Motor-symptom Laterality Affects Acquisition in Parkinson's disease: a Cognitive and fMRI study

Journal:	*Movement Disorders*
Manuscript ID	MDS-16-0758.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
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Keywords:	Parkinson's disease, Hemispheric asymmetry, Dopamine, Association learning, Resting-state functional MRI

Title Page

Motor-symptom Laterality Affects Acquisition in Parkinson's disease: a Cognitive and fMRI study

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Word Count for Manuscript: 3856; **Word Count for Abstract:** 244; **Character Count for Title:** 96.

Keywords

Parkinson's disease; hemispheric asymmetry; dopamine; association learning; resting-state functional MRI

Running title: Lateral Parkinsonism affects acquisition

Funding sources:

Dr. Tan is in charge of National Natural Science Foundation of China (81100952). Dr. Liu is in charge of National Natural Science Foundation of China (81201083). Prof. Zang is in charge of National Natural Science Foundation of China (81520108016). Prof. Chen is in charge of National Natural Science Foundation of China (30872729, 30971031). Prof. Gluck received grants from Program for Outstanding Medical Academic Leader (LJ 06003), and the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health (1 P50 NS 071675-02). The other co-authors report no disclosures relevant to the manuscript.

Conflict of interests: None.

Abstract

Objective: Asymmetric onset of motor symptoms in PD can affect cognitive function. We examined whether motor-symptom laterality could affect feedback-based associative learning and explored its underlying neural mechanism by fMRI in PD patients.

Methods: We recruited 63 early-stage medication-naïve PD patients (29 left-onset medication-naïve patients, 34 right-onset medication-naïve patients) and 38 matched normal controls. Subjects completed an acquired equivalence task (including acquisition, retention and generalization) and resting-state fMRI scans. Learning accuracy and response time in each phase of the task was recorded for behavioral measures. Regional homogeneity was used to analyze resting-state fMRI data, with regional homogeneity lateralization to evaluate hemispheric functional asymmetry in the striatum.

Results: Left-onset patients made significantly more errors in acquisition (feedback-based associative learning) than right-onset patients and normal controls, while right-onset patients performed as well as normal controls. There was no significant difference among these three groups in the accuracy of either retention or generalization phase. The three groups did not show significant differences in response time. In the left-onset group, there was an inverse relationship between acquisition

errors and regional homogeneity in the right dorsal rostral putamen. There were no significant regional homogeneity changes in either the left or the right dorsal rostral putamen in right-onset patients when compared to controls.

Conclusions: Motor-symptom laterality could affect feedback-based associative learning in PD, with left-onset medication-naïve patients being selectively impaired. Dysfunction in the right dorsal rostral putamen may underlie the observed deficit in associative learning in patients with left-sided onset.

102

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by resting tremor, rigidity, bradykinesia and postural instability. The onset and progression of motor symptoms in PD are usually asymmetric, reflecting asymmetric contralateral dopamine depletion in the basal ganglia.[1-4] Relationships between cognitive performance and symptom asymmetry have been revealed that left-onset PD patients performed worse on cognitive measures, such as spatial attention and tasks of orientation and mental imagery, than right-onset PD patients.[5-7] Cognitive processes that are closely related with dopamine, such as cognitive flexibility and motivation, showed different deficits between right-onset and left-onset PD patients.[8] For example, left-onset PD patients with greater loss of dopamine in the right hemisphere had impaired cognitive flexibility.[5, 8]

Feedback-based associative learning, which involves learning through corrective feedback provided on each trial, has been correlated to striatal dopamine release[9] and the function of the basal ganglia.[10, 11] Previous studies have reported that feedback-based associative learning was impaired in PD patients.[12, 13] However, the impairment in feedback-based learning in PD is not a universal finding, and many factors could contribute to this variability. Wilkinson L et al. did not find a selective impairment in PD in probabilistic feedback-based learning, but reported that there was a significant correlation between disease severity and the impairment in feedback-based learning.[14] It has also been reported that off-medication PD patients

learned stimulus-response associations equally well compared to healthy controls but learning was impaired by dopaminergic medication.[13] Thus, in the underlying disease process, disease severity and dopaminergic medication might be all involved in modulating feedback-based learning. However, it is still not clear how the dopamine asymmetry affects feedback-based associative learning in PD patients.

To control and minimize the effects of medication involvement and disease severity, we recruited early-stage medication-naïve right-handed PD patients including Left-onset medication-naïve patients (L-naïve) and Right-onset medication-naïve patients (R-naïve). We used a computer-based cognitive task of learning and generalization based on the acquired equivalence paradigm to test the effects of dopamine asymmetry on feedback-based associative learning. The acquired equivalence task, which includes tests of acquisition, retention and generalization, was repetitively used to evaluate feedback-based associative learning in patients with PD and other neurodegenerative disorders.[10, 11] In the acquisition phase, learning through trial-by-trial feedback learning was shown to correlate to striatal function, while generalization without feedback was shown to correlate with hippocampal and medial temporal lobe (MTL) functionality.[10, 11] Although striatal involvement in associative learning has been consistently reported in the previous task-based fMRI studies, such as caudate nucleus, amygdala and ventral striatum, the specific loci are not always the same.[15-17] Thus, which subdivision of the basal ganglia is associated with feedback-based associative learning remains unclear, and resting-state fMRI (rs-fMRI)

data were collected to explore the underlying neural mechanism of feedback-based learning.

In the present study, we examined whether motor-symptom laterality could affect feedback-based associative learning and explored its underlying neural mechanism using rs-fMRI in PD patients.

Subjects and Methods

Subjects. We recruited 63 right-handed, early stage (H-Y scores between 1 and 2), medication-naïve PD patients and 38 right-handed normal controls (NC) during 2012-2015. Subjects in the PD and NC groups were matched for age, gender, education and general cognitive status.[18] According to the motor-symptom laterality, PD patients were divided into L-naïve (N = 29) and R-naïve (N = 34) subgroups. All participants were non-demented (Mini Mental State Examination, MMSE $\geq$ 24) and scored less than 15 on the Beck Depression Inventory II (BDI-II).[19, 20] Participants were also screened for history of cerebral trauma, cerebrovascular diseases, head surgery, severe sleep disorders, hyperthyroidism, insulin-dependent diabetes, psychiatric or neurological disorders, abuse of alcohol, tobacco use, use of hormonal contraceptives, anticholinergic drugs, or antidepressants. For subjects with PD, only patients with unilateral side of onset and asymmetrical motor symptoms were involved and diagnosis was confirmed by two movement disorder specialists according to the UK Brain Bank criteria for the diagnosis of PD.[21] The side of onset was determined by medical history

and physical examination. The severity of motor symptoms was evaluated by Unified Parkinson's Disease Rating Scale III (UPDRS-III).[22] Motor asymmetry index (MAI) was calculated as (right side symptoms - left side symptoms) / (right side symptoms + left side symptoms).

Standard protocol approvals, registrations, and patient consents. We received approval from the Ethics Committee of Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine. We obtained written informed consents from all patients and controls prior to their participation in the study.

Behavioral data acquisition and evaluation. We used an Apple MacBook to run the Acquired Equivalence Task.[11] This task is composed of two phases: acquisition and transfer. In the acquisition phase, participants acquire associations of colored faces and fish through trial-by-trial feedback-based learning. Following this acquisition, the transfer phase includes two types of trials: tests of previously learned associations (retention) and tests of new associations that are presented without feedback (generalization). The mean number of incorrect choices and the mean response time in each phase was recorded for behavioral measures. For detailed procedure, please refer to eMethod 1 and eTable 1 in the Supplement.

MRI data acquisition and processing. MRI scan was performed after the acquired

equivalence test with an interval of 8.6 ± 1.5 days. A subgroup of 70 subjects (23 L-naïve, 25 R-naïve and 22 NC) participated in resting-state fMRI on a 3.0 T GE Medical System scanner based on the subjects' willingness. During the scan, the subjects were asked to remain motionless and awake with their eyes closed. For each participant, 210 functional images were collected using echo planar imaging T2*-weighted sequence (repetition time = 2000 ms, echo time = 30 ms, flip angle = 90 ^{o}, 33/35/37 slices, matrix = 64 × 64, voxel size = 3.75 × 3.75 × 4 mm^3). Then the high-resolution, three-dimensional T1-weighted structural images (repetition time = 5.78 ms, echo time = 1.77 ms, flip angle = 12 ^{o}, 196 slices, matrix = 256 × 256, voxel size = 1 × 1 × 1 mm^3) were acquired for registration and normalization of the functional images.

