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Functional Networks in Motor Sequence Learning: Abnormal Topographies in Parkinson's Disease

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Abstract: We examined the neural circuitry underlying the explicit learning of motor sequences in normal subjects and patients with early stage Parkinson's disease (PD) using ^{15}O-water ($H_2{}^{15}O$) positron emission tomography (PET) and network analysis. All subjects were scanned while learning motor sequences in a task emphasizing explicit learning, and during a kinematically controlled motor execution reference task. Because different brain networks are thought to subserve target acquisition and retrieval during motor sequence learning, we used separate behavioral indices to quantify these aspects of learning during the PET experiments. In the normal cohort, network analysis of the PET data revealed a significant covariance pattern associated with acquisition performance. This topography was characterized by activations in the left dorsolateral prefrontal cortex (PFdl), rostral supplementary motor area (preSMA), anterior cingulate cortex, and in the left caudate/putamen. A second independent covariance pattern was associated with retrieval performance. This topography was characterized by bilateral activations in the premotor cortex (PMC), and in the right precuneus and posterior parietal cortex. The normal learning-related topographies failed to predict acquisition performance in PD patients and predicted retrieval performance less accurately in the controls. A separate network analysis was performed to identify discrete learning-related topographies in the PD cohort. In PD patients, acquisition performance was associated with a covariance pattern characterized by activations in the left PFdl, ventral prefrontal, and rostral premotor regions, but not in the striatum. Retrieval performance in PD patients was associated with a covariance pattern characterized by activations in the right PFdl, and bilaterally in the PMC, posterior parietal cortex, and precuneus. These results suggest that in early stage PD sequence learning networks are associated with additional cortical activation compensating for abnormalities in basal ganglia function. *Hum. Brain Mapping 12:42–60, 2001.* © 2001 Wiley-Liss, Inc.

Key words: sequence learning; brain networks; PET; Parkinson's disease

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of dopamine producing cells in the substantia nigra. Although the neurochemical lesion is mainly localized to the nigrostriatal dopamine system, the effects of dopamine loss are widespread and affect non-dopaminoceptive neurons in brain regions constituting crucial elements of motor control and other pathways [Wichmann and DeLong, 1996]. Indeed, other than the known motor abnormalities encountered in PD, this disorder can also result in a degradation of cognitive function, including visuomotor learning and memory [e.g., Taylor et al., 1986; Levin et al., 1989; Brown and Marsden,

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1990; Dubois and Pillon, 1997]. Previous PET studies in PD have emphasized functional abnormalities associated with the execution of movement sequences of varying complexity [Brooks, 1995; Samuel et al., 1997; Catalan et al., 1999]. Nevertheless, although a body of behavioral and anatomical data exists to implicate the striatum in the learning of sequences [Graybiel, 1995; Brown, 1999], little is actually known about the functional substrates of this behavior in PD and other diseases of the basal ganglia.

The learning of motor sequences is mediated by two distinct processes: implicit learning represents the ability to acquire skills through repeated practice without conscious awareness; explicit learning involves conscious recollection of episodic events. During the learning of motor sequences in a serial reaction time paradigm [Nissen and Bullemer, 1987], both forms of learning may take place concurrently [Willingham et al., 1989]. In this paradigm, subjects are instructed to respond differentially to an array of stimuli: unbeknownst to them, the stimuli are ordered as repeating sequences. Learning is manifest as a progressive reduction in reaction time, which does not occur when the stimuli are in random order. Although subjects are initially unaware of the presence of a repeating sequence, the learning may be implicit or explicit.

The neural basis of sequence learning has been studied in normal subjects performing serial reaction tasks using ^{15}O-water ($H_2{}^{15}O$) and positron emission tomography (PET) [Grafton et al., 1995; Rauch et al., 1995; Honda et al., 1998]. However, in its standard form [Nissen and Bullemer, 1987], this task is not well suited for imaging in patients with neurological diseases. Firstly, the sequences are long and complex, presumably to favor implicit learning. Thus, learning may take four to six blocks of 100 movements each, an amount of time far too long for PET recording. Secondly, the time at which explicit knowledge is achieved varies substantially from subject to subject. This is likely to be due to the varied attention given by subjects to the conscious identification of a recurring pattern in the stimuli. Thus, the PET images obtained in the serial reaction time paradigm represent the implicit and explicit learning processes in varying proportions. In the present study we used a motor task derived from basic paradigms described in previous reports [Hening et al., 1988; Ghez et al., 1997; Krakauer et al., 1999; Ghilardi et al., 2000], in which simple repeating sequences may be learned in ninety seconds or less. Instructions emphasized detection and explicit learning of the sequence. Moreover, with this study we analyzed two functional components involved in all forms of learning: acquisition, or the encoding of initial information that initiates a memory trace, and retrieval, which relies upon prior encoding [e.g., Tulving et al., 1994]. The parcellation of learning into discrete components may be critical for the elucidation of abnormal learning mechanisms in pathological situations.

PET studies in normal subjects have demonstrated activation of the prefrontal, premotor, and posterior parietal cortices in association with explicit learning of movement sequences [Jenkins et al., 1994; Grafton et al., 1995; Rauch et al., 1995; Jueptner et al., 1997; Honda et al., 1998]. Additionally, recent studies using functional magnetic resonance imaging (fMRI) have described the time course of this activation [Sakai et al.. 1998; Toni et al., 1998]. Nonetheless, previous studies have not explored how patterns of brain activation relate to the specific components of sequence learning performance nor how these patterns might be altered in parkinsonism.

In this study, we sought to identify the specific brain regions that are associated with acquisition and with retrieval performance during explicit motor sequence learning in both normal volunteers and in unmedicated age-matched early stage PD patients. In addition to univariate brain-behavior correlational analyses that do not necessarily address regional connectivity, we utilized a novel network modeling approach to brain activation data [Moeller et al., 1998; Alexander et al., 1999]. We hypothesized that the acquisition and retrieval functions are associated with discrete brain networks. Moreover, we examined the possibility that these performance-related network topographies are abnormal in parkinsonism.

MATERIALS AND METHODS

We studied eight right-handed normal volunteer subjects (five men and three women; age 56.3 ± 11.0 years [mean ± SD]). These subjects were recruited by advertisement among local PD support groups, and among North Shore University Hospital personnel. The following exclusion criteria were used: (a) past history of neurological or psychiatric illness; (b) prior exposure to neuroleptic agents or drug use; (c) past medical history of hypertension, cardiovascular disease and diabetes mellitus; and (d) abnormal neurological examination.

We also studied 16 right-handed age-matched patients with mild [Hoehn & Yahr (H&Y) Stage I] idiopathic PD (12 men and four women; age 59.6 ± 10.1 years; disease duration, 3.3 ± 3.0 years). A diagnosis of PD was made if the patient had "pure" parkinson-

ism without a history of known causative factors such as encephalitis or neuroleptic treatment; did not have dementia, supranuclear gaze abnormalities or ataxia; and had a convincing response to a single oral dose of dopaminergic therapy (≥20% improvement in Unified Parkinson's Disease Rating Scale [UPDRS]; motor ratings [items 19 to 31; Fahn and Elton, 1984]). The PD cohort was comprised of two clinical subgroups: eight patients had right upper limb involvement; eight patients had left limb involvement. Parkinsonian signs and symptoms were primarily akinetic-rigid. Mild resting tremor (UPDRS tremor ratings 1–2 in affected limbs) was present in seven of the patients (three in the left hemi-PD subgroup and four in the right hemi-PD subgroup), and did not interfere with the execution of the motor tasks (see below). At the time of recruitment into this study, seven of the patients had been chronically unmedicated, and three others had been treated with deprenyl alone. The six remaining patients had been chronically treated with levodopa/carbidopa, two of whom also received dopamine agonist therapy.

All patients and normal volunteers had scores >27 on Mini-Mental Examination [Folstein et al., 1975], and underwent magnetic resonance imaging (MRI) to exclude potential structural brain lesions (e.g., stroke, mass lesion, or hydrocephalus/atrophy), and for three-dimensional (3D) PET-MRI image coregistration and region of interest (ROI) placement (see below). Written informed consent was obtained from all participants under a protocol approved by the Institutional Review Board of North Shore University Hospital.

Behavioral tasks

The motor tasks required subjects to move a handheld cursor on a 12" × 18" digitizing tablet (Numonics Corporation) while their hand and target locations were displayed on a 15" computer screen. A Macintosh computer (Apple computer) controlled the experiment, generated screen displays and acquired kinematic data from the digitizing tablet at 200 Hz. On the days prior to PET scan, all subjects were given two sessions of training to become familiar with all tasks and furthermore to achieve stable levels of accuracy in a motor reference task. During practice, subjects sat facing the computer screen and moved their dominant right hand on a horizontal surface at waist height. During scanning, subjects were supine and moved their hand on the digitizing tablet supported over their chest. The computer monitor was placed vertically within the subject's field of vision.

