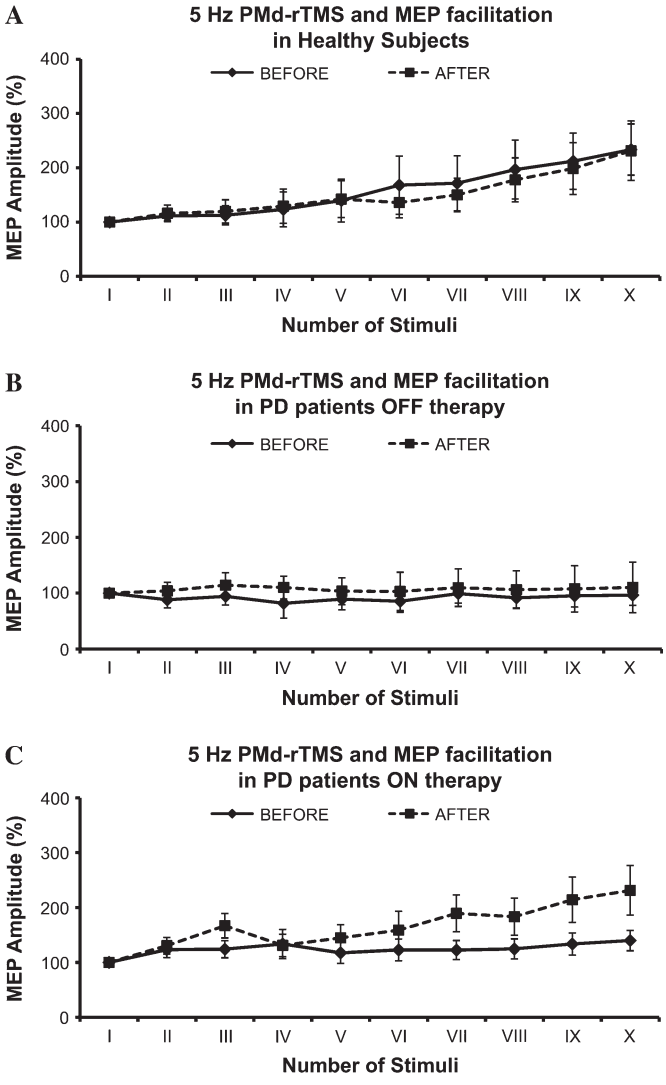


**Figure 2.** MEP facilitation evoked by 5 Hz rTMS over the primary motor cortex (M1) before and after conditioning 5 Hz rTMS over the dorsal premotor cortex (PMd) in healthy subjects (*A*) and in PD patients off (*B*) and on (*C*) therapy. Note the significant difference in responses before and after 5 Hz PMd-rTMS in patients on therapy. Each point corresponds to the mean MEP amplitude expressed as a percentage of the first MEP amplitude evoked by 15 trains of M1-rTMS. Vertical bars denote standard error.

$\text{Condition}_{\text{Eyes closed}}$: $P < 0.01$, $\text{Condition}_{\text{Nontarget hand}}$ vs. $\text{Condition}_{\text{Eyes closed}}$: $P = 0.41$). MEPs were significantly facilitated in the target hand ($P < 0.01$) but not in the nontarget hand ($P = 0.12$) or in the eyes closed condition ($P = 0.35$).

In experiment 3, real but not sham 1 Hz PMd-rTMS induced significant changes in the attention-induced STP in PD patients on therapy (Fig. 4). The 3-way repeated measures ANOVA disclosed a significant 3-way interaction between factors Stimulation, Time, and Number of Stimuli ($F_{9,36} = 2.91$, $P = 0.01$). Post hoc 2-way ANOVA testing the effect of real 1 Hz PMd-rTMS showed a significant 2-way interaction between factors Time and Number of Stimuli ($F_{9,36} = 5.33$, $P < 0.01$). Conversely, the 2-way ANOVA testing the effect of sham 1 Hz PMd-rTMS showed a significant effect of factor Number of Stimuli ($F_{9,36} = 8.37$, $P < 0.01$) but no significant effect of factor Time ($F_{1,4} = 0.26$, $P = 0.64$). Real 1 Hz PMd-rTMS significantly reduced MEP amplitude ($P < 0.01$ before and $P = 0.1$ after real 1 Hz PMd-rTMS), whereas sham 1 Hz PMd-rTMS did not ($P < 0.01$ before and after sham 1 Hz PMd-rTMS) (Fig. 4).

