Early Stage Parkinson's Disease Patients and Normal Volunteers: Comparative Mechanisms of Sequence Learning

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Abstract: Early-stage nondemented Parkinson's disease (PD_(es)) patients can learn short but not long sequences as well as controls. We have previously shown that to achieve normal performance, PD_(es) patients activated the same right-sided cortical regions as controls plus the homologous left sided cortex and bilateral cerebellum. In this study, we evaluated two related hypotheses to explain the behavioral abnormalities and the increased bilateral brain activation observed in the PD_(es) group. Hypothesis 1 proposed that PD_(es) patients recruit regions from a normal bilateral network specialized for sequence learning that healthy controls would activate if performing difficult tasks. Thus, PD_(es) patients can learn short sequences as well as controls. Hypothesis 2 proposed that information processing within the network in the PD_(es) group is impaired. Thus, PD_(es) patients cannot learn as difficult a sequence as controls. To test hypothesis 1, we increased task difficulty and statistical power in the control group and showed that the control and the PD_(es) groups activated the same regions. To test hypothesis 2, we analyzed the equal performance data using two partial least squares (PLS) multivariate analyses. The task-PLS analysis showed that to perform equally with controls, the PD_(es) group expressed the normal bilateral network more than the control group. The behavior-PLS analysis showed that the correlation between learning performance and regional activation was significantly different between the groups. We conclude that PD_(es) patients have near normal learning if task difficulty is moderate because they can recruit additional regions from a normal bilateral network specialized for sequence learning. However, when a difficult task would normally require bilateral activation, PD_(es) patients fail to learn because information processing within the network is impaired. *Hum. Brain Mapp.* 20:246–258, 2003. © 2003 Wiley-Liss, Inc.

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INTRODUCTION

Early-stage nondemented Parkinson's disease patients (PD_(es)) have difficulties with many aspects of cognition [Brown and Marsden, 1990; Dubois and Pillon, 1997]. Our declarative, trial-and-error motor sequence learning task (TEseq) has executive and visuo-spatial components that make sequence learning more difficult for PD_(es) subjects than healthy controls [Mentis et al., 2003]. However, if the sequence to be learned is not too long (difficult), and if they are given time, PD_(es) patients can eventually perform as well as controls [Mentis et al., 2003]. Therefore, within certain bounds, the adverse effects of parkinsonism on brain function can be overcome and cognitive dysfunction can be minimized. The brain mechanism by which this is achieved is largely unknown.

A video accompanies this article online (see abstract page).

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Our modified equal performance study [Mentis et al., 2003] was the first such attempt to identify the brain mechanism by which PD patients overcome the effects of disease to minimize behavioral dysfunction. In that study, the PD_(es) group achieved equal learning performance with the control group by the end of a Positron Emission Tomography (PET) scan. Thus, the early part of the PET scan, when most signal is acquired, captured brain function in the PD_(es) group while they were in the process of successfully overcoming the adverse effects of Parkinsonism. Objectively, both groups learned sequences of equal length. However, subjectively the control group found the task easy, whereas the PD_(es) patients found the task difficult. The control group learning a subjectively easier task activated predominantly right-sided cortical regions. These brain areas appeared to be a subset of a larger bilateral sequence learning network that has previously been observed when normal subjects learn difficult sequences [Ghilardi et al., 2000; Nakamura et al., 2001]. The PD_(es) patients, learning a subjectively difficult task but achieving a degree of sequence learning performance equivalent to that of the controls, utilized greater volumes of the same brain regions that the control group activated and, in addition, recruited regions not utilized by the control group including contralateral left-sided cortical regions and bilateral lateral cerebellum. These recruited regions appeared to be similar to those previously identified as belonging to the normal subject bilateral sequence learning network. These results are reprinted in Figure 1A (with permission from Lippincott Williams & Wilkins) [Mentis et al., 2003] to facilitate a direct comparison with findings from the current analyses.

We test two hypotheses to explain the equal performance findings reported in Mentis et al. [2003]. These hypotheses aim to deepen our understanding of the mechanism by which PD_(es) subjects minimize the effects of disease on sequence learning. Our first hypothesis states that to achieve equal performance with healthy controls, the PD_(es) group will recruit additional regions from within a normal bilateral sequence learning network. To test this hypothesis we make the following two predictions: (1) When the same healthy controls from Mentis et al. [2003] learn a more difficult sequence, and when we increase our statistical power to detect brain activation, we will reproduce previous findings of a normal bilateral sequence learning network in this group of healthy controls. The right-sided regions in this group of healthy controls which are activated when learning subjectively easier sequences (see Fig. 1A) will be a subset of the normal bilateral sequence learning network. It is instructive to use the same healthy controls as in Mentis et al. [2003] because a within-subject rather than a between-subject design more strongly supports the notion that successful performance of an easy version of a task may utilize part of a network that a difficult version of the task utilizes in its entirety. (2) The bilateral network utilized by the PD_(es) group to learn sequences that they found difficult (see Fig. 1A) will be similar to the normal sequence

learning network activated by the healthy controls in (1). These predictions from hypothesis 1 are illustrated in Figure 1B.

However, sequence learning by PD_(es) patients is not normal. On average, the maximum sequence length PD_(es) patients can learn is shorter than the maximum sequence length matched healthy controls can memorize [Mentis et al., 2003]. There is a level of task difficulty above which compensation by PD_(es) patients fails. Furthermore, PD_(es) patients exhibit subtle abnormalities of learning even with tasks of moderate difficulty and often require more time to achieve equal performance with controls. To account for these behavioral observations, we propose hypothesis 2 to constrain hypothesis 1.