All motor tasks required out and back movements of the hand from a central start area to one of eight radial targets (45° apart). The start area and target locations on the screen were displayed on a white background as 2 cm diameter circles (Fig. 1). At target presentation (1 per sec) one circle turned black, in synchrony with a 160 ms tone. All trial blocks lasted 90 sec. At the start of a trial block, subjects positioned the screen cursor within the central start area and a series of three tones were sounded at 1 Hz to provide the required tempo of the movements to follow. With the fourth and subsequent tones, successive targets turned black and subjects were instructed to move their hand smoothly out and back to each target without corrections and with sharp reversal. Movement extent was the same across motor tasks. As illustrated in Figure 1, if the movement reached the target within the set time window, the target turned gray, signaling a successful hit. The number of hits was displayed to the subjects on the screen after each block of trials.

The experimental paradigm consisted of three tasks during PET imaging: (1) a motor reference execution task (M*pred*) where subjects reached *pred*ictable targets in synchrony with a tone [Ghilardi et al., 2000]; (2) a sequence learning task (RT*learn*) which started in reaction time mode; and (3) a sensory reference task (S). A reaction time task with random sequences (RT*ran*) was performed in the scanner before each PET imaging session.

M*pred*

In this task, targets appeared in a predictable counterclockwise order, starting from the target at 3 o'clock. Subjects were instructed to reach for each successive target and to synchronize the reversal of their hand movements as closely as possible with the tone as the target appeared. Thus, subjects anticipated each target and initiated movements before it appeared as in 'timed response' tasks [Hening et al., 1988; Ghez et al., 1997; Ghilardi et al., 2000]. If the movement reached the target within a time window defined as 250 ms prior to and after each tone, the target turned gray, signaling a successful hit.

RT*learn*

In this task, subjects were again instructed to reach for successive targets and to synchronize their reversal with target appearance and tone. However, the eight targets appeared in a pseudo-random repeating sequence without repeating elements. Subjects were informed of this and instructed to discover and learn the

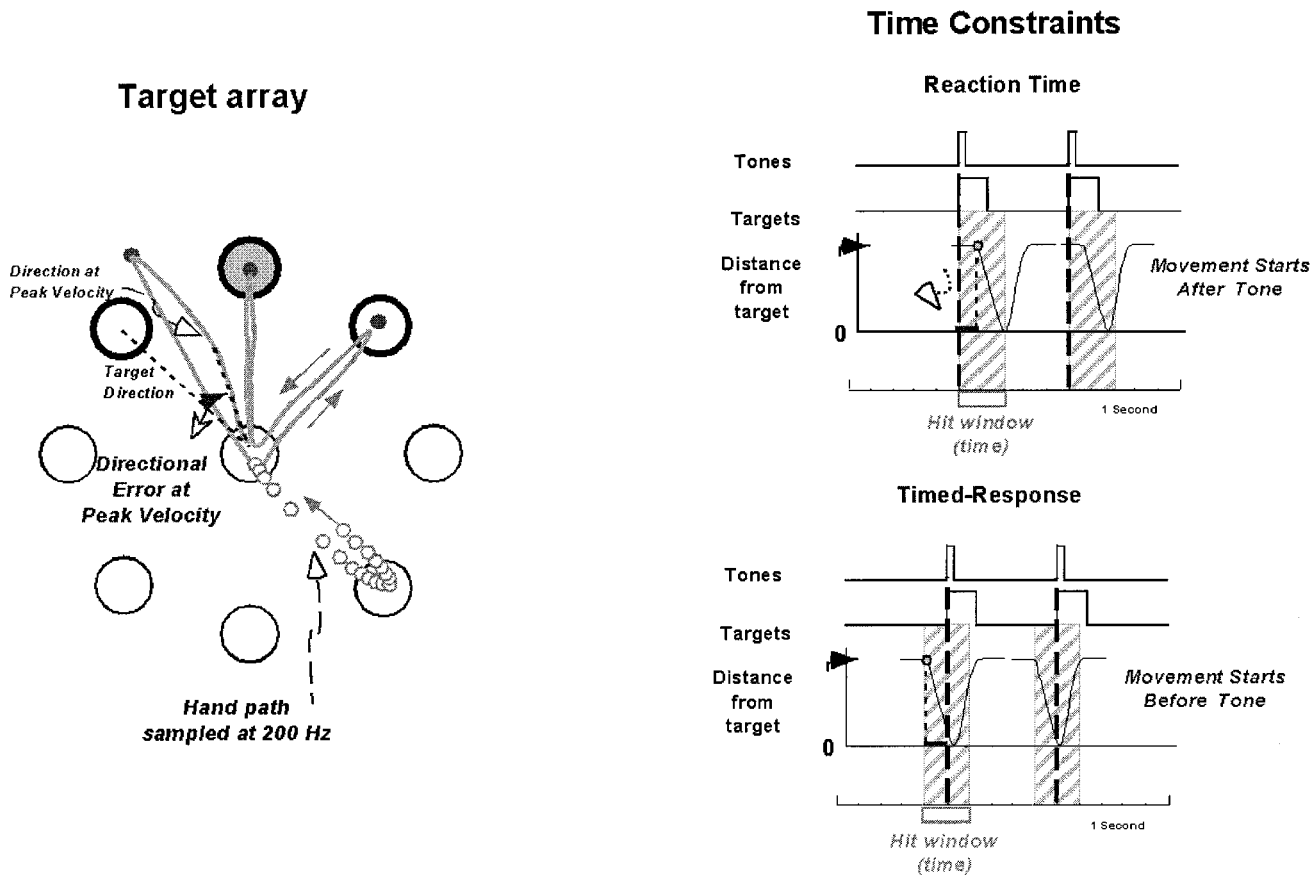

Figure 1.

Left: Task design: Array of eight targets displayed on a screen together with three trajectories. In the psychophysical experiments, targets become gray when hit in the appropriate time window, as shown for the target at 12 o'clock. Vectors for target direction and movement direction at peak velocity are indicated by dotted line. The directional error at the peak velocity is the directional difference between the two vectors (see text). **Right**: Time constraints for the reaction time (top) and the time response (bottom) paradigms.

sequence order so as to anticipate the target and reach it as it appeared. At the end of the trial, the subjects were asked to indicate the order of the sequence verbally.

During training sessions conducted before imaging, each subject experienced two or three different sequences; during PET imaging experiments, entirely different sequences were used. The same sequences were used for both the normal control and the PD experiments.

RT*ran*

This task was performed before PET scanning. Targets were presented in pseudo-randomized, non-repeating, and unpredictable order. Subjects were required to reach for each target "as soon as possible," minimizing both reaction time and movement time, and as accurately as possible.

In the sensory reference task, used only during scanning, subjects remained immobile but experienced the same visual and auditory stimuli as during the motor activation tasks. Screen targets, cursor images and tones were presented to the subjects asynchronously and irregularly in equal numbers to those used in the motor tasks. Subjects were instructed to attend to the screen and, at the end of the trial, to report, which of the targets did not turn gray at the end of the trial. This occurred randomly to one of the eight targets. This task was designed to serve as a sensory reference condition for M*pred*.

Psychophysical data analysis

Automatic routines plotted cursor (and thus hand) positions and digitally differentiated the data to identify the location and time bins of movement onset,

peak velocity, peak acceleration, and movement reversal. The marking for each movement trajectory was then checked visually by one of the experimenters and, if not appropriate, was manually changed. For each movement the cursor position at the velocity zero cross, calculated backward from the peak, was defined as movement onset; the location of the velocity minimum at the direction reversal was taken as the movement end point. We computed the following performance variables for each movement: (1) Spatial error (cm), the shortest distance of the reversal point from the center of the target; (2) Movement time (msec), the time from the onset of the outward motion to the reversal point; and (3) Onset time (msec), the time from target and tone presentation to movement onset. Depending upon the experimental time constraint (timed response or reaction time), this measure corresponds to the movement latency or the reaction time. Negative values signify anticipatory responses that are initiated before the tone. The more negative the onset time, the greater the subject's ability to initiate movement in advance of the tone [Hening et al., 1988; Ghez et al., 1997; Ghilardi et al., 2000]. For all motor tasks, we computed means and variances across the entire trial block, as well as for each complete cycle of eight movements.

In RT*learn*, we calculated the mean onset time and the number of the correct responses for each cycle (i.e., every eight target presentations). We defined correct responses movements with directional error of 22° or less at peak velocity, as illustrated in Figure 1. We also computed other behavioral measures to quantify separately the acquisition of the target sequence and its retrieval during the execution of the movements. Because subjects were instructed to identify the sequence explicitly and to reach for the correct target before it appeared, anticipatory movements to the correct target were considered to reflect explicit learning. All the movements initiated below the lowest onset time in RT*ran* were considered anticipatory. Examination of the reaction time distributions in all cases for RT*ran* showed that these lowest values represented the floors and not anticipatory responses or outliers. (This is supported by the fact that none of the movements in any of the subjects was directed to the wrong target in this fully randomized set).