None of the patients who participated in the attentional experiment had cognitive impairment (MMSE mean value ± SD: 28.6 ± 1.9, cutoff ⩾ 23.85), executive dysfunction (WCST mean value ± SD: 53.1 ± 1.5, cutoff ⩾ 35), or attention deficits (AM mean value ± SD: 53.6 ± 3.1, cutoff ⩾ 31).

One-way ANOVA in healthy subjects and in PD patients off and on therapy, testing whether the first MEP elicited by M1-rTMS progressively increased or decreased in amplitude from the 1st to the 15th train, showed no significant effect of factor Number of Train in any group or condition.

The Wilcoxon matched pairs test showed that UPDRS values significantly differed in PD patients on and off therapy participating in experiments 1 and 2.

Spearman rank test showed no significant correlation between changes in UPDRS values and changes in the amplitude of MEPs evoked by M1-rTMS in the experiment testing PMd-rTMS and attention-induced changes in MEP facilitation.

## Discussion

We found that in patients with mild-to-moderate PD on but not off therapy, 5 Hz conditioning rTMS applied to the PMd improves STP as tested by the MEP facilitation. Similarly, in PD patients on therapy, attention partly restored the reduced STP as tested by the MEP facilitation. In addition, real 1 Hz PMd-rTMS reduced the attention-induced changes in STP in patients on therapy, whereas sham rTMS did not. Our study provides new information showing that dopaminergic therapy restores altered STP in PD and does so by modulating functional PMd-to-M1 connectivity.

### *MEP Facilitation in Healthy Subjects and in Patients with PD: The Effect of PMd-rTMS*

In healthy subjects, we confirmed our previous finding that 5 Hz PMd-rTMS failed to enhance the M1 5 Hz rTMS-induced

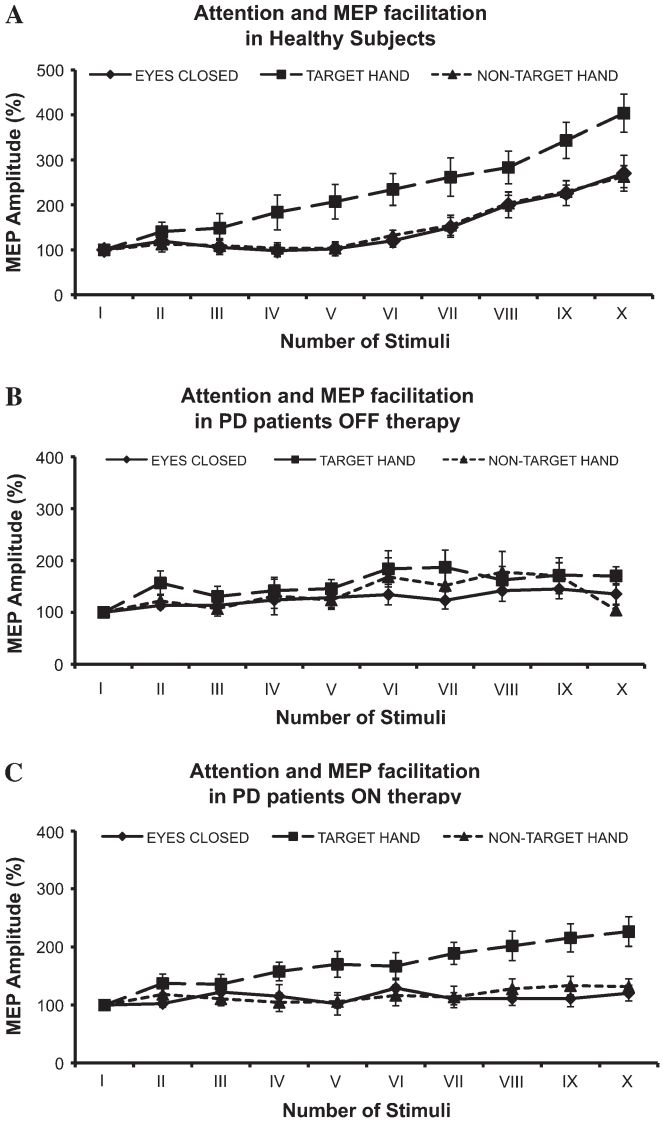


**Figure 3.** MEP facilitation evoked by 5 Hz rTMS over the primary motor cortex (M1) in the 3 attention-demanding tasks in healthy subjects (*A*) and in PD patients off (*B*) and on (*C*) therapy. Note the significant difference in responses in the 3 attention-demanding tasks in patients on therapy. Each point corresponds to the mean MEP amplitude expressed as a percentage of the first MEP amplitude evoked by 15 trains of M1-rTMS. Vertical bars denote standard error.

