Abnormal excitability of premotor–motor connections in de novo Parkinson’s disease

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Summary
The dorsal premotor cortex (PMd) is abnormally active in patients with idiopathic Parkinson’s disease. This has been interpreted as a functional correlate of adaptive plasticity within the motor system to compensate for deficient activation of striato-mesial–frontal projections in these patients. Whether abnormal PMd activity influences excitability in the primary motor cortex (M1) in untreated Parkinson’s disease patients and how this premotor–motor interaction might be altered by l-dopa is unclear. To this end, we studied the effects of 1 Hz prefrontal repetitive transcranial magnetic stimulation (rTMS) on M1 excitability in 10 previously untreated non-tremulous Parkinson’s disease patients before (day 1) and after (day 8) their first ever l-dopa treatment and compared the results with those of a group of nine age- and sex-matched healthy controls. In each rTMS session, 1200 pulses of 1 Hz rTMS were applied at an intensity of 80% active motor threshold (AMT) to the PMd contralateral to the clinically more affected side in Parkinson’s disease patients and to the left PMd in healthy controls. Intracortical paired pulse excitability of ipsilateral M1 was probed using a TMS paired pulse paradigm where subthreshold conditioning pulses (80% of AMT) were given 2–15 ms prior to a suprathreshold test pulse. In Parkinson’s disease patients, abnormal baseline intracortical excitability at an interstimulus interval (ISI) of 5 ms was normalized by premotor rTMS. In contrast, rTMS led to an increased excitability at an ISI of 5 ms in healthy controls. Premotor rTMS effects lasted longer (for at least a week) in patients. These results show that the modifiability of premotor–motor connections is abnormal in untreated Parkinson’s disease. A single dose of l-dopa reversed, i.e. normalized, the direction of excitability changes in M1 following premotor rTMS in Parkinson’s disease patients, suggesting that dopamine depletion directly or indirectly influences premotor–motor interactions in Parkinson’s disease. The rTMS conditioning approach described here provides a promising tool to delineate further the excitability changes in frontal motor areas in response to progressive degeneration of nigrostriatal dopaminergic neurons and also to chronic l-dopa treatment in Parkinson’s disease.

Keywords: Parkinson’s disease; premotor cortex; transcranial magnetic stimulation; repetitive transcranial magnetic stimulation; premotor–motor connections

Abbreviations: AMT = active motor threshold; FDI = first dorsal interosseous muscle; FST = finger sequence test; 20-FWT = 20-feet walking test; 9-HPT = nine-hole peg test; ISI = interstimulus interval; LTP = long-term potentiation; M1 = primary motor cortex; MEP = motor evoked potential; PMd = dorsal premotor cortex; PST = pronation–supination test; 2-PT = two-point test; rTMS = repetitive transcranial magnetic stimulation; SICF = short interval intracortical facilitation; SICI = short interval intracortical inhibition; TMS = transcranial magnetic stimulation; UPDRS = Unified Parkinson’s Disease Rating Scale


Introduction
According to the basal ganglia–thalamocortical circuit model (Alexander and Crutcher, 1990), striatal dopamine depletion in Parkinson’s disease reduces activity in thalamic relay nuclei projecting to the frontal lobe, leading to
functional cortical deafferentation in these areas. In accordance with this view, Parkinson’s disease patients ‘off’ medication show an attenuation of neuronal activity in frontal motor areas, particularly in the supplementary motor area but also in the primary motor cortex (M1) when performing simple or complex finger movements (Playford et al., 1992; Buhmann et al., 2003; Sabatini et al., 2000). Additionally, amplitudes of the contingent negative variation in a Go/No go task are reduced over central medial regions in Parkinson’s disease (Cunnington et al., 2001). There is also some evidence for reduced coupling between prefrontal and premotor cortex when Parkinson’s disease patients attend to their actions (Rowe et al., 2002). In contrast, movement-related neuronal activity [as indexed by regional blood flow or blood oxygen level-dependent (BOLD) signal] is increased in the dorsal premotor cortex (PMd) and posterior parietal areas compared with healthy controls (Samuel et al., 1997; Catalan et al., 1999; Sabatini et al., 2000). This has been interpreted as a neural correlate of adaptive plasticity within the motor system to compensate for deficient activation of striato-mesial-frontal projections (Sabatini et al., 2000).

In which way abnormal PMd activity influences M1 excitability in untreated Parkinson’s disease patients and how this premotor–motor interaction might be altered by L-dopa is unclear. The physiological interactions between the PMd and M1 in humans have been explored recently in healthy volunteers using transcranial magnetic stimulation (TMS) (Civardi et al., 2001; Gerschlager et al., 2001; Münchau et al., 2002; Bäumer et al., 2003a; Rizzo et al., 2004). Depending on the stimulation parameters, conditioning of the PMd with repetitive TMS (rTMS) leads to specific excitability changes in M1 as indexed by a change in the amplitude of motor evoked potentials (MEPs) in contralateral hand muscle (Gerschlager et al., 2001; Münchau et al., 2002; Bäumer et al., 2003b; Rizzo et al., 2004).

The activity of inhibitory circuits in M1 is deficient in Parkinson’s disease but can, at least partly, be normalized by dopaminergic medication (Ridding et al., 1995; Pierantozzi et al., 2001). Given intense functional connectivity between PMd and M1 in humans, it is conceivable that abnormal premotor activity in Parkinson’s disease drives some of the changes in motor cortical excitability found in these patients.

The questions we pose in the present study are (i) whether the shaping of excitability in M1 through modulation of PMd activity by ‘inhibitory’ 1 Hz rTMS of the PMd is altered in Parkinson’s disease off medication and (ii) in which way this premotor–motor connectivity is affected by L-dopa treatment. To avoid potentially confounding effects of chronic L-dopa treatment on the excitability and modifiability of the motor system (i.e. central adaptation and reorganization phenomena; Marsden, 1994), we only studied drug-naïve Parkinson’s disease patients. Along with measuring motor cortical excitability, we also carried out a number of clinical tests to assess possible behavioural consequences of both premotor rTMS and L-dopa intake.

