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Motor loop dysfunction causes impaired cognitive sequencing in patients suffering from Parkinson's disease

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ABSTRACT

Cognitive impairment in Parkinson's disease (PD) is often attributed to dopamine deficiency in the prefrontal-basal ganglia–thalamo-cortical loops. Although recent studies point to a close interplay between motor and cognitive abilities in PD, the so-called "motor loop" connecting supplementary motor area (SMA) and putamen has been considered solely with regard to the patients' motor impairment. Our study challenges this view by testing patients with the serial prediction task (SPT), a cognitive task that requires participants to predict stimulus sequences and particularly engages premotor sites of the motor loop. We hypothesised that affection of the motor loop causes impaired SPT performance, especially when the internal sequence representation is challenged by suspension of external stimuli. As shown for motor tasks, we further expected this impairment to be compensated by hyperactivity of the lateral premotor cortex (PM).

We tested 16 male PD patients ON and OFF dopaminergic medication and 16 male age-matched healthy controls in an functional Magnetic Resonance Imaging study. All subjects performed two versions of the SPT: one with on-going sequences (SPT0), and one with sequences containing non-informative wildcards (SPT+) increasing the demands on mnemonic sequence representation. Patients ON (compared to controls) revealed an impaired performance coming along with hypoactivity of SMA and putamen. Patients OFF compared to ON medication, while showing poorer performance, exhibited a significantly increased PM activity for SPT+ vs. SPT0. Furthermore, patients' performance positively covaried with PM activity, corroborating a compensatory account. Our data reveal a contribution of the motor loop to cognitive impairment in PD, and suggest a close interplay of SMA and PM beyond motor control.

1. Introduction

Parkinson's disease (PD) results from the degeneration of dopaminergic neurons in the substantia nigra. While motor symptoms are very prominent (Rodriguez-Oroz et al., 2009), patients also suffer from cognitive deficits even at very early stages of the disease (Muslimović et al., 2005). These cognitive impairments are commonly assumed to result from frontal lobe dysfunction consecutive to dopamine depletion (Saint-Cyr et al., 1988; Taylor et al., 1990; Dubois and Pillon, 1997) of cortico-basal ganglia–thalamo-cortical circuits (Alexander et al., 1986; Sawamoto et al., 2008). In particular, circuits connecting the caudate nucleus and the prefrontal lobe are supposed to contribute to cognitive deficits (Rinne et al., 2000; Brück et al., 2001; Cools et al., 2001; Owen, 2004; Zgaljardic et al., 2006; Grahn et al., 2008; Polito et al., 2012).

In contrast to the caudate–prefrontal loops, the "motor loop" (Alexander et al., 1986) that connects the putamen to the lateral premotor cortex (PM) and the supplementary motor area (SMA) is scarcely considered to underlie cognitive dysfunction in PD. However, evidence has accumulated that some cognitive functions draw specifically on premotor loops: For example, imagining, planning or observation of actions have been shown to activate the same motor network as used when performing an action (Decety et al., 1994; Stephan et al., 1995; Jeannerod, 2001). Moreover, the premotor system contributes to predictions of upcoming events even if they are not related to actions which we are able to perform ourselves. Therefore, the premotor system contributes to

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processes beyond classical motor functions (Schubotz, 2007).

A motor–cognitive relation has also been established in PD, as recent studies report an intra-individual correlation between severity of freezing of gait and lower scores of frontal tests (Amboni et al., 2008; Vandenbossche et al., 2011; Vercruysse et al., 2012). Furthermore, we found the severity of motor impairment to significantly correlate with patients' deficits in the serial prediction task (SPT), a cognitive sequencing task (Schönberger et al., 2013). The SPT requires subjects to monitor a structured stimulus sequence for structure-violating items in a non-speeded response regime (Schubotz, 1999). It reliably engages PM and SMA in healthy participants (Schubotz and Von Cramon, 2003; Schubotz, 2007), although it does not imply any motor abilities. Hence, the question arises whether the observed deficits of patients suffering from PD when performing the SPT result from premotor or prefrontal dysfunction.

In PD, the most significant premotor dysfunction is a SMA hypoactivity when patients perform motor tasks (Playford et al., 1992; Haslinger et al., 2001). SMA hypoactivity is often accompanied by PM hyperactivity (Samuel et al., 1997; Haslinger et al., 2001) which implies a compensatory mechanism (Haslinger et al., 2001). Moreover, external cues such as auditory rhythms may be used to ameliorate motor impairment (Martin, 1967; McIntosh et al., 1997; Azulay et al., 1999). Hanakawa et al. (1999) showed that improved motor function under external guidance co-occurs with increased PM activity compared to conditions where external help is lacking. These findings can be explained in terms of a functional dichotomy within the premotor cortex as proposed by Goldberg (1985): According to his review, the SMA accounts for internal motor control rather than for an external or stimulus-driven one, whereas the opposite holds for the PM. This assumption was largely (but not always, cf. Weeks et al., 2001; Cunnington et al., 2002) confirmed by imaging studies comparing externally versus internally guided movements (Debaere et al., 2003; Heuninckx et al., 2010), although these may constitute a relative functional bias rather than a strict double-dissociated functional dichotomy (Jahanshahi et al., 1995). Taken together, findings suggest that patients' difficulties in internally initiating movements reflect the hypoactive SMA, whereas the hyperactive PM ameliorates this deficit by exploiting external cues.

Using functional Magnetic Resonance Imaging (fMRI) we investigated whether this interplay of impairment in internal motor control and compensatory external control extends to cognitive functions supported by the premotor network and thus adds to cognitive impairment in PD. To this end, patients suffering from PD were tested ON and OFF dopaminergic medication in the SPT. Two versions of the SPT were implemented which differed by their demand on keeping an internal representation of a stimulus sequence: In “SPT0”, sequences of stimuli were continuing while in “SPT+” parts of the sequence were replaced by void stimuli, thereby increasing the demand for keeping track of the sequence based on an internal sequence representation (Schönberger et al., 2013). Previously, we found patients suffering from Parkinson's disease to be particularly impaired in SPT+ after withdrawal of their medication (Schönberger et al., 2013) suggesting a relation of internal sequence representation to SMA and striatal hypoactivity. Using fMRI allows to extend these results and to examine if compensational brain hyperactivity of the PM is found where no behavioural deficit is yet evolving. Note that PM hyperactivity during SMA hypoactivity can only be interpreted as effective functional compensation as long as there are no behavioural differences between PD and healthy controls (Samuel et al., 1997; Sabatini et al., 2000; Haslinger et al., 2001; Mallol et al., 2007).

Drawing on our previous findings we expected patients' performance to be impaired when compared to healthy controls. Furthermore, we expected the patients' performance to be worse during OFF medication compared to ON medication, most notably in SPT+ where internal sequence representation is necessary. We assumed this impairment to be accompanied by SMA and putamen hypoactivity. This pathological activity pattern should be more pronounced for SPT+ than for SPT0, and should be attenuated ON as compared to OFF medication. Moreover, in case that patients' performance is preserved, we expected SMA hypoactivity to be accompanied by PM hyperactivity. In case we should observe preserved SMA activity as well as compensating PM activity, they should be related to better performance. Finally, we expected the degree of hypoactivity of SMA and putamen to depend on the severity of motor impairment.

2. Materials and methods

2.1. Participants

Sixteen male patients suffering from PD and sixteen healthy male controls participated in the study. Patients had a mean age of 60.1 years (range: 44–72 years; see Table 1 for further demographical, clinical and neuropsychological data). Diagnosis of idiopathic PD was made according to the UK Parkinson's disease Society Brain Bank Criteria (Hughes et al., 1992). No subject had undergone a surgical treatment for the disease or had a history of any psychiatric or any other neurological disease. The severity of clinical symptoms defined according to Hoehn and Yahr (1967) ranged between I and III under regular medication. The motor score of the UPDRS (unified Parkinson's disease rating scale; Fahn and Elton, 1987) was assessed in each of the two experimental sessions independently by two movement disorder specialists blinded to the state of medication.