Hypothesis 2 states that information processing among regions within the bilateral dedicated sequence learning network in PD_(es) patients is different from and less efficient than information processing among those same regions in healthy controls. We tested this hypothesis using two partial least squares (PLS) multivariate analyses. These analyses were performed on the same equal performance and equal powered data as was used in Mentis et al. [2003]. Therefore, these multivariate results are directly comparable to the univariate analyses in Mentis et al. [2003] and in Figure 1A. We utilized task-PLS to confirm that both groups activated a common bilateral network to learn sequences (further confirmation of hypothesis 1), and then extended the previous analyses by evaluating the extent to which each group expressed the network. If the PD_(es) group had to express the common network more than the control group to achieve equal performance, the PD_(es) group was utilizing the network less efficiently than the controls. We used behavior-PLS to evaluate information processing within the same brain network in both groups. In this analysis, the success each individual had in learning the sequence (individual learning scores) was correlated with the extent to which each individual expressed a common group of regions (individual brain scores). Because these regions were common to both groups, if each group activated the regions similarly when learning, behavior-network correlations would be similar across groups. Conversely, if each group activated the regions differently when learning, behavior-network correlations would be significantly different across groups.

SUBJECTS AND METHODS

Subjects

Brain activation from five male and three female early-stage PD patients, mean age 60.4 ± 11.2 (SD) years, were compared with three male and five female healthy control subjects, mean age 58.6 ± 9.4 years. There was no difference in age ($P = 0.74$, t -test) or sex ($P = 0.20$, χ^2) between the eight PD and eight control subjects. Parkinsonian and control subjects with prior histories of unrelated neurological or psychiatric illness, hypertension, cardiovascular disease, diabetes mellitus, or abnormal

Figure 1.

Brain regions activated during sequence learning (TEseq-TEseq_c) in Parkinson's Disease and Control groups are color coded and overlaid on a structural MRI that conforms to the space of the Talairach and Tournoux [1988] atlas. yellow, PD but not Control; blue, PD and Control; red, Control but not PD. **A:** The two hypotheses tested in this report were generated from data that are printed from Mentis et al. [2003]. Panel A demonstrates brain regions activated when PD_(es) patients were in the process of achieving equal performance with controls. That is, when performance between the groups was equal by the end of the PET scan. Power to detect regional activation was

equivalent between groups. **B:** Testing hypothesis 1. The normal bilateral sequence learning circuit was observed when the control group learned longer sequences and when the power to detect activation was increased (Supporting prediction a). The PD_(es) group activated a network similar to the normal bilateral sequence learning network (supporting prediction b). Numbers represent level of axial slice in mm relative to the anterior-posterior commissure line. Left side of the brain is on left of image. Figure reproduced with permission of Lippincott, Williams & Wilkins [Mentis et al., 2003].

diagnostic MRI scans were excluded. All subjects were right-handed and had scores greater than 27 on the Mini-Mental State Examination [Folstein et al., 1975]. PD patients had pure hemiparkinsonism (Hoehn and Yahr Stage 1) without known causative factors, supranuclear gaze abnormalities, or ataxia.

Six of the eight PD patients had left hemiparkinsonism. All subjects signed informed consent in accordance with the North Shore University Institutional Review Board. Univariate equal performance data from this cohort has been presented previously [Mentis et al., 2003].

Tasks

The TEseq task and its motor control TEseq_c were identical to those reported previously [Mentis et al., 2003]. The required equipment, an example of a subject performing the task, and the strategy taught to subjects so that all learn the sequences in a comparable manner were recorded on a video (online at <http://www.interscience.wiley.com/humanbrainmapping>. A free download of Real Player V8.0 is available from <http://proforma.real.com/real/player/blackjack.html>). A figure illustrating the task is presented in Mentis et al. [2003].

During both TEseq and TEseq_c, subjects moved a hand-held puck on a digitizing tablet (12-inch $\times$ 18-inch; Numonics Corporation). The digitizing tablet was connected to a computer and monitor such that the puck moved a cursor on the computer monitor. The aim was to move the cursor out-and-back from a central position to one of a set of radially arranged circles called targets in synchrony with a 1-Hz tone. If a subject moved the cursor such that it entered the correct peripheral target between 250 msec prior to and after the tone, the target turned gray providing the subject with immediate positive reinforcement that a "hit" had been made. If a subject moved the cursor to an incorrect target or to the correct target but outside the time window with respect to the tone, the target did not turn gray providing negative reinforcement. TEseq is a declarative task because subjects are aware of its three basic rules: (1) The length of the sequence to be learned is equal to the number of visible peripheral targets. (2) Each target will be visited once and only once during each sequence cycle. (3) The complete sequence cycles continuously through its veridical order for the duration of the scan. A single strategy for sequence learning based on these three rules was taught to all subjects (see TEseq video).

Subjects also performed a control task TEseq_c that had movements of identical length and timing to TEseq (kinematically controlled) but required no sequence learning. Eight peripheral targets were placed in a circle around a center target. The subject made out-and-back movements in synchrony with a 1-Hz tone from the center target to each peripheral target in counterclockwise order starting with the target at 3 o'clock. Subjects received positive and negative feedback as with TEseq.