Methods

Study design

Main experiment

The main experiment consisted of two sessions which were performed 1 week apart to avoid carry-over effects (Fig. 1). In healthy young subjects, repeated 1 Hz rTMS of the left PMd can prolong the after effects on M1 excitability when given on consecutive days, but not when applied with an interval of 1 week (Bäumer et al., 2003a). TMS measurements, clinical assessment (see below) and rTMS conditioning on day 1 were identical to procedures on day 8. Patients were studied without dopaminergic medication on day 1, whereas on day 8, patients received a single oral dose of L-dopa for the first time ever before rTMS was applied.

In each session, we applied 1200 pulses of 1 Hz rTMS to the PMd. Left PMd was stimulated in healthy controls and PMd contralateral to the clinically more affected side in Parkinson’s disease patients. TMS and clinical measurements were assessed before and after rTMS and L-dopa treatment, respectively (Fig. 1). TMS measurements lasted for ~15 min and were carried out before clinical testing.

We used the same rTMS protocol that previously produced specific changes of paired pulse excitability in ipsilateral M1 in healthy young subjects when applied to the left PMd (Münchau et al., 2002). Because this rTMS protocol did not alter motor cortical excitability when given directly to M1 (Münchau et al., 2002), we did not include 1 Hz motor rTMS as a control condition in the present study.

Control experiment

To exclude that the after effects of rTMS in Parkinson’s disease patients observed in the second session (day 8) of the main experiment were influenced by rTMS ‘pre-conditioning’ on day 1, we conducted a control experiment. In a separate group of previously untreated Parkinson’s disease patients, premotor rTMS was applied only following the first ever L-dopa treatment (protocol of day 8 of the main experiment).

Day 1

| M | rTMS 20 min | M |

Day 8

| M | 30 min | M | rTMS 20 min | M |

L-Dopa

Fig. 1 Study design. Time points where TMS and clinical measurement (M) were carried out are represented by the boxes. On day 1, measurements were performed before and immediately after 1 Hz premotor rTMS and on day 8 before and after L-dopa intake and then again following rTMS.
**Subjects**

In the main experiment, we studied 10 patients with idiopathic Parkinson’s disease [five men; age 58.4 ± 10.5 years (mean ± SD)] and nine age- and sex-matched right-handed healthy subjects (four men; mean age 54.6 ± 12.5 years). All subjects participated on day 1 of the study. All Parkinson’s disease patients and six healthy subjects (mean age 58.0 ± 7.0 years) were also studied on day 8. In a control experiment (see below), another group of six idiopathic Parkinson’s disease patients (four men; mean age 54.4 ± 10.6 years) was studied.

Patients were recruited from our movement disorder clinic. Idiopathic Parkinson’s disease was diagnosed according to the criteria of the UK Parkinson’s Disease Society Brain Bank (Gibb and Lees, 1988). Patients were mildly to moderately affected with a mean stage of 2.1 ± 0.6 according to Hoehn and Yahr (1967) and a mean motor score of 16.0 ± 6.9 according to part III of the Unified Parkinson’s Disease Rating Scale (UPDRS) (Fahn et al., 1987). We only included non-tremulous akinetic-rigid Parkinson’s disease patients who had not received any antiparkinsonian drug treatment before (de novo patients). One patient was left-handed; the remaining 15 patients and all healthy controls were consistent right-handers according to the Edinburgh Handedness Inventory (Oldfield, 1971). All female subjects in our study were post-menopausal so that hormonal changes that can affect M1 excitability measurements across longer periods (Smith et al., 1999, 2002) are unlikely to have confounded our results. All participants gave their written informed consent prior to participation in the study that was approved by the Hamburg Ethics Committee.

**TMS measurements and recording system**

These were identical to a previous TMS/rTMS study in young healthy subjects (Bäumer et al., 2003a). Using a belly-tendon montage, EMG was recorded with two silver disc surface electrodes placed over the first dorsal intersosseous (FDI) muscle. The right FDI muscle was studied in healthy subjects and the FDI muscle of the clinically more affected side in Parkinson’s disease patients. EMG signals were amplified, analogue filtered (5 Hz to 1 kHz; Toennies amplifier, Toennies, Würzburg, Germany) and acquired at a sampling rate of 5 kHz using a laboratory interface (1401 Micro, Cambridge electronic design, Cambridge, UK). Measurements were performed with two high power Magstim 200 magnetic stimulators, connected by a bistim module. A figure-of-eight-shaped coil with an outer winding diameter of 70 mm (Magstim Company, Whitland, Dyfed, UK) was used. The magnetic stimulus had a nearly monophasic pulse configuration. The coil was placed tangentially to the scalp with the handle pointing back-wards and laterally at a 45° angle away from the midline, approximately perpendicular to the line of the central sulcus inducing a posterior–anterior current in the brain.

The ‘motor hot spot’ of the contralateral FDI muscle was marked with a wax pen. Resting motor threshold, expressed as a percentage of maximum stimulator output, was defined as the minimum stimulus intensity that produced an MEP of >50 μV in five out of 10 consecutive trials, and active motor threshold (AMT) as the lowest stimulus intensity at which MEPs of 150 μV amplitude were elicited in the tonically contracting FDI muscle (~10% of maximum voluntary contraction).

Short interval intracortical inhibition and intracortical facilitation (SICI and SICF) were evaluated using paired magnetic pulses as described by Kujirai et al. (1993). The intensity of the first (conditioning) stimulus was set at 80% AMT to avoid floor or ceiling effects. The second (test) stimulus had an intensity that, when given alone, evoked an EMG response of ~0.5–1.5 mV peak to peak size. This stimulation intensity was kept constant throughout the experiments. All subjects received paired pulses at 10 different interstimulus intervals (ISIs), ranging from 2 to 15 ms with an inter-trial interval of 5 s. In addition, the test pulse alone (control condition) was also given to assess the relative magnitude of SICI/SICF. These 11 conditions were applied randomly in two blocks (A and B) of 70 trials. In block A, the control condition (test pulse alone) was tested 20 times and each of the following five conditioning test stimuli 10 times: 2, 4, 6, 8 and 10 ms. In block B, the control condition (test pulse alone) was again tested 20 times and the ISIs of 3, 5, 7, 9 and 15 ms 10 times each. The order of blocks was pseudo-randomized. The mean peak to peak amplitude of the conditioned MEP, at each ISI, was expressed as a percentage of the mean peak to peak size of the unconditioned test pulse.