All patients received dopaminergic medication. Patients were tested once ON their regular medication and once OFF medication. OFF-state was defined as at least 14 h of withdrawal of dopaminergic medication; long-acting dopamine agonists were discontinued up to 36 h. Four additional patients were excluded from the analysis: One due to a depression score above the cut-off-value in the Beck depression inventory-II (BDI-II; cut-off for depression: $\geq$ 20 points; Hautzinger et al., 2006) and the other three due to behavioural performance at chance level for SPT0 and SPT+ both ON and OFF.

Sixteen healthy male participants comparable to the patients regarding age and level of school education served as control subjects. All 32 analysed participants scored between 18 and 30 points in the Parkinson neuropsychometric dementia assessment (PANDA; Kalbe et al., 2008), i.e., showed an age-adequate cognitive performance, and scored below the cut-off for depression (see above).

All participants gave their written informed consent prior to participation. The study was performed according to the Declaration of Helsinki and approved by the local ethics committee of the Medical Faculty, University of Cologne, Germany.

PANDA = Parkinson neuropsychometric dementia assessment; BDI-II = Beck depression inventory-II; LEDD = Levodopa-equivalent daily dose calculated according to Tomlinson et al. (2010); UPDRS ON = UPDRS III scores ON dopaminergic medication; UPDRS OFF- = UPDRS III scores OFF dopaminergic medication.

2.2. Stimuli and tasks

We employed three tasks: The serial prediction task in two versions (SPT0 and SPT+) and a serial match-to-sample task (SMTS) which served as a control task (Fig. 1). The SMTS requires holding a particular target stimulus in working memory while watching a series of non-target stimuli. It does not enable a

Table 1
Subject demographics and neuropsychological test data.

Characteristic	Mean ± standard error		p[a]
	Patients ($n=16$)	Controls ($n=16$)	
Age, years	60.13 ± 2.12	59.56 ± 1.42	0.567
Education, years	11.12 ± 2.06	11.81 ± 1.90	0.385
PANDA	25.81 ± 0.98	26.25 ± .75	0.595
BDI-II	6.69 ± 1.46	3.94 ± 1.04	0.109
Time since diagnosis, years	5.81 ± 3.52	–	–
LEDD	600.91 ± 125.57	–	–
UPDRS ON	15.3 ± 1.48	–	–
UPDRS OFF	23.6 ± 2.69	–	–

[a] Significance of differences between groups, computed with paired t-tests.

prediction of the order of stimuli as in the serial prediction task and thus controls for working memory processes apart from serial prediction.

In both versions of the serial prediction task, participants had to decide whether a sequence of 15 stimuli ended orderly or with a sequential violation. Stimuli consisted of concentric circles that differed in size (12 sizes, diameters ranging from 0.5° to 3.5° of visual angle). Three different consecutively presented stimuli formed a triplet (1-2-3) which was shown five times per trial. In 50% of the trials, the position of two stimuli in the last triplet was switched: instead of the original triplet (1-2-3) a new triplet (1-3-2 or 2-1-3) was presented. The first four presentations always showed the original triplet to allow learning of the sequence. Only the last triplet could be switched. In a forced-choice-response phase of 3.5 s participants had to indicate whether the sequence contained a switch or not. Overall, one trial lasted 18.75 s including response and feedback. The inter-trial-interval varied from 4.0 to 5.5 s depending on jitter times (0, 500, 1000, or 1500 ms).

The SPT+ condition was identical to SPT0 except for a parametric modulation of the necessity of internal sequential recall: In SPT+ trials one, two, three or four stimuli were replaced by so-called "occluders", i.e., non-informative stimuli that replaced standard circle-stimuli of the sequence. Similar to SPT0 a switch could only appear in the last triplet which the participants had to indicate in the response phase.

To ensure an overall high level of attention, catch trials were presented in all tasks: 12 SPT-trials and four SMTS-trials were added that ended after six, nine or twelve stimuli. These trials were answered like standard trials but did not enter either the fMRI or the behavioural analysis. For further details regarding the paradigms, see our previous behavioural study (Schönberger et al., 2013).

Further, 11 empty trials (null-events) were implemented. Each of them lasted 18.75 s like standard trials. All conditions (i.e., SMTS, SPT0, SPT+, and null-events) were presented in a randomized order (mixed trial design). Between trials there were no cues to signalise which trial started next. The experiment lasted in total 34.8 min with a total of 99 trials (19 SMTS trials, 24 SPT0 trials, 40 SPT+ trials, 10 for each number of occluders per trial, and 16 catch trials) and 11 null-events.

SPT0 (serial prediction task): Subjects were asked to monitor a sequence of three circles (1st-2nd-3 rd). In this example the order of circle-sizes is ascending, but it could as well be descending or intermingled. Each trial was preceded by a fixation cross (1 s). Every stimulus was presented for 600 ms with an inter stimulus interval of 125 ms. Subjects had to indicate whether the sequence ended as predicted (50%: 1st–2nd–3rd) or not (25%: 1st–3rd–2nd; 25%: 2nd–1st–3rd) in a forced-choice-response phase with maximum 3.5 s to deliver a response. Two response buttons were provided: one for answering "correct sequence" and one for responding "a sequential switch occurred". Answers were delivered with the right index and middle fingers, with finger response association balanced across subjects. A valid feedback indicated a correct, false or missing answer.

SPT+ (serial prediction task with occluders): Subjects had to perform in the same manner as in SPT0, except that 1-4 stimuli of every trial were replaced by so-called occluders: instead of a circle a blank card was shown. The first three stimuli of a trial were never replaced by an occluder, and never two consecutive stimuli

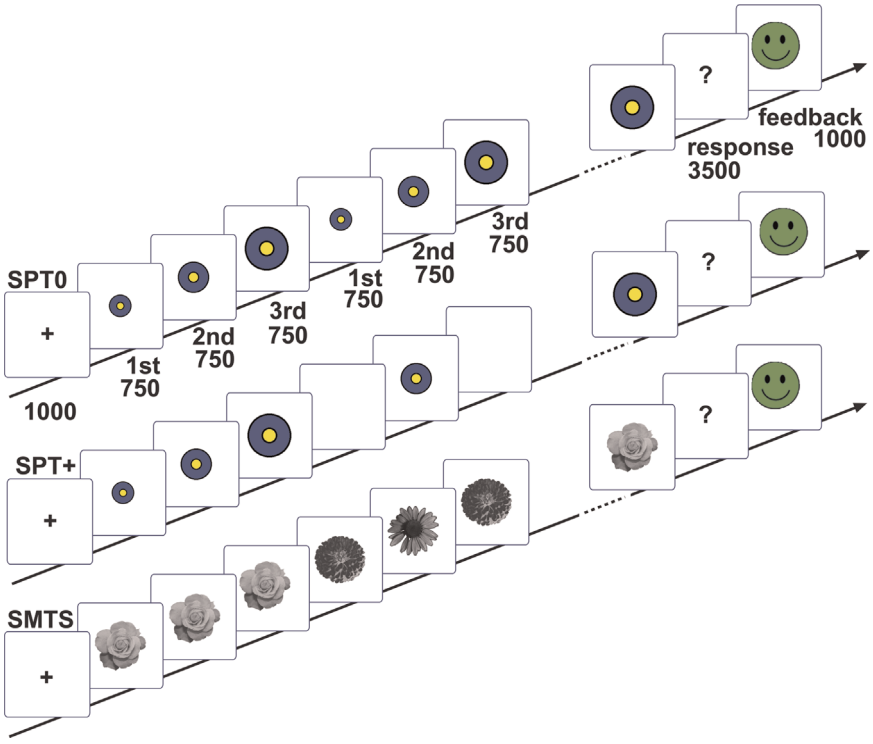

Fig. 1. Stimulus material and trial structure.

were replaced. The last triplet contained maximal one occluder.

SMTS (serial match-to-sample task as control task): Subjects were instructed to memorise the first stimulus, one out of 50 monochrome blossoms, which was presented three times. Subsequently, twelve (or less in case of a catch trial) other randomized blossoms, sometimes replaced by an occluder, were shown with presentation parameters identical to those of the SPT. Subjects had to indicate whether the last blossom was identical to the very first one. Occluders were used to make the perceptual effects similar to those of the SPT, but did not have any relevance for correctly answering the task as the last blossom never was an occluder.