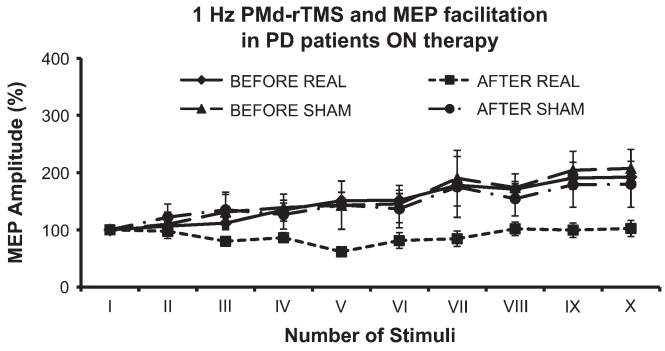


**Figure 4.** MEP facilitation evoked by 5 Hz rTMS over the primary motor cortex (M1) in PD patients on therapy looking at the stimulated hand ("target hand" condition). Note that real but not sham 1 Hz PMd-rTMS significantly reduced the attention-induced MEP facilitation. Each point corresponds to the mean MEP amplitude expressed as a percentage of the first MEP amplitude evoked by 15 trains of M1-rTMS. Vertical bars denote standard error.

MEP facilitation, probably owing to a "ceiling effect" in M1 interneurons (Suppa et al. 2008). The novel finding in the present study is that in PD patients off therapy, conditioning PMd with 5 Hz rTMS leaves the absent MEP facilitation unchanged. Conversely, conditioning 5 Hz rTMS over PMd facilitates MEPs only when patients are on therapy.

We took several precautions in evaluating possible changes in STP. For example, to exclude target muscle contraction, we continuously monitored EMG activity and excluded from the analysis all trials showing background muscle contraction. Similarly, we continuously monitored patients' arousal and attention levels and kept them constant throughout the experiments. In addition, we found no differences in RMT and AMT in healthy subjects and in patients with PD on and off therapy. M1 5 Hz rTMS also induced a similar amplitude first MEP in all groups and under all experimental conditions. Finally, the amplitude of the first MEP elicited by M1 5 Hz rTMS remained unchanged from the 1st to the 15th M1-rTMS train in all groups and conditions, confirming that a 1- to 2-min M1 intertrain interval is sufficiently long to prevent M1-rTMS–induced plasticity from building up.

Because RMT, AMT, and the first MEP amplitude elicited by M1-rTMS remained unchanged before and after PMd-rTMS, we also consider it unlikely that our findings reflect an unspecific PMd-rTMS–induced enhancement in M1 excitability. The apparent inconsistency between our findings and previous reports concerning the lack of effects on the first MEP amplitude in healthy subjects and in patients with PD probably depends on the TMS-induced current polarity used for testing M1 excitability (Suppa et al. 2008). To evoke the MEP facilitation in healthy subjects and in PD patients on and off therapy, we used a biphasic PA–AP stimulation. In a previous study, we showed that although PMd-rTMS modulates MEPs evoked by monophasic PA and biphasic AP–PA single-pulse TMS over M1, it has virtually no effect on MEPs evoked by monophasic AP and biphasic PA–AP TMS (Suppa et al. 2008). Direct recordings from the cervical spinal epidural space of TMS-evoked descending corticospinal activity have shown that monophasic PA and biphasic AP–PA preferentially recruit $I_1$-waves, whereas monophasic AP and biphasic PA–AP preferentially recruit $I_3$-waves together with a "proximal D-wave" (Di Lazzaro et al. 2001). We therefore suggest that PMd-rTMS may influence M1 excitability predominantly by modulating the I-wave inputs to corticospinal neurons. PMd-rTMS could, for example, produce a larger effect on MEPs evoked by monophasic PA and biphasic AP–PA than on MEPs produced by monophasic AP and biphasic PA–AP stimuli. Monophasic AP and biphasic PA–AP stimuli contain excitatory input from the proximal D-wave that would be uninfluenced by changes in I-wave recruitment.

Another reason why M1-rTMS failed to induce an MEP facilitation in patients with PD is the finding that PD patients have a steeper input–output (I/O) curve at rest (Valls-Solé et al. 1994). Accordingly, the first MEP size might have reached its maximal amplitude in the early part of the train, thus preventing a further increase in MEP amplitude during the train (ceiling effect). A ceiling effect nevertheless seems unlikely given the similar-amplitude first MEPs in PD patients off and on therapy and also in healthy subjects, suggesting that in patients off therapy, the first MEPs in the trains did not saturate in size over the train.