After exclusion due to vascular diseases (1 L-naïve, 2 R-naïve and 3 NC) and obvious head motion (2 L-naïve and 2 R-naïve, with the translation and rotation head motion parameters larger than 2 mm or 2°), MRI data from 60 subjects (20 L-naïve, 21 R-naïve, and 19 NC) qualified for analysis. MRI data were processed with Data Processing Assistant for Resting-State fMRI (DPARSF) programs.[23] Regional homogeneity (ReHo), as a commonly used method to analyze rs-fMRI data, was used with the rs-fMRI Data Analysis Toolkit (REST, http://www.restfmri.net) by calculating the Kendall's coefficient of concordance (KCC) of the time series of a given voxel with its 26 nearest neighboring voxels.[24, 25] With the assumption that PD patients with unilateral onset of motor symptoms had asymmetric functional impairments in the

brain, ReHo lateralization index was used to evaluate hemisphere asymmetry in neural activity as previously reported.[26, 27] Striatum subregions including the putamen, caudate and pallidum were chosen as regions of interest (ROIs). For detailed methods, please refer to eMethod 2 in the Supplement.

Statistical analysis. The normality of clinical and demographic data distribution was checked by the Kolmogorov-Smirnov test. One-way ANOVA was used to compare the normally distributed continuous variables (age), and the chi-square test was employed to analyze categorical variables (gender). The continuous variables that were not normally distributed (education, disease duration, H-Y, BDI-II, MMSE, UPDRS-III score, MAI) were analyzed by Kruskal-Wallis test. Mix-model ANOVAs with group as between-subject factor and phase as within-subject factor were used to analyze the behavioral data. Post-hoc analysis was done using Tukey HSD test. In the correlational analysis, Spearman rho was calculated. The alpha level was set at 0.05. All P values less than the alpha level were considered statistically significant. SPSS version 17.0 (IBM, Chicago, IL, USA) was used for statistical analysis.

For the fMRI data analysis, comparison of the hemispheric asymmetry among groups was performed using one-way ANCOVA. Age, gender, the mean frame-wise displacement (FD) corresponding to the temporal derivative of the head motion parameters, and mean gray matter (GM) volume of ROI were used as nuisance covariates.[28, 29] Post-hoc analysis was performed within the significant regions. To

control for family-wise error rates, Monte Carlo simulations were performed (3dClustSim; 10,000 iterations) using all brain voxels within the half-striatum ROI.[30] The cluster threshold for a corrected alpha level of P = 0.05 was 27 voxels for ANCOVA and 7 voxels for post-hoc t-test, respectively.

Results

Clinical and demographic characteristics of subjects

The clinical and demographic characteristics of subjects are presented in Table 1. All the groups were matched for age, gender, education, MMSE, and BDI-II ($P \geq 0.190$). There were no significant differences in disease duration, H-Y score, and UPDRS-III scores (including tremor, rigidity, bradykinesia sub-scores and total scores) between the L-naïve and R-naïve PD groups ($P \geq 0.104$). MAI was calculated as (right side symptoms - left side symptoms) / (right side symptoms + left side symptoms). The mean value of MAI for L-naïve and R-naïve groups were -0.9 (0.2) vs. 0.9 (0.2) respectively, indicating that motor-symptom asymmetry was also matched. In addition, subjects participating in the MRI examination were also matched across the three groups (see eTable 2 in the Supplement).

Behavioral performance

Acquisition was impaired in L-naïve but normal in R-naïve patients

Mix-model ANOVAs showed that both group [$F(2, 294) = 7.228$, $P = 0.001$] and phase

[F (2, 294) = 52.680, P < 0.001] had significant effects on accuracy. The interaction between group and phase was at a trend-level [F (4, 294) = 2.127, P = 0.077]. Fig.1A indicated that the group effect is primarily driven by the acquisition phase. Post-hoc analysis showed that accuracy was significantly different among L-naïve, R-naïve and NC. L-naïve patients made significantly more errors than R-naïve patients (P = 0.003) and NC (P = 0.002), while R-naïve patients performed as well as NC (P = 0.996) (Fig. 1A). There were no significant differences between groups in retention or generalization phase, indicating that retrieval function and generalization were preserved in both L-naïve and R-naïve patients (retention, P = 0.201 vs. generalization, P = 0.331) (Fig. 1A). Thus, L-naïve patients, rather than R-naïve patients, were selectively impaired in feedback-based associative learning, indicating a potential effect of asymmetric dopamine depletion on associative learning.

Phase had significant effect on the response time [F (2, 294) = 12.564, P < 0.001]. However, there was neither an effect of group [F (2, 294) = 0.733, P = 0.481] nor an interaction between group and phase [F (4, 294) = 0.782, P = 0.538] (Fig. 1B).

Functional MRI results

Dorsal rostral putamen was impaired in the right side in L-naïve but intact in R-naïve patients

One-way ANCOVA analysis showed that L-naïve, R-naïve and NC groups were significantly different in ReHo lateralization in the dorsal rostral putamen (voxel level

P < 0.05, cluster size > 27 voxels，corresponding to cluster-level corrected P < 0.05) (Fig. 2A and Table 2). Post-hoc analysis showed that L-naïve patients had higher ReHo lateralization in the dorsal rostral putamen compared with NC group (Fig. 2B and Table 2) and R-naïve group (Fig. 2C and Table 2) (voxel level P < 0.05, cluster size > 7 voxels，corresponding to cluster-level corrected P < 0.05). Further graphing using a scatterplot in Fig. 2D indicated that higher ReHo lateralization in L-naïve patients was due to decreased ReHo activity in the right side of the dorsal rostral putamen. There was no significant difference between the left and the right dorsal rostral putamen in R-naïve patients, suggesting neural function of the left dorsal rostral putamen in R-naïve patients was relatively preserved in early stage PD. Our results showed that the right dorsal rostral putamen of L-naïve patients had reduced neural activity compared with the left dorsal rostral putamen in R-naïve patients, indicating that the right side of dorsal rostral putamen might be more sensitive to dopamine depletion than the left side.

Dorsal rostral putamen was correlated with feedback-based associative learning

Correlational analysis in the L-naïve group showed that the mean number of errors in acquisition was inversely correlated with ReHo activity of the right dorsal rostral putamen (r = -0.535, P = 0.015) (Fig. 2E). This suggests that reduced activity in the right dorsal rostral putamen might be associated with poor feedback-based associative learning in L-naïve patients. In addition, poor performance in acquisition was also inversely correlated with ReHo activity of the left dorsal rostral putamen in L-naïve

patients (r= -0.479， P = 0.033) (Fig. 2E). However, there was no significant ReHo activity change in the left dorsal rostral putamen of L-naïve patients. Thus, this inverse correlation between acquisition and ReHo activity of the left dorsal rostral putamen has no clinical significance in those early-stage L-naïve PD patients.

Since only 60 cases (20 L-naïve, 21 R-naïve and 19 NC) underwent resting-state fMRI examination based on subjects' willingness, performance in the acquired equivalence task was also analyzed in these 60 cases. Results were consistent with the earlier results from all 101 subjects (See eResult 1 and eFigure 1 in the Supplement). Our results showed that the dorsal rostral putamen activity might be specifically implicated in acquisition learning.

Function of the dorsal rostral putamen did not correlate with the severity of motor symptoms

In order to test whether the dorsal rostral putamen was associated with motor function, correlation analysis was done between UPDRS-III scores and ReHo activity in the dorsal rostral putamen in L-naïve patients. No significant correlation was found between UPDRS-III score and neural activity of either the right or the left dorsal rostral putamen (right r = -0.141, P = 0.554 vs. left r = -0.092, P = 0.701) (Fig. 3). In addition, neither the right nor the left dorsal rostral putamen was significantly associated with left-side motor symptom scores in L-naïve patients (right r = -0.067, P = 0.779 vs. left r = -0.046, P = 0.848) (see eFigure 2 in the Supplement), suggesting that activity of the

dorsal rostral putamen did not correspond consistently with motor-symptom severity.

Discussion

The present study confirms previous reports regarding impaired acquisition and normal generalization in PD. The novel finding in our study in medication-naïve PD patients indicated that impaired acquisition was only detected in L-naïve patients, while R-naïve patients learned equally well as healthy controls. Results from rs-fMRI results indicated that there was a correlation between the impairment in acquisition and the activity in the dorsal rostral putamen, but not with motor-symptom severity. Dysfunction of the right dorsal rostral putamen was associated with acquisition deficit in L-naïve patients, which confirms the earlier reports that the dorsal rostral putamen is mainly involved in cognitive function rather than in motor function.[31-33]

Dorsal rostral putamen resting-state activity correlated with performance in acquisition not motor symptom severity in Parkinson's disease

In the present study, the impairment in acquisition was inversely correlated with ReHo activity in the right dorsal rostral putamen, identifying that the dorsal rostral putamen could be an important region involved in feedback-based associative learning. The hypothesis of ReHo measurement postulates that significant brain activities would more likely occur in clusters rather than in a single voxel. ReHo measures the functional coherence of a given voxel with its nearest neighbors and can be used to

evaluate resting-state brain activities.[25] Wu T et al. reported that ReHo, which was negatively correlated with UPDRS, decreased in extensive motor function-related brain regions, including the putamen, thalamus, and supplementary motor area, etc. in off-medication PD patients compared with normal controls. Administration of levodopa relatively normalized ReHo.[34] Thus, changes in ReHo can happen secondary to dopamine deficiency, and can be related to the motor symptom severity of the disease. In the present study, decreased ReHo in the dorsal rostral putamen, which might also be secondary to dopamine deficiency, was associated with the impairment in acquisition learning, but not with motor-symptom severity. Decreased ReHo reflects asynchronous neural activity of the dorsal rostral putamen and might lead to impaired performance in associative learning.