2.3. Procedure

Every patient attended the study on three consecutive days and each control subject on two consecutive days. On the first day, every subject received training on SPT0, SPT+ and SMTS outside the scanner and all patients were on their regular dopaminergic medication. On the second day, 50% of patients were tested ON and 50% OFF. Healthy controls did not receive any medication. The third day was arranged in the same way as day two, except that patients who had been tested ON the day before were now tested OFF and vice versa. Patients were randomly assigned to measuring ON or OFF first.

2.4. Functional MRI data acquisition

In the fMRI sessions, participants lay on the scanner bed in a supine position with their right index and middle fingers placed on two different buttons of a response box. The visual stimuli were presented on a 30-inch MRI display (OptoStim, medres GmbH, Cologne, Germany). Imaging was performed at a 3T scanner (Siemens TRIO, Erlangen, Germany) equipped with a standard birdcage head coil. Thirty axial slices (210 mm field of view, 64×64 pixel matrix, 3 mm thickness; 1 mm spacing) positioned parallel to the bicomissural plane (AC-PC) were acquired using a single-shot gradient echo-planar imaging (EPI) sequence (TE 30 ms, flip angle 90°, TR 2000 ms; serial recording) sensitive to blood oxygenation level dependent (BOLD) contrast. Prior to functional imaging, 29 anatomical T1-weighted modified driven equilibrium Fourier transform (MDEFT) images (Uğurbil et al., 1993; Norris, 2000) were acquired.

In a separate session prior to the fMRI sessions, high-resolution whole-brain images were recorded for every participant using a T1-weighted 3D-segmented MDEFT sequence (128 slices, field of view 256 mm, 256×256 pixel matrix, thickness 1 mm, spacing 0.25 mm).

2.5. Behavioural analysis

The statistical software package SPSS (SPSS Statistic 20.0, IBM, Chicago, IL) was used for statistical analyses. Behavioural performance was assessed by probability of recognition (P_R, Snodgrass and Corwin, 1988) defined as the difference of hit rate and false alarm rate (cf. Schönberger et al., 2013). Chance performance level relates to 0.25 in SPT0 (24 trials) and 0.225 in SPT+ (40 trials). All participants included in the statistical analysis performed above these chance levels in at least one SPT version. As patients and controls were matched for their gender, age and level of education all comparisons between these groups were carried out as paired *t*-tests or repeated measures ANOVA.

Paired *t*-tests were conducted comparing age, years of school education, PANDA and BDI-II of patients and controls. Further, a paired *t*-test was calculated for UPDRS III scores ON and OFF to ensure a significant effect of medication.

Performance in SMTS was compared between groups (patients ON vs. controls) and within patients (OFF vs. ON) in two paired-t-tests to control for differences in working memory capacity. Differences in SPT performance were calculated in two 2×2 ANOVAs, first between patients ON and controls [within-subject factors TASK (SPT0 vs. SPT+) and GROUP (patients ON vs. controls)] and second between performance of patients ON and OFF [within-subject factors TASK (SPT0 vs. SPT+) and MEDICATION (ON vs. OFF)]. Note that response times are not of interest as all participants were instructed to give correct, but non-speeded responses. Nevertheless, to rule out the possibility of a speed-accuracy trade off, we also conducted these two 2×2 ANOVAs with response time as the dependent variable. Furthermore, the number of missing responses was used as dependent variable in the two 2×2 ANOVAs to suspend that group differences in brain activity were caused by differences in the number of motor responses. Finally, all comparisons with the factor MEDICATION were also conducted with a covariate which coded if a patient was ON or OFF medication on the first day of testing. By this means possible practice effects that could mask differences between ON and OFF state were controlled. An interaction between medication, task and testing day could not be directly tested since the testing days' influence is not independent of medication state since patients were either ON or OFF medication on their first day of testing.

Additionally, correlation analyses for UPDRS III, age, and PANDA with performance in SPT0, SPT+, and SMTS were carried out for ON- and OFF-state, respectively. Controls' age and PANDA-scores were correlated with performance in SPT0, SPT+ and SMTS. All correlation analyses were computed using standard Pearson's correlation coefficient, with p-Values < 0.05 regarded as significant.

2.6. fMRI data processing

Motion correction of the functional data was performed with the Siemens motion correction protocol (Siemens, Erlangen, Germany). All further analyses were conducted with the software package LIPSIA (Lohmann et al., 2001). A high-pass filter with a cut-off frequency of 1/120 Hz was applied and spatial smoothing was performed with a Gaussian filter of 5.65 mm FWHM (full width half maximum). Functional data sets were aligned with a three-dimensional (3D) stereotactic coordinate reference system by co-registration of the low-resolution MDEFT datasets onto the individual high-resolution 3D MDEFT reference set. Due to technical problems some of the low-resolution anatomical datasets were not recorded properly; in these cases functional data were aligned with the individual 3D MDEFT reference sets by taking the 20th time-step of each fMRI time course. The resulting parameters formed a transformation matrix with three rotational and three translational degrees of freedom. This matrix was normalised to a standardized Talairach brain size (Tailarach and Tournoux, 1988) and applied to the functional slices using trilinear interpolation. The generated output had a spatial resolution of $3 \times 3 \times 3$ mm^3.

Statistical evaluation was based on a least-squares estimation using GLM (general linear model) for serially auto-correlated observations (Worsley and Friston, 1995). The design matrices were generated with a delta function, convolved with the hemodynamic response function (gamma function).

In the first analysis, brain activations were analysed time-locked to onset of the trials, and the analysed epoch comprised the duration of a complete fifteen-stimuli-sequence without the response and feedback time window, i.e. 11.25 s. In the employed GLM, the conditions SMTS, SPT0, SPT+, and null-events were modelled. Null-events were also modelled with a length of 11.25 s. Six contrast images were generated for each participant (SMTS > resting, SPT0 > resting, SPT+ > resting, SPT0 > SMTS, SPT+ > SMTS, SPT+ > SPT0). These contrast images entered

paired-*t*-tests for analyses of group differences (patients ON vs. controls) and medication effects (patients ON vs. OFF). Furthermore, the contrasts SPT0 > SMTS and SPT+ > SMTS were used in one-sample-*t*-tests to verify the expected premotor involvement in serial prediction beyond working memory processes in all participants.

To strengthen the ability to detect differences in sequential processing in SPT0 and SPT+ a second, event-related analysis was carried out. Instead of the whole epoch of SPT+ and SPT0 trials only one event per trial was modelled where the processing in both tasks should be maximally different. In every SPT+ trial one stimulus was selected which was presented directly after an occluder. At this time point the processing in SPT+ is diverging from the processing in SPT0 while the stimuli themselves are identical. In case of more than one occluder within a SPT+ trial it was randomly determined which stimulus after an occluder was chosen; occluders at the end of a trial were not considered. Likewise, one stimulus out of every SPT0 trial was selected which matched the position of a selected SPT+ stimulus. The resulting SPT+and SPT0 events were modelled with the duration of 1 s. Because the remaining stimuli in both SPT tasks were presented too densely to enter them in the GLM, SPT trials were additionally modelled as epochs of 11.25 s. SMTS trials and null events were also modelled as epochs of 11.25 s. Complementing the contrast of SPT+ > SPT0 in the first analysis, the contrast of SPT+ events > SPT0 events was computed for each participant and compared in controls vs. patients ON medication and patients ON vs. OFF medication.

The comparison of controls with patients ON medication demanded consideration of the day of data acquisition: Half of the patients were measured ON medication on their second day and the other half on their third day of participation, while the matched controls' data were all collected on the second day. Differences between controls and patients ON medication resulting from more frequent execution of SPT, in the case of patients who were ON medication on the third day, should not be considered as reliable group difference. Therefore the effect of the day of data acquisition was calculated in patients (second vs. third day) to be controlled in the comparison of controls and patients ON medication: activations less pronounced on the third day than on the second day of patients' participation should not be interpreted in favour of our hypothesis of hypoactivation in comparison to controls, and activations more pronounced on the third day than on the second day could not support our hypothesis of hyperactivation.

Finally, *t*-values were transformed into *z*-scores. To correct for false positive results data were whole-brain corrected at $p < 0.05$ by setting an initial voxelwise *z*- threshold to $z = 2.33$ ($p \leq 0.01$, uncorrected) and correcting for multiple comparisons using cluster-size and cluster-value thresholds obtained by Monte Carlo simulations at a significance level of $p = 0.05$ (Lohmann et al., 2008).