**rTMS conditioning**

The conditioning rTMS protocol was identical to our previous study (Bäumer et al., 2003a). rTMS was carried out with a MagPro stimulator (Medtronic-neuromuscular, Skovlunde, Denmark) and a slightly angled figure-of-eight coil. The magnetic stimulus had a biphasic waveform with a pulse width of ~300 μs. The coil was held in an identical way to that described above for the TMS measurements. The intensity of rTMS was referenced to the individual AMT of M1 as assessed with the MagPro stimulator. A single rTMS session consisted of a 20 min train of 1 Hz rTMS of the PMd (1200 stimuli). The intensity of premotor rTMS was set at 80% of AMT because this is the optimum intensity to modulate intracortical excitability in ipsilateral M1 (Münchau et al., 2002; Rizzo et al., 2004). The coil position for rTMS of the PMd was defined relative to the position of the motor ‘hot spot’ for the FDI muscle. We calculated for each subject 8% of the distance between nasion and inion (typically ~3 cm) and defined the PMd site as this distance anterior to the M1 hand area ‘hot spot’. On the basis of various control experiments in a previous study, we can be relatively certain that at this site we actually stimulated the PMd without a spread of excitation to ipsilateral M1 (Münchau et al., 2002). The rTMS protocol was in accordance with published safety recommendations for rTMS (Wassermann, 1998).

**Clinical assessment**

Apart from a full neurological examination at baseline, motor symptoms were assessed in all patients using the motor section (part III) of the UPDRS. Subjects were also asked to perform the 20-feet walking test (20-FWT), pronation–supination test (PST), two-point test (2-PT), finger sequence test (FST) and nine-hole peg test (9-HPT) (for details see Appendix). Subjects performed each manual motor test twice separately for the right and left arm in a counterbalanced order. For each test, the time (in s) required to complete the task was measured. In addition, the number of steps to complete the task was counted during the 20-FWT. Only the time of the faster trial was taken for further analysis. For the 20-FWT, we considered the trial where fewer steps were needed.

**L-Dopa application and pre-treatment**

On day 8, participants received a single oral dose of water-soluble L-dopa (250 mg). Subjects were pre-treated with 3 × 10 mg of domperidone for 3 days to avoid nausea.
Statistical analysis

Baseline TMS and clinical measurements were compared between groups using one-factorial analysis of variance (ANOVA) and Student’s t test. The effects of rTMS and L-Dopa on motor cortical excitability and motor symptoms were assessed in separate repeated measures ANOVAs. In particular, to prove that rTMS had differential effects on paired pulse intracortical excitability across time in Parkinson’s disease patients and healthy controls, we performed a two-factor between-subjects repeated measures ANOVA with the factors ISI (five levels; baseline (day 1), post-rTMS (day 1), baseline (day 8), post-L-Dopa (day 8) and post-rTMS (day 8)), ISI (conditioned MEP size at all ISIs; 10 levels) and group. To determine whether rTMS and L-Dopa affected a certain part of the paired pulse curve, we separated the paired pulse curve into three bins, the period of early inhibition (ISIs of 2, 3 and 4 ms), the period of late facilitation (ISIs of 9, 10 and 15 ms) and an intermediate period (ISIs of 5, 6, 7 and 8 ms) for the following reasons. Pharmacological studies showed that SICI and SICF are mediated by different neuronal populations (Liepert et al., 1997; Di Lazzaro et al., 1998; Ziemann et al., 1998; Schwenkreis et al., 2000; Reis et al., 2002). In addition, there is good evidence from previous rTMS experiments that stimulation of the PMd influences paired pulse excitability in M1 at intermediate ISIs of 6 and 7 ms (Civardi et al., 2001; Münchau et al., 2002; Bäumer et al., 2003a; Rizzo et al., 2004). Moreover, in this study, significant between-group differences at baseline were only present at ISIs of 5 and 8 ms (see Results). Because we were interested specifically in the premotor–motor interaction in Parkinson’s disease patients and its modulation by premotor rTMS (expected to occur at ISIs of 6 and 7 ms) and L-Dopa (that was expected to normalize altered baseline excitability at ISIs of 5 and 8 ms), we analysed this intermediate period of the paired pulses curve (ISIs of 5–8 ms) separately from the early inhibitory (SICI) and late facilitatory period (SICF). Thus, for each bin of the intracortical excitability curve, we calculated separate additional ANOVAs. Statistical thresholds were Bonferroni corrected for multiple non-independent comparisons ($P < 0.017$).

The Greenhouse–Geisser method was used to correct for non-sphericity. Conditional on a significant F-value, paired samples t tests were employed to characterize the patterns of time-dependent changes as revealed by previous ANOVA. For all statistical analyses, a $P$ value of $<0.05$ was considered to be significant.

Results

Two healthy subjects complained of mild, short lasting nausea after L-Dopa intake which did not interfere with their ability to participate in the study. None of the subjects reported adverse effects after rTMS.

TMS measurements

Motor cortical excitability at baseline (day 1)

Resting motor threshold, AMT and unconditioned peak to peak MEP amplitude did not differ between healthy subjects and Parkinson’s disease patients (Table 1). In contrast, paired pulse excitability curves were different. Two-way ANOVA with the factors ISI (10 levels) and group (two levels) revealed a main effect of the factor ISI [$F(4,466.4) = 28.8; P < 0.0001$] and an interaction between ISI and group [$F(4,466.4) = 3.3; P = 0.013$]. SICI was reduced at an ISI of 5 ms [$t(16) = 3; P = 0.008$, Student’s $t$ test] and SICF increased at 8 ms [$t(10.8) = 2.6; P = 0.024$] in Parkinson’s disease patients (Fig. 2A).