The putamen was classically regarded as motor-related structure. However, recent studies have revealed that the putamen's subdivisions were involved in comprehensive connectivity with motor, cognitive and emotional function.[35-37] For example, caudal putamen exhibited co-activation with primary sensorimotor cortex, caudal supplementary motor cortices, and anterior cerebellum, demonstrating its role in motor function;[31, 38] The rostral putamen had connectivity with dorsolateral prefrontal cortex (DLPFC), rostral anterior cingulate cortex and posterior parietal cortex, suggesting its participation in higher-level cognitive functions.[31, 32] Moreover, the rostral putamen combined with most of the head of the caudate, referred to as associative striatum,[31] played a dominant role in instrumental learning.[33, 39] The dorsal striatum was involved

in both motor function and associative cognition, and has been implicated in maintaining information about reward outcomes and consequences.[40, 41] Our results were consistent with previous studies, but more specifically, the dorsal rostral putamen was identified as the region closely related to feedback-based associative learning.

In our study, no correlation was found between the dorsal rostral putamen and the severity of motor symptoms, which further substantiated that the dorsal rostral putamen was mainly associated with cognitive function rather than motor function. ReHo in both sides of the putamen was associated with acquisition errors in L-naïve, but not in R-naïve patients. However, in the scatterplot of Figure 2D, there was no significant ReHo activity change in the left dorsal rostral putamen of L-naïve patients. Thus, this inverse correlation between acquisition errors and ReHo activity of the left dorsal rostral putamen does not have much clinical significance in those early-stage L-naïve PD patients. But we believe, with the disease progression, ReHo activity of the left dorsal rostral putamen will decrease and finally lead to associative learning impairment in R-naïve patients. This suggests a greater role for the dorsal rostral putamen in associative learning in left-onset rather than right-onset patients and may have implications for understanding of disease progression in relation to motor-symptom laterality.

Right and left dorsal rostral putamen might function differently following dopaminergic denervation

ReHo activity of the right dorsal rostral putamen was inversely correlated with the mean number of errors in acquisition in L-naïve. R-naïve, with intact function of the dorsal rostral putamen, performed equally well to NC in acquisition. The possible reason for different acquisition performance between L-naïve and R-naïve could be that the left and the right dorsal rostral putamen might function differently following dopaminergic denervation. Generally speaking, L-naïve had more dopaminergic neuronal loss in the right substantia nigra,[2] resulting in more severely reduced dopamine release in the right striatum.[3] The reverse was true in R-naïve patients. Based on the fact that L-naïve and R-naïve patients were well matched in UPDRS-III scores in our study, we assumed that the degree of dopaminergic denervation contralateral to the onset side should be similar in the two groups. However, our fMRI results showed reduced neural activity in the right dorsal rostral putamen in L-naïve patients, while the activity of the left dorsal rostral putamen in R-naïve was similar to that of controls. This indicates that the right and the left dorsal rostral putamen might function differently after PD-related dopaminergic loss. The right dorsal rostral putamen might be more sensitive to dopamine depletion and finally led to impaired feedback-dependent associative learning function.

There is no pathological or anatomical evidence to explain why the right and the left dorsal rostral putamen function differently following dopaminergic denervation. However, recent studies reported asymmetric dopamine signaling in the striatal and frontal regions caused by genetic variants of dopamine transporter and dopamine D2

receptor,[42] and left-right asymmetric dopamine D2/3 receptor availability in the dorsal putamen in healthy population.[43] Asymmetric dopamine receptors availability and asymmetric dopamine signaling might contribute to asymmetric functional changes to dopamine depletion between the right and the left dorsal rostral putamen. Asymmetric or uneven function between the left and the right striatum has been evidenced from ^{18}F-dopa PET-scan, where tower of London scores correlated with activity in the right caudate nucleus. On the other hand, activity in the left putamen was related to verbal working memory task.[44] A task-related fMRI study of healthy subjects observed activity in the right striatum and the right inferior prefrontal cortexes during earlier phases of probability learning.[45] A study by Postuma et al. provided stronger evidence for asymmetric function of the putamen by analyzing the functional connectivity between the cortex and the striatum in a meta-analysis of 126 published functional neuroimaging studies.[37] The right and the left putamen co-activated in conjunction with different brain regions with different laterality. Briefly, the left putamen showed a large ipsilateral coactivation essentially with the entire primary motor and somatosensory cortex, while the right putamen showed a peak co-activation with the right DLPFC. These functional connectivity pictures delineated by the above study fully illustrated asymmetric function of the right and the left putamen. Therefore, the left and the right dorsal rostral putamen might co-activate with different regions and function unevenly and differently in feedback-based associative learning. Early-stage R-naïve patients would be spared in acquisition as long as the right dorsal rostral

putamen was not affected. But with the progression of the disease, deficits in acquisition of learning will appear because both sides of the dorsal rostral putamen will be affected.

Our results also showed that both retrieval and generalization function were normal in PD. It has been reported that PD might exhibit impaired retrieval function, which could benefit substantially from cueing.[46] Normal retrieval function in our study might be due to the fact that these early-stage PD patients had relatively normal executive function at that point, or because the faces shown on the computer screen acted as efficient cueing to help patients recall what they had learned. Normal generalization in our study indicated that in the early stage of PD, asymmetric dopamine depletion didn't affect MTL function and patients had normal cognitive flexibility to use learned knowledge in a new context, which is consistent with the previous studies.[10, 11, 47]

Limitations and Conclusions

Although the relationship between asymmetric motor symptoms and laterality of dopaminergic depletion is well founded,[3, 48] there are a considerable proportion of PD patients who have ipsilateral or bilateral deficits of dopaminergic function. Erro R et al. analyzed a dataset of 46 [^{123}I] FP-CIT scans of PD patients and reported a prevalence of 4.3% of scans with predominant ipsilateral dopaminergic deficit.[49] The limitation in this study is that severity of motor symptoms instead of neuroimaging was used as the

indicator for the extent of relative dopamine depletion between the hemispheres. In a study by Kaasinen V, motor asymmetry index and DAT binding asymmetry index showed good accordance with each other in both right-handed and left-handed PD patients, which provided evidence that motor asymmetry index could be used as an indicator for asymmetry evaluation.[50] Future studies should apply neuroimaging to directly measure dopaminergic asymmetry and analyze its associations with associative learning.

In conclusion, our study showed that motor-symptom laterality could affect feedback-based associative learning in L-naïve PD patients. We conjecture that dysfunction of the right dorsal rostral putamen in L-naïve but not in R-naïve patients might be a rationale to explain why L-naïve patients performed worse than R-naïve patients in acquisition. Left and right dorsal rostral putamen might function differently or respond differently to dopamine depletion, which needs further exploration.

Authors' Roles:

Dr. Huang, Dr. Tan and Dr. Chen had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. PH: provision of study material, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. YYT: conception and design, subjects' recruitment and execution, administrative support, data analysis and interpretation, manuscript writing, final approval of manuscript. DL, YZ: fMRI data

analysis and interpretation, revised manuscript writing, final approval of manuscript. MMH, EL, MAG: learning data analysis and interpretation, manuscript editing, final approval of manuscript. YW: subjects' recruitment and execution, final approval of manuscript. SC: conception and design, subjects' recruitment and execution, administrative support, manuscript writing, final approval of manuscript.

Acknowledgements

The authors also thank Dr. Na-Ying He, Dr. Ke-Min Chen, Dr. Fu-Hua Yan and Dr. Hua-Wei Ling at Department of radiology, Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine for the contribution of the MRI scanner. We thank all the subjects who participated in the study. Financial support was generously provided by grants from the National Natural Science Foundation of China (30872729, 30971031, 81100952, 81201083, 81520108016), Program for Outstanding Medical Academic Leader (LJ 06003), and the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health (1 P50 NS 071675-02).

Conflict of interests: None.

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Figure legends

Fig.1 Learning performance on the Acquired Equivalence Task.

(A) L-naïve patients made significantly more errors than R-naïve patients and NC in the acquisition phase. R-naïve patients performed as well as NC in acquisition. No significant difference in accuracy was found either in the retention phase or in the generalization phase among three groups.

(B) There was no significant difference in response time in the acquisition phase, retention phase and generalization phase among L-naïve, R-naïve and NC groups.

L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls.

Fig. 2 Difference in neural activity asymmetry and its correlation with learning.

(A) The ANCOVA analysis among L-naïve, R-naïve and NC groups in the ReHo lateralization within the striatum (the left striatum was shown). A significant difference was found in the dorsal rostral putamen.

(B) Post-hoc analysis between L-naïve and NC group. Increased ReHo lateralization was detected in the dorsal rostral putamen in L-naïve group.

(C) Post-hoc analysis between L-naïve and R-naïve group. Increased ReHo lateralization was detected in the dorsal rostral putamen in L-naïve group compared to R-naïve group.