2.7. Region of interest analysis: correlation between BOLD, performance and UPDRS III

Beta-values of four regions of interest (ROI), left and right SMA and left and right PM, were calculated to assess the hypothesised relationships between severity of disease as well as SPT performance level with hypoactivity of SMA and compensating PM hyperactivity. ROI were derived from a previous fMRI study that tested young healthy participants performing SPT0 and SPT+ (Schubotz and von Cramon, 2004). Execution of both SPT versions compared to a control task was found to significantly increase activity in left SMA (Talairach coordinates of peak voxel: $x = -5$, $y = -1$, $z = 52$), right SMA ($x = 1$, $y = 5$, $z = 52$), left PM ($x = -56$, $y = 7$, $z = 23$) and right PM ($x = 52$, $y = 4$, $z = 34$). Based on these coordinates a ROI was defined as the peak voxel and its six adjacent voxels (inferior, superior, left, right, anterior and posterior to the peak voxel). The four resulting ROI were used to extract beta-values of activation during execution of SMTS (compared to resting), SPT0 (compared to resting), SPT+ (compared to resting), and during SPT+ compared to SPT0 (SPT+ > SPT0). Note that this procedure avoids double dipping as the ROI were derived from an independent data set (Kriegeskorte et al., 2009).

To analyse the relationship of SMA and PM activity with performance, beta-values of each ROI were correlated with performance (P_r) as follows. For patients ON, patients OFF and controls performance in SPT0 was correlated with beta-values of the contrast SPT0 > resting. Likewise, performance in SPT+ was correlated with beta-values of the contrast SPT+ > resting and SPT+ > SPT0. To test if the activity of SMA and PM exclusively predict SPT performance and not working memory capacity, correlations of SMTS performance with beta-values of the contrasts SMTS > resting, SPT0 > resting, SPT+ > resting and SPT0 > SPT+ were also calculated.

Finally, for each ROI UPDRS III scores ON were correlated with beta-values of patients ON and UPDRS III scores OFF with beta-values OFF (SPT0 > resting, SPT+ > resting, SPT+ > SPT0 and SMTS > resting).

In order to control for the number of comparisons, *p*-Values were multiplied with the number of ROI, i.e. 4, and considered significant when $p < 0.05$.

3. Results

3.1. Behavioural results

No significant differences were found between patients and healthy controls with regard to age, level of school education, BDI-II or PANDA scores (Table 1). The comparison of the UPDRS III motor-scores ON and OFF showed a highly significant difference ($t = -4.74$, $p < 0.001$).

Performance in SMTS did not differ significantly between ON and OFF ($t = 1.01$, $p = 0.33$) or between patients ON and controls ($t = 1.53$, $p = 0.146$; see Fig. 2). There also was no influence of medication when controlling in which state the patient was first [$F(1,15) < 1$]. The repeated-measures ANOVA with the two 2-level factors GROUP (patients ON, controls) and TASK (SPT0, SPT+) yielded a main effect for TASK [$F(1,15) = 12.66$, $p = 0.003$] as well as an almost significant main effect for GROUP [$F(1,15) = 4.49$, $p = 0.051$], but no interaction [$F(1,15) < 1$]. The repeated-measures ANOVA with the two 2-level factors MEDICATION (ON vs. OFF) and TASK (SPT0 vs. SPT+) exhibited a main effect of TASK [$F(1,15) = 13.45$, $p = 0.002$], but no main effect for MEDICATION [$F(1,15) < 1$] and no interaction [$F(1,15) < 1$]. Taking into account if patients were in ON or OFF

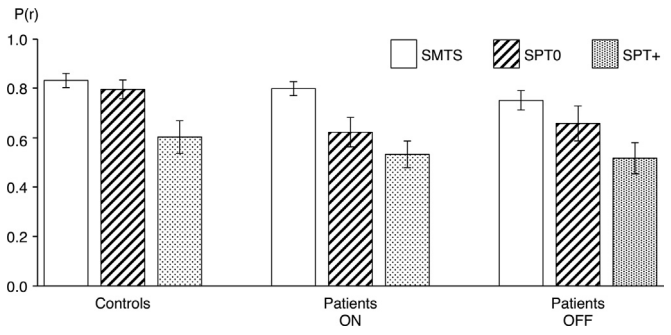

Fig. 2. Behavioural performance in all tasks. Bars show mean performance (± standard error) calculated as probability of recognition (Snodgrass and Corwin, 1988).

state on the first day of testing, there was a significant effect of MEDICATION [F(1,15)=6.45, p=0.024], but no effect of TASK [F (1,15) < 1] and no interaction [F(1,15)=1.91, p=0.188]. These results *mostly* met our expectations, as patients tended to perform worse than controls and showed poorer performance in SPT+ than SPT0. In contrast, the controls' advance in performance was not more pronounced in SPT+ (Fig. 2). Furthermore, patients showed the expected difference in performance OFF compared to ON when controlling for practice effects.

Regarding response times the analysis showed that patients ON (mean=956 ms, standard deviation=235 ms) responded significantly slower than controls [mean=796 ms, standard deviation=211 ms;$F(1,15)=8.823$, $p=0.01$]. There was no significant effect of TASK [$F(1,15) < 1$] and no interaction of GROUP and TASK [$F(1,15)=1.12$, $p=0.307$]. The ANOVA with the two 2-level factors MEDICATION (ON vs. OFF) and TASK (SPT0 vs. SPT+) exhibited no significant effects [all *F*-values (1,15) < 1]. When controlling for practice effects, the tendency of SPT+ patients to respond faster when OFF (mean=937 ms, standard deviation=300 ms) than ON [mean=965 ms, standard deviation=235 ms) did not reach significance [F(1,15)=4.05, p=0.064]. Neither were the factor TASK [F (1,15) < 1] or the interaction [F(1,15)=1.39,p=0.257] significant.

There was no effect of GROUP [$F(1,15)=1.667$, $p=.216$] or TASK [F (1,15) < 1] and no interaction [$F(1,15) < 1$] on the number of missing responses when comparing controls and patients ON. Further, there was no main effect of MEDICATION and TASK or interaction of both factors when comparing patients ON with patients OFF and when controlling for possible practice effects [all *F*-values (1,15) < 1]. This means that controls and patients regardless of medication state exhibited the same number of responses.

Correlations between age and performance revealed a trend for patients to be more impaired with increasing age both in SPT+ ($r=-0.473$; $p=0.065$) and in SPT0 ($r=-0.475$; $p=0.063$) in OFF- but not ON-state. Correlations of patients' BDI, PANDA, and UPDRS III with their performance were not significant. Like patients OFF, controls performed worse in SPT0 with increasing age ($r=-0.53$; $p=0.035$), while good performance in SPT+ was related to higher PANDA scores ($r=0.586$; $p=0.017$).

SMTS=serial match-to-sample task; SPT0=serial prediction task; SPT+=serial prediction task with occluders.

3.2. fMRI results

3.2.1. BOLD during serial prediction in all participants

To ensure that the premotor network's activity in the SPT generally exceeded activation during the SMTS we calculated one-sample-*t*-tests of the contrasts SPT0 > SMTS and SPT+ > SMTS in all groups and conjoined them [patients ON (SPT0>SMTS) ∩ patients ON (SPT+>SMTS) ∩ controls (SPT0>SMTS) ∩ controls (SPT+>SMTS) ∩ patients OFF (SPT0>SMTS) ∩ patients OFF (SPT+>SMTS)]. Indeed, this analysis revealed the expected higher activation in SMA, PM and inferior parietal lobule and additionally in the superior temporal gyrus during the SPT than the SMTS (Table 2).

3.2.2. BOLD differences of patients and healthy controls

We expected that patients ON, when engaged in the SPT, would show a hypoactivity of SMA and putamen when compared to healthy controls. This hypothesis was tested by calculating differences between patients ON and controls during the SPT vs. SMTS and during the SPT vs. resting: First, patients ON and controls were compared in SPT0 > SMTS and in SPT+ > SMTS separately and the results were conjoined in a second step [patients ON (SPT0 > SMTS) vs. controls (SPT0>SMTS) ∩ patients ON (SPT+ > SMTS) vs. controls (SPT+ > SMTS)]. This analysis did not reveal any significant differences.