The total number of correct anticipatory movements achieved during the scanning epoch was considered to be an objective measure of overall retrieval of previously acquired targets, i.e., a global retrieval index. Additionally, in each cycle, we identified the movements to targets that were correctly anticipated in that cycle but which were not correctly anticipated in the preceding cycle. The total number of these movements was considered to reflect successful acquisition of *new* targets, i.e., a global acquisition index. We also quantified the number of accurate target locations reported by the subject at the end of this task (0 = unawareness of a repeating sequence to 8 = complete correct sequence). This declarative score represented an additional measure of the explicit learning achieved.

MRI

These studies were performed on a 1.5T GE Signa scanner (at 5.4 software level; General Electric, Milwaukee, WI). Subjects were scanned with T1 and T2 weighted sequences. T1 images were acquired in approximately 6 min with a 3D-gradient echo sequence with matrix size 128 × 180 × 256 giving 1–1.5 mm resolution in each dimension. We also acquired T2 weighted images in approximately 3 min with a whole-brain multislice fast spin echo (FSE) sequence (TR = 3400 ms, TE = 120 ms, 4 mm slice thickness, 250 × 256 matrix size, in-plane resolution 0.8 × 0.8 mm).

PET

All patients and normal volunteers fasted at least six hours before PET scanning. All antiparkinsonian medications were discontinued at least 12 hours before PET investigations. Motor tasks were performed with the dominant right arm and an intravenous catheter was placed in the left arm for administration of $H_2{}^{15}O$. Each subject was scanned in randomized order while performing the sequence learning task (RT*learn*,1 run), and the motor and sensory reference tasks (M*pred* and S, two runs each). (The learning task was performed once during the PET session to avoid potential confounding effects of task repetition on learning performance. The reference tasks were performed twice to provide a stable baseline for image subtraction [see below]). PET studies were performed using a GE Advance tomograph (General Electric, Milwaukee, WI) in 3D mode. The performance characteristics have been described elsewhere [DeGrado et al., 1994]. This 18-ring bismuth germanate scanner produced 35 slices with an axial field of view of 14.5 cm and a resolution of 4.2 mm (FWHM) in all directions. To minimize head movement during the scan subjects were positioned in a stereoadaptor (Sandstrom Medical, Windsor, Ontario) [Hariz and Eriksson, 1986] with 3D laser alignment. Reconstructed PET images were corrected for random coincidences, electronic dead time and tissue attenuation by transmission scans, and 2D Gaussian-

fit correction was used to compensate for scatter effects [Dhawan et al., 1998].

Relative rCBF was estimated using a modification of the slow bolus method of Silbersweig et al in which 10 mCi of $H_2{}^{15}O$ in 3 ml saline was injected by automatic pump in 18 sec (10 ml/min) followed by a manual 3 ml saline flush [Silbersweig et al., 1993]. Using this injection protocol there was a time delay of approximately 17 sec before onset of brain radioactivity, and the time from onset to peak count rate was 45–50 sec. The timing of task initiation was individually adjusted so that the arrival of radioactivity occurred approximately 10 sec after the start of each task. PET data acquisition began at the time of radioactivity arrival in the brain and continued for 80 sec. The end of task thus coincided with the end of data acquisition. In this slow bolus $H_2{}^{15}O$/PET method, images reflect rCBF during the rising phase of the brain radioactivity, corresponding to the 2nd–8th cycles in our tasks. The interval between successive $H_2{}^{15}O$ administrations was 10 min to allow for the decay of radioactivity.

Imaging data analysis

Brain-behavior relationships were analyzed with both univariate and multivariate approaches. The former approach used voxel-based Statistical Parametric Mapping (SPM). The latter employed a ROI-based network modeling approach utilizing principal component analysis (PCA) in conjunction with the Scaled Subprofile Model (SSM).

Statistical parametric mapping

Data processing was performed using SPM96 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Mathworks, Sherborn, MA). The scans from each subject were realigned using the first scan as a reference. After realignment, all images were proportionally rescaled to a global CBF of 50 ml/min/dl and stereotaxically normalized into a standard anatomical space developed at Montreal Neurological Institute [Collins et al., 1994]. This space closely approximates the Talairach coordinate system [Talairach and Tournoux, 1988] and has been adopted by the International Consortium for Brain Mapping. The images were smoothed with an isotropic Gaussian kernel (FWHM 10 mm for all directions) to allow for inter-individual gyral variation and to improve the signal to noise ratio.

Subtraction analysis. To identify the regions related to motor execution and to motor learning (mean differences between conditions), we performed subtraction analysis of the M*pred* and S images (execution) and of the RT*learn* and M*pred* images (learning), using a threshold of $p < 0.001$ for activation height. These analyses were performed separately in each of the two groups (normal and PD). Additionally, we compared differences in activation between the groups to detect regions that were activated more in one group than the other. For between-group comparisons we set the threshold at $p < 0.01$. Differences in activation between- and within-group were considered significant for $p < 0.05$, corrected for spatial extent.

Behavioral correlation analysis. We performed an exploratory parametric analysis to characterize the relationship between task performance in motor learning and rCBF activation. The results, thresholded at $p < 0.05$ without correction for multiple independent comparisons, indicate voxels wherein rCBF activation was correlated with individual differences in each covariate. In addition to evaluating the main effects of the covariates in each group, we also examined an interaction of group and covariate. This analysis allowed us to detect the regions where slopes of the regressions in one group were steeper than in the other.

In this study, we sought to examine rCBF correlations with specific aspects of explicit learning measured within the PET epoch. Thus, in assessing brain-behavior relationships, we employed the global acquisition index and the global retrieval index as analytical covariates. Only positive correlations were analyzed for each covariate.

Scaled subprofile model

We used PCA with the subtraction Scaled Subprofile Model (SSM) [Alexander and Moeller, 1994; Eidelberg et al., 1996; Alexander et al., 1999] to identify rCBF activation covariance patterns associated with the acquisition and the retrieval processes in sequential movement learning, and to quantify the individual expression of these patterns in individual subjects. RT*learn* and M*pred* images from each subject were coregistered with individual MRI scans using SPM96 software. MRI slices for each subject were used to place ROI borders, which in turn were transferred to the PET images using Scan/VP software [Spetsieris et al., 1995] adapted for Windows NT. We employed a system of 39 standardized ROIs representing a modification of that described by us previously [Eidelberg et al., 1997]. This ROI system was comprised of 17 cortical gray matter ROIs in each cerebral hemisphere,

and three cerebellar (vermis, right, and left cerebellar hemispheres) and two brainstem (pons and midbrain) ROIs. Mean ROI size ranged between 90 pixels for the caudate nucleus to 680 pixels for the dorsolateral prefrontal cortex (1 pixel = 4 mm^2). To reduce partial volume effects, we applied a thresholding algorithm that averaged the upper 20% of voxel values in each ROI [Rottenberg et al., 1991].

SSM/PCA was performed on the rCBF ROI data from RT*learn* and M*pred* image pairs as described previously [Moeller et al., 1998]. This technique of network analysis characterizes patterns of brain function across all regions sampled and produces subject scores that quantify the degree to which each pattern is expressed by each subject. Principal components (PCs) obtained from the analysis represent regional covariance patterns reflecting aspects of functional neural connectivity. Subject differences in the expression of the identified patterns can be independently validated with measures of task performance or behavior. SSM/PCA analysis performed with the subtraction of image scans during different experimental conditions provides regional covariance patterns that represent between-condition changes in the functional interactions of brain regions.

We performed SSM/PCA analysis for the subtraction of the RT*learn* minus the averaged M*pred* control scans to extract patterns of regional interactions for explicit motor sequence learning throughout the brain. SSM/PCA subject scores were computed that quantified the between-condition (i.e., RT*learn*—M*pred*) change in rCBF pattern expression for each subject. These subject scores were subsequently evaluated in relation to behavioral measures of task performance (i.e., the global acquisition and retrieval indices) obtained during the PET scans using multiple regression analysis. This procedure was restricted to the first five independent PCA eigenvalues accounting for greater than 50% of the total subject × region variance [Moeller et al., 1996, 1998]. We identified the PC subject scores that either singly or in linear combination best predicted each of the two behavioral performance indices at a significance threshold of $p < 0.05$. Region weights on each of the two performance-related networks were calculated by linearly combining the region weights on the individual PCs according to the multiple regression coefficients of the subject scores [Moeller et al., 1996; Alexander et al., 1999]. This analytical procedure was applied separately to the normal and the PD groups to identify acquisition- and retrieval-related network topographies in each cohort.

Topographic profile rating

We also computed the subject scores for the *normal* acquisition- and retrieval-related networks in each PD patient on a prospective case-by-case basis. This was achieved using the Topographic Profile Rating (TPR) algorithm [Eidelberg et al., 1995; Moeller et al., 1996]. TPR quantifies the degree to which an individual subject expresses a previously identified covariance pattern in their PET data. We assessed the correlations between these prospectively computed subject scores and each of the two learning indices by computing Pearson-product moment correlation coefficients. SPM, SSM/PCA, and TPR analyses were performed using programs written in MATLAB for Windows NT 4.0.