(D) Scatter plot of ReHo activity in bilateral dorsal rostral putamen among groups. The

horizontal line represents the mean level of ReHo. Group differences in ReHo lateralization were due to the decrease of ReHo in the right dorsal rostral putamen of L-naïve group.

(E) The mean number of errors in acquisition was inversely correlated with the ReHo activity both of the right dorsal rostral putamen and the left dorsal rostral putamen in L-naïve group.

L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls; ReHo = regional homogeneity.

Fig. 3 Correlation between ReHo activity of the dorsal rostral putamen and UPDRS-III score. No significant correlation was found between UPDRS-III score and neural activity of either the right or the left dorsal rostral putamen in L-naïve patients. L-naïve = left-onset medication-naïve patients with Parkinson's disease; ReHo = regional homogeneity.

Tables

Table 1 Clinical and demographic characteristics of medication-naïve Parkinson's patients and normal controls

	L-Naïve (n = 29)	R-Naïve (n = 34)	NC (n = 38)	P Value
Age (y)	57.3 (8.6)	59.6 (9.2)	58.5 (8.5)	0.580
Gender (M/F)	16 / 13	19 / 15	14 / 24	0.190
Education (y)	11.6 (3.4)	12.9 (3.3)	12.6 (2.6)	0.288
Disease duration (y)	2.7 (2.2)	2.2 (1.4)	-	0.799
UPDRS-III				
Tremor	1.9 (1.9)	1.6 (1.7)	-	0.545
Rigidity	3.6 (2.2)	3.9 (1.6)	-	0.203
Bradykinesia	5.0 (3.6)	4.1 (2.0)	-	0.204
Left side symptoms	9.2 (4.0)	0.6 (1.2)	-	-
Right side symptoms	0.8 (2.7)	8.4 (2.8)	-	-
Dominant side symptoms	9.2 (4.0)	8.4 (2.8)	-	0.565
Non-dominant side symptoms	0.8 (2.7)	0.6 (1.2)	-	0.415

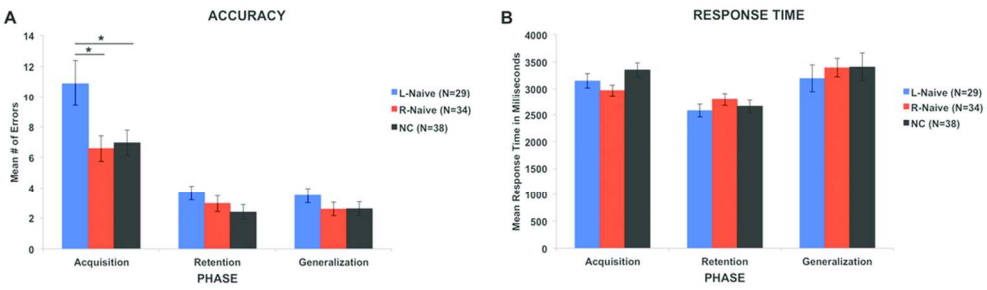
Total score	14.9 (8.5)	13.2 (5.6)	-	0.391
Motor asymmetry index	-0.9 (0.2)	0.9 (0.2)	-	-
H-Y score	1.2 (0.3)	1.4 (0.4)	-	0.104
BDI-II	5.2 (3.0)	5.6 (3.3)	5.0 (2.7)	0.740
MMSE	28.8 (1.0)	28.7 (1.4)	29.0 (1.2)	0.363

Data are mean (standard deviation). L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls; UPDRS-III = Unified Parkinson's Disease Rating Scale III; Motor asymmetry index = (right side symptoms - left side symptoms) / (right side symptoms + left side symptoms); H-Y = Hoehn and Yahr; BDI-II = Beck Depression Inventory II; MMSE = Mini Mental State Examination.

Table 2 Differences in the asymmetry of ReHo in the striatum

Brain Regions	Voxels	Peak MNI coordinates			Maximum F/T Values
		X	Y	Z	
ANCOVA					
Dorsal rostral putamen	27	-24	12	-3	5.229 (F)
L-naïve > NC					
Dorsal rostral putamen	22	-24	12	-3	2.811 (T)
L-naïve > R-Naïve					
Dorsal rostral putamen	7	-12	12	-9	3.141 (T)

L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls; ReHo = regional homogeneity.



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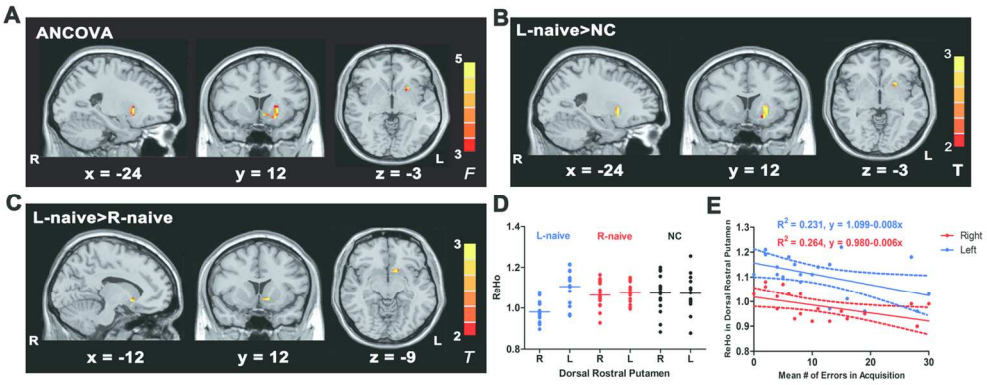

Fig. 2 Difference in neural activity asymmetry and its correlation with learning.
(A) The ANCOVA analysis among L-naïve, R-naïve and NC groups in the ReHo lateralization within the striatum (the left striatum was shown). A significant difference was found in the dorsal rostral putamen.
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(C) Post-hoc analysis between L-naïve and R-naïve group. Increased ReHo lateralization was detected in the dorsal rostral putamen in L-naïve group compared to R-naïve group.
(D) Scatter plot of ReHo activity in bilateral dorsal rostral putamen among groups. The horizontal line represents the mean level of ReHo. Group differences in ReHo lateralization were due to the decrease of ReHo in the right dorsal rostral putamen of L-naïve group.
(E) The mean number of errors in acquisition was inversely correlated with the ReHo activity both of the right dorsal rostral putamen and the left dorsal rostral putamen in L-naïve group.
L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls; ReHo = regional homogeneity.

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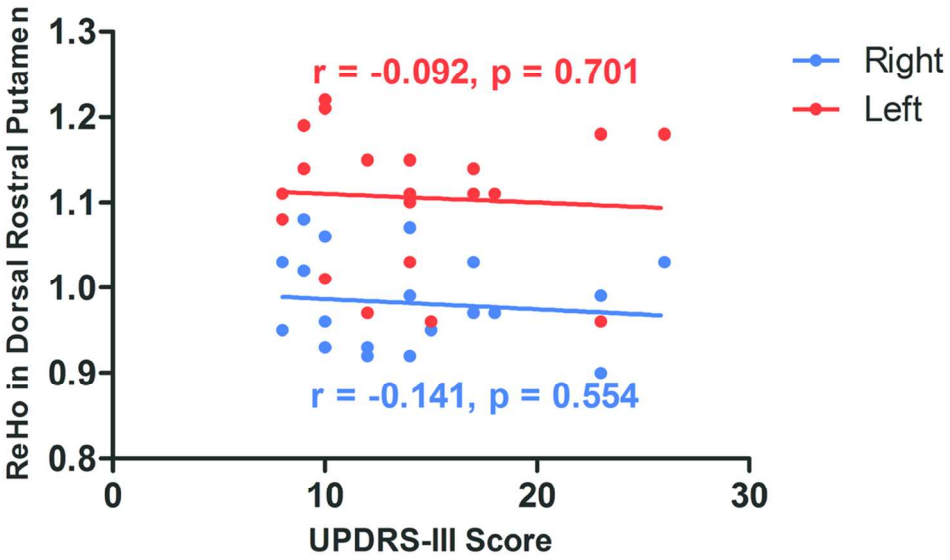

Fig. 3 Correlation between ReHo activity of the dorsal rostral putamen and UPDRS-III score. No significant correlation was found between UPDRS-III score and neural activity of either the right or the left dorsal rostral putamen in L-naïve patients. L-naïve = left-onset medication-naïve patients with Parkinson's disease; ReHo = regional homogeneity.

49x30mm (600 x 600 DPI)

Supplementary Online Content

Supplemental Methods:

eMethod 1 Procedure of Acquired Equivalence Task

Stimuli

We used an Apple MacBook to run the acquired equivalence task.[1] Four drawings of faces (girl, boy, woman, man) were set as antecedent stimuli while the consequents were four drawings of colored fish (red, blue, purple, green). The girl and man had brown hair while the boy and woman wore yellow hair. So each antecedent stimulus shared one feature with another in "**A**ge (adult vs. child), **G**ender (male vs. female), and **H**air color (brown vs. yellow)". For each participant, the antecedent (A1G1H1, A1G2H2, A2G1H2, A2G2H1) and consequent stimuli (X1, X2, Y1, Y2) were randomly assigned.