Given that dopamine makes focal excitatory inputs to cortical networks more effective by boosting cortical plasticity in human M1 (Kuo et al. 2008; Rodrigues et al. 2008), the PMd-rTMS–induced MEP facilitation we observed in patients with PD on therapy might reflect direct dopaminergic changes in M1 cortical plasticity rather than restored PMd-to-M1 connectivity. Dopaminergic changes alone nonetheless seem unlikely to explain our findings insofar as M1-rTMS delivered before PMd-rTMS evoked no MEP facilitation in PD patients on therapy, thus confirming our previous observation (Gilio et al. 2002). Conversely, one hypothesis for explaining the effect of PMd-rTMS on STP in PD patients on therapy is that PMd-rTMS induced lasting changes in PMd and indirectly in the tonic output from PMd to M1, consequently changing the excitability of M1 intracortical circuits involved in the MEP facilitation and thus allowing STP to develop. A further hypothesis is that PMd-rTMS directly activates PMd-to-M1 connections, thus inducing aftereffects on similar neural circuits involved in STP.

What we find difficult to state is why dopamine restores the abnormal long-term plasticity tested with PAS in patients with PD (Morgante et al. 2006; Ueki et al. 2006; Kuo et al. 2008; Rodrigues et al. 2008) but leaves the altered STP tested with the 5 Hz rTMS in patients on therapy almost unchanged (Gilio et al. 2002). PAS is considered a Hebbian form of spike timing–dependent plasticity since the PAS-induced long-term potentiation–like and long-term depression–like phenomena emerge in M1 after repetitive activation of specific sensorimotor circuits within a restricted time window (Stefan et al. 2000). Conversely, the 5 Hz rTMS-induced MEP facilitation reflects STP in M1, resembling NMDA-dependent short-term potentiation of synaptic connections described in animal experiments (Inghilleri et al. 2004, 2005; Cooke and Bliss 2006). Dopamine could conceivably have a stronger impact on PAS than on 5 Hz rTMS because the physiological mechanisms underlying long-term plasticity differ from those responsible for STP.

Several functional magnetic resonance imaging (fMRI) studies in patients with PD off therapy have shown a decreased activation of supplementary motor area (SMA) and dorsolateral prefrontal cortex (DLPC) and an increased activity in lateral premotor cortex during finger movements (Sabatini et al. 2000; Haslinger et al. 2001). In patients with PD, SMA and DLPC underactivation might be the functional substrate underlying akinesia owing to reduced motor input from the basal ganglia–thalamocortical motor loop. In patients with PD on therapy, dopaminergic treatment almost normalizes the abnormal fMRI brain activation patterns in the SMA, DLPC, and premotor areas during voluntary movements (Haslinger et al. 2001; Buhmann et al. 2003). Accordingly, in our patients with PD on therapy, dopamine may partly restore normal PMd-to-M1 connectivity patterns and thus enhance the facilitatory effect of PMd on mechanisms of STP.

### *Attention Levels and MEP Facilitation in Healthy Subjects and in Patients with PD*

Our experiments investigating whether attention restores the MEP facilitation to normal provide new insight into the role of PMd-to-M1 connections in healthy subjects and in patients with PD. The significantly higher MEP facilitation when healthy subjects directed their attention to the stimulated hand than when they looked at the nonstimulated hand or during the eyes closed condition again shows that in healthy subjects, attention modulates mechanisms of cortical plasticity (Stefan et al. 2004; Conte et al. 2007, 2008). Insofar as we previously found that conditioning PMd with 1 Hz rTMS disrupts the attention-induced changes in MEPs elicited by M1-rTMS in healthy subjects (Conte et al. 2007), our findings overall indicate that the PMd-to-M1 connectivity as part of the frontoparietal network involved in the self-recognition process mediates the attention-induced effect on MEP facilitation (Van den Bos and Jeannerod 2002; Chouinard et al. 2003; MacDonald and Paus 2003; Conte et al. 2007).