RESULTS

Task performance

Mpred

We did not identify significant differences between the normal and the PD groups in any of the analytical parameters measured in this task. (Spatial error: normal 0.26 ± 0.13 cm, PD 0.24 ± 0.15 cm [$p = 0.6$, Mann-Whitney Rank Sum test]; Movement time: normal 440.5 ± 60.8 msec, PD 458.6 ± 45.7 msec [$p = 0.6$]; Onset time: normal −433.1 ± 72 msec, PD −445.1 ± 44.6 msec [$p = 0.5$]). The two hemi-PD subgroups did not differ from one another in these parameters, nor did the values for each subgroup differ from normal control measurements. (Spatial error: left hemi-PD 0.17 ± 0.06 cm, right hemi-PD 0.31 ± 0.18 cm [$p = 0.07$]; Movement time: left hemi-PD 439.1 ± 36.1 msec, right hemi-PD 477.0 ± 47.1 msec [$p = 0.1$]; Onset time: left hemi-PD −426.5 ± 36.8 msec, right hemi-PD −463.3 ± 42.6 msec [$p = 0.9$]). Thus, there were no between-group performance differences in the execution of the M*pred* control task to confound comparisons of motor learning between PD patients and normals.

RTlearn

Target acquisition for the normal and the PD groups is presented as a function of cycles in Figure 2A. Two-way repeated measures analysis of variance (ANOVA) identified a significant main effect of cycles ($F[10,220] = 4.3$, $p < 0.0001$), but no main effect of group ($F[1,22] = 1.2$, $p = 0.29$), and no significant interaction between group and cycles ($F[10,220] = 1.3$,

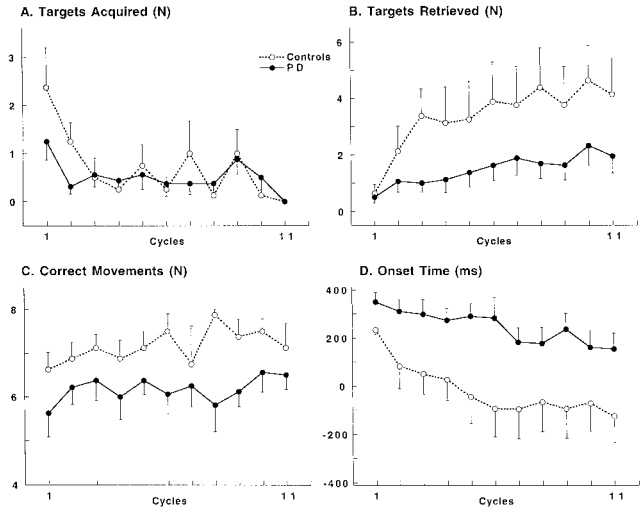

Figure 2.
Means and standard errors (SE, bars) for the number of: (**A**) targets acquired, (**B**) targets retrieved, (**C**) movements to correct targets, and (**D**) movement onset time plotted for each cycle of the 90 sec motor sequence learning PET epoch. Data from the normal subjects appear as open circles (dotted lines); data from PD patients appear as filled circles (solid lines).

$p = 0.22$). Target retrieval increased progressively across cycles in both groups (Fig. 2B). Two-way repeated measures ANOVA identified a significant main effect of both cycles ($F[10,220) = 7.2$, $p < 0.0001$) and groups ($F[1,22] = 4.4$, $P < 0.05$), and no significant interaction between group and cycles ($F[10,220] = 1.7$, $p = 0.09$).

Figure 2C depicts the number of correct movements for each group as a function of cycles. The percentage of the total correct movements within the block for the normal and the PD groups was 89.9 ± 8.4% and 77.2 ± 13.8%, respectively. Two-way repeated measures ANOVA across the 11 cycles identified a significant main effect of groups ($F[1,22] = 5.2$, $p < 0.04$), but no main effect of cycles ($F[10,220] = 0.8$, $p = 0.63$), and no significant interaction between group and cycles ($F[10,220] = 1.7$, $p = 0.78$). Reductions in onset time for the correct movements are presented in Figure 2D. Two way repeated ANOVA identified significant main effects of groups ($F[1,22] = 6.9$, $p < 0.02$) and of cycles ($F[10,220] = 7.9$, $p < 0.0001$), but no significant interaction between group and cycle ($F[10,220]=1.7$, $p = 0.17$).

In every subject global acquisition and retrieval indices were determined for the entire 90-second PET epoch. These global values were utilized for correlation with the imaging data. The mean global acquisition index was 7.6 ± 4.5 and 5.6 ± 4.1 for the normal and PD groups, respectively. The mean global retrieval index was 37.0 ± 32.3 and 16.1 ± 17.2, respectively for the two groups. A significant correlation was noted between the global acquisition and retrieval indices ($R^2 = 0.34$, $p < 0.005$). We considered these mutual effects in SPM correlational analysis (see below) by analyzing partial correlation coefficients in a general linear model incorporating both indices.

The declarative scores for the normal and the PD groups were 4.9 ± 3.5 and 2.1 ± 3.1, respectively ($p < 0.05$, Mann-Whitney Rank Sum test). The declarative scores correlated significantly ($p < 0.0001$) with both the global retrieval index ($R^2 = 0.77$) as well as the global acquisition index ($R^2 = 0.53$). This suggested that both these behavioral indices were descriptors of the explicit learning process. There were no significant differences between the two hemi-PD subgroups in any of the learning performance parameters measured during RT*learn*. Thus, the two hemi-PD subgroups did not differ behaviorally with respect to psychophysical measures of motor learning and execution and were therefore combined for subsequent behavioral data analysis.

RTran

The mean reaction time in RT*ran* for the PD group (303.2 ± 66.3 msec) was significantly higher than in the normal controls (234.0 ± 40.1 msec; $p < 0.01$, Mann-Whitney Rank Sum test). There was also a significant difference in the lowest reaction time in RT*ran* between the PD group (166.2 ± 24.3 msec) and the normal controls (143.0 ± 14.1 msec; $p < 0.005$). The reaction time minimum in RT*ran* was used in each individual subject as the criterion for determining the number of anticipatory movements in RT*learn* (see above).

Statistical parametric mapping

Subtraction analysis

The results of the SPM subtraction analyses are presented in Table I.

***M*pred-*S*.** Subtraction analysis of these two tasks was performed to identify regions associated with motor execution (Table IA). We identified significant activation of the left primary sensorimotor cortex (SMC; BA4), dorsocaudal premotor cortex (PMdc; BA6) and supplementary motor area (SMA; BA6), posterior putamen and thalamus, and of the right cerebellar hemisphere and of the cerebellar vermis. These activations

TABLE I. Subtraction analysis of regional brain activation*

A. Brain regions activated during motor execution in normal subjects and PD patients

	Normal controls				PD patients			
	Coordinates (mm)				Coordinates (mm)			
Brain region	x	y	z	Z-max	x	y	z	Z-max
Left SMC	−32	−24	74	6.06	−36	−34	62	6.79
Left PMdc	−22	−18	74	6.32	−22	−18	74	6.82
Left SMA	−10	−14	54	5.85	−8	−10	50	6.70
Left putamen (posterior)	−30	−10	2	3.94	−34	−4	2	4.35
Left thalamus	−20	−16	18	4.47	−22	−16	16	4.19
Right cerebellum	14	−50	−14	6.82	18	−50	−14	6.87
Cerebellar vermis	6	−56	−22	6.88	2	−56	−18	6.76

B. Brain regions activated during motor learning in normal subjects and PD patients

	Normal controls				PD patients			
	Coordinates				Coordinates (mm)			
Brain region	x	y	z	Z-max	x	y	z	Z-max
Left PFdl					−46	12	32	4.71
Left PFv					−32	52	10	4.33
PreSMA					0	16	48	4.84
Left CMAr	−16	14	36	4.89				
Left PMdr	−26	6	58	4.15	−26	6	60	4.44
Right PMdr	32	12	52	4.64	32	4	56	3.60
Right precuneus					2	−66	62	4.80
Left parietal (posterior)					−30	−60	52	4.33
Right parietal (posterior)	26	−74	56	4.11	42	−56	52	3.94
Cerebellar vermis					4	−74	−38	3.90

* SMC, sensorimotor cortex; PMdc, premotor cortex (dorsocaudal); SMA, supplementary motor area; PFdl, prefrontal cortex (dorsolateral); PFv, prefrontal cortex (ventral); CMAr, cingulate motor area (rostral); PMdr, premotor cortex (rostrodorsal).

did not differ significantly between the PD and the control groups, or between the two hemi-PD subgroups. These findings, as well as the similarity of the behavioral data performance in the PD and the normal control cohorts, supports the use of M*pred* as a kinematically controlled motor reference task for image subtraction in the motor learning experiments.