Acquisition phase

At the beginning of the test, instruction (Chinese Version) was presented on the screen as follows: "Welcome to the experiment. We will show you several drawings of people who have some pet fish. Remember that every person has his own pet fish, which may be different from others. Your task is to find the preference of each person. At the start, you will have to guess." These words were read aloud to the subject by the tester and then acquisition phase started.

On each trial, participants were shown a face and two colored fish with the cue: "Which fish does this person prefer?" The left-right sequence of fish was randomized across each trial. Subjects must learn the association between face and fish according to the corrective feedback that was given after each choice.

The acquisition phase was composed of three stages, each had growing numbers of associations as shown in Table e-1. Since there were eight kinds of relationship between face and fish, stage 1 consisted of two associations and terminated after the subject had made four consecutive correct choices. Similarly, stage 2 and 3 that each included four and six associations ended after eight and twelve consecutive correct responses, respectively. However, the start of a new stage was not informed to the participant.

Transfer phase

The transfer phase followed the end of acquisition phase with instruction: "Good job!

In the following phase of the experiment, you will use what you have learned. Correct answers will not be given. But we will show you how many trials you got right at last."

In this part, forty-eight trials including thirty-six old associations (retention) that had been trained during the acquisition phase and twelve new associations (generalization) were tested for the examination of retrieval and learned equivalence. The presence of new associations was not informed to the participant.

Eventually, the mean number of incorrect choices and the mean response time in each phase was recorded for behavioral measures.

eMethod 2 MRI data Processing

MRI data were processed with Data Processing Assistant for Resting-State fMRI (DPARSF) programs.[2] The first 10 frames were discarded and the remaining volumes were slice-timing corrected. Then they were realigned to the first image to correct for head motion. Individuals with the translation and rotation head motion parameters larger than 2 mm or 2 ^{o} were excluded. Then functional images were coregistered to the corresponding T1 images. The coregistered T1 images were segmented using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm), which takes more information outside the brain into account, then the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) tool was employed to spatially normalize both the T1 images and the functional images into MNI space. Finally， functional images were resampled to 3 × 3 × 3 mm^3 voxels.[3]

ReHo analysis was performed with the rs-fMRI Data Analysis Toolkit (REST, http://www.restfmri.net).[4] It is based on the hypothesis that the time courses of neighboring voxels are similar with each other and such local synchronization can be modulated by different states.[5] First, the linear trend was removed and each voxel was temporally band-pass filtered (0.01-0.08 Hz) to reduce the impact of low-frequency drift and high-frequency noise. Second, the ReHo analysis was accomplished by calculating the Kendall's coefficient of concordance (KCC) of the time series of a given voxel with its 26 nearest neighboring voxels.

Given that patients with unilateral onset of PD motor symptoms have asymmetric functional impairments in the striatum, it might influence hemi-striatal asymmetry in neural activity. For instance, left-onset patients have dysfunction in the right striatum, reverse for right-onset patients. In those early-stage patients with Parkinson's disease,

the left striatum of left-onset patients and right striatum of right-onset patients should be relatively spared. Thus, motor symptoms laterality might cause hemi-striatal asymmetry in neural activity. In order to evaluate this impact, we used the ReHo lateralization index.[6, 7] In consideration of the anatomical asymmetry of human brain, a symmetric template was firstly made by averaging the gray matter probability template in SPM and its mirror copy that was left-right flipped. In this symmetrical template, each side of the image represents an average of both hemispheres. And individual ReHo maps were then spatially normalized to this symmetrical template and further divided by the global mean ReHo for the purpose of standardization.[5, 8] Next, the resulting ReHo maps were left-right flipped to obtain the mirror copies. The nonflipped ReHo map was subtracted by flipped ReHo map, and then smoothed with a 4-mm full-width at half-maximum (FWHM) Gaussian kernel. This produced a final ReHo lateralization map, in which left side (meaning image of the left hemisphere minus the right) had the same absolute value with the right side (meaning image of the right hemisphere minus the left), but had different plus-minus sign. Therefore, both sides could reflect hemispheric differences in the same patient. As the template was symmetric, we can restrict statistical analyses to any half of hemisphere. Due to the potential importance of the striatum in Parkinson's disease, a striatum mask was also made. In detail, the gray matter concentration maps obtained during segmentation were firstly thresholded by 0.5, and multiplied with the mask including putamen, caudate and pallidum in the AAL template. Then these regions of interests (ROIs) were normalized to the symmetric template and the intersection was made across subjects.

Supplemental Results:

eResult 1 Behavioral performance of MRI subjects

Acquisition was impaired in L-naïve but normal in R-naïve patients

Mix-model ANOVAs showed that both the group [F (2, 171) = 10.575, P < 0.001] and phase [F (2, 171) = 21.119, P < 0.001] had significant effects on accuracy, and there was significant interaction between group and phase [F (4, 171) = 2.603, P = 0.038]. Post hoc analysis showed that accuracy was significantly different among L-naïve, R-naïve and NC. L-naïve patients made significantly more errors than R-naïve patients (P = 0.002) and NC (P < 0.001), while R-naïve patients performed as well as NC (P = 0.140) (Fig. e-1A). Significant differences were in the acquisition phase as the post hoc analysis indicated, rather than retention or generalization phase, indicating retrieval function and generalization were well preserved in both L-naïve and R-naïve patients (acquisition vs. retention, P < 0.001; acquisition vs. generalization, P < 0.001; retention vs. generalization, P = 0.985) (Fig. e-1A). Thus, L-naïve patients, rather than R-naïve patients, were selectively impaired in feedback-based associative learning, indicating that asymmetric dopamine depletion could affect feedback-based associative learning.

Phase had significant effect on the response time [F (2, 171) = 6.552, P = 0.002], but neither the effect of group [F (2, 171) = 0.638, P = 0.530] nor the interaction between group and phase [F (4, 171) = 0.816, P = 0.517] was significant (Fig. e-1B).

Supplemental Tables

eTable 1 Acquired Equivalence Task[1]

Acquisition Stage 1: Stimulus-outcome associations	Acquisition Stage 2: Stimulus-stimulus associations	Acquisition Stage 3: New stimulus-outcome associations	Transfer Phase: Old and new associations testing
A1G1H1→X1	A1G1H1→X1	A1G1H1→X1	A1G1H1→X2?
	A1G2H2→X1	A1G2H2→X1	
		A1G1H1→X2	
A2G1H2→Y1	A2G1H2→Y1	A2G1H2→Y1	A2G1H2→Y2?
	A2G2H1→Y1	A2G2H1→Y1	
		A2G1H2→Y2	

Transfer phase interleaved trials with old associations (a measure of retention) as well as new associations (a measure of generalization) without feedback. A = age, G = gender, H = hair color.

eTable 2 Clinical and demographic characteristics of the MRI subjects

	L-Naïve (n = 20)	R-Naïve (n = 21)	NC (n = 19)	P Value
Age (y)	56.6 (8.8)	61.4 (8.5)	60.8 (5.9)	0.117
Gender (M/F)	7 / 13	13 / 8	8 / 11	0.201
Education (y)	11.4 (2.8)	12.9 (3.3)	12.7 (2.6)	0.193
Disease duration (y)	2.9 (2.5)	2.2 (1.5)	-	0.717
UPDRS-III				
Tremor	1.8 (1.5)	1.4 (1.3)	-	0.641
Rigidity	3.3 (1.4)	3.9 (1.6)	-	0.231

Bradykinesia	4.8 (2.4)	4.4 (2.3)	-	0.334
Left side symptoms	9.1 (3.1)	0.8 (1.4)	-	-
Right side symptoms	0.4 (1.2)	8.3 (2.3)	-	-
Dominant side symptoms	9.1 (3.1)	8.3 (2.3)		0.545
Non-dominant side symptoms	0.4 (1.2)	0.8 (1.4)		0.199
Total score	14.2 (5.2)	13.4 (5.5)	-	0.565
Motor asymmetry index	-0.9 (0.2)	0.9 (0.2)	-	-
H-Y score	1.2 (0.3)	1.4 (0.4)	-	0.066
BDI-II	5.0 (3.0)	4.9 (3.2)	4.7 (2.1)	0.991
MMSE	28.8 (0.9)	28.9 (0.9)	29.3 (1.0)	0.127

Data are mean (standard deviation). L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls; UPDRS = Unified Parkinson's Disease Rating Scale; Motor asymmetry index = (right side symptoms - left side symptoms) / (right side symptoms + left side symptoms); H-Y = Hoehn-Yahr; BDI = Beck Depression Inventory; MMSE = Mini Mental State Examination.

Supplemental Figure Legends

eFig.1 Learning performance of MRI subjects on the Acquired Equivalence Task.

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eFig.2 Correlation between ReHo activity of dorsal rostral putamen and left-side symptom scores.