In patients off therapy, attention levels might have failed to modulate MEPs evoked by 5 Hz rTMS owing to several possible confounding factors. We excluded the possible influence of different levels of muscle contraction. We also found no difference in the RMT, AMT, and first MEP amplitude in healthy subjects and in PD patients off and on therapy. In addition, the neuropsychological assessment in PD patients made unlikely the possibility that attention left MEPs amplitude unchanged owing to cognitive deterioration, executive dysfunction, or attention deficit. The observation that in PD patients on therapy M1-rTMS did not induce a significant MEP facilitation when patients were tested in the eyes closed condition and when they directed their attention to the nontarget hand suggests that dopamine does not restore STP. Evidence that attention plays a role in restoring STP comes from the observation that in PD patients on therapy, M1-rTMS induced a significant facilitation only when they directed attention to the target hand. The finding that dopamine is able to restore STP only when patients direct attention to the target hand agrees with our previous study demonstrating that M1 participates in processes of motor attention through a complex neural circuit including premotor-to-motor connectivity (Conte et al. 2007, 2008). Consistent with this hypothesis, the experiment testing the effect of 1 Hz real and sham PMd-rTMS on the MEP facilitation in PD patients on therapy showed that, as previously described in healthy subjects (Conte et al. 2007), real 1 Hz rTMS significantly reduced the attention-induced changes in STP, whereas sham rTMS did not. A further possibility for explaining the attention-induced changes in the MEP facilitation in healthy subjects and in PD patients on therapy concerns an involvement of the "mirror system." We consider mirror system involvement unlikely, however, for several reasons. First, the mirror system plays a role preferentially during goal-directed actions performed by another individual or when the individual itself performs a motor action (Rizzolatti et al. 2002). The participants in this study were not required to look at actions performed by another individual, and the rTMS-evoked muscle twitch in no way resembled a goal-directed action. Second, the mirror system is related to the activation of neurons in the ventral premotor cortex (PMv), the area supposed to be the human homologue of the macaque area F5 (Rizzolatti et al. 2002). In experiment 3, we disrupted the attention-induced MEP facilitation in PD patients on therapy by delivering 1 Hz rTMS over the PMd and not over the PMv. PMd is thought to be located 2.5 cm anterior to the FDI motor hot spot (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004; Suppa et al. 2008), whereas the PMv is thought to be located 3 cm anterior and 2.5 cm lateral to the FDI motor hot spot (Bäumer et al. 2009).

Overall, our findings provide evidence that in addition to dopamine, PMd is at least in part involved in the

attention-induced MEP facilitation in PD patients on therapy. This conclusion fits well with recent fMRI findings investigating brain activation patterns during attention-demanding tasks and showing that compared with simple motor execution, attention to action led in healthy subjects to increased activation of prefrontal, premotor, and SMA, whereas in patients with PD off therapy, it did not (Rowe et al. 2002). Our findings are also in line with experimental data in animals and humans, showing that dopamine intervenes in attention processes (Nieoullon 2002; Nieoullon and Coquerel 2003; Remy and Samson 2003) and improves Parkinsonian patients' performance in attention-demanding tasks (Cools 2006; Moustafa et al. 2008; Koerts et al. 2009).

## Conclusion

Overall, these experiments suggest that in patients with PD off therapy, dopaminergic denervation might affect STP not only by reducing M1 plasticity per se but also by altering the functional connectivity between PMd and M1 (Buhmann et al. 2004; Mir et al. 2005). Conversely, in patients with PD on therapy, dopamine promotes STP not only restoring directly M1 plasticity but also restoring premotor-to-motor cortex connectivity. In conclusion, dopamine restores altered STP in PD and does so by modulating functional PMd-to-M1 connectivity.

A question that remains unclear is whether PMd-rTMS directly activates PMd-to-M1 connections thus inducing after-effects on specific neural circuits involved in STP or conversely induces changes in PMd and then indirectly alters the PMd-to-M1 tonic output, thereby inducing similar overall aftereffects in the same target circuits (Suppa et al. 2008). Despite this limitation, the present findings provide new insight into the role of functional connectivity between PMd and M1 in the pathophysiology of PD.