***RT*learn − *M*pred.** Subtraction analysis of these two tasks was performed to identify regions associated with sequence learning (Table IB, Fig. 3). In normal controls, we found significant bilateral activation of the rostrodorsal premotor cortex (PMdr; BA6), of the left rostral anterior cingulate area (CMAr; BA24/32), and of the right posterior parietal cortex (BA7). In the PD group, RT*learn* − M*pred* subtraction revealed significant bilateral activation of the PMdr, preSMA, and posterior parietal cortex (BA40), of the left dorsolateral prefrontal cortex (PFdl; BA46/9) and the ventral prefrontal cortex (PFv; BA10), of the right precuneus (BA7), and of the inferoposterior cerebellar vermis. There was no significant difference between the PD and normal groups in RT*learn* − M*pred* activation. Neither of the two hemi-PD subgroups differed significantly from normals in this activation, nor were there significant activation differences between the two. Thus the two patient subgroups were combined for further imaging analysis.

Behavioral correlation analysis

The results of the SPM correlational analyses are presented in Table II.

Acquisition. In normal subjects, the global acquisition index was positively correlated with activation in the PFdl (BA 46/9) bilaterally, in the left PFv (BA 45/47), and in the right PMdr, precuneus, preSMA, and SMC.

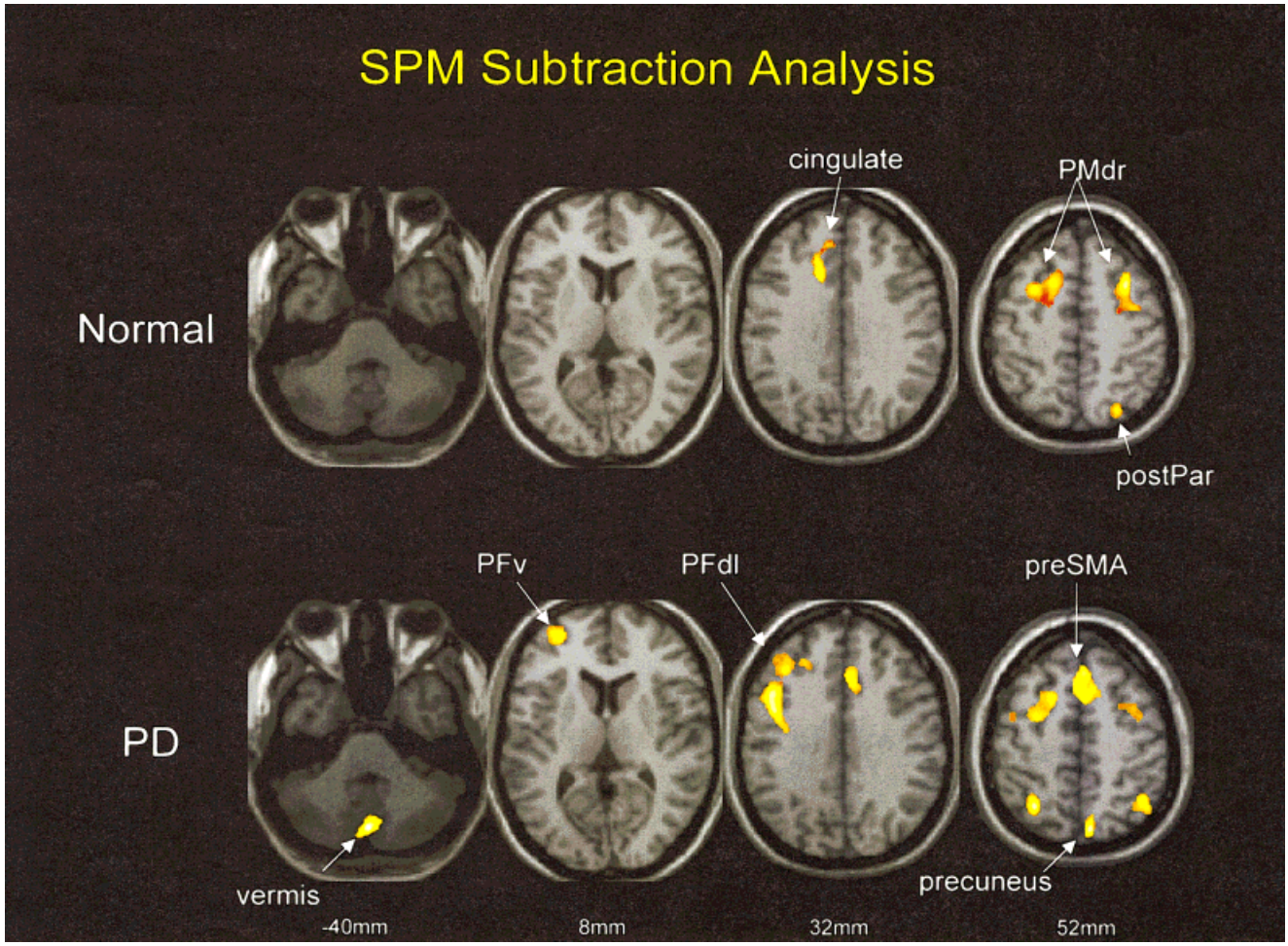

Figure 3.

Brain regions associated with sequence learning (RT*learn*-M*pred*) in normal controls and in Parkinson's disease (PD) patients. No significant difference in sequence learning activation was evident between the PD and normal groups. PMdr, rostrodorsal premotor cortex (Brodmann area [BA] 6); postPar, posterior parietal cortex (BA 7); cingulate, cingulate cortex (BA 24/32); preSMA, rostral supplementary motor area (BA 6); precuneus, precuneus area (BA 7); PFdl, dorsolateral prefrontal cortex (BA 46/9); PFv, ventral prefrontal cortex (BA 10). (*Z* maps were thresholded at $p < 0.001$. Numbers represent millimeters relative to anterior-posterior commissure line).

In PD patients, this measure was positively correlated with bilateral activation in the PFdl, and in the left PMdr and PFv (BA 10 and 45/47). A significant interaction of group and parameter ($p < 0.05$) was seen in the PFv bilaterally, in the left PMdr, and in the right preSMA. For acquisition, the normal controls had a steeper regression slope in the right preSMA. By contrast, in PD patients, the regression slope was steeper in the left PMdr and PFv.

Retrieval. In normal subjects, the global retrieval index was positively correlated with activation in the left caudal PM (PMc)/SMA, and in the right PMdr, posterior parietal cortex, preSMA, and PFdl. In the PD group, this index was positively correlated with increased activation in the PMdr and precuneus bilaterally, in the left preSMA/rostral cingulate motor area (CMAr, BA 32), and in the right PFdl, PFv, and posterior parietal cortex. A significant interaction of group and parameter ($p < 0.05$) was seen bilaterally in the posterior parietal cortex and the precuneus, in the left PMc/SMA, PMdr, and preSMA/CMAr, and in the right PFdl and PFv. For retrieval, normal volunteers had a steeper regression slope in the left PMc/SMA. By contrast, in PD the slope was steeper than normal in the posterior parietal cortex bilaterally, in the left PMdr, preSMA/CMAr, and precuneus, and in the right PFdl and PFv.

TABLE II. Behavioral correlational analysis of regional brain activation during motor learning*

A. Brain regions with significant correlations between the acquisition index and activation

	Normal controls				PD patients			
	Coordinates				Coordinates (mm)			
Brain region	x	y	z	Z-max	x	y	z	Z-max
Left PFv					−34	52	−2	3.28
Left PFdl	−46	26	34	2.93	−44	26	34	3.65
Right PFdl	40	30	26	3.15				
Right preSMA	8	18	52	2.50				
Left PMdr					−32	12	64	3.04
Right precuneus	0	−76	56	2.80				

B. Brain regions with significant correlations between the retrieval index and activation

	Normal controls				PD patients			
	Coordinates				Coordinates (mm)			
Brain region	x	y	z	Z-max	x	y	z	Z-max
Right PFdl	48	42	26	3.46	34	34	24	3.22
Left CMAr					−6	24	44	3.45
Left preSMA					−12	20	58	3.21
Left SMA	−16	−10	60	2.46				
Left PMdr					−28	8	58	2.92
Right PMdr	30	12	50	2.50	34	12	52	2.86
Left precuneus					−4	−78	62	3.05
Right precuneus					6	−76	52	3.16
Left parietal (posterior)					−32	−42	50	4.17
Right parietal (posterior)	24	−74	54	3.01	28	−74	48	3.54

* SMC, sensorimotor cortex; PMdc, premotor cortex (dorsocaudal); SMA, supplementary motor area; PFdl, prefrontal cortex (dorsolateral); PFv, prefrontal cortex (ventral); CMAr, cingulate motor area (rostral); PMdr, premotor cortex (rostrodorsal).