Table 2
Areas more activated during the SPT than SMTS in all groups [patients ON (SPT0>SMTS) ∩ patients ON (SPT+>SMTS) ∩ controls (SPT0>SMTS) ∩ controls (SPT +>SMTS) ∩ patients OFF (SPT0>SMTS) ∩ patients OFF (SPT+>SMTS)].

Localisation	BA	Size	Coordinates			Z
			x	y	z	
SMA (L)	6	1701	−8	3	54	3.04
Lateral premotor cortex (L)	6	2052	−50	−3	42	3.53
Precentral gyrus/lateral premotor cortex (R)	6 / 44	5400	49	6	15	3.35
Inferior parietal lobule (R)	40	4752	46	−36	45	3.26
Superior temporal gyrus (L)	42 / 13	3429	−50	−39	15	3.71
Superior temporal gyrus (R)	42	1566	55	−33	12	3.45

Talairach coordinates of cluster peak voxel ($p < 0.05$, whole-brain corrected for multiple comparisons). BA=Brodmann Area; *L*=left hemisphere; *R*=right hemisphere; Size=cluster in mm^3; *Z*=maximal *Z*-scores.

Comparing SPT vs. resting [patients ON (SPT0 > resting) vs. controls (SPT0>resting) ∩ patients ON (SPT+ > resting) vs. controls (SPT+ > resting)] we found bilateral hypoactivity for patients ON as compared to controls in SMA and in right striatum; further hypoactivity was recorded in left primary motor cortex, left primary somatosensory cortex, bilateral inferior temporal gyrus, inferior occipital gyrus, precuneus and cuneus (Table 3 and Fig. 3). Against our expectation we did not observe a concurrent hyperactivity in PM. No area revealed a significantly higher BOLD response in patients ON than controls.

To support our hypothesis, the observed hypoactivations in patients must not have been caused by practice effects accompanying the day of patients' data acquisition. To suspend this possibility, second vs. third day's BOLD response of patients were compared for the contrast SPT0 > resting and SPT+ > resting in two paired-*t*-tests. The conjunction of both *t*-tests [second day (SPT0 > resting) vs. third day (SPT0>resting) ∩ second day (SPT +>resting) vs. third day (SPT+>resting)] showed no differences in activation between the second and third day of patients' participation while performing the SPT.

To examine if the hypoactivity in putamen and SMA was specific for the engagement in the SPT, we conducted a paired *t*-test of the contrast SMTS > resting in patients ON vs. controls and conjoined it with the conjunction of SPT0 > resting and SPT+ > resting. The same pattern of hypoactivity in right putamen, SMA, left and right primary somatosensory and motor cortex, left middle temporal gyrus, inferior occipital gyrus and cuneus in patients ON compared to controls was observed (Table 4 and Fig. 3). Therefore, the hypoactivity of putamen and SMA emerged when the patients were engaged in either the SPT or the SMTS.

To test for a pronounced hypoactivity of SMA in patients performing SPT+, the contrast SPT+ > SPT0 was compared between

Table 3
Areas more activated in controls than in patients ON medication during performance of SPT compared to resting [controls (SPT0>resting) vs. patients ON (SPT0>resting) ∩ controls (SPT+>resting) vs. patients ON (SPT+>resting)].

Localisation	BA	Size	Coordinates			Z
			x	y	z	
SMA (L+R)/primary motor cortex (L)/ primary somatosensory cortex (L)	6/4/3	4401	−5	−12	54	3.46
Putamen (R)		3564	22	9	−6	3.77
Precuneus (L)	7/19	2403	−20	−78	30	3.30
Cuneus (L)	18	8775	−14	−84	21	4.21
Middle temporal gyrus (L)	21	1863	−50	−15	−3	3.60
Inferior temporal gyrus (R)	19/37	2187	43	−60	−9	3.26
Inferior temporal gyrus (L)	19	2187	−56	−66	0	3.30

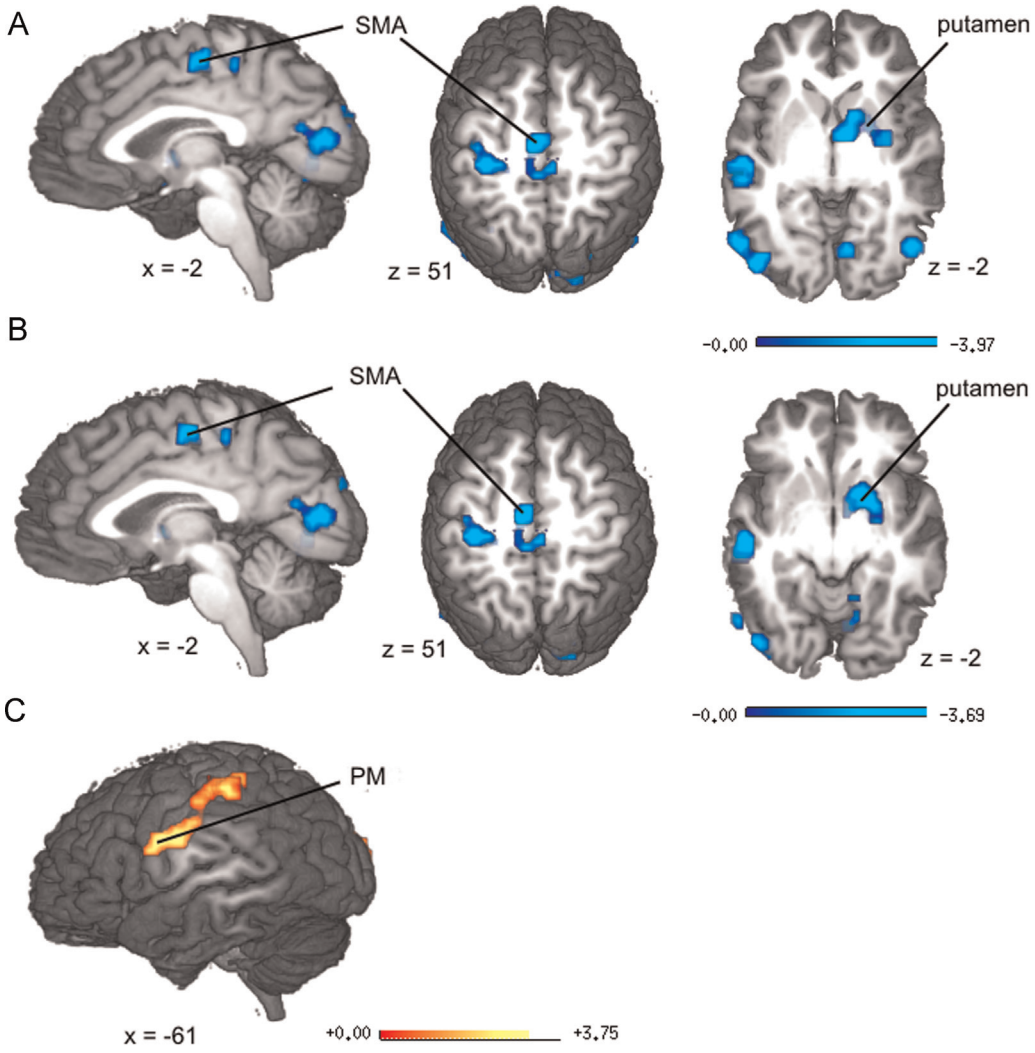

Fig. 3. Significant activation differences in serial prediction tasks. (A) Hypoactivity of patients ON compared to controls during the SPT [(controls (SPT0 > resting) vs. patients ON (SPT0 > resting)) ∩ controls (SPT+ > resting) vs. patients ON (SPT+ > resting)]. (B) Hypoactivity of patients ON compared to controls during the SMTS [(controls (SMTS > resting) vs. patients ON (SMTS > resting)) ∩ controls (SMTS > resting) vs. patients ON (SMTS > resting)]. (C) Hyperactivity in patients OFF compared to ON medication [patients OFF (SPT+ > SPT0) vs. patients ON (SPT+ > SPT0)]. Paired *t*-tests ($p < 0.05$) whole-brain corrected for multiple comparisons. Significant areas of hypoactivity (in blue) and hyperactivity (in red) are superimposed on 3D brain rendering.