All subjects were given training and practice sessions prior to PET. Subjects used these sessions to practice the 1-Hz out-and-back movements and to familiarize themselves with the learning strategy. This training ensured equivalent movement parameters and a common method of sequence learning among all subjects. During practice sessions, we determined the longest sequence each PD patient was able to learn. This was defined as the longest sequence for which the PD patient correctly hit all targets in the sequence at least once within each of three successive blocks of 80 movements. During PET, each PD patient performed his or her personal longest sequence. In con-

trast, during these training sessions we evaluated the control group learning novel sequences at multiple sequence lengths. This enabled us to identify longer sequence lengths that the healthy controls could learn but that were too long for the PD_(es) patients. Data from healthy controls learning long sequences during PET were used in testing hypothesis 1 and are presented in Figure 1B. Further, we used the training sessions to identify the sequence length for each healthy control subject such that learning performance would be equal to the PD patients between 50 and 80 sec after starting the task, that is, for the last 30 sec of the PET scan. The univariate analysis of this data set was presented in Mentis et al. [2003] and reproduced in Figure 1A. We used the multivariate analysis of this dataset (partial least squares analysis), to deepen our understanding of hypothesis 1 (see Fig. 2 and Discussion), and to test hypothesis 2 (Fig. 3). To clarify, a PD patient had only one TEseq PET scan during which his or her personal longest sequence was learned. A control subject had three TEseq PET scans, one of which was paired with a PD subject such that learning was equivalent for the final 30 sec of the task. The remaining two TEseq scans were at different sequence lengths but equal to or longer than those learned by members of the PD group. All subjects had one control TEseq_c scan.

Previously [Mentis et al., 2003], we described several learning parameters that can be calculated from TEseq data including "correct" movements, acquired data, retrieved data, forgotten data, and perseveration data. As the number of correct movements is a general measure of learning that includes indices of sequence acquisition and sequence retrieval, it is suitable for evaluating hypothesis 2 (see Introduction and Discussion). Thus, for this study we used the number of "correct" movements as the behavioral correlate with brain function. We defined a movement as correct when the directional error was less than 22 degrees on either side of the appropriate target midpoint.

PET

Regional cerebral blood flow (rCBF) was measured using H₂¹⁵O with an 18-ring bismuth germanate GE Advance tomograph (General Electric, Milwaukee, WI) in 3-D mode that has reconstructed transverse and axial resolutions of 4.3 mm (FWHM) at the center, and an axial field of view of 14.5 cm [Dhawan et al., 1998]. Relative rCBF was estimated using a modification of the slow bolus method of Silbersweig et al. [1993], as described elsewhere [Ghilardi et al., 2000; Nakamura et al., 2001].

All patients and controls fasted for 6 hours prior to PET scanning. All antiparkinsonian medications were discontinued at least 12 hours before the studies. Motor tasks (Mseq and TEseq_c) were performed with the dominant right hand, and an intravenous catheter was placed in the left to administer H₂¹⁵O.

Figure 2.

Testing hypotheses 1 and 2. **A:** The same brain network is associated with sequence learning in both groups (supporting hypothesis 1 prediction b). The network is overlaid on horizontal sections from a structural MRI that conforms to the space of Talairach and Tournoux [1988]. Numbers represent level of axial slice in mm relative to the anterior–posterior commissure line. Left side of the

brain is on left of image. **B:** Degree to which each group expresses the brain network during TEseq and TEseq_c. To achieve equal performance, the PD_(es) group had to express the normal bilateral sequence learning network 15% more than the control group. This suggests less efficient utilization of the network by the PD_(es) group (supporting hypothesis 2).

Analysis

Univariate equal performance equal powered analysis performed in Mentis et al. [2003] and presented in Figure 1A

One TEseq scan for each of the eight PD patients was matched with one TEseq scan for each of the eight control subjects such that the number of correct movements during the final 30 sec of the scan was equivalent between groups. We used statistical parametric mapping (SPM99) software (MRC Cyclotron Unit, London, UK) to determine the statistical score at each voxel for the contrast TEseq-TEseq_c within each group. The regions within each group activated by sequence learning were color coded and superimposed upon the same structural Magnetic Resonance Image (MRI) in Figure 1A. Statistical scores were thresholded at $P < 0.001$. Thus, number of subjects, number of scans, and statistical threshold was equivalent between groups. We performed no correction for multiple independent comparisons on rCBF voxel changes as all regions activated have previously been identified by this and other sequence learn-

ing tasks [Boecker et al., 1998; Grafton et al., 1995; Honda et al., 1998; Jenkins et al., 1994; Nakamura et al., 2001]. These regions were considered to be hypothesis-driven in that they comprise the distributed networks known to be activated in sequence learning tasks and are, therefore, not independent of each other [Boecker et al., 1998].

Univariate analysis of expanded control data to test hypothesis 1 (Fig. 1B)

To test hypothesis 1, we made the control data set larger by adding two scans per subject (16 scans) to the original eight equal performance scans for a total of 24 scans. During these additional 16 scans, the 8 control subjects learned longer sequences. As this expanded control data set had three times as many scans as the equal powered, equal performance control data in Mentis et al. [2003], we had greater statistical power to detect regional activation. We performed the same within-group TEseq-TEseq_c analysis as presented above and in Mentis et al. [2003]. To reduce type 2 errors (detect truly active regions), we lowered the significance threshold for significant activation at each voxel to $P < 0.01$ (see Introduction and

Figure 3.