No significant correlation was found between left-side symptom scores and neural activity of either right or left dorsal rostral putamen in L-naïve patients. L-naïve = left-onset medication-naïve patients with Parkinson's disease; ReHo = regional homogeneity.

eReferences

e1. Myers CE, Shohamy D, Gluck MA, et al. Dissociating hippocampal versus basal

ganglia contributions to learning and transfer. Journal of cognitive neuroscience 2003;15(2):185-193.

e2. Chao-Gan Y, Yu-Feng Z. DPARSF: A MATLAB Toolbox for "Pipeline" Data Analysis of Resting-State fMRI. Frontiers in systems neuroscience 2010;4:13.

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e5. Zang Y, Jiang T, Lu Y, He Y, Tian L. Regional homogeneity approach to fMRI data analysis. NeuroImage 2004;22(1):394-400.

e6. Blumenfeld H, McNally KA, Vanderhill SD, et al. Positive and negative network correlations in temporal lobe epilepsy. Cerebral cortex (New York, NY : 1991) 2004;14(8):892-902.

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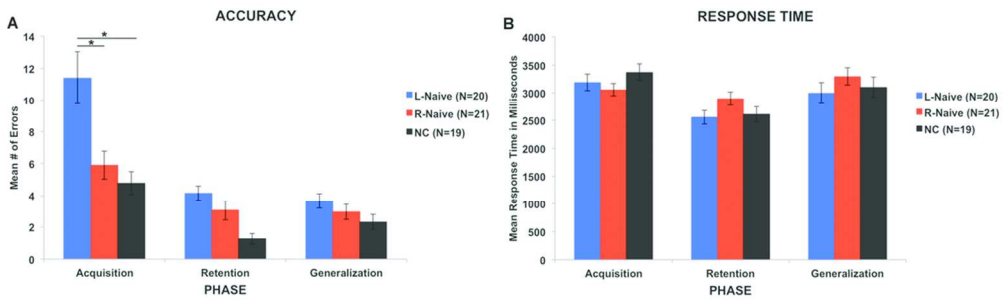
e8. Yan CG, Craddock RC, Zuo XN, Zang YF, Milham MP. Standardizing the intrinsic brain: towards robust measurement of inter-individual variation in 1000 functional connectomes. NeuroImage 2013;80:246-262.



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(A) L-naïve patients made significantly more errors than R-naïve patients and NC in the acquisition phase. R-naïve patients performed as well as NC in acquisition. No significant difference in accuracy was found either in the retention phase or in the generalization phase among three groups.
(B) There was no significant difference in response time in the acquisition phase, retention phase and generalization phase among L-naïve, R-naïve and NC groups.
L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls.

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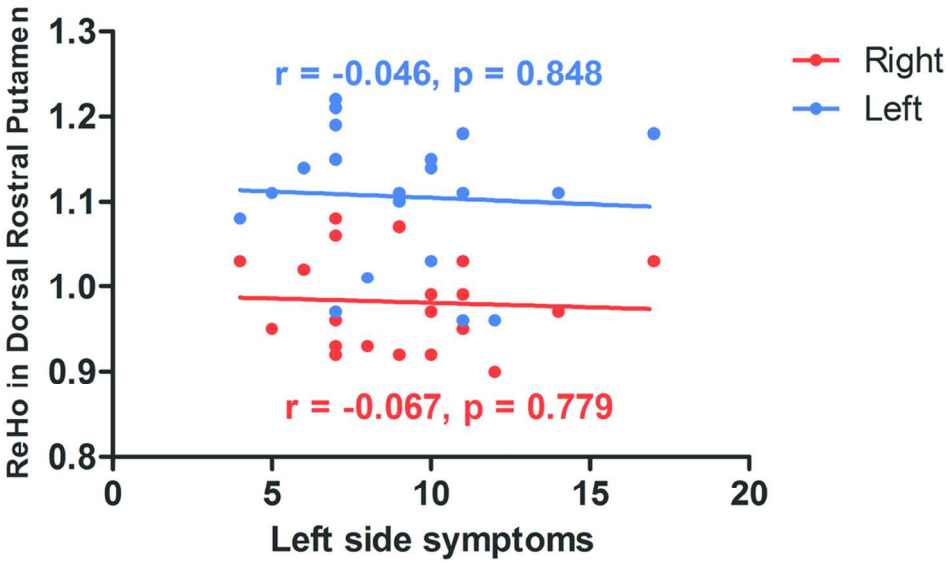

eFig.2 Correlation between ReHo activity of dorsal rostral putamen and left-side symptom scores. No significant correlation was found between left-side symptom scores and neural activity of either right or left dorsal rostral putamen in L-naïve patients. L-naïve = left-onset medication-naïve patients with Parkinson's disease; ReHo = regional homogeneity.

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Title Page

Motor-symptom Laterality Affects Acquisition in Parkinson's disease: a Cognitive and fMRI study

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Word Count for Manuscript: 3856; **Word Count for Abstract:** 244; **Character Count for Title:** 96.

Keywords

Parkinson's disease; hemispheric asymmetry; dopamine; association learning; resting-state functional MRI

Running title: Lateral Parkinsonism affects acquisition

Funding sources:

Dr. Tan is in charge of National Natural Science Foundation of China (81100952). Dr. Liu is in charge of National Natural Science Foundation of China (81201083). Prof. Zang is in charge of National Natural Science Foundation of China (81520108016). Prof. Chen is in charge of National Natural Science Foundation of China (30872729, 30971031). Prof. Gluck received grants from Program for Outstanding Medical Academic Leader (LJ 06003), and the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health (1 P50 NS 071675-02). The other co-authors report no disclosures relevant to the manuscript.

Conflict of interests: None.

Abstract

Objective: Asymmetric onset of motor symptoms in PD can affect cognitive function. We examined whether motor-symptom laterality could affect feedback-based associative learning and explored its underlying neural mechanism by fMRI in PD patients.

Methods: We recruited 63 early-stage medication-naïve PD patients (29 left-onset medication-naïve patients, 34 right-onset medication-naïve patients) and 38 matched normal controls. Subjects completed an acquired equivalence task (including acquisition, retention and generalization) and resting-state fMRI scans. Learning accuracy and response time in each phase of the task was recorded for behavioral measures. Regional homogeneity was used to analyze resting-state fMRI data, with regional homogeneity lateralization to evaluate hemispheric functional asymmetry in the striatum.

Results: Left-onset patients made significantly more errors in acquisition (feedback-based associative learning) than right-onset patients and normal controls, while right-onset patients performed as well as normal controls. There was no significant difference among these three groups in the accuracy of either retention or generalization phase. The three groups did not show significant differences in response time. In the left-onset group, there was an inverse relationship between acquisition

errors and regional homogeneity in the right dorsal rostral putamen. There were no significant regional homogeneity changes in either the left or the right dorsal rostral putamen in right-onset patients when compared to controls.

Conclusions: Motor-symptom laterality could affect feedback-based associative learning in PD, with left-onset medication-naïve patients being selectively impaired. Dysfunction in the right dorsal rostral putamen may underlie the observed deficit in associative learning in patients with left-sided onset.

102

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by resting tremor, rigidity, bradykinesia and postural instability. The onset and progression of motor symptoms in PD are usually asymmetric, reflecting asymmetric contralateral dopamine depletion in the basal ganglia.[1-4] Relationships between cognitive performance and symptom asymmetry have been revealed that left-onset PD patients performed worse on cognitive measures, such as spatial attention and tasks of orientation and mental imagery, than right-onset PD patients.[5-7] Cognitive processes that are closely related with dopamine, such as cognitive flexibility and motivation, showed different deficits between right-onset and left-onset PD patients.[8] For example, left-onset PD patients with greater loss of dopamine in the right hemisphere had impaired cognitive flexibility.[5, 8]

Feedback-based associative learning, which involves learning through corrective feedback provided on each trial, has been correlated to striatal dopamine release[9] and the function of the basal ganglia.[10, 11] Previous studies have reported that feedback-based associative learning was impaired in PD patients.[12, 13] However, the impairment in feedback-based learning in PD is not a universal finding, and many factors could contribute to this variability. Wilkinson L et al. did not find a selective impairment in PD in probabilistic feedback-based learning, but reported that there was a significant correlation between disease severity and the impairment in feedback-based learning.[14] It has also been reported that off-medication PD patients

learned stimulus-response associations equally well compared to healthy controls but learning was impaired by dopaminergic medication.[13] Thus, in the underlying disease process, disease severity and dopaminergic medication might be all involved in modulating feedback-based learning. However, it is still not clear how the dopamine asymmetry affects feedback-based associative learning in PD patients.

To control and minimize the effects of medication involvement and disease severity, we recruited early-stage medication-naïve right-handed PD patients including Left-onset medication-naïve patients (L-naïve) and Right-onset medication-naïve patients (R-naïve). We used a computer-based cognitive task of learning and generalization based on the acquired equivalence paradigm to test the effects of dopamine asymmetry on feedback-based associative learning. The acquired equivalence task, which includes tests of acquisition, retention and generalization, was repetitively used to evaluate feedback-based associative learning in patients with PD and other neurodegenerative disorders.[10, 11] In the acquisition phase, learning through trial-by-trial feedback learning was shown to correlate to striatal function, while generalization without feedback was shown to correlate with hippocampal and medial temporal lobe (MTL) functionality.[10, 11] Although striatal involvement in associative learning has been consistently reported in the previous task-based fMRI studies, such as caudate nucleus, amygdala and ventral striatum, the specific loci are not always the same.[15-17] Thus, which subdivision of the basal ganglia is associated with feedback-based associative learning remains unclear, and resting-state fMRI (rs-fMRI)

data were collected to explore the underlying neural mechanism of feedback-based learning.