Network analysis

Normal networks

Acquisition. An SSM/PCA analysis was performed on the RT*learn*—M*pred* rCBF activation data of the normal controls. The first five PCs accounted for 70% of the subject × region variance; each accounted for more than 10% of the subject × region variance. In the PCA, we identified a covariance pattern (3rd and 5th PCs, accounting for 12.8% of the subject × region variance) whose subject scores correlated significantly with the global acquisition index ($R^2 = 0.58$, $p < 0.02$, Fig. 4A, solid line). This covariance pattern was characterized by significant increases in the activation of the left PFdl, CMAr, preSMA, caudate, and putamen, and of the right SMC (Fig. 5A, right hand boxes). [These regions had pattern weights with values greater than or equal to +1. In each of these regions, at least 35% of the variability in globally normalized rCBF activation was predicted by subject differences in pattern expression ($p < 0.010$) [Eidelberg et al., 1997].

The expression of this normal acquisition-related pattern was computed in each of the PD patients using TPR. In the PD cohort, subject scores for this pattern did not differ significantly from corresponding values in normals ($p = 0.3$). Additionally, this measure failed to predict the global acquisition index in the PD group ($R^2 = 0.19$, $p = 0.09$, Fig. 4A, dotted line), suggesting that a different network may subserve this function in disease.

Retrieval. In the SSM/PCA of the normative data, we also identified an orthogonal pattern (2nd and 4th PCs, accounting for 17.1% of the subject × region variance) whose subject scores correlated significantly with the global retrieval index (R2 = 0.74, $p < 0.005$, Fig. 4B, solid line). This covariance pattern was characterized by significant increases in the activation of

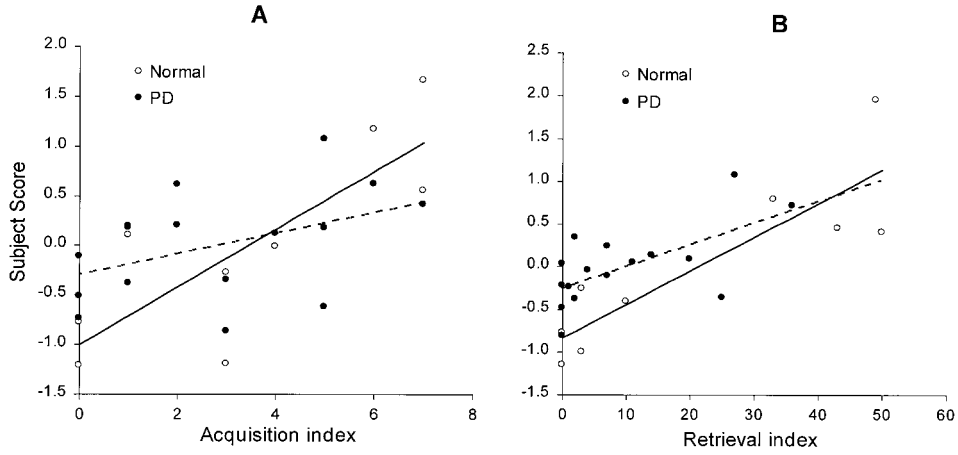

Figure 4.

A: Network analysis revealed normal subjects the presence of a significant covariance pattern associated with target acquisition (see text). In the normals (open circles), subject scores for this pattern correlated significantly with the acquisition index (R^2 = 0.58, $p < 0.02$; solid line). By contrast, subject scores for this pattern computed prospectively in the PD patients (filled circles) did not correlate significantly with target acquisition performance (R^2 = 0.19, $p = 0.09$; dotted line). **B**: Network analysis in normal subjects revealed the presence of different covariance pattern associated with the target retrieval (see text). In normals (open circles), subject scores for this pattern correlated significantly with the retrieval index (R^2 = 0.74, $p < 0.005$; solid line). In PD patients (filled circles), subject scores for this pattern also correlated with retrieval performance although the magnitude of this correlation was comparatively lower in the disease group (R^2 = 0.40, $p < 0.01$; dotted line).

the PMdr bilaterally, and of the right posterior parietal cortex and precuneus (Fig. 5B, right hand boxes).

The expression of this normal retrieval-related pattern was computed in each of the PD patients using TPR. In the PD cohort, computed subject scores for this pattern did not differ significantly from corresponding values in normals ($p = 0.4$). In contrast with subject scores for the normal acquisition pattern which did not predict performance in PD, prospectively computed subject scores for the normal retrieval pattern did predict the corresponding behavioral index in the patient cohort, albeit with a smaller magnitude of correlation ($R^2 = 0.40$, $p < 0.001$, Fig. 4B, dotted line). This suggests the presence of a retrieval-related network in PD that is topographically similar, though not identical, to the network subserving this function in normal subjects.

PD-related networks

Acquisition. SSM/PCA analysis was performed on the rCBF activation data from the PD group. The first three PCs accounted for 50% of the subject × region variance; each accounted for more than 10% of the subject × region variance. We identified an independent covariance pattern (2nd PC, accounting for 13.4% of the subject × region variance) whose subject scores correlated significantly with the acquisition index ($R^2 = 0.27$, $p < 0.04$). This covariance pattern was characterized by significant increases in the activation of the left PFdl, PFv, and PMdr (Fig. 5A, upper boxes).

Retrieval. We also identified another independent covariance pattern (1st PC, accounting for 26.3% of the subject × region variance) whose subject scores correlated significantly with the retrieval index ($R^2 = 0.75$, $p < 0.001$). This covariance pattern was characterized by significant increases in the activation of the PMdr, posterior parietal cortex and precuneus bilaterally, of the left preSMA and CMA, and of the right PFdl (Fig. 5B, upper boxes).

DISCUSSION

In this study we demonstrate that the different components of explicit motor sequence learning are associated with distinct patterns of brain activation. In normal subjects, the acquisition of targets is associated mainly with activation of the left PFdl, preSMA, CMAr, as well as the caudate and putamen. Target retrieval is associated with bilateral activation of the PMdr, and of the right PFdl, posterior parietal cortex and precuneus. By contrast, in PD patients these activation patterns differ for both learning components. Our findings suggest that even at early stages of disease, PD patients differ from normals in the brain-

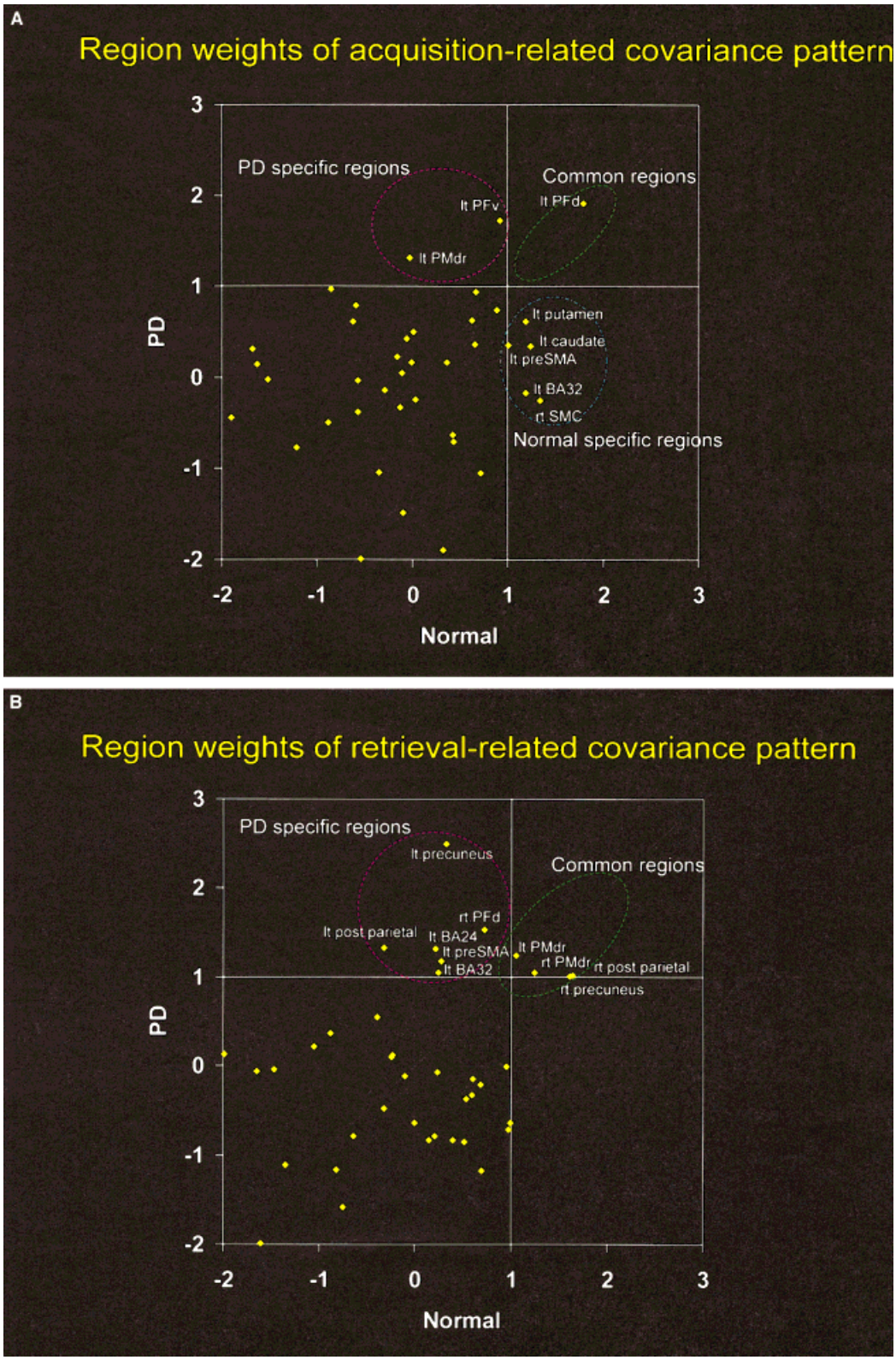

Figure 5.
(Legend on facing page)

behavior relationships subserving the learning of motor sequences.