Table 1 Motor thresholds and MEP amplitudes

<table>
<thead>
<tr>
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<th>Healthy subjects</th>
<th>Parkinson patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMT (%) day 1 baseline</td>
<td>39 ± 2</td>
<td>40.3 ± 1</td>
</tr>
<tr>
<td>Post-rTMS</td>
<td>40 ± 2</td>
<td>40 ± 1.3</td>
</tr>
<tr>
<td>Day 2 baseline</td>
<td>39.2 ± 2</td>
<td>38.1 ± 1</td>
</tr>
<tr>
<td>Post-L-Dopa</td>
<td>40.2 ± 2</td>
<td>37 ± 1.4</td>
</tr>
<tr>
<td>Post-rTMS</td>
<td>40.6 ± 1</td>
<td>37.5 ± 1.5</td>
</tr>
<tr>
<td>AMT (%) day 1 baseline</td>
<td>33.7 ± 2.5</td>
<td>36.8 ± 1.8</td>
</tr>
<tr>
<td>Post-rTMS</td>
<td>33.8 ± 2.2</td>
<td>36.3 ± 1.8</td>
</tr>
<tr>
<td>Day 2 baseline</td>
<td>34.6 ± 3</td>
<td>35.8 ± 1.9</td>
</tr>
<tr>
<td>Post-L-Dopa</td>
<td>34.2 ± 3</td>
<td>35.8 ± 2.1</td>
</tr>
<tr>
<td>Post-rTMS</td>
<td>34.4 ± 2.8</td>
<td>35.8 ± 2.1</td>
</tr>
<tr>
<td>MEP (mV) day 1 baseline</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Post-rTMS</td>
<td>0.8 ± 0.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Day 2 baseline</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Post-L-Dopa</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Post-rTMS</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
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</tbody>
</table>

AMT = active motor threshold; RMT = resting motor threshold. Values are the mean ± SEM.

Changes in motor cortical excitability induced by rTMS and L-Dopa

Repeated measures ANOVA did not reveal an effect of time [five levels: baseline (day 1), post-rTMS (day 1), baseline (day 8), post-L-Dopa (day 8) and post-rTMS (day 8)] on thresholds and size of unconditioned MEPs, no effect of group and no interaction between time and group (see Table 1).

Two-factor between-subjects repeated measures ANOVA with the factors time (five levels), ISI (conditioned MEP size at all ISIs; 10 levels) and group showed a significant effect of ISI [$F(4,54,33) = 23; P < 0.0001$]. The effect of time failed to reach statistical significance [$F(4,52) = 2.1; P = 0.095$]. However, there was a significant interaction time × ISI [$F(3,64,468) = 1.6; P = 0.011$] and, importantly, a significant interaction time × ISI × group [$F(3,64,468) = 1.93; P = 0.001$]. This can be taken as evidence that rTMS effects were significantly different in the two groups across time.

To determine whether rTMS and L-Dopa affected a certain part of the paired pulse curve, we calculated separate ANOVAs for the three parts of the curve (see Methods). Repeated measures ANOVA for the intermediate period with the withinsubject factors time (five levels) and ISI (four levels; 5, 6, 7 and 8 ms) and the between-subjects factor group revealed a significant effect of time [$F(4,56) = 5.9; P = 0.01$] and ISI [$F(3,42) = 20; P < 0.0001$]. The interaction time × ISI × group was also significant [$F(12,168) = 2.6; P = 0.004$], illustrating that, for the intermediate period, changes in paired pulse excitability differed significantly between Parkinson’s disease patients and healthy subjects across time. There was also an effect of ISI (three levels;
2, 3 and 4 ms) for the inhibitory period \(F(2,26) = 5.8; P = 0.008\], but no effect of time and no interaction time ISI group. There was no effect of ISI (three levels; 9, 10 and 15 ms) for the facilitatory period, no effect of time and no interaction time ISI group.

In view of these selective effects of rTMS and L-dopa on intermediate ISIs, we restricted further statistical analyses to this part of the intracortical excitability curve (see Figs 2–5). For illustration, data on the early inhibitory and late facilitatory part of the excitability curve are also shown in Figs 2, 3 and 5.

Effects of rTMS on day 1

Figure 3 shows that after rTMS, conditioned MEP amplitudes at ISIs of 5 and 8 ms were reduced in Parkinson’s disease patients \((n = 10)\) and increased in healthy subjects \((n = 9)\). ANOVAs for the different ISIs with the factors group and time [two levels; baseline (day 1), post-rTMS (day 1)] assessing the conditioning effects of the first rTMS session showed no significant effect of time at any of the different ISIs. However, there was a significant interaction time × group at 5 ms \([F(1,16) = 11.3; P < 0.004]\). This interaction was not significant at 8 ms. Conditioned MEP amplitudes at an ISI of 5 ms were significantly reduced after rTMS in healthy subjects \([t(7) = −3; P = 0.019, \text{ paired sample } t \text{ test}; \text{Fig. 3A}]\). In contrast, abnormal baseline facilitation at 5 ms was reversed to inhibition in patients \([r(9) = 2.6; P = 0.029; \text{Fig. 3B}]\). As a net result, there was no difference any more between healthy subjects and Parkinson’s disease patients at an ISI of 5 ms following premotor rTMS (Student’s \(t\) test). Post-rTMS, there was also no difference at 8 ms (Fig. 2B).

Long-lasting effects of rTMS on day 1

We restricted our analysis to conditioned MEP amplitudes at an ISI of 5 ms because premotor rTMS on day 1 had induced significant changes at this ISI only. We computed a repeated measures ANOVA for conditioned MEPs at an ISI of 5 ms with the within-subject factor time [three levels; baseline (day 1), post-rTMS (day 1), baseline (day 8)] and the between-factor group. There was no effect of time but a significant interaction time × group.
time × group \[ F(2,28) = 3.7; P = 0.037 \]. In Parkinson’s disease patients, baseline conditioned MEPs at 5 ms ISI on day 8 were still significantly smaller compared with baseline values on day 1 \[ t(9) = 2.6; P = 0.026, \text{ paired samples } t \text{ test} \] but not significantly different from post-rTMS values on day 1 (Figs 3 and 4). In contrast, there was no difference of baseline MEP amplitudes on day 1 and baseline of day 8 in healthy subjects.

**Effects of L-dopa (day 8)**

We compared baseline and post-L-dopa conditioned MEP amplitudes for the intermediate ISIs using separate ANOVAs with the factors group and time [two levels; baseline(day 8) and post-L-dopa(day 8)]. There was a main effect of time at ISIs of 6 ms \[ F(1,14) = 10.9; P = 0.005 \], 7 ms \[ F(1,14) = 10.8; P < 0.005 \] and 8 ms \[ F(1,14) = 8.1; P = 0.013 \]. An interaction between time and group was present only at an ISI of 8 ms \[ F(1,14) = 4.7; P = 0.047 \]. L-Dopa treatment abolished MEP facilitation at 6 and 7 ms \[ t(5) = 3.1; P = 0.027 \text{ and } t(5) = 6.1; P = 0.002, \text{ respectively} \] in healthy subjects (Fig. 5A). It also reduced excitability at these ISIs in Parkinson’s disease, but this was not significant. However, in patients, but not in healthy subjects, L-dopa significantly reduced paired pulse facilitation at 8 ms \[ t(9) = 3.6; P = 0.005 \] (Fig. 5A).