In the present study, we examined whether motor-symptom laterality could affect feedback-based associative learning and explored its underlying neural mechanism using rs-fMRI in PD patients.

Subjects and Methods

Subjects. We recruited 63 right-handed, early stage (H-Y scores between 1 and 2), medication-naïve PD patients and 38 right-handed normal controls (NC) during 2012-2015. Subjects in the PD and NC groups were matched for age, gender, education and general cognitive status.[18] According to the motor-symptom laterality, PD patients were divided into L-naïve (N = 29) and R-naïve (N = 34) subgroups. All participants were non-demented (Mini Mental State Examination, MMSE ≥ 24) and scored less than 15 on the Beck Depression Inventory II (BDI-II).[19, 20] Participants were also screened for history of cerebral trauma, cerebrovascular diseases, head surgery, severe sleep disorders, hyperthyroidism, insulin-dependent diabetes, psychiatric or neurological disorders, abuse of alcohol, tobacco use, use of hormonal contraceptives, anticholinergic drugs, or antidepressants. For subjects with PD, only patients with unilateral side of onset and asymmetrical motor symptoms were involved and diagnosis was confirmed by two movement disorder specialists according to the UK Brain Bank criteria for the diagnosis of PD.[21] The side of onset was determined by medical history

and physical examination. The severity of motor symptoms was evaluated by Unified Parkinson's Disease Rating Scale III (UPDRS-III).[22] Motor asymmetry index (MAI) was calculated as (right side symptoms - left side symptoms) / (right side symptoms + left side symptoms).

Standard protocol approvals, registrations, and patient consents. We received approval from the Ethics Committee of Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine. We obtained written informed consents from all patients and controls prior to their participation in the study.

Behavioral data acquisition and evaluation. We used an Apple MacBook to run the Acquired Equivalence Task.[11] This task is composed of two phases: acquisition and transfer. In the acquisition phase, participants acquire associations of colored faces and fish through trial-by-trial feedback-based learning. Following this acquisition, the transfer phase includes two types of trials: tests of previously learned associations (retention) and tests of new associations that are presented without feedback (generalization). The mean number of incorrect choices and the mean response time in each phase was recorded for behavioral measures. For detailed procedure, please refer to eMethod 1 and eTable 1 in the Supplement.

MRI data acquisition and processing. MRI scan was performed after the acquired

equivalence test with an interval of 8.6 ± 1.5 days. A subgroup of 70 subjects (23 L-naïve, 25 R-naïve and 22 NC) participated in resting-state fMRI on a 3.0 T GE Medical System scanner based on the subjects' willingness. During the scan, the subjects were asked to remain motionless and awake with their eyes closed. For each participant, 210 functional images were collected using echo planar imaging T2*-weighted sequence (repetition time = 2000 ms, echo time = 30 ms, flip angle = 90 ^{o}, 33/35/37 slices, matrix = 64 × 64, voxel size = 3.75 × 3.75 × 4 mm^3). Then the high-resolution, three-dimensional T1-weighted structural images (repetition time = 5.78 ms, echo time = 1.77 ms, flip angle = 12 ^{o}, 196 slices, matrix = 256 × 256, voxel size = 1 × 1 × 1 mm^3) were acquired for registration and normalization of the functional images.

After exclusion due to vascular diseases (1 L-naïve, 2 R-naïve and 3 NC) and obvious head motion (2 L-naïve and 2 R-naïve, with the translation and rotation head motion parameters larger than 2 mm or 2°), MRI data from 60 subjects (20 L-naïve, 21 R-naïve, and 19 NC) qualified for analysis. MRI data were processed with Data Processing Assistant for Resting-State fMRI (DPARSF) programs.[23] Regional homogeneity (ReHo), as a commonly used method to analyze rs-fMRI data, was used with the rs-fMRI Data Analysis Toolkit (REST, http://www.restfmri.net) by calculating the Kendall's coefficient of concordance (KCC) of the time series of a given voxel with its 26 nearest neighboring voxels.[24, 25] With the assumption that PD patients with unilateral onset of motor symptoms had asymmetric functional impairments in the

brain, ReHo lateralization index was used to evaluate hemisphere asymmetry in neural activity as previously reported.[26, 27] Striatum subregions including the putamen, caudate and pallidum were chosen as regions of interest (ROIs). For detailed methods, please refer to eMethod 2 in the Supplement.

Statistical analysis. The normality of clinical and demographic data distribution was checked by the Kolmogorov-Smirnov test. One-way ANOVA was used to compare the normally distributed continuous variables (age), and the chi-square test was employed to analyze categorical variables (gender). The continuous variables that were not normally distributed (education, disease duration, H-Y, BDI-II, MMSE, UPDRS-III score, MAI) were analyzed by Kruskal-Wallis test. Mix-model ANOVAs with group as between-subject factor and phase as within-subject factor were used to analyze the behavioral data. Post-hoc analysis was done using Tukey HSD test. In the correlational analysis, Spearman rho was calculated. The alpha level was set at 0.05. All P values less than the alpha level were considered statistically significant. SPSS version 17.0 (IBM, Chicago, IL, USA) was used for statistical analysis.

For the fMRI data analysis, comparison of the hemispheric asymmetry among groups was performed using one-way ANCOVA. Age, gender, the mean frame-wise displacement (FD) corresponding to the temporal derivative of the head motion parameters, and mean gray matter (GM) volume of ROI were used as nuisance covariates.[28, 29] Post-hoc analysis was performed within the significant regions. To

control for family-wise error rates, Monte Carlo simulations were performed (3dClustSim; 10,000 iterations) using all brain voxels within the half-striatum ROI.[30] The cluster threshold for a corrected alpha level of P = 0.05 was 27 voxels for ANCOVA and 7 voxels for post-hoc t-test, respectively.

Results

Clinical and demographic characteristics of subjects

The clinical and demographic characteristics of subjects are presented in Table 1. All the groups were matched for age, gender, education, MMSE, and BDI-II ($P \geq 0.190$). There were no significant differences in disease duration, H-Y score, and UPDRS-III scores (including tremor, rigidity, bradykinesia sub-scores and total scores) between the L-naïve and R-naïve PD groups ($P \geq 0.104$). MAI was calculated as (right side symptoms - left side symptoms) / (right side symptoms + left side symptoms). The mean value of MAI for L-naïve and R-naïve groups were -0.9 (0.2) vs. 0.9 (0.2) respectively, indicating that motor-symptom asymmetry was also matched. In addition, subjects participating in the MRI examination were also matched across the three groups (see eTable 2 in the Supplement).

Behavioral performance

Acquisition was impaired in L-naïve but normal in R-naïve patients

Mix-model ANOVAs showed that both group [$F(2, 294) = 7.228$, $P = 0.001$] and phase

[F (2, 294) = 52.680, P < 0.001] had significant effects on accuracy. The interaction between group and phase was at a trend-level [F (4, 294) = 2.127, P = 0.077]. Fig.1A indicated that the group effect is primarily driven by the acquisition phase. Post-hoc analysis showed that accuracy was significantly different among L-naïve, R-naïve and NC. L-naïve patients made significantly more errors than R-naïve patients (P = 0.003) and NC (P = 0.002), while R-naïve patients performed as well as NC (P = 0.996) (Fig. 1A). There were no significant differences between groups in retention or generalization phase, indicating that retrieval function and generalization were preserved in both L-naïve and R-naïve patients (retention, P = 0.201 vs. generalization, P = 0.331) (Fig. 1A). Thus, L-naïve patients, rather than R-naïve patients, were selectively impaired in feedback-based associative learning, indicating a potential effect of asymmetric dopamine depletion on associative learning.

Phase had significant effect on the response time [F (2, 294) = 12.564, P < 0.001]. However, there was neither an effect of group [F (2, 294) = 0.733, P = 0.481] nor an interaction between group and phase [F (4, 294) = 0.782, P = 0.538] (Fig. 1B).

Functional MRI results

Dorsal rostral putamen was impaired in the right side in L-naïve but intact in R-naïve patients

One-way ANCOVA analysis showed that L-naïve, R-naïve and NC groups were significantly different in ReHo lateralization in the dorsal rostral putamen (voxel level

P < 0.05, cluster size > 27 voxels，corresponding to cluster-level corrected P < 0.05) (Fig. 2A and Table 2). Post-hoc analysis showed that L-naïve patients had higher ReHo lateralization in the dorsal rostral putamen compared with NC group (Fig. 2B and Table 2) and R-naïve group (Fig. 2C and Table 2) (voxel level P < 0.05, cluster size > 7 voxels，corresponding to cluster-level corrected P < 0.05). Further graphing using a scatterplot in Fig. 2D indicated that higher ReHo lateralization in L-naïve patients was due to decreased ReHo activity in the right side of the dorsal rostral putamen. There was no significant difference between the left and the right dorsal rostral putamen in R-naïve patients, suggesting neural function of the left dorsal rostral putamen in R-naïve patients was relatively preserved in early stage PD. Our results showed that the right dorsal rostral putamen of L-naïve patients had reduced neural activity compared with the left dorsal rostral putamen in R-naïve patients, indicating that the right side of dorsal rostral putamen might be more sensitive to dopamine depletion than the left side.