Table 4
Areas more activated in controls than in patients ON medication during performance of SMTS and SPT compared to resting [controls (SMTS>resting) vs. patients ON (SMTS>resting) ∩ controls (SPT0>resting) vs. patients ON (SPT0>resting) ∩ controls (SPT+>resting) vs. patients ON (SPT+>resting)].

Localisation	BA	Size	Coordinates			*Z*
			x	*y*	*z*	
SMA (bilateral)	6	1566	−8	−18	60	3.37
Primary motor cortex (L)/primary somatosensory cortex (L)	4 / 3	1566	−29	−21	48	3.23
Putamen (R)		2214	31	−3	6	2.88
Cuneus (L)	18	6966	−14	−84	18	3.88
Middle temporal gyrus (L)	21	1296	−50	−18	−3	3.50

patients ON and controls in a paired-*t*-test [patients ON (SPT+ > SPT0) vs. controls (SPT+ > SPT0)]. No significant differences were found here. There also were no significant differences in the contrast SPT+ events > SPT0 events in the event-related analysis.

3.2.3. BOLD differences in patients ON and OFF

Regarding the comparison of patients ON vs. OFF, we expected that hypoactivity in SMA and putamen would be attenuated by medication. The conjunction of two paired-t-tests comparing the effect of medication in SPT0 > SMTS and SPT+ > SMTS yielded no significant activations [patients ON (SPT0 > SMTS) vs. OFF (SPT0 > SMTS)∩patients ON (SPT+ > SMTS) vs. OFF (SPT+ > SMTS)]. Further, there was no medication effect during the SPT compared to resting [patients ON (SPT0 > resting) vs. OFF (SPT0>resting) ∩ patients ON (SPT+ > resting) vs. OFF (SPT+ > resting)]. However, a paired-t-test calculating differences between patients ON and OFF in SPT+ compared to SPT0 [patients ON (SPT+ > SPT0) vs. OFF (SPT+ > SPT0)] revealed a significantly enhanced BOLD effect OFF in the left ventral PM, extending into the parietal lobe via primary motor and somatosensory areas (Table 5 and Fig. 3). Parietal areas are usually co-activated with the premotor system during serial prediction (Schubotz, 2007), reflecting premotor-parietal loops (Rizzolatti and Luppino, 2001).

Furthermore, the event-related analysis also showed more activity in the left ventral PM of patients OFF than ON when comparing SPT+ with SPT0 [patients ON (SPT+ events > SPT0 events) vs. OFF (SPT+ events > SPT0 events)]. The dorsolateral prefrontal cortex and anterior cingulate cortex were also significantly higher activated (Table 5).

Table 5
Areas significantly more activated in patients OFF than patients ON medication during performance of SPT+ compared to SPT0 (A) in the epoch based analysis [patients ON (SPT+ > SPT0) vs. patients OFF (SPT+ > SPT0)] and (B) the event-related analysis [patients ON (SPT+ event > SPT0 events) vs. patients OFF (SPT+ events > SPT0 events)].

Localisation	BA	*k*	Coordinates			*Z*
			x	*y*	*z*	
(A) Epoch based analysis						
Lateral premotor cortex extending into primary motor and somatosensory areas	6/4/2/3/40	1809	−62	−8	27	4.05
Lateral occipital gyrus (R)	18/19	2376	34	−92	9	3.64
(B) Event-related analysis						
Lateral premotor cortex extending into primary motor cortex	6/4	1755	−62	−8	27	4.17
Dorsolateral prefrontal cortex extending into anterior cingulate cortex	8/9/32	3078	25	10	33	3.93

These analyses implicate that medication did not attenuate hypoactivity in SMA and putamen; instead PM was more active OFF than ON medication, as expected, but only when load on the SMA-putamen loop was increased. Our assumption that this PM hyperactivity represents a compensational mechanism is tested in the following section by relating it to the patients' performance.

3.3. Regions of interest analysis

We expected the patients' SMA activity to be positively correlated with performance, especially in SPT+. Furthermore, activity of PM should be related to preserved performance provided that SMA is hypoactive and compensation via PM is effective.

Considering results in SPT0 first, neither controls nor patients ON showed a significant relation between ROI beta-values (SPT0 > resting) and performance (Table 6). In contrast, patients OFF exhibited a significant positive correlation of performance with beta-values in left PM ($r=0.593$, $p=0.03$). Conducting analyses for SPT+, patients OFF exposed a similar positive correlation of performance with beta-values (SPT+ > resting) in left PM ($r=0.591$, $p=0.032$). This relation also applied to patients ON, whose performance in SPT+ was positively correlated with activity in left PM ($r=0.558$, $p=0.05$), and additionally with activity in left SMA ($r=0.557$, $p=0.05$). Beta-values of controls showed no significant correlation with performance in SPT+ (Table 6). Patients' OFF-performance depended on SMA activity in a specific way: The analysis of beta-values derived from the contrast SPT+ > SPT0 revealed significant positive correlations of left SMA activity ($r=0.676$, $p=0.008$) and right SMA activity ($r=0.607$, $p=0.026$) with performance in SPT+. This relation of better performance to increased activity in SPT+ than SPT0 was not found in patients ON and controls, and did not apply to left or right PM in any of the groups (Table 6).

There were no significant correlations of either SMA or PM activity with performance in the SMTS in any group (Table 7).

Finally, we expected that SMA hypoactivity varies as a function of individual motor impairment according to UPDRS III. Indeed, UPDRS III scores in ON-state were negatively correlated with beta-values (SPT+ > SPT0) in left SMA ($r=-0.584$, $P=0.034$) and right SMA ($r=-0.689$, $P=0.006$). Unexpectedly, this relation was also found in left PM ($r=-0.572$, $P=0.042$). Correlations of UPDRS III with beta-values derived from the other contrasts (SPT0 > resting, SPT+ > resting, SMTS > resting) did not gain significance in medicated patients, pointing to a specific negative relation between severity of PD symptoms and the ability to intensify activity in SMA and left PM during SPT+. No correlations between beta-values OFF and UPDRS III scores OFF were found. All significant correlations of performance and UPDRS III scores with beta-values are diagrammed in Fig. 4.

4. Discussion

In the present study, we investigated how the so-called "motor loop" connecting SMA and striatum (Alexander et al., 1986) contributes to cognitive dysfunction in Parkinson's disease (PD). Drawing on a compensational mechanism implemented by PM, which is known to accompany pathological hypoactivity of SMA and striatum in motor tasks (Samuel et al., 1997; Haslinger et al., 2001; Mallol et al., 2007), we tested whether in a cognitive task PD patients' SMA hypoactivity could also be compensated by PM hyperactivity. We tested 16 male patients suffering from PD ON and OFF dopaminergic medication and 16 male healthy controls in the serial prediction task (SPT), which engages both PM and SMA in healthy subjects (Schubotz and Von Cramon, 2003; Schubotz, 2007). We applied two versions of the SPT, one with on-going sequences of stimuli (SPT0), and one with sequences containing non-informative stimuli, so-called "occluders", increasing the demand to build an internal representation of the sequence (SPT+).

Indeed, the pattern of our behavioural and functional findings show the interplay of SMA hypoactivity disrupting serial prediction performance and point to PM hyperactivity restoring performance: On the one hand, we found less activity in SMA and putamen during SPT and a serial match-to-sample task (SMTS) combined with poor SPT performance when patients were compared to controls. On the other hand, PM was hyperactive after withdrawal of medication in SPT+ while patients OFF showed poorer performance relative to ON medication. Importantly, SMA as well as PM activity positively correlated with the patients' performance.

Considering the group comparison first, patients exhibited poorer SPT performance than controls while the patients'

Table 6
ROI analysis: Correlation (* when significant at $p<0.05$, corrected for multiple comparisons) of beta-values and performance (P_R=probability of recognition) in SPT.

		P_R(SPT0)		P_R(SPT+)			
Group	ROI	with beta-values (SPT0 > Resting)		with beta-values (SPT+ > Resting)		with beta-values (SPT+ > SPT0)	
Hemisphere		*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
Controls	SMA	0.283	0.163	0.396	−0.162	−0.263	−0.306
	PM	0.177	0.278	0.018	0.017	0.109	0.155
Patients ON	SMA	0.440	0.092	0.558*	0.389	0.347	0.065
	PM	0.489	0.396	0.557*	0.498	0.136	0.159
Patients OFF	SMA	0.384	0.305	0.502	0.331	0.676*	0.607*
	PM	0.593*	0.234	0.591*	0.210	0.477	0.311

Table 7
ROI analysis: Correlation (* when significant at $p < 0.05$, corrected for multiple comparisons) of beta-values and performance (P_R=probability of recognition) in SMTS.