**Effects of rTMS following pre-conditioning with L-dopa (day 8)**

After pre-medication with L-dopa, ANOVAs with the factors group and time [two levels; post-L-dopa(day 8) and post-rTMS(day 8)] for the intermediate ISIs showed an effect of time on conditioned MEP amplitudes at 5 ms \[ F(1,14) = 4.7; P = 0.048 \] and 6 ms \[ F(1,14) = 4.6; P = 0.048 \]. There was no significant interaction time × group for any of the ISIs, i.e. there was no differential effect of rTMS in the two groups.

Comparable with day 1, rTMS led to a significant reduction in paired pulse inhibition at an ISI of 5 ms following pre-treatment with L-dopa in healthy subjects \[ t(5) = -4.3; P = 0.008, \text{ paired samples } t \text{ test} \] (Fig. 5B). In contrast to rTMS...
on day 1, rTMS produced extra-facilitation at 6 ms in Parkinson’s disease patients \( t(9) = -2.9; P = 0.016 \) but left conditioned MEP amplitudes at other ISIs unchanged (Fig. 5B).

To validate whether rTMS had differential effects depending on the dopaminergic state in the two groups, we carried out an additional two-factor repeated measures ANOVA focusing on paired pulse excitability at ISIs of 5 and 6 ms which was modulated by rTMS. There was an effect of time [four levels; before and after rTMS(day 1); after L-dopa intake(day 8) and following rTMS(day 8); \( F(3,42) = 4.4, P = 0.009 \)] but not of ISI (two levels; 5 and 6 ms). There was no interaction time \( \times \) group or ISI \( \times \) group. However, the interaction time \( \times \) group \( \times \) ISI was significant \( F(3,42) = 3.9; P = 0.016 \), showing that the effects of rTMS at these ISI were significantly different across time in the two groups.

In healthy controls, there was an effect of time \( F(3,15) = 7.8, P = 0.002 \) but no effect of ISI and no interaction between time and ISI, i.e. the direction of rTMS-induced effects was not different on day 1 and day 8. Additionally, the magnitude of change (expressed as the difference of pre- and post-TMS conditioned MEP values at an ISI of 5 ms) was also not different (paired samples \( t \) test). In contrast, there was both an effect of time \( F(3,27) = 3.6; P = 0.026 \) and an interaction between time and ISI \( F(3,27) = 4.1; P = 0.016 \) in Parkinson’s disease, reflecting the differential response to rTMS on day 1 and day 8.

**Control experiment**

Separate ANOVAs for the inhibitory, intermediate and facilitatory periods of the intracortical excitability curve revealed no differences in baseline excitability between Parkinson’s disease patients on day 1 in the main experiment and Parkinson’s disease patients who participated in the control experiment. Next, we focused on significant findings on day 8 of the main experiment, i.e. changes at 8 ms after administration of L-dopa and at 6 ms following rTMS. ANOVA with the factors time (three levels; baseline and baseline day 8, respectively; post-L-dopa and post-rTMS) and ISI (two levels; 6 and 8 ms) did not reveal an interaction time \( \times \) group, ISI \( \times \) group or time \( \times \) ISI \( \times \) group, indicating that L-dopa and rTMS effects did not differ between the two Parkinson’s disease groups. As in the main experiment, L-dopa administration led to a significant decrease of baseline SICF at 8 ms in the Parkinson’s disease control group \( t(5) = 3.7; P = 0.013 \), paired samples \( t \) test; Fig. 6]. The magnitude of L-dopa-induced change (expressed as the difference of pre- and post-L-dopa conditioned MEP amplitudes at an ISI of 8 ms) was not significantly different between both Parkinson’s disease groups (Student’s \( t \) test). Similar to patients in the main experiments, premotor rTMS following L-dopa administration also led to an extra-facilitation at an ISI of 6 ms in the Parkinson’s disease control group \( t(5) = -3.3; P = 0.023 \), Fig. 6]. Again, there was no difference between Parkinson’s disease groups regarding the magnitude of this effect.
Clinical assessment

Baseline performance

Baseline UPDRS was 16 ± 1.6 SEM in patients. Patients took significantly longer to complete the 20-FWT (11.8 ± 0.6 s SEM) compared with healthy subjects (9.3 ± 1 s) [t(15) = 3.6; P = 0.003] although the number of steps (20.5 ± 1.9 in patients and 19.4 ± 0.7 in healthy controls) was not different. Baseline motor performance for the other test is shown in Table 2. Patients performed all tests significantly more slowly on their more affected side and 2-PT and 20-FST also on their less affected side compared with healthy subjects.

Changes in motor performance induced by rTMS and l-dopa

In patients, there was a significant effect of time on part III of the UPDRS [F(4,20) = 21; P < 0.001]. UPDRS was improved after rTMS on day 1 [t(8) = 2.8; P = 0.03, paired samples t test], after intake of l-dopa on day 8 [t(8) = 6.6; P = 0.001] and also following rTMS on day 8 [t(8) = 4.1; P = 0.009] (Fig. 7A). For each motor task, we carried out separate repeated measures ANOVAs with the factors group, time [five levels: baseline(day 1), post-rTMS(day 8), baseline(day 8), post-l-dopa(day 8) and post-rTMS(day 8)] and side (two levels; right and left in healthy subjects; clinically more and less affected side in patients). There was no significant interaction time × side or time × side × group for any of the tests, indicating that there were no side-specific effects of rTMS or l-dopa and no differential effects of the interventions in the two groups (Fig. 8).

Consequently, we did not perform additional ANOVAs for these clinical tests for day 1 and day 8, but restricted our analysis to calculating the effects of l-dopa in patients on day 8 to prove that l-dopa was clinically effective.