Although dopamine may induce changes in PMd-to-M1 connectivity in humans by acting indirectly through the cortico–basal ganglia–thalamocortical loop, we cannot fully exclude the possibility that dopamine influences PMd-to-M1 connectivity also by acting directly at cortical level through the mesocortical projections (Remy and Samson 2003). The MEP facilitation is thought to reflect recruitment through cortico-cortical connections of higher threshold cortical columns in the target muscle representation, probably via lateral spread of excitation through reciprocal connections to layer II/III pyramidal neurons (Suppa et al. 2008). Experimental studies in animals have shown that excitatory cortico-cortical connections from PMd project predominantly to layer II/III M1 interneurons (Ghosh and Porter 1988; Tokuno and Nambu 2000), supporting the hypothesis that the PMd-to-M1 output might modulate M1 responses to other inputs, thus affecting the way M1 cortical columns modulate ongoing motor processing (Chouinard and Paus 2006; Suppa et al. 2008). Interestingly, experimental studies in animals have also shown that dopamine promotes NMDA-dependent short-term and long-term plasticity in layer II/III pyramidal neurons in the frontal cortex (Wang and O'Donnell 2001; Tseng and O'Donnell 2004; Molina-Luna et al. 2009). Knowing more about how dopamine modulates PMd-to-M1 connections and M1 cortical plasticity is important in understanding the pathophysiologic mechanisms underlying PD. The present findings may provide useful information for developing possible new therapeutic neurostimulation approaches for Parkinsonian patients.

## Notes

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## References

Bagnato S, Agostino R, Modugno N, Quartarone A, Berardelli A. 2006. Plasticity of the motor cortex in Parkinson's disease patients on and off therapy. Mov Disord. 21:639–645.

Bäumer T, Schippling S, Kroeger J, Zittel S, Koch G, Thomalla G, Rothwell JC, Siebner HR, Orth M, Münchau A. 2009. Inhibitory and facilitatory connectivity from ventral premotor to primary motor cortex in healthy humans at rest—a bifocal TMS study. Clin Neurophysiol. 120:1724–1731.

Berardelli A, Inghilleri M, Rothwell JC, Romeo S, Currà A, Gilio F, Modugno N, Manfredi M. 1998. Facilitation of muscle evoked responses after repetitive cortical stimulation in man. Exp Brain Res. 122:79–84.

Boussaoud D. 2001. Attention versus intention in the primate premotor cortex. Neuroimage. 14:S40–S45.

Buhmann C, Glauche V, Stürenburg HJ, Oechsner M, Weiller C, Büchel C. 2003. Pharmacologically modulated fMRI-cortical responsiveness to levodopa in drug-naive hemiparkinsonian patients. Brain. 126:451–461.

Buhmann C, Gorsler A, Bäumer T, Hidding U, Demiralay C, Hinkelmann K, Weiller C, Siebner HR, Münchau A. 2004. Abnormal excitability of premotor-motor connections in de novo Parkinson's disease. Brain. 127:2732–2746.

Caminiti R, Ferraina S, Mayer AB. 1998. Visuomotor transformations: early cortical mechanisms of reaching. Curr Opin Neurobiol. 8:753–761.

Chouinard PA, Paus T. 2006. The primary motor and premotor areas of the human cerebral cortex. Neuroscience. 12:143–152.

Chouinard PA, Van Der Werf YD, Leonard G, Paus T. 2003. Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices. J Neurophysiol. 90:1071–1083.

Cisek P, Kalaska JF. 2005. Neural correlates of reaching decisions in dorsal premotor cortex: specification of multiple direction choices and final selection of action. Neuron. 45:801–814.

Conte A, Belvisi D, Iezzi E, Mari F, Inghilleri M, Berardelli A. 2008. Effects of attention on inhibitory and facilitatory phenomena elicited by paired-pulse transcranial magnetic stimulation in healthy subjects. Exp Brain Res. 186:393–399.

Conte A, Gilio F, Iezzi E, Frasca V, Inghilleri M, Berardelli A. 2007. Attention influences the excitability of cortical motor areas in healthy humans. Exp Brain Res. 182:109–117.

Cooke SF, Bliss TVP. 2006. Plasticity in the human central nervous system. Brain. 129:1659–1673.

Cools R. 2006. Dopaminergic modulation of cognitive function—implications for l-DOPA treatment in Parkinson's disease. Neurosci Biobehav Rev. 30:1–23.

Corbetta M, Akbudak E, Conturo TE, Snyder AZ, Ollinger JM, Druhy HA, Linenweber MR, Petersen SE, Raichle ME, Van Essen DC, et al. 1998. A common network of functional areas for attention and eye movements. Neuron. 21:761–773.

Coull JT, Nobre AC. 1998. Where and when to pay attention: the neural systems for directing attention to spatial locations and to time intervals as revealed by both PET and fMRI. J Neurosci. 18:7426–7435.

Courtney SM, Petit L, Maisog JM, Ungerleider LG, Haxby JV. 1998. An area specialized for spatial working memory in human frontal cortex. Science. 279:1347–1351.