Testing hypothesis 2. **a:** In both groups, this brain network is significantly correlated with the number of “correct” movements. The network is overlaid on horizontal sections from a structural MRI that conforms to the space of Talairach and Tournoux [1988]. Numbers represent level of axial slice in mm relative to the anterior-posterior commissure line. Left side of the brain is on left

of image. **b:** The brain network-behavior correlation is significant for each group, but the direction of the correlation is significantly different across groups. This suggests information processing within the bilateral sequence learning network is different in the PD_(es) than in the Control group (supporting hypothesis 2).

Discussion). Increasing statistical power by increasing the number of scans, increasing task difficulty, and reducing statistical threshold were the three maneuvers we used on the expanded control data set to maximize our ability to detect the bilateral normal sequence learning circuit and test prediction (1) of hypothesis 1 (see Introduction). We illustrated predictions (1) and (2) of hypothesis 1 in Figure 1B by superimposing both the activated regions from this expanded healthy control data set and the original regions activated by the eight PD_(es) patients upon the same MRI. Note that the regions activated by the PD_(es) group in Figure 1B are the regions activated in the equal-powered, equal performance analysis that is presented in Figure 1A.

Multivariate partial least squares (PLS) analysis to further understanding of hypothesis 1 and test hypothesis 2

Partial least squares is a family of multivariate analyses that describe the relationship between any set of indepen-

dent variables like experimental tasks or behaviors with a set of dependent variables like brain images. PLS is similar to principal components [Moeller et al., 1987] or eigenimage analyses [Friston et al., 1993], but an important feature of PLS is that the solutions are constrained by including prior knowledge of experimental task (task-PLS) or behavior (behavior-PLS) into the primary data matrix. Two PLS analyses were performed on the equal performance dataset: on the eight control and eight PD TEseq scans matched for performance over the last 30 sec of the scan. Task-PLS (Fig. 2A,B) extended the SPM data on hypothesis 1 by allowing us to directly compare the extent to which each group utilized a common network of regions while learning a sequence. Behavior-PLS (Fig. 3a,b) provided data for hypothesis 2 by allowing us to directly compare brain-behavior correlations between a network of regions common to both groups and a general measure of sequence learning (“correct” movements). Because the network and its regional loadings were identical in both groups, the extent to which the network-

behavior correlation was similar or different between groups is an index of the extent to which information processing within the network was similar or different between groups. The mathematical details of this analysis have been described previously [McIntosh et al., 1996] (online at ftp.rotman-baycrest.on.ca/pub/Randy/pls). We present the essential details of the analysis here.

A data matrix containing prior information is generated for each analysis. For each task (task-PLS), each scans' difference from the grand mean of all scans was calculated across all voxels. To create a matrix of zero difference means the resultant (summed) images for the control group *TEseq* and *TEseq_c* were stacked on top of each other. These two images were then stacked on top of those images derived in similar fashion for the PD group. Scan-specific correlations (behavior-PLS) were computed between rCBF at each voxel within an image and the mean number of correct movements over the duration of the PET scan (from 0–80 sec). In both PLS analyses, the data matrix was analyzed with a singular value decomposition (SVD) to produce mutually orthogonal latent variables (LVs) that account for decreasing amounts of matrix variance. Each latent variable is associated with a vector of relationships among tasks or behavior (singular profile) on the one hand and an image-wide pattern of voxel activity (brain network) on the other hand. A singular vector quantifies the association between the singular profile and the brain network. The brain network can be displayed superimposed upon an anatomical image. The numerical weight for each element in the singular profile vector is called a task weight or a behavior weight, and the numerical weight at each voxel in the brain network is called a salience. In task-PLS, for each LV the brain network identifies that group of voxels that is most related to the difference between task (*TEseq*) and control (*TEseq-c*). In behavior-PLS, for each LV the brain network identifies that group of voxels that is most related to a measurement of behavior.

In behavior-PLS, we calculate a second piece of information similar to factor scores called brain scores. Brain scores quantify how strongly each subject expresses the brain network. Brain scores for each LV are derived from the inner product of the matrix of original rCBF images for each subject with the matrix of brain network images. To calculate network–behavior correlations we regress original behavior scores on brain scores.

Statistical assessment of brain- and behavior-PLS is performed at two levels: at the level of the entire latent variable and at the voxel or behavior measure level. A permutation test was used to determine the overall significance of each latent variable [Edgington, 1980]. Five hundred random permutations of the data were performed. At each permutation, the singular values for the permuted data were compared to the original analysis. We considered a brain network significant if the latent variable it represented was significant at permutation $P < 0.05$, i.e., the permuted data produced a singular value greater than or equal to the original analysis less than 5% of the time. There is no need to correct for multiple comparisons in this assessment since the permutation

P values were determined across the entire latent variable rather than at the voxel level.

We then assessed the stability of voxel salience values (i.e., whether they should be considered reliable members of a brain network) and the stability of brain–behavior correlations using bootstrap estimation of standard errors [Efron and Tibshirani 1986; McIntosh et al., 1999]. Bootstrapping entails resampling the entire group with replacement. One hundred bootstrap samples were performed. If the salience value of a voxel depended greatly on particular subjects in the group, the salience value of such a voxel would have a high standard error. Conversely, if the salience value of a voxel remained the same regardless of which subjects were included in the group, the salience value of such a voxel would have a low standard error. Individual voxels were considered stable and part of a brain network if they had a ratio of salience value to salience standard error (bootstrap ratio) greater than 2. That is, the voxels presented as part of the network were those identified with a consistent salience value throughout the bootstrap procedures. The network–behavior correlations were considered stable if the sign of the bootstrap correlation did not change compared to the original correlation 95% of the time (i.e., the 95% confidence interval [CI] for r did not cross zero). To clarify, the bootstrap procedure evaluates the stability of a voxel's contribution to a pattern (voxelwide index) or the stability of a network–behavior correlation. The statistical assessment of the LV and its associated brain network is provided by the permutation test (imagewise index). In both permutations and bootstrapping, orthogonal procrustes rotations of the resampled estimates to the original PLS estimates were performed to correct for arbitrary axis reflection and rotations [Milan and Whittaker 1995].