Apart from an improvement of part III of the UPDRS by l-dopa (see above; Fig. 7A), a single dose of l-dopa also improved the 20-FWT [time taken to complete the task; t(8) = 2.5; P = 0.042, paired samples t test; Fig. 7B]. Additionally, two-factor repeated measures ANOVA with the factors time (two levels; before and after intake of l-dopa) and side (two levels) showed a significant effect of time on 9-HPT [F(1,7) = 13; P = 0.008], and side on PST [F(1,8) = 5.8; P = 0.042], FST [F(1,8) = 9.7; P = 0.014] and 9-HPT [F(1,7) = 13; P = 0.008].

The effects of side in the ANOVAs are explained by slower performance on the clinically more affected side (see also Table 2). There was no significant interaction of time × side. Thus, as expected and in accordance with the main ANOVAs (across time), clinical improvements after l-dopa were not side specific.

Fig. 5 Day 8 paired pulse excitability curves in the healthy subjects (n = 6; left) and Parkinson’s disease patients (n = 10; right). Curves following l-dopa are superimposed on baseline curves in (A) and post-rTMS curves are superimposed on post-l-dopa curves in (B). On the x-axis, the different interstimulus intervals are shown. On the y-axis, the mean amplitude of the conditioned MEP is shown as a percentage of the mean test pulse amplitude. Error bars indicate the SEM. Values differing significantly from baseline (P < 0.05) are marked with an asterisk.
Discussion

The present study shows that subthreshold 1 Hz rTMS over the PMd can induce excitability changes in ipsilateral M1 via premotor–motor connections in drug-naive Parkinson’s disease patients and age-matched healthy controls. Using the Kujirai paradigm (Kujirai et al., 1993) to assess changes in intracortical excitability in ipsilateral M1, both groups exhibited a selective change in paired pulse excitability at an ISI of 5 ms after rTMS conditioning. However, the after effects were opposite in sign. In drug-naive Parkinson’s disease patients in whom paired pulse excitability was abnormally increased at ISIs of 5 and 8 ms at baseline 1 Hz rTMS to PMd led to a decrease of excitability at 5 ms. In contrast, premotor 1 Hz rTMS increased excitability at 5 ms in healthy controls. Additionally, rTMS effects lasted much longer (for at least a week) in patients compared with healthy controls. These results demonstrate an abnormal modifiability of ipsilateral M1 via premotor–motor connections in untreated Parkinson’s disease patients. A single dose of l-dopa reversed, i.e. normalized, the direction of excitability changes in M1 following premotor rTMS in Parkinson’s disease patients. This finding suggests that dopamine...
depletion directly or indirectly influences premotor–motor interactions in Parkinson’s disease.

We discuss the implications of these data in terms of our understanding of the pathophysiology of Parkinson’s disease in four sections: (i) changes in motor cortical excitability; (ii) abnormal modifiability of motor cortex excitability via premotor–motor connections; (iii) the ‘normalizing’ effects of L-dopa; and (iv) the functional relevance of altered modifiability of premotor–motor interactions.

**Changes in motor cortical excitability in drug-naïve patients with Parkinson’s disease**

This is, to the best of our knowledge, the first study that used the Kujirai paradigm (Kujirai et al., 1993) to study the intracortical excitability curve exclusively in de novo Parkinson’s disease patients. Therefore, measurements were not confounded by well-known long-term reorganization induced by dopaminergic therapy (Marsden, 1994). Parkinson’s disease patients had a selective increase in paired pulse excitability at intermediate ISIs (5 and 8 ms) compared with age-matched healthy controls. The fact that SICI at ISIs of 2–4 ms and SICF at ISIs of 9–15 ms were normal in drug-naïve Parkinson’s disease patients strongly suggests that paired pulse excitability at intermediate intervals ranging from 5 to 8 ms are mediated by neuronal circuits that are distinct from those mediating SICI at 2–4 ms and SICF at 9–15 ms. Another specific feature of these circuits is that premotor rTMS at 80% of AMT can provoke a bidirectional shift in their excitability depending on the rate of stimulation (Münchau et al., 2002; Rizzo et al., 2004). This indicates that they are closely linked to the PMd, presumably via premotor–motor connections. Therefore, we hypothesize that the abnormal baseline increase in paired pulse excitability at intermediate intervals in de novo Parkinson’s disease patients reflects an increased facilitatory input from the PMd to M1.

Previous studies have reported increases in paired pulse excitability at 2, 4 and 5 ms (Ridding et al., 1995; Pierantozzi et al., 2001) and 2, 3 and 6 ms (Pierantozzi et al., 2001). Of note, paired pulse excitability at an ISI of 8 ms was not included in these studies. This discrepancy may be attributed to differences in TMS stimulus intensities. Ridding et al. (1995) and Pierantozzi et al. (2001) adjusted the stimulus intensity of the conditioning pulse to 95% of AMT, whereas the intensity was set at 80% of AMT in the present study. This might have decreased the sensitivity to pick up changes of SICI. Alternatively, it is possible that an abnormal decrease in SICI can only be observed in patients who are chronically treated with L-dopa but not in drug-naïve patients.
Abnormal modifiability of motor cortical excitability via premotor–motor connections

Similarly to healthy young subjects (Münchau et al., 2002; Bäumer et al., 2003a; Rizzi et al., 2004), premotor 1 Hz rTMS had a net facilitatory effect on paired pulse excitability of ipsilateral M1 in older controls. This is in good agreement with previous studies in healthy humans (Civardi et al., 2001) and primates (Ghosh and Porter, 1988; Tokuno and Nambu, 2000; Shimazu et al., 2004) that have shown a strong modulatory influence of the lateral premotor cortex on motor cortex excitability. In drug-naïve Parkinson’s disease patients, premotor 1 Hz rTMS normalized the pre-existing increase in paired pulse excitability at an ISI of 5 ms, i.e. the direction of the change in excitability in Parkinson’s disease was opposite to that in healthy controls. This reversal of the ‘normal’ conditioning rTMS effects indicates that in de novo Parkinson’s disease patients, repetitive activation of premotor–motor connections induced an abnormal pattern of excitability in ipsilateral M1. At least three alternative mechanisms could have caused the opposite effect of PMd conditioning in Parkinson’s disease patients.

First, the pre-existing increase in M1 excitability or abnormal activity in premotor–motor connections, or both, might have increased the tendency of intracortical circuits in M1 to give an inhibitory response to PMd conditioning. This view is supported by a recent rTMS study showing that depending on the pre-existing level of excitability, the direction of excitability changes induced by 1 Hz rTMS over the M1 can be reversed (Siebner et al., 2004).