Di Lazzaro V, Oliviero A, Berardelli A, Mazzone P, Insola A, Pilato F, Saturno E, Dileone M, Tonali PA, Rothwell JC. 2002. Direct demonstration of the effects of repetitive transcranial magnetic stimulation on the excitability of the human motor cortex. Exp Brain Res. 144:549–553.

Di Lazzaro V, Oliviero A, Mazzone P, Insola A, Pilato F, Saturno E, Accurso A, Tonali P, Rothwell JC. 2001. Comparison of descending

volleys evoked by monophasic and biphasic magnetic stimulation of the motor cortex in conscious humans. Exp Brain Res. 141:121–127.
Dum RP, Strick PL. 2002. Motor areas in the frontal lobe of the primate. Physiol Behav. 77:677–682.
Dum RP, Strick PL. 2005. Frontal lobe inputs to the digits representations of the motor areas on the lateral surface of the hemisphere. J Neurosci. 25:1375–1386.
Gerschlager W, Siebner HR, Rothwell JC. 2001. Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. Neurology. 57:449–455.
Ghosh S, Porter R. 1988. Corticocortical synaptic influences on morphologically identified pyramidal neurones in the motor cortex of the monkey. J Physiol. 400:617–629.
Gibb WR, Lees AJ. 1988. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry. 51:745–752.
Gilio F, Conte A, Vanacore N, Frasca V, Inghilleri M, Berardelli A. 2007. Excitatory and inhibitory after-effects after repetitive magnetic transcranial stimulation (rTMS) in normal subjects. Exp Brain Res. 176:588–593.
Gilio F, Curra A, Inghilleri M, Lorenzano C, Manfredi M, Berardelli A. 2002. Repetitive magnetic stimulation of cortical motor areas in Parkinson's disease: implications for the pathophysiology of cortical function. Mov Disord. 17:467–473.
Halligan PW, Kischka U, Marshall JC. 2003. Handbook of clinical neuropsychology. Oxford: Oxford University Press.
Haslinger B, Erhard P, Kämpfe N, Boecker H, Rummeny E, Schwaiger M, Conrad B, Ceballos-Baumann AO. 2001. Event-related functional magnetic resonance imaging in Parkinson's disease before and after levodopa. Brain. 124:558–570.
Hobson DE, Lang AE, Martin WR, Razmy A, Rivest J, Fleming J. 2002. Excessive daytime sleepiness and sudden-onset sleep in Parkinson disease: a survey by the Canadian Movement Disorders Group. JAMA. 287:455–463.
Inghilleri M, Conte A, Frasca V, Currà A, Gilio F, Manfredi M, Berardelli A. 2004. Antiepileptic drugs and cortical excitability: a study with repetitive transcranial stimulation. Exp Brain Res. 154:488–493.
Inghilleri M, Conte A, Frasca V, Gilio F, Lorenzano C, Berardelli A. 2005. Synaptic potentiation induced by rTMS: effect of lidocaine infusion. Exp Brain Res. 163:114–117.
Jennum P, Winkel H, Fuglsang-Frederiksen A. 1995. Repetitive magnetic stimulation and motor evoked potentials. Electroencephalogr Clin Neurophysiol. 97:96–101.
Kammer T, Beck S, Thielscher A, Laubis-Herrmann U, Topka H. 2001. Motor threshold in humans: a transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. Clin Neurophysiol. 112:250–258.
Koerts J, Leenders KL, Brouwer WH. 2009. Cognitive dysfunction in non-demented Parkinson's disease patients: controlled and automatic behavior. Cortex. 45:922–929.
Kuo MF, Paulus W, Nitsche MA. 2008. Boosting focally-induced brain plasticity by dopamine. Cereb Cortex. 18:648–651.
Lebedev MA, Wise SP. 2001. Tuning for the orientation of spatial attention in dorsal premotor cortex. Eur J Neurosci. 13:1002–1008.
MacDonald PA, Paus T. 2003. The role of parietal cortex in awareness of self-generated movements: a transcranial magnetic stimulation study. Cereb Cortex. 13:962–967.
Mir P, Matsunaga K, Gilio F, Quinn NP, Siebner HR, Rothwell JC. 2005. Dopaminergic drugs restore facilitatory premotor-motor interactions in Parkinson's disease. Neurology. 64:1906–1912.
Molina-Luna K, Pekanovic A, Röhrich S, Hertler B, Schubring-Giese M, Rioult-Pedotti MS, Luft AR. 2009. Dopamine in motor cortex is necessary for skill learning and synaptic plasticity. PLoS One. 4:e7082.