RESULTS

Psychophysical Results

In those 16 scans (one per subject) used for the equal performance analyses (Figs. 1A, 2, and 3), the correct learning parameter was no different between-groups for the time period 50 through 80 sec during the PET scan ($P = 0.97$ repeated measures analysis of variance [rANOVA]). We have previously shown [Mentis et al., 2003] that there was no between-group difference for any of the learning parameters during this time period in the PET scan. However, there were modest differences in several learning parameters during the first 50 sec of the PET scan. Thus, the fundamental premise of this modified equal performance design was satisfied. That is, that during the first 50 sec of the scan (when most PET signal is recorded), PD subjects were in the process of doing whatever was necessary to achieve equal learning performance by 50 through 80 sec.

There were no between group differences in movement psychophysics for the duration of the PET scan. The movement parameters measured included; the time difference between movement onset and the 1-Hz tone ($P = 0.15$ rANOVA), the time taken to complete the whole out-and-

back movement ($P = 0.39$ rANOVA), and the time difference between out-and-back reversal and the 1-Hz tone ($P = 0.30$ rANOVA). Four of the six PD patients had left hemiparkinsonism and, therefore, were making movements with their clinically normal hand. Interestingly, values of movement parameters of the two right hemiparkinsonism patients were close to the mean of the four left hemiparkinsonism patients. We also demonstrated [Mentis et al., 2003] that there were no between-group differences in either brain function or movement psychophysics during *TEsec_c*. Thus, although we are evaluating a group with Parkinson's disease, any between-group differences in brain function are unlikely to be the result of differences in movement, or differences in the baseline control task, and are likely to be the result of differences in learning.

The extended control data set used for the analysis shown in Figure 1B consisted of three scans per control subject and included those scans where the controls learned more difficult sequences than in the equal performance data set. We found that all control subjects but only one of the PD patients were able to successfully learn a six-element sequence ($P < 0.0001$). This supported the notion that the performance of PD patients can approximate that of controls up to a moderate level of difficulty after which there is a dramatic fall-off in performance.

Positron Emission Tomography Results

Univariate equal performance equal powered analysis performed in Mentis et al. [2003] and presented in Figure 1A

We present Figure 1A as the two hypotheses tested in the current study were developed from this analysis. Further, the PLS analyses (Figs. 2 and 3) were performed on the same data set as that which generated Figure 1A, so it is instructive to directly compare the results of hypothesis testing (Figs. 1B, 2, and 3) with the results of the original analysis (Fig. 1A). The univariate results of the equal performance analysis have been presented in detail [Mentis et al., 2003] and will be briefly summarized here. Figure 1A is color coded such that brain regions activated by *TEseq* in both groups are blue, in controls but not PD are red, and in PD but not controls are yellow. Brain regions activated by both groups (blue) included right dorsolateral prefrontal cortex (DLPFC Brodmann Area (BA) 46/10), bilateral premotor cortex (PMC BA 6/8), rostral supplementary motor area (preSMA BA 6), precuneus (BA 7), and right parietal cortex (PC BA 7/40). The PD_(es) group activated a total of 9,621 voxels while the control group activated 2,397 voxels. The PD_(es) activation included all regions activated by the control group (yellow around blue, yellow next to red), as well as regions not activated by the control group (yellow). The latter included a very large activation in left DLPFC extending from 6 mm through 38 mm relative to the anterior commissure–posterior commissure (ACPC) line (BA 46/10/9), anterior cingulate (BA 32), left PC (BA 7/40), left insula/inferior frontal (BA 47), left orbitofrontal cortex (OFC BA

10), and bilateral lateral cerebellum. Thus, regions activated by both groups were predominantly right sided, whereas regions activated by the PD_(es) group alone were predominantly the homologous left-sided regions and bilateral cerebellum.

Univariate analysis of expanded control data to test hypothesis 1 (Fig. 1B)

Figure 1B is color coded as in Figure 1A. The control group activation (red and blue voxels) represents those regions activated by a more difficult *TEseq* task, and after statistical power to detect activated regions was increased (see Materials and Methods). The regions activated included bilateral OFC, bilateral DLPFC, bilateral PMC, bilateral PC, preSMA, precuneus, anterior cingulate and bilateral lateral cerebellum. Thus, supporting the first prediction of hypothesis 1, this control group did indeed activate the large bilateral network we have seen previously with other healthy subjects learning difficult sequences [Ghilardi et al., 2000; Nakamura et al., 2001].

To facilitate the comparison of regions activated by the expanded control data and regions activated by the PD_(es) group, we superimposed the networks activated by each group onto the same MRI in Figure 1B. Color coding in Figure 1B is the same as in Figure 1A. The similarity of the regions activated by each group is apparent. This observation supports the second prediction of hypothesis 1, that the PD_(es) group learning their most difficult sequences (albeit sequence lengths that are relatively easy for controls to learn) utilized the normal large bilateral sequence learning network.