Group	ROI	SMTS > Resting		SPT0 > Rest		SPT+ > Rest		SPT+ > SPT0	
		Left	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
Controls	SMA	0.273	0.343	0.344	0.439	0.348	0.431	0.134	0.219
	PM	−0.164	−0.001	0.042	−0.054	0.030	−0.013	−0.028	0.147
Patients ON	SMA	0.064	0.004	0.052	0.115	0.027	0.204	−0.081	0.286
	PM	0.245	0.004	0.058	0.012	0.114	0.169	0.309	0.369
Patients OFF	SMA	0.201	0.023	0.144	−0.281	0.247	−0.203	0.416	0.163
	PM	0.213	0.069	0.229	0.146	0.295	0.256	0.372	0.346

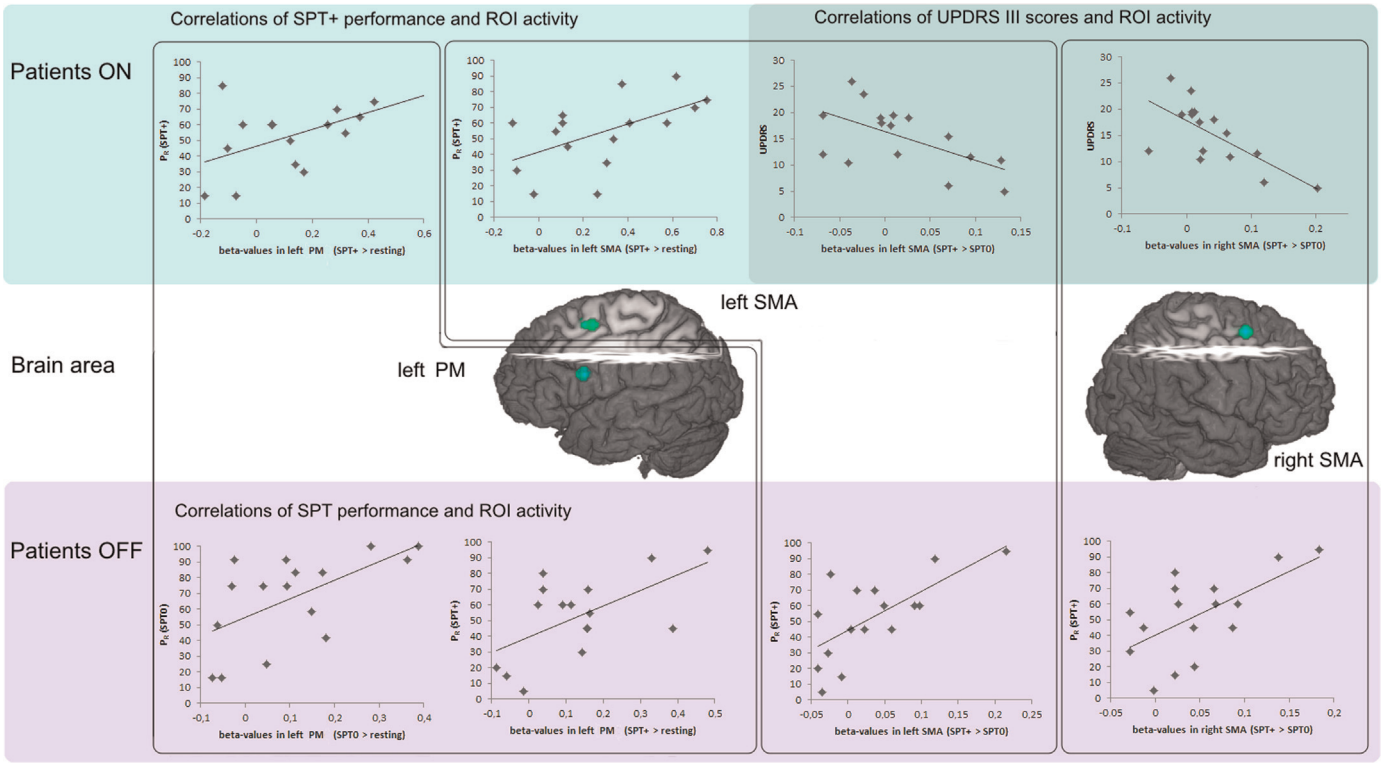

Fig. 4. Scatter plots of significant correlations between SPT performance and beta-values and UPDRS III scores and beta-values. Abbreviations: UPDRS=UPDRS III scores ON dopaminergic medication; P_R=probability of recognition.

performance in SMTS as well as in PANDA was not significantly worse than performance of controls. This suspends the possibility that patients' deficits in SPT resulted from global cognitive dysfunction or working memory deficits. Even more important, the SPT does not rely on prefrontal functions, but recruits primarily the premotor network (Schubotz and Von Cramon, 2003), and we did not observe differential prefrontal activations in controls and patients ON medication**.** There was only one contrast showing enhanced activity in dorsolateral prefrontal cortex and anterior cingulate cortex in concert with PM when we conducted the event-related analysis**.**

Relative to healthy controls, PD patients showed a decreased activity in SMA and striatum during both the SPT and the SMTS. We suppose that this hypoactivity emerged in both tasks as they recruited both of these areas due to their serial composition. Note that activation differences in motor areas between groups were not due to differences in the motor responses of controls and patients as there were no differences in the number of responses given by either group. Moreover, it can also be ruled out that activation differences in SMA were due to abnormal resting state activity in PD patients. Skidmore et al. (2013) found that PD patients show a hypoactive SMA during resting conditions and no abnormal pattern in the striatum. This means that we rather underestimated SMA hypoactivation in PD patients, relative to healthy controls, when contrasting SPT and SMTS with resting state.

In contrast to the SMTS, the SPT additionally recruited SMA, PM and parietal projection areas as shown in previous studies (e.g., Schubotz and von Cramon, 2004; Schubotz, 2007). This confirms that all participants showed a specific premotor involvement in serial prediction beyond working memory engagement. Most importantly, the hypoactivity of SMA and putamen affected performance in the SPT whereas patients showed an equal performance to healthy controls in the serial match-to-sample task. These results indicate that accomplishing the serial match-to-sample task draws on other, presumably also prefrontal areas, while successful serial prediction particularly relies on SMA and striatum. We therefore assume that the motor loop's affection caused the observed deficits in serial prediction. This is additionally corroborated with the finding that correlations between BOLD response, UPDRS III scores and task performance showed no significant relation of SMA and PM activity to the serial match-to-sample task,

but significant and specific relations with serial prediction (see Fig. 4): Correlations of UPDRS III scores and SMA activity revealed a close relationship of bilateral SMA activity during the SPT with the individual degree of the motor loop's affection. The more patients suffered from motor impairment, the less their SMA activity increased during SPT+ compared to SPT0. Thus, patients with severe motor symptoms were unable to intensify SMA activity during engagement in SPT+, the task that was significantly more difficult than SPT0 for all participants.

Interestingly, higher motor impairment also came along with reduced enhancement of left PM activity in SPT+ compared to SPT0. Possibly, medicated patients whose SMA was working to capacity in SPT0, so to say, made use of left PM as compensational mechanism, leading to the situation that SMA as well as PM activity could not be further intensified in SPT+. If this interpretation holds true, PM activity should serve as compensational mechanism already in SPT0 in those PD patients whose SMA function via the motor loop is severely impaired. Indeed, this pattern was reflected in the correlations between BOLD response and SPT performance: Controls and medicated patients did not exhibit a significant relation between activity in SMA or PM and performance in SPT0, whereas patients OFF with higher left PM activity showed increased performance in SPT0 as well as SPT+. However, since we found patients to produce more errors OFF than ON medication, it seems that compensatory mechanisms could not fully restore performance. Moreover, performance ON correlated with left PM activity only in SPT+, probably because SMA activity in most patients under medication was still sufficient to perform well in SPT0. The importance of SMA activity for successful engagement in SPT+, especially without medication, was demonstrated by its particular correlation with performance in SPT+: patients OFF performed the better the more they were able to recruit left and right SMA in SPT+ compared to SPT0. Concurrently, intermission of medication caused hyperactivity in left PM during execution of SPT+ that probably compensated the inability to further increase SMA activity despite heightened demand on internal sequence representation. Notably, patients' performance dropped OFF medication in SPT0 as well as SPT+ when controlling for practice effects, whereas in our previous behavioural study (Schonberger et al., 2013) withdrawal of medication led to particularly impaired performance *only* in the SPT+. Possibly, the observed hyperactivity in left PM during SPT+ prevented the patients in the present study from even poorer performance in this more difficult task.