Methodological considerations

We designed these experiments to emphasize the explicit learning of motor sequences and to assess the substrates of differences in this process that occur in non-demented PD patients [Brown, 1999]. Our paradigm reflected the explicit learning process: verbal instruction promoted explicit learning, and the sequences presented during the imaging experiment were relatively simple. Indeed, most of the subjects reported awareness of elements of the sequence within one trial block. Additionally, our task design allowed us to characterize the course of learning within each block, and to obtain quantitative descriptors of acquisition and retrieval as psychophysical components of explicit learning. By contrast, when no advanced information is given as in a classical serial reaction time task [Nissen and Bullemer, 1987], implicit and explicit processes may be engaged differently (i.e., serially or in parallel) and unpredictably in the course of learning. Thus, classical subtraction analyses of mean differences between conditions do not necessarily delineate specific regions involved with different aspects of the learning process. The identification of specific associations between these individual parameters and regional brain activation supports the results of prior studies utilizing other experimental approaches [Jueptner et al., 1997; Honda et al., 1998; Sakai et al., 1998]. The high correlation between the declarative score and the retrieval index suggests that these two measures reflect similar features of the explicit learning process. By contrast, the relatively lower correlation of the acquisition index with the declarative score likely stems from the forgetting and re-encoding of targets by subjects during the experimental block (see Fig. 2A). Nonetheless, we note that the acquisition and retrieval indices are merely intercorrelated approximations of the corresponding psychophysical processes.

Prior investigations have compared brain activation in PD patients and normal control subjects during the execution of sequential movements of varying complexity [e.g., Samuel et al., 1997; Catalan et al., 1999]. However, no comparative imaging studies have been performed during the learning of new motor sequences. In the current study, we implemented an experimental design that minimized differences in motor execution between the clinical groups. This was made possible by limiting our study to early stage PD patients with minimal clinical signs. By selecting minimally impaired early stage PD patients, we were able to utilize identical experimental task parameters for both the PD and the normal control cohorts, and the two groups did not differ significantly with respect to any of the measured psychophysical parameters of motor execution. This established the M*pred* reference task as a suitable imaging reference for group comparisons in the learning experiments. Although the PD cohort comprised equal numbers of patients with and without clinically discernible involvement of the executing dominant right hand, the two hemi-PD subgroups did not differ from one another with respect to any of the psychophysical performance measures or to the patterns of brain activation that were identified with PET. We therefore combined the two hemi-PD subgroups to enhance the statistical power of the data analysis.

In this study, we designed a simple kinematically controlled task (RT*learn*) which emphasized explicit learning of motor sequences. Although implicit learning may play a role in this task, the prior instruction given the subjects and the high correlation between the global retrieval index and the subjects' verbal report (declarative scores) suggest that sequence learning in RT*learn* represents primarily an explicit process. Most previously reported PET studies of explicit sequence learning [e.g., Jenkins et al., 1994; Jueptner et al., 1997; Ghilardi et al., 2000] have utilized paradigms with dual task requirements, forcing subjects to divide attentional and processing resources between reaching targets in a narrow time window and explicitly memorizing the target sequence. Thus, attentional differences between patients and controls may give rise to potentially differing activation responses between the two groups. We therefore designed the RT*learn* task to be sufficiently easy so that both normal subjects and PD patients could learn elements of the sequences

Figure 5.

Region weight correlations between early stage PD (y axis) and age-matched control subjects (x axis) for SSM networks associated with sequence acquisition (**A**) and retrieval (**B**). The upper right hand box contains significant regions (network region weight >1, $P < 0.01$) [Eidelberg et al., 1997] common to both the PD and the normal networks. The upper left hand box contains regions contributing significantly to PD networks but not to normal networks. The lower right hand box contains regions contributing significantly to normal but not to PD networks (see text). For acquisition, there has been a disease-related shift from subcortical to cortical contributions within the associative cortico-striato-pallido-thalamocortical (CSPTC) loop. For retrieval, disease-related contributions from homologous regions in the left hemisphere augment the normal network topography.

by the end of the 90-second trial. Because of the reduced cognitive demand of this task, it is not surprising that in normal subjects regions such as the PFdl and lateral cerebellum are activated to a lower degree (significant only at a $p < 0.01$ threshold) than in previous sequence learning paradigms. Additionally, we note that the M*pred* and RT*learn* tasks cannot be simply dichotomized into self-initiated or externally triggered movements [Jahanshahi et al., 1995, 2000]. Movements in both motor tasks, though self-initiated, are externally paced by the same predictable 1/second tone. Regardless of classification, we detected small but significant decrements in the PD group relating mainly to the retrieval process. This finding is consistent with prior neuropsychological studies in nondemented parkinsonians [Foti and Cummings, 1997].

The absence of significant mean differences in RT*learn*—M*pred* activation between the PD and the normal groups with SPM analysis is compatible with the minor behavioral abnormalities noted in the comparison of the two groups. Additionally, the lack of a significant group difference in this activation cannot be readily attributed to disparities in group size or to the clinical heterogeneity of the combined PD cohort. Indeed, significant SPM between-group differences were not attained on comparison of each hemi-PD subgroup with the normal control cohort. These findings suggest that although the normal and disease cohorts may differ only minimally in terms of mean effects, significant differences between the two may exist with regard to the relationship between the individual differences in brain activation and motor learning performance.

We tested this notion through a univariate SPM correlational analysis to relate the individual learning measures to regional activation in each of the two groups on a voxel basis. We also employed a ROI-based SSM/PCA to identify regional activation covariance patterns associated with different aspects of motor sequence learning [Moeller et al., 1998]. Because this analysis included only portions of the brain bounded within the ROIs, our analysis may have omitted network contributions from voxels outside these regions. We considered this possibility and performed additional image analyses utilizing voxel-based SSM/PCA [Alexander et al., 1999; Moeller et al., 1999], as well as SPM eigenimage analysis [Friston et al., 1995]. No significant behavioral correlation was found with either of these multivariate analytical approaches. These observations suggested that the application of SSM/PCA to ROI data may have superior signal-to-noise characteristics compared with voxel-based covariance analyses. We did however detect significant behavioral correlations utilizing simple univariate SPM correlational analysis. Nonetheless, while detecting voxel clusters associated with each learning component, this univariate approach does not consider the mutual functional interactions that may or may not exist between these regions.

In this study we sought to compare the functional correlates of acquisition and retrieval performances during sequence learning in PD patients with normal volunteer subjects. We enhanced the statistical power of correlational analysis in the PD group by combining the two hemi-PD subgroups. This was justified by the absence of significant subgroup differences in the behavioral parameters as well as the SPM mean activation contrasts for RT*learn*—M*pred* and M*pred*—S. Additionally, SSM/PCA of each of the two subgroups (data not presented) yielded learning-associated networks that were topographically similar to those identified in the combined group (region weight correlation: $R^2 >^2 0.7$, $p < 0.001$). The use of a larger patient sample for prospective TPR calculations was helpful in assessing whether the normal learning networks were indeed predictive of performance in the PD group. Moreover, the larger PD sample enabled greater precision in the estimation of region weights on the disease-related learning networks. However, given the inequalities in sample size between the PD and the normal groups, a comprehensive network comparison will require the acquisition of activation PET data from a larger normal sample. This will permit a rigorous correlation of region weights on learning-related patterns extracted from equal-sized cohorts. Additionally, the acquisition of additional data from a prospective normal cohort can be utilized to validate the reported relationships between network expression and learning performance.