Multivariate partial least squares (PLS) analysis support hypothesis 1 and test hypothesis 2 (Fig. 2)

Task-PLS. There was a high level of covariance (74%) between the task and brain function in the task-PLS analysis. Figure 2B shows that salience values for *TEsec_c* were negative in both controls (−4.6) and in PD patients (−5.4), while values for *TEseq* were positive in both controls (4.6) and PD patients (5.4). This confirms that the same pattern of regional activation in each group was associated with learning (positive values for *TEseq*) but not associated with movements (negative values for *TEsec_c*). Those brain regions in Figure 2A that make up the pattern of regional activation associated with learning had voxelwise bootstrap-derived ratios $> +2.0$ and < -2.0 . Thus, the voxels making up the network were stable in that they consist only of those voxels that were consistently present in the bootstrap procedures. Positive bootstrap ratios (regions of brain activation) were observed in bilateral DLPFC (BA 46/10), bilateral PMC (BA 6/8), bilateral PC (BA 7/40), preSMA (BA 6), precuneus (BA 7), anterior cingulate (BA 32/24), and right insula/inferior frontal lobe (BA 47) (Fig. 2A). That is, in regions similar to those identified by univariate analyses when controls and PD_(es) subjects learn subjectively difficult sequences (compare activations in Fig. 2A with those in Fig. 1B).

However, although the task salience values for the common pattern of regional activation were high in both groups, they were not identical. Values were 15% higher in the PD than in the control group (Fig. 2B) indicating that the bilateral sequence learning network was less expressed by the control group than by the PD_(es) group. This suggests that while they were in the process of achieving equal performance, the PD_(es) group utilized the normal bilateral sequence learning network less efficiently than the healthy controls.

Behavior-PLS. There was a high level of covariance (41%) between the number of correct movements and brain function in the behavior-PLS analysis. Figure 3a shows brain regions associated with the first latent variable that have voxelwise bootstrap-derived ratios above +2 and below -2. Regions with positive ratios (brain activation) included left DLPFC (BA 46/10), anterior cingulate (BA 32/24), preSMA (BA 6), and precuneus (BA 7). These regions are part of the normal bilateral sequence-learning network. Figure 3b shows that in the control group, the correlation between number of correct movements and this network of brain regions was positive ($r = +0.88$), significant (95% CI did not cross zero) and stable (bootstrap ratio 5.4). In contrast, in the PD group the correlation was negative ($r = -0.82$), significant (95% CI did not cross zero), and stable (bootstrap ratio -4.8). Thus, a subgroup of regions from the bilateral sequence learning network significantly correlated with the learning parameter correct in both groups. However, the slope of the correlations was significantly different between groups (no overlap between 95% CI for each correlation). This suggests that although both groups utilized the same regions to learn, the manner in which those regions were utilized in the learning process was significantly different between the groups.

DISCUSSION

A network of brain regions has been shown to be associated with normal sequence learning [Ghilardi et al., 2000; Grafton et al., 1995; Honda et al., 1998; Jenkins et al., 1994; Nakamura et al., 2001; Rauch et al., 1995; Sakai et al., 1998; Toni et al., 1998]. Although much remains unknown, a plausible functional anatomy for some of the regions observed in the network can be summarized as follows. The initial seconds of data acquisition activate first left DLPFC followed by right DLPFC [Sakai et al., 1998]. The right DLPFC, right dorsal PMC, and right PC are involved in spatial working memory [Goldman-Rakic, 1987] and in integrating visual and sensory information into an internal representation of external space [Mesulam, 1990]. Contributing to this network, and more important in acquisition of new (rather than retrieval of old) sequences, are rostral supplementary motor area (preSMA), anterior cingulate, caudate head, and anterior putamen. By contrast, the retrieval of old sequences (but not acquisition of new ones) involves primarily precuneus and posterior striatum [Boecker et al., 1998; Hikosaka et al., 1998; Honda et al., 1998; Jueptner et al., 1997a,b]. Rostral dorsal-PMC prepares and selects the motor movements

while caudal dorsal-PMC executes the movements through connections with the primary motor strip [Wise and Kurata, 1989]. Although less well studied, activation within limbic-associated regions (anterior cingulate, OFC, striatum) might be associated with attention and reward for successful learning [Brown, 1999; Francis et al., 1999; Graybiel, 1995]. Activation in lateral cerebellum may also play a role in motor sequence learning via lateral cerebellar loops that project to DLPFC [Middleton and Strick, 2000].

Little is known concerning the applicability of these notions to PD [Brown, 1999; Doyon et al., 1998]. Because of the emphasis on motor rather than cognitive symptoms in PD, many imaging studies on PD patients have concentrated on motor sequence execution [e.g., Catalan et al., 1999; Sabatini et al., 2000; Samuel et al., 1997] and appropriately have minimized the effect of new learning by ensuring that the sequences were overlearned prior to performance in the scanner. However, sequence learning in PD has been shown to activate many regions observed in the normal bilateral sequence learning network [Nakamura et al., 2001], and brain regions associated with the planning of visuospatial sequences in PD have been reported [Owen et al., 1998]. We used a modified equal performance design to identify brain mechanisms by which PD patients overcome the effects of disease to minimize behavioral dysfunction [Mentis et al., 2003]. To do this we compared brain function in a PD_(es) group with brain function in a matched control group while the PD_(es) patients were in the process of achieving a degree of sequence learning performance equivalent to that of the controls. PD and healthy control subjects were matched to have equal learning performance during the final 30 sec of a ^{15}O -labelled water (H_2^{15}O) positron emission tomography (PET) scan. That is, by the end of the scan PD patients had successfully overcome any effects of pathology and were learning as well as controls. Therefore, data collected during the initial 50 sec of the PET scan, when most signal is acquired, recorded this successful mechanism.