Dorsal rostral putamen was correlated with feedback-based associative learning

Correlational analysis in the L-naïve group showed that the mean number of errors in acquisition was inversely correlated with ReHo activity of the right dorsal rostral putamen (r = -0.535, P = 0.015) (Fig. 2E). This suggests that reduced activity in the right dorsal rostral putamen might be associated with poor feedback-based associative learning in L-naïve patients. In addition, poor performance in acquisition was also inversely correlated with ReHo activity of the left dorsal rostral putamen in L-naïve

patients (r= -0.479, P = 0.033) (Fig. 2E). However, there was no significant ReHo activity change in the left dorsal rostral putamen of L-naïve patients. Thus, this inverse correlation between acquisition and ReHo activity of the left dorsal rostral putamen has no clinical significance in those early-stage L-naïve PD patients.

Since only 60 cases (20 L-naïve, 21 R-naïve and 19 NC) underwent resting-state fMRI examination based on subjects' willingness, performance in the acquired equivalence task was also analyzed in these 60 cases. Results were consistent with the earlier results from all 101 subjects (See eResult 1 and eFigure 1 in the Supplement). Our results showed that the dorsal rostral putamen activity might be specifically implicated in acquisition learning.

Function of the dorsal rostral putamen did not correlate with the severity of motor symptoms

In order to test whether the dorsal rostral putamen was associated with motor function, correlation analysis was done between UPDRS-III scores and ReHo activity in the dorsal rostral putamen in L-naïve patients. No significant correlation was found between UPDRS-III score and neural activity of either the right or the left dorsal rostral putamen (right r = -0.141, P = 0.554 vs. left r = -0.092, P = 0.701) (Fig. 3). In addition, neither the right nor the left dorsal rostral putamen was significantly associated with left-side motor symptom scores in L-naïve patients (right r = -0.067, P = 0.779 vs. left r = -0.046, P = 0.848) (see eFigure 2 in the Supplement), suggesting that activity of the

dorsal rostral putamen did not correspond consistently with motor-symptom severity.

Discussion

The present study confirms previous reports regarding impaired acquisition and normal generalization in PD. The novel finding in our study in medication-naïve PD patients indicated that impaired acquisition was only detected in L-naïve patients, while R-naïve patients learned equally well as healthy controls. Results from rs-fMRI results indicated that there was a correlation between the impairment in acquisition and the activity in the dorsal rostral putamen, but not with motor-symptom severity. Dysfunction of the right dorsal rostral putamen was associated with acquisition deficit in L-naïve patients, which confirms the earlier reports that the dorsal rostral putamen is mainly involved in cognitive function rather than in motor function.[31-33]

Dorsal rostral putamen resting-state activity correlated with performance in acquisition not motor symptom severity in Parkinson's disease

In the present study, the impairment in acquisition was inversely correlated with ReHo activity in the right dorsal rostral putamen, identifying that the dorsal rostral putamen could be an important region involved in feedback-based associative learning. The hypothesis of ReHo measurement postulates that significant brain activities would more likely occur in clusters rather than in a single voxel. ReHo measures the functional coherence of a given voxel with its nearest neighbors and can be used to

evaluate resting-state brain activities.[25] Wu T et al. reported that ReHo, which was negatively correlated with UPDRS, decreased in extensive motor function-related brain regions, including the putamen, thalamus, and supplementary motor area, etc. in off-medication PD patients compared with normal controls. Administration of levodopa relatively normalized ReHo.[34] Thus, changes in ReHo can happen secondary to dopamine deficiency, and can be related to the motor symptom severity of the disease. In the present study, decreased ReHo in the dorsal rostral putamen, which might also be secondary to dopamine deficiency, was associated with the impairment in acquisition learning, but not with motor-symptom severity. Decreased ReHo reflects asynchronous neural activity of the dorsal rostral putamen and might lead to impaired performance in associative learning.

The putamen was classically regarded as motor-related structure. However, recent studies have revealed that the putamen's subdivisions were involved in comprehensive connectivity with motor, cognitive and emotional function.[35-37] For example, caudal putamen exhibited co-activation with primary sensorimotor cortex, caudal supplementary motor cortices, and anterior cerebellum, demonstrating its role in motor function;[31, 38] The rostral putamen had connectivity with dorsolateral prefrontal cortex (DLPFC), rostral anterior cingulate cortex and posterior parietal cortex, suggesting its participation in higher-level cognitive functions.[31, 32] Moreover, the rostral putamen combined with most of the head of the caudate, referred to as associative striatum,[31] played a dominant role in instrumental learning.[33, 39] The dorsal striatum was involved

in both motor function and associative cognition, and has been implicated in maintaining information about reward outcomes and consequences.[40, 41] Our results were consistent with previous studies, but more specifically, the dorsal rostral putamen was identified as the region closely related to feedback-based associative learning.

In our study, no correlation was found between the dorsal rostral putamen and the severity of motor symptoms, which further substantiated that the dorsal rostral putamen was mainly associated with cognitive function rather than motor function. ReHo in both sides of the putamen was associated with acquisition errors in L-naïve, but not in R-naïve patients. However, in the scatterplot of Figure 2D, there was no significant ReHo activity change in the left dorsal rostral putamen of L-naïve patients. Thus, this inverse correlation between acquisition errors and ReHo activity of the left dorsal rostral putamen does not have much clinical significance in those early-stage L-naïve PD patients. But we believe, with the disease progression, ReHo activity of the left dorsal rostral putamen will decrease and finally lead to associative learning impairment in R-naïve patients. This suggests a greater role for the dorsal rostral putamen in associative learning in left-onset rather than right-onset patients and may have implications for understanding of disease progression in relation to motor-symptom laterality.

Right and left dorsal rostral putamen might function differently following dopaminergic denervation

ReHo activity of the right dorsal rostral putamen was inversely correlated with the mean number of errors in acquisition in L-naïve. R-naïve, with intact function of the dorsal rostral putamen, performed equally well to NC in acquisition. The possible reason for different acquisition performance between L-naïve and R-naïve could be that the left and the right dorsal rostral putamen might function differently following dopaminergic denervation. Generally speaking, L-naïve had more dopaminergic neuronal loss in the right substantia nigra,[2] resulting in more severely reduced dopamine release in the right striatum.[3] The reverse was true in R-naïve patients. Based on the fact that L-naïve and R-naïve patients were well matched in UPDRS-III scores in our study, we assumed that the degree of dopaminergic denervation contralateral to the onset side should be similar in the two groups. However, our fMRI results showed reduced neural activity in the right dorsal rostral putamen in L-naïve patients, while the activity of the left dorsal rostral putamen in R-naïve was similar to that of controls. This indicates that the right and the left dorsal rostral putamen might function differently after PD-related dopaminergic loss. The right dorsal rostral putamen might be more sensitive to dopamine depletion and finally led to impaired feedback-dependent associative learning function.

There is no pathological or anatomical evidence to explain why the right and the left dorsal rostral putamen function differently following dopaminergic denervation. However, recent studies reported asymmetric dopamine signaling in the striatal and frontal regions caused by genetic variants of dopamine transporter and dopamine D2

receptor,[42] and left-right asymmetric dopamine D2/3 receptor availability in the dorsal putamen in healthy population.[43] Asymmetric dopamine receptors availability and asymmetric dopamine signaling might contribute to asymmetric functional changes to dopamine depletion between the right and the left dorsal rostral putamen. Asymmetric or uneven function between the left and the right striatum has been evidenced from ^{18}F-dopa PET-scan, where tower of London scores correlated with activity in the right caudate nucleus. On the other hand, activity in the left putamen was related to verbal working memory task.[44] A task-related fMRI study of healthy subjects observed activity in the right striatum and the right inferior prefrontal cortexes during earlier phases of probability learning.[45] A study by Postuma et al. provided stronger evidence for asymmetric function of the putamen by analyzing the functional connectivity between the cortex and the striatum in a meta-analysis of 126 published functional neuroimaging studies.[37] The right and the left putamen co-activated in conjunction with different brain regions with different laterality. Briefly, the left putamen showed a large ipsilateral coactivation essentially with the entire primary motor and somatosensory cortex, while the right putamen showed a peak co-activation with the right DLPFC. These functional connectivity pictures delineated by the above study fully illustrated asymmetric function of the right and the left putamen. Therefore, the left and the right dorsal rostral putamen might co-activate with different regions and function unevenly and differently in feedback-based associative learning. Early-stage R-naïve patients would be spared in acquisition as long as the right dorsal rostral

putamen was not affected. But with the progression