Secondly, apart from striatal dopamine deficiency, dopaminergic innervation is also depleted in the frontal cortex of Parkinson’s disease patients, particularly in superficial cortical layer 1 (Gaspar et al., 1991). This layer comprises many dendrites of underlying pyramidal cells and receives input from cortico-cortical afferents (Sliper, 1973; Jones, 1984) that form facilitatory and inhibitory circuits modulating the excitability of pyramidal cells. Degeneration of dopaminergic fibres in these cortical areas in Parkinson’s disease patients might affect their responsiveness to premotor rTMS. However, because L-dopa substitution normalized the direction of premotor rTMS effects, it is unlikely that such fibre degeneration is the main cause for abnormal rTMS responses in Parkinson’s disease.

Fig. 8 Performance during PST (pronation–supination test), 2-PT (two-point test), FST (finger sequence test) and 9-HPT (nine-hole peg test) in Parkinson’s disease patients and healthy subjects across time. On the x-axis, the five different time points are shown: B-1 = baseline on day 1; rTMS-1 = post-rTMS on day 1; B-8 = baseline on day 8; LD = post-L-dopa on day 8; rTMS-8 = post-rTMS on day 8. Error bars indicate the SEM.
Finally, it is possible that 1 Hz premotor rTMS altered motor cortex activity more indirectly through cortico-basal ganglia–cortical loops. Of note, 10 Hz rTMS over the left mid dorsolateral prefrontal cortex gave rise to a lasting increase in endogenous dopamine release in the ipsilateral caudate nucleus in healthy volunteers (Stratella et al., 2001). Thus, dysfunction of the cortico-basal ganglia–cortical loops in Parkinson’s disease might have reduced endogenous dopamine release in the ipsilateral caudate nucleus and contributed to the differential response to premotor conditioning in Parkinson’s disease patients.

The absence of pre-conditioning effects of the first rTMS session (day 1) on rTMS responses on day 8 in elderly healthy controls is in concordance with a previous study in young healthy subjects (Bäumer et al., 2003a). In contrast, excitability changes induced by premotor rTMS on day 1 were still present at baseline on day 8 in Parkinson’s disease patients (Figs 3B and 4), implying that cellular responses responsible for ‘storing’ of rTMS effects are abnormally enhanced in Parkinson’s disease.

Basic mechanisms of excitability changes outlasting rTMS trains in humans are unresolved. It has been suggested that they resemble long-term potentiation (LTP) and long-term depression in animal models (Chen et al., 1997) which can induce early genes, e.g. c-fos, leading to protein synthesis. Interestingly, in rats, expression of immediate early genes in the striatum in response to chemical stimulation of the neocortex can be augmented by blocking striatal D2 receptors (Berretta et al., 1999). This suggests that striatal D2 class receptor stimulation can negatively regulate gene induction at an extended time scale, in response to contemporaneous cortical stimulation. As a corollary, dopamine deficiency in Parkinson’s disease could therefore result in a net facilitation of postsynaptic effects induced by cortico-striatal terminals and lead to increases of gene expression producing long-term changes in cortico-basal ganglia–thalamo-cortical networks (Berretta et al., 1999). Interestingly, similar modulation of gene expression by D2 class dopamine receptors apparently also occurs within intracortical connections, at least in rats (Berretta et al., 1999).

The role of dopamine in regulating plasticity in the brain is supported by a recent study in hemiparkinsonian rats chronically treated with l-dopa (Picconi et al., 2003). Corticostrial LTP was impaired by dopamine denervation but could be restored by l-dopa. Physiologically, corticostrial LTP can be reversed to pre-LTP levels by low frequency stimulation which has been referred to as depotentiation (Bashir and Collingridge, 1994). In dyskinetic hemiparkinsonian rats, such a depotentiation was absent, indicating a loss of bidirectional plasticity which may lead to pathologic storage of information and may favour the development of maladaptive motor plasticity causing dyskinesia. Here we found an altered direction and marked prolongation of rTMS induced after effects in previously untreated Parkinson’s disease patients. The implication could be that abnormal plasticity as indexed by an abnormal modifiability of intracortical excitability is already present at early stages of Parkinson’s disease.

### Effects of l-dopa on motor cortex excitability and premotor–motor interactions

One has to bear in mind that excitability changes measured with paired pulse TMS reflect the net sum of l-dopa–dopamine receptor interactions in different parts of the brain. For instance, in the striatum, in addition to dopamine receptor expressing medium spiny neurons that project from the striatum to the internal and external segment of the globus pallidus (direct and indirect way, respectively) (Albin et al., 1989), dopamine receptors are also abundant on striatal cholinergic interneurons (Nicola et al., 2000). In primate frontal cortex, dopaminergic nerve terminals synapse onto dendrites of GABA neurons (Sesack et al., 1998) and in the rat prefrontal cortex, dopamine exerts complex effects on GABA-A-mediated inhibitory postsynaptic currents in pyramidal cells (Seamans et al., 2001).

Given these widespread and complex actions of dopamine, it cannot be stated with certainty whether changes of cortical excitability and rTMS responsiveness following l-dopa intake in the present study were caused by direct stimulation of basal ganglia–frontal cortex loops and/or cortical neurons bearing dopamine receptors or were instead mediated more indirectly, e.g. through dopamine effects on cholinergic or GABA-ergic interneurons in the striatum and cerebral cortex, respectively.

In young healthy subjects, orally administered l-dopa did not alter SICI or SICF, whereas dopamine agonists such as bromocriptine or pergolide increased SICI (Ziemann et al., 1996, 1997). This has been explained by an intact l-dopa storing capacity of nigro-striatal nerve terminals in young people preventing significant stimulation of dopamine receptors by oral administration of l-dopa (Ziemann et al., 1997). In this study, a single dose of l-dopa induced a robust decrease of paired pulse excitability at ISIs of 6 and 7 ms in older healthy controls (Fig. 5A). This suggests incomplete storage of l-dopa in striatal nerve terminals in older controls with some degree of ‘spill over’ of l-dopa to dopamine receptors, increased sensitivity of striatal dopamine receptors, or both. In fact, there is good evidence, at least in non-human primates, that dopamine sensitivity is significantly higher at older age (Paule et al., 1998).