Taken together, medicated patients showed a deficit in serial prediction when compared to controls accompanied by the expected hypoactivity in SMA and striatum which appeared during the serial prediction and the serial match-to-sample task. Further, patients revealed an impaired serial prediction performance after withdrawal of medication together with PM hyperactivity when the necessity to build an internal presentation of the sequence was heightened in SPT+. We assume that PM hyperactivity was compensatory and prevented the patients without medication from an even stronger drop in performance, as correlations between performance and brain activity indicate: Patients' performance in SPT+ depended on the level of left PM and SMA activity and, without medication, on the bilateral increase of SMA activity compared to SPT0 while performance. In contrast, the performance in the serial match-to sample task did not correlate with PM or SMA activity. These results demonstrate for the first time a significant correlation of compensatory PM activity with performance and confirm the assumption of a close interplay between SMA hypoactivity and PM hyperactivity.

By which mechanisms does the interplay of SMA and PM support performance in internal sequence representation in the SPT? SMA has been shown to conduct memory-guided sequential movements in monkeys (Mushiake et al., 1990; Halsband et al., 1994) by activity of specified types of neurons which Shima and Tanji (2000) found in SMA and pre-SMA. One type of these neurons revealed preparatory activity before performing a specific order of movements which can be interpreted as retrieval of the particular sequence. A second type was activated during intervals between movements, thus providing a link to the next movement, and a third type was selective for the rank order (1st, 2nd or 3rd element of the sequence). There was no preparatory and interval-selective neuronal activity under visual guidance, but it developed gradually with trial repetition and sequence learning. In this manner SMA is capable of generating internal sequence representations and controlling a sequence's progress in time. Importantly, many SMA neurons are multimodal (Ikeda et al., 1999) pointing to the possibility that SMA not only codes motor sequences, but also sequences of perceptual events. Therefore, we assume SMA to monitor the sequences of stimuli presented in the SPT and to be crucial for detection of violations of their serial order.

How can we think of PM to support cognitive sequencing when SMA function is deficient? Comparisons between BOLD activation induced by the SPT and, for instance, serial-to-match or serial detection tasks, have shown that PM is more sensitive to the sequential order of stimuli then to the mere occurrence of the stimuli themselves (Schubotz and Von Cramon, 2003). Thus in the current study, PM may promote deficient SMA sequence representation by providing a bias to an expected transition of two stimuli. When presented with a void stimulus, direct cortical input from PM to SMA may even help bridging two missing transitions, though not in the same stable manner warranted by operating striatal loops. The striatal loops' contribution to cognitive sequencing can be understood in the framework put forward by Graybiel (1998), according to which the striatum chunks motor performance in order to allow implicit learning of sequences. This chunking is thought of as an evidence-based probabilistic weighting of the transition between consecutive cortical activation patterns. Importantly, this striatal learning mechanism depends on dopaminergic projections from substantia nigra and should be particularly impaired in patients without medication which we observed to exhibit PM hyperactivity. Since SMA and PM maintain direct reciprocal connections, PM may help generating an internal sequence representation in SMA by providing stimulus information not pre-processed by striatum. Concurrently, input of PM to striatum may be strengthened and result in enhanced input to SMA via the motor loop despite dopaminergic depletion.

As an alternative explanation for our findings, one could assume PM activity to contribute to performance in SPT independent of SMA function. In this case, PM may have improved performance by providing information for other cortical areas, e.g., prefrontal regions which store and update stimulus information in working memory. This interpretation is in line with finding PM and the dorsolateral prefrontal cortex co-activated in the event-related analysis. Maybe, the prefrontal activations represent a working a memory process helping to maintain the internal sequence representation in PM (Curtis and D'Esposi, 2003). However, even in this scenario, the SMA's importance for sequence processing is corroborated, as the possibly stabilising prefrontal function is found to be increased OFF medication when the motor loop's function is impaired.

Our results do not support the common view that cognitive dysfunction in non-demented PD patients, often subsumed under the notion of “executive dysfunction”, can be utterly attributed to dopamine deficiency in basal ganglia–prefrontal circuits (Kudlicka et al., 2011; Ray and Strafella, 2012; Dirnberger and Jahanshahi, 2013; Kehagia et al., 2012). The contribution of an impaired dorsolateral circuit to cognitive deficits in PD is well described (Brück

et al., 2001; Cools et al., 2001; Polito et al., 2012), but the role of the motor circuit was neglected so far even though recent studies suggest a close interplay of motor and cognitive decline in PD (Amboni et al., 2008; Vercruysse et al., 2012). Furthermore, akinetic-rigid compared to tremor-dominant patients reveal a higher risk of cognitive decline (Alves et al., 2006; Burn et al., 2006) accompanied by a greater dopamine loss in putamen, i.e., the striatal component of the "motor loop" (Eggers et al., 2011). Thus, associating cognitive dysfunction in PD with executive dysfunction may be an oversimplification.

Rather, our current findings point to a contribution of SMA hypoactivity to deficits in cognitive sequencing. Patients are known to reveal deficits in sequence learning (Jackson et al., 1995; Doyon et al., 1997; Shin and Ivry, 2003; Siegert et al., 2006), but most of the studies investigating sequencing in PD employ motor learning, e.g., the serial reaction time task (Nissen and Bullemer, 1987). However, it is possible to disentangle cognitive sequencing abilities from motor sequence learning by presenting sequences of perceptual stimuli independent of motor responses (Hoffmann and Koch, 1997). The SPT applied in the current study refines this principle and merely draws on perceptual sequencing, thus showing that patients suffering from PD are impaired in a purely cognitive task due to affection of the motor loop. This dovetails with the recruitment of SMA and PM in healthy individuals performing the SPT (Schubotz and von Cramon, 2004; Schubotz, 2007). Further findings point to the fact that impaired cognitive abilities are responsible for reduced performance in the serial reaction time task, since it correlates with scales for outcomes in PD cognition (SCOPA-COG), an assessment tool for cognitive deficits in PD (Vandenbossche et al., 2009). Interestingly, the SCOPA-COG (Marinus et al., 2003) contains four out of ten items that require sequencing (repeating a digit span backwards, pointing to cubes in a given sequential order, naming the months backwards, performing a given sequence of hand movements) and another three items at least partly depend on sequencing abilities (i.e., verbal recall of a sequence of 10 words, counting backwards, delayed recall of the 10 words). As our results suggest, performance in SCOPA-COG is related to motor impairment: patients suffering from freezing of gait compared to those without freezing of gait were found to be significantly impaired in SCOPA-COG (Vercruysse et al., 2012). Another tool often used to assess cognitive abilities in PD is the Trial-Making Test (TMT; Bowie and Harvey, 2006). It also involves cognitive sequencing since it requires switching between two sequences (1-A-2-B-3-C…), and was proven to activate the premotor system (Moll et al., 2003), thus supporting the notion of the motor loops' contribution to cognitive sequencing.

5. Conclusions

Cognitive impairment in Parkinson's disease cannot be explained by a single mechanism. Considering that the basal ganglia are at the core of functional decline, it is plausible to expect that difficulties arise from dysfunction in more than one cortico-striatal–thalamo-cortical loop. Since Alexander and colleagues used the label "motor loop" in their seminal 1986 paper to address the circuit connecting SMA to the putamen, researchers have been prone to interpret motor loop dysfunction, by definition, as solely affecting motor functions. Current findings, together with fMRI studies in healthy subjects, reveal that the PD-associated decline of serial prediction results from motor loop rather than prefrontal loop dysfunction.

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