Behaviorally, on the one hand PD_(es) patients perform well when sequence learning tasks are of mild to moderate difficulty. Evaluation of learning parameters revealed only subtle abnormalities of behavioral learning rates during the first 50 sec of scanning [Mentis et al., 2003]. On the other hand, PD_(es) patients perform poorly when task difficulty is increased. In the present study, all control subjects but only one of the PD patients were able to learn a six-element sequence within 80 sec ($P < 0.0001$). Further, those PD_(es) subjects who were able to learn four or five element sequences with near normal parameters did not perform five or six element sequences with parameters moderately subnormal, but rather were unable to perform the task at all. It appears that PD_(es) patients learn easy and moderate sequences almost as well as controls, but then reach a performance ceiling and perform very much worse than controls on tasks that are only slightly more difficult.

Anatomically, in Mentis et al. [2003] it was demonstrated that the control group, learning sequences of moderate difficulty (for them), activated right dorsolateral prefrontal cor-

tex (DLPFC) (Brodmann Area (BA) 46/10), bilateral premotor cortex (PMC) (BA 6/8), anterior supplementary motor area (preSMA) (BA 6), precuneus (BA 7), and right parietal cortex (PC) (BA 7/40). In contrast, to achieve equal performance, the PD_(es) patients had to activate four times as much brain as the control group. They utilized greater volumes of the same brain regions activated by the control group, and in addition activated regions not utilized by the control group including contralateral cortical regions and bilateral lateral cerebellum (see Fig. 1A). We note that the regions activated by the PD_(es) group in the equal performance experiment were similar to the regions we have previously seen activated by healthy controls learning more difficult sequences [Ghilardi et al., 2000; Nakamura et al., 2001]. Further, these regions have also been identified during sequence learning in tasks different from TEseq [Boecker et al. 1998; Catalan et al., 1999; Grafton et al., 1995; Honda et al. 1998; Jahanshahi et al., 1995; Jenkins et al., 1994; Jueptner et al., 1997a,b; Rauch et al., 1995; Sakai et al., 1998; Samuel et al., 1997; Toni et al., 1998]. We used these anatomical and behavioral observations to generate two hypotheses to further understand the mechanism by which PD_(es) patients overcome the effects of Parkinsonism (see Introduction).

Testing hypothesis 1. To achieve equal performance with healthy controls, PD_(es) patients recruit additional regions from within a large bilateral network normally specialized for sequence learning

Implicit in this hypothesis is the notion that under certain conditions any healthy control will activate a large bilateral network to learn sequences. Thus, the first prediction of this hypothesis was that the healthy controls in Mentis et al. [2003] who had activated a predominantly right-sided subset of regions while performing a sequence learning task of moderate difficulty (for them) would activate the bilateral network under different circumstances. We changed the circumstances of the control group in three ways: (1) We increased the length of sequences to be learned on the assumption that the controls may need to recruit more of the learning network to learn a more difficult task. (2) We increased the number of control TEseq scans to be analyzed from 8 to 24 to increase our statistical power to detect activated regions. (3) We decreased the statistical threshold for considering a regional activation significant from $P < 0.001$ to $P < 0.01$. We did this to reduce the chance of failing to consider a true regional activation significant (reduce type 2 errors). Obviously, this will simultaneously increase the risk that we might falsely consider a spurious region part of the learning network (increase type 1 errors). However, we were only interested in establishing whether the regions previously identified as constituting the bilateral sequence learning network would also be activated by the original control group. We were not interested in claiming that any new regions that might be falsely activated were also part of the learning network. Thus, our interpretation strategy would not result in type 1 errors. (The same argument applies to the comparison of the control network with

the PD_(es) network below). After implementing the above three measures to improve our ability to identify regions activated by TEseq, the control group activated bilateral OFC, bilateral DLPFC, bilateral PMC, bilateral PC, preSMA, precuneus, anterior cingulate and bilateral lateral cerebellum (Fig. 1B). As these are all regions in the bilateral sequence learning network, this data supports the first prediction from hypothesis 1.

The second prediction derived from hypothesis 1 was that the additional regions (yellow regions in Fig. 1A) recruited by the PD_(es) patients to learn sequences that for them were difficult, would be part of the normal bilateral sequence learning network. That is, part of the network observed in the original healthy control group learning sequences that were difficult for them. In Figure 1B, we superimposed the networks derived from the healthy control group and the PD_(es) group each learning appropriately difficult sequences on the same MRI. The substantial overlap observed between these two networks supports the second prediction. The data are consistent with both predictions from the first hypothesis. Therefore, we suggest that the mechanism by which PD_(es) patients achieve equal performance with healthy controls is to recruit regions from within a large bilateral network that is normally specialized for sequence learning.

Testing hypothesis 2. Information processing among regions within the bilateral dedicated sequence learning network in PD_(es) patients is different from and less efficient than information processing among the same regions in healthy controls

In contrast to the SPM analysis presented in Figure 1B, task-PLS and behavior-PLS analyses were performed on the same equal performance, equal powered dataset as in Mentis et al. [2003] (i.e., eight PD_(es) TEseq scans, eight control TEseq scans where learning was equivalent with the PD_(es) group for the last 30 sec of the scan, and eight TEseq_c scans from each group). It is important to appreciate that the PLS analyses calculated brain networks across both groups combined. No attempt was made to model the regions activated by the PD_(es) and control groups separately as in the within-group SPM t -tests. We chose this approach to evaluate how function in a set of regions common to both groups relates to task differences or to behavior differences in each group. This allows for a direct head-to-head comparison of mechanisms the PD_(es) group might have used to successfully overcome the effects of parkinsonism and perform equivalently with controls.