Normal functional networks

Both SPM and SSM analytical strategies verified our hypothesis that the acquisition and retrieval components of sequence learning are associated with distinct patterns of regional activation. In normal subjects, both methods revealed acquisition to be associated with left PFdl activation. The role of this region in motor sequence learning is widely accepted, although hemispheric specialization for this form of processing may vary according to task. Grafton et al. [1995] demonstrated a longitudinal increase of rCBF in the right PFdl during a serial reaction time task. Honda et al. [1998] also showed that this activation in this region correlated positively with declarative measures of explicit learning. By contrast, Jenkins et al. [1994] noted

bilateral activation in the PFdl during sequence learning. Differences in the lateralization of PFdl cortical activation during motor learning may also stem from an inherent dichotomy in the acquisition and retrieval processes. Tulving et al. [1994] proposed a model of hemispheric asymmetry for episodic memory in which there is preferential involvement of the left prefrontal region during acquisition and of the right prefrontal region during explicit retrieval. In this vein, the correlational analysis used by Grafton was relatively sensitive to the retrieval component, which was associated with continuous temporal change during the study [Grafton et al., 1995]. On the other hand, simple analyses of mean differences between conditions are not likely to discern patterns associated with individual behavioral components. Using their original trial and error task, Sakai et al. [1998] showed that the left PFdl was activated in the earlier stages of learning, whereas the right PFdl has prolonged activation. This difference in the time course of activation, as well as our current findings, supports Tulving's model in the explicit learning of a motor sequence.

PreSMA was also associated with acquisition in normal subjects. Indeed, Hikosaka et al also found a correlation between subject performance and the cumulative sum of the learning-related activation in this region [Hikosaka et al., 1996]. Corticocortical connections between PFdl and preSMA are critical for the transfer of information in working memory and the generation of movements based on it [Dum and Strick, 1991; Luppino et al., 1993]. CMAr (BA32) is also connected with PFdl [Vogt and Pandya, 1978]; both regions are often coactivated in motor learning studies [e.g., Jenkins et al., 1994].

Using SSM/PCA we also detected network-related activation associated with target acquisition in the left caudate and putamen. Although a ROI analysis was used, which precluded sub-parcellation of these nuclei, the functional covariance of the caudate with the putamen suggested that the network activity may be referable to the anterior portion of the latter nucleus. The anterior striatum receives massive projections from PFdl which subserve spatial working memory and motor learning processes [e.g., Sawaguchi and Goldman-Rakic, 1991]. Indeed, Jueptner et al. [1997] reported activation in the caudate during trial and error motor learning. In primate experiments, Miyachi et al. [1997] demonstrated deficits in new motor learning following the injection of muscimol into the anterior basal ganglia including the caudate head and anterior putamen. Our finding implicating the basal ganglia in the acquisition process is consistent with these results. The significance of activation in the ipsilateral SMC is unclear, although several studies have suggested that this region was involved in implicit motor learning and the control of complex sequential movements [Shibasaki et al., 1993; Boecker et al., 1998; Honda et al., 1998].

The acquisition-related network in normals involves mainly left hemisphere regions; whereas the normal retrieval-related network shows a transition to right hemisphere processing, with involvement of more posterior cortical regions. Our data suggest that the right PMdr, posterior parietal cortex, and precuneus comprise a retrieval-related neural network in normal subjects. Animal studies have demonstrated that the dorsal premotor cortex (PMd) is composed of rostral (PMdr) and caudal (PMdc) areas, which receive projections from distinct portions in the posterior parietal cortex, and which subserve different functions [Wise et al., 1989; Lacquaniti et al., 1995]. PMdr is associated with preparation or selection of movements, and PMdc is related to movement execution based on positional or kinematic information. Mushiake et al. [1991] reported numerous sequence-specific neurons in PMdr. Indeed, PMdr activation, accompanied mainly by right posterior parietal activation, has been noted in a number of PET studies of explicit motor sequence learning [Jenkins et al., 1994; Grafton et al., 1995; Honda et al., 1998], as well as in studies of complex sequential movements [Sadato et al., 1996; Catalan et al., 1998]. These findings suggest that the PMdr, especially on the right, is involved in integrating spatial information with motor programming to produce the precise sequential movement map which is necessary to facilitate complex movements.

Engagement of the precuneus has been reported in a variety of $H_2{}^{15}O$/PET activation studies involving verbal memory tasks [Grasby et al., 1993; Petrides et al., 1993]. Importantly, precuneus activation had been detected in tasks relating to retrieval but not acquisition [Shallice et al., 1994; Fletcher et al., 1996]. Roland et al. [1990] reported activation in the precuneus associated with visual imagery. The precuneus has also been activated in the motor tasks involving complex sequential movements [Sadato et al., 1996; Boecker et al., 1998] and explicit motor learning [Honda et al., 1998]. Furthermore, fMRI experiments suggest that activation of this region is most pronounced during the intermediate stage of explicit learning in which retrieval dominates [Sakai et al., 1998]. These data and ours support a role for the precuneus in declarative retrieval irrespective of its type. This is further supported by the presence of corticocortical connections between the PFdl and precuneus [Goldman-Rakic, 1987]. A number of experimental investigations sug-

gest that the posterior parietal cortex integrates visual, somatic, and other information, and well as providing a sensory representation of extrapersonal space [Mesulam, 1990]. In addition, this region may play a role in motor intention, and not perception [Snyder et al., 1997]. Deiber et al described the activation of this region during a motor reaction time task with preparatory cues, and suggested a role for the posterior parietal cortex in motor selection processes [Deiber et al., 1997]. Our finding of a retrieval-related activation of this area is consistent with other PET studies associating this region with spatial memory, awareness of sequences, and sequence complexity [e.g., Grafton et al., 1995; Catalan et al., 1998].

Abnormal functional networks in parkinsonism

In the SPM correlational analyses we generally noted increases in the slopes of activation of the PD patients relative to the normal controls. Nonetheless, the slopes of the PD patients were lower than normal in the right preSMA for acquisition, and in the left caudal SMA for retrieval. These findings are in keeping with the notion of an impairment of SMA function in PD during motor execution tasks, especially when involving internally generated movement selection [Marsden and Obeso, 1994; Brooks, 1995; Jahanshahi et al., 1995]. Nevertheless, this univariate approach was insufficient to determine whether these PD-related alterations in the relationship of SMA activity to performance are primary, or whether they are related to an underlying abnormality in striatal function. We found that, in contrast to the controls, PD patient subject scores for the normal acquisition-related topography did not correlate with the corresponding learning index. This suggests that the acquisition function is subserved by a separate, abnormal brain network in parkinsonism. Indeed, an SSM/PCA restricted to activation data from the PD cohort alone revealed a disease-related acquisition network that was topographically distinct from its counterpart in normal subjects. This pathological network exhibited topographic similarities and differences with the normal acquisition network (Fig. 5A). We found that the left PFdl is common to the acquisition networks of both groups, indicating that this region is essential for this function. As discussed above, the normal acquisition-related topography is defined by functional interactions between the left striatum, preSMA, and PFdl. By contrast, in PD the normal pattern of striatal-frontal connectivity may have become disrupted. In this circumstance, localized striatal dysfunction may be compensated for in acquisition by network contributions from cortical areas functionally and anatomically connected to the left PFdl. Hence in PD, successful target acquisition may be accomplished by deploying a disease-related brain network involving abnormal cortical-cortical interactions, in conjunction with a shift away from normal subcortical-cortical functional relationships. The PMdr has reciprocal connections with the PFdl [Luppino et al., 1993], while those from PFv are unclear. Nevertheless, it is possible that the PFv may be required for acquisition in pathological situations distinct from those in which the region is typically involved.

By contrast, subject scores for the normal retrieval-related network computed by TPR in the PD patients did predict their retrieval indices, albeit to a lesser degree than in controls. Here too, a second SSM/PCA limited to the patient group, disclosed an abnormal network subserving retrieval in PD. Nonetheless, this network was topographically related to that identified in the age-matched normals, with common regions identified in the right PMdr, posterior parietal cortex, and precuneus. These findings suggest that although PD patients are able to deploy the normal retrieval-related network, the expression of this network is not as well linked to behavior as in normals. To achieve maximal learning, PD patients require additional network contributions from the right PFdl, and from the left PMdr, preSMA, CMAr, and the posterior parietal cortex (Fig. 5B). In a recent study of sequential movements of varying length, Catalan et al. [1999] demonstrated overactivity of the PMdr and parietal cortices, as well as additional activation of preSMA/CMAr in the PD patients compared with normal controls. These results are compatible with our study of new sequence learning.

The need for a more extensive retrieval-related activation network in parkinsonism might be explained as follows. In PD, the deployment of the normal retrieval network is inadequate; a greater degree of brain activation is needed to achieve a near normal level of performance. One way for this to occur is by enhancing the normally lateralized activation network through functional contributions from homotypic areas in both hemispheres. In this vein, we have recently found that normal subjects also recruit a bilateralized network when the difficulty of sequence learning increases [Nakamura et al., 1999]. We do not expect this form of network enhancement to be necessarily present throughout the disease process. It is likely that with advancing neurodegeneration, these functional mechanisms will decompensate, and that patient performance will likewise decline. This notion can be assessed by measuring performance over the course of the illness, and by contrasting the relationships between performance and network expression during early and late disease stages.

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