Morgante F, Espay AJ, Gunraj C, Lang AE, Chen R. 2006. Motor cortex plasticity in Parkinson's disease and levodopa-induced dyskinesias. Brain. 129:1059–1069.
Moustafa AA, Sherman SJ, Frank MJ. 2008. A dopaminergic basis for working memory, learning and attentional shifting in parkinsonism. Neuropsychologia. 46:3144–3156.
Munchau A, Bloem BR, Irlbacher K, Trimble MR, Rothwell JC. 2002. Functional connectivity of human premotor and motor cortex explored with repetitive transcranial magnetic stimulation. J Neurosci. 22:554–561.
Nieoullon A. 2002. Dopamine and the regulation of cognition and attention. Prog Neurobiol. 67:53–83.
Nieoullon A, Coquerel B. 2003. Dopamine: a key regulator to adapt action, emotion, motivation and cognition. Curr Opin Neurol. 16:S3–S9.
Pascual-Leone A, Valls-Solè J, Wassermann EM, Hallet M. 1994. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain. 117:847–858.
Picard N, Strick PL. 2001. Imaging the premotor areas. Curr Opin Neurobiol. 11:663–672.
Remy P, Samson Y. 2003. The role of dopamine in cognition: evidence from functional imaging studies. Curr Opin Neurol. 16:S37–S41.
Rizzo V, Siebner HR, Modugno N, Pesenti A, Münchau A, Gerschlager W, Webb RM, Rothwell JC. 2004. Shaping the excitability of the human motor cortex with premotor rTMS. J Physiol. 554:483–495.
Rizzolatti G, Fogassi L, Gallese V. 2002. Motor and cognitive functions of the ventral premotor cortex. Curr Opin Neurobiol. 12:149–154.
Rodrigues JP, Walters SE, Stell R, Mastaglia FL, Thickbroom GW. 2008. Spike-timing-related plasticity is preserved in Parkinson's disease and is enhanced by dopamine: evidence from transcranial magnetic stimulation. Neurosci Lett. 448:29–32.
Rowe J, Stephan KE, Friston K, Frackowiak R, Lees A, Passingham R. 2002. Attention to action in Parkinson's disease: impaired effective connectivity among frontal cortical regions. Brain. 125:276–289.
Sabatini U, Boulanouar K, Fabre N, Martin F, Carel C, Colonnese C, Bozzao L, Berry I, Montastruc JL, Chollet F, et al. 2000. Cortical motor reorganization in akinetic patients with Parkinson's disease: a functional MRI study. Brain. 123:394–403.
Simon SR, Meunier M, Piettre LS, Berardi AM, Segebarth CM, Boussaoud D. 2002. Spatial attention and memory versus motor preparation: premotor cortex involvement as revealed by fMRI. J Neurophysiol. 88:2047–2057.
Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. 2000. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain. 123:572–584.
Stefan K, Wycislo M, Classen J. 2004. Modulation of associative human motor cortical plasticity by attention. J Neurophysiol. 92:66–72.
Suppa A, Bologna M, Gilio F, Lorenzano C, Rothwell JC, Berardelli A. 2008. Preconditioning repetitive transcranial magnetic stimulation of premotor cortex can reduce but not enhance short-term facilitation of primary motor cortex. J Neurophysiol. 99:564–570.
Tokuno H, Nambu A. 2000. Organization on nonprimary motor cortical inputs on pyramidal and non pyramidal tract neurons of primary motor cortex: an electrophysiological study in the macaque monkey. Cereb Cortex. 10:58–68.
Tseng KY, O'Donnell P. 2004. Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci. 24:5131–5139.
Ueki Y, Mima T, Kotb MA, Sawada H, Saiki H, Ikeda A, Begum T, Reza F, Nagamine T, Fukuyama H. 2006. Altered plasticity of the human motor cortex in Parkinson's disease. Ann Neurol. 59:60–71.
Valls-Solé J, Pascual-Leone A, Brasil-Neto JP, Cammarota A, McShane L, Hallett M. 1994. Abnormal facilitation of the response to transcranial magnetic stimulation in patients with Parkinson's disease. Neurology. 44:735–741.
Van den Bos E, Jeannerod M. 2002. Sense of body and sense of action both contribute to self-recognition. Cognition. 85:177–187.
Wang J, O'Donnell P. 2001. D(1) dopamine receptors potentiate nmda-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. Cereb Cortex. 11:452–462.