Task-PLS. There was a 74% correlation between the first latent variable and the tasks. In both groups, task weights were large and positive for TEseq and large and negative for TEseq_c (Fig. 2B). Therefore, this network was associated with sequence learning (TEseq) in both groups. This data confirms the SPM finding (Fig. 1B) that sequence learning in both groups activates a large bilateral dedicated network that includes DLPFC, PMC, anterior cingulate, preSMA, PC,

and precuneus. However, the observation that the task weights were 15% higher in the PD_(es) than the control group extends the SPM findings. To achieve equal performance, the PD_(es) group expressed this large bilateral network 15% more than the control group. Indeed, when a univariate SPM analysis was performed on the same data (see Mentis et al. [2003] and Fig. 1A), most of the left-sided regions in the control group did not meet the statistical threshold of $P < 0.001$ to be considered activated. We interpret the SPM and PLS data together as supporting the notion that both groups were in fact, utilizing the large normal bilateral network to learn sequences. However, the PD_(es) subjects had to express the left-sided regions in particular more strongly than the control group to achieve equal performance. Thus, PD pathology resulted in the PD_(es) patients utilizing the bilateral sequence learning network less efficiently than healthy subjects and activating left-sided cortical regions relatively more than control subjects.

Behavior-PLS. Because it directly correlates individual scores of learning success with a network of activation that is common to both groups, behavior-PLS is well suited for a direct comparison of information processing between the groups. There was a 41% correlation between the number of “correct” movements made by each subject and brain function. The regions identified as stable contributors to this correlation included left DLPFC, anterior cingulate, preSMA, and precuneus (Fig. 3a). These are regions identified by the univariate SPM analysis (Fig. 1A,B), by the task-PLS analysis (Fig. 2A), and by the literature [Boecker et al. 1998; Catalan et al. 1999; Ghilardi et al. 2000; Grafton et al. 1995; Honda et al. 1998; Jahanshahi et al., 1995; Jenkins et al., 1994; Jueptner et al., 1997a,b; Nakamura et al. 2001; Rauch et al., 1995; Sakai et al. 1998; Samuel et al. 1997; Toni et al. 1998] as important for encoding of new sequences and retrieval of learned sequences. As the “correct” measure of learning is a composite index of encoding and retrieval [Mentis et al., 2003], is it a good general measure of learning for this analysis. Both groups had significant correlations between function within this network of regions and correct movements (Fig. 3b, see Results) suggesting that these regions were significantly involved in sequence learning in both groups. However, the direction of the correlation between correct movements and the network was significantly different between the groups (Fig. 3b) suggesting that information processing among these regions was different between the groups.

The behavior-PLS analysis was not designed to extract the best Parkinsonian or the best control network associated with learning. A within-group multivariate analysis would be appropriate for such a goal [e.g., Nakamura et al. 2001]. Rather, the present analysis in which individual measures of learning were correlated with rCBF changes in a common group of regions was designed for a direct head-to-head between-group comparison of information processing among those regions. Therefore, the interpretation should be restricted to an evaluation of whether information processing within regions was similar or different between the

groups. More specific interpretations, for example, that increases in activity in preSMA was associated with improved learning in controls but impaired learning in PD_(es) are beyond the scope of this “common-region” design and should be approached differently.

Further Analyses

In Mentis et al. [2003], data and analyses were presented supporting the assertion that between-group differences in PET brain function were due to between-group differences in learning and not to differences in motor psychophysics, or brain function associated with movement. This assertion is equally applicable to the present analysis because the multivariate PLS analyses (Figs. 2 and 3) were performed on the identical dataset as that in Mentis et al. [2003]. The control group univariate SPM analysis (Fig. 1B) used the same control subjects.

Conclusion

The analyses in this study have attempted to deepen our understanding of the brain mechanisms used by PD_(es) subjects to overcome the effects of pathology and achieve performance levels approximating normal. A discussion on what underlying pathology might be responsible for these mechanisms has been presented in Mentis et al. [2003].

Nondemented Parkinson's Disease patients minimize the effects of PD pathology on sequence learning by increasing the level of function within left-sided brain regions that form part of a normal bilateral dedicated sequence learning network. When the level of task difficulty does not normally (i.e., for healthy controls) require bilateral activation, this mechanism results in near normal learning behavior in the PD_(es) group. However, although the regions activated by the PD_(es) group are the same as those utilized by healthy controls, information processing among these regions by PD_(es) patients is different from and less efficient than that of healthy control subjects. Therefore, when PD_(es) patients attempt to learn a sequence that would normally require bilateral activation of the network, PD_(es) learning fails dramatically.

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LEGEND TO THE VIDEO

A videotape accompanies this article in the online issue (www.interscience.wiley.com/humanbrainmapping).

TEseq_fast or TEseq_slow.

Segment 1. Equipment.

Segment 2. Subject performing TEseq task.

Segment 3. Strategy for learning TEseq task.

The video is 4 minutes long. A free download of Real Player v. 8.0 is available from <http://proforma.real.com/real/player/blackjack.html>.

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