In Parkinson’s disease, dopaminergic nigro-striatal neurons degenerate. This causes both reduced dopamine storage capacity and dopamine deficiency, leading to a compensatory increase in dopamine receptor binding potentials in the caudate nucleus and putamen (denervation supersensitivity) (Ichise et al., 1999). Therefore, one might expect that l-dopa would have a stronger impact on paired pulse excitability compared with healthy controls. On the other hand, it is also conceivable that reduced dopaminergic innervation in prefrontal, premotor and motor areas in Parkinson’s disease attenuates the ‘normal’ l-dopa effect on paired pulse
excitability present in elderly subjects (Gaspar et al., 1991). We found that L-dopa reduced paired pulse excitability at different ISIs (8 ms in patients compared with 6 and 7 ms in healthy controls), but the magnitude of this suppressive effect of L-dopa on paired pulse excitability was similar.

Given the complex action of L-dopa and manifold structural and functional changes of the dopamine system in Parkinson’s disease, we can only infer that the responsiveness of motor-cortical circuits to acute L-dopa replacement is altered in Parkinson’s disease. However, the present data do not allow us to draw definite conclusions as to the underlying mechanisms.

Similar to rTMS effects on day 1, premotor rTMS also caused a reduction of excitability at 5 ms following pre-conditioning with L-dopa in healthy controls (Fig. 5B). In contrast, compared with rTMS effects in a dopamine-depleted state, the direction of premotor rTMS-induced changes of motor cortex excitability was reversed in Parkinson’s disease patients after their first ever L-dopa intake. This rapid switch from abnormal to more physiological premotor–motor interaction cannot be explained by pre-conditioning with rTMS (on day 1) as rTMS applied following L-dopa intake had the same effect in a Parkinson’s disease control group who had not received rTMS before their first L-dopa treatment. The implication is that abnormal excitability patterns and modifiability of M1 can, at least in part, be normalized by acute L-dopa replacement.

It remains to be clarified why premotor rTMS induced facilitation at 6 ms in Parkinson’s disease (following L-dopa treatment) and 5 ms in older healthy controls, whereas previous studies using an identical rTMS protocol found an increased facilitation at 6 or 7 ms in young subjects (Münchau et al., 2002; Bäumer et al., 2003a; Rizzo et al., 2004).

**Functional consequences of L-dopa and rTMS**

As expected, after intake of L-dopa, part III of the UPDRS and the 20-FWT were improved and performance of the 9-HPT was faster bilaterally in Parkinson’s disease patients. UPDRS also improved after rTMS on both day 1 and day 8 in patients. However, there were no side- and group-specific changes of motor performance across time. Given this lack of side specificity, we think that improvement of UPDRS following premotor rTMS in Parkinson’s disease patients was most probably due to training, a placebo effect, or both. Thus, direct conclusions as to the functional significance of abnormal baseline premotor–motor interactions and ‘resetting’ of this abnormal interaction by L-dopa in Parkinson’s disease patients cannot be drawn on the basis of the present study. This notwithstanding, the theme of altered premotor cortex activity in Parkinson’s disease is supported by previous neuroimaging studies. These studies have consistently demonstrated that mesial frontal areas, particularly the rostral supplementary motor area, are hypoactive during self-selected, externally triggered single joystick movements (Playford et al., 1992), simple finger movements (Rascol et al., 1992; Samuel et al., 1997; Buhmann et al., 2003) or more complex sequential movements (Sabatini et al., 2000), whereas movement-related activity was increased in the lateral premotor and parietal cortices during finger movements, and also during paradoxical gait (Samuel et al., 1997; Catalan et al., 1999; Hanakawa et al., 1999; Sabatini et al., 2000). Both the parietal and premotor cortex participate in the control of complex externally guided movements in extrapersonal space (Wise et al., 1997; Rizzolatti et al., 1998). It has been hypothesized that overactivity of premotor–parietal circuits in Parkinson’s disease represents a compensatory mechanism for deficient activation of impaired striato-mesial–frontal projections (Sabatini et al., 2000). In fact, motor control in Parkinson’s disease is improved by sensory cues rather than prediction and forward planning (Flowers, 1978; Brown and Marsden, 1988). It remains to be shown how the abnormal premotor–motor interaction as determined with combined TMS/rTMS in the present study is linked to the ‘overactivity’ of premotor–parietal circuits in motor activation studies.

Although behavioural correlates of this abnormal interaction are as yet unclear, the TMS/rTMS paradigm tested in this study can be used to examine in vivo how different motor disorders, including basal ganglia diseases, affect the functional cross-talk between the premotor and motor cortex. Rather than solely determining motor cortex excitability using TMS measurements directly over the motor cortex, the advantage of the premotor–motor TMS/rTMS paradigm used here may be that it can capture cortical network abnormalities, i.e. alterations of cortico-cortical connectivity in frontal motor cortex. It may also prove useful to delineate dynamic responses of the cortico motor system to ongoing neurodegeneration and therapeutic interventions such as chronic L-dopa treatment.

**Conclusions**

This study shows that intracortical excitability as determined with the Kujirai paired pulse paradigm is abnormally increased in untreated Parkinson’s disease patients. Following 1 Hz premotor rTMS, paired pulse excitability at an ISI of 5 ms was decreased, i.e. normalized, in Parkinson’s disease but increased in healthy controls. This abnormal modifiability of premotor–motor connectivity in untreated Parkinson’s disease patients could be switched towards a more normal premotor–motor interaction by a single dose of L-dopa. This implies that abnormal excitability in premotor–motor connections can be attributed to dopamine depletion in Parkinson’s disease.

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Appendix: description of tests used for clinical assessment

20-feet walking test (20-FWT): subjects were seated in a chair and stood up in response to the instruction ‘Stand up and go’. Subjects were required to rise from the chair without using their arms, walk 20 feet, turn around, walk back and then sit down again.

Pronation–supination test (PST): 20 cycles of rapid alternating pronation/supination hand movements on the knees with the largest amplitude possible.

Two-point test (2-PT): 10 cycles of consecutive horizontal arm movements. Subjects had to alternately touch two marks on a table 30 cm apart with their index finger while holding the arm extended.

Finger sequence test (FST): 10 consecutive cycles of a rapid finger sequence movement involving a sequential opposition of the thumb to digit II, III, IV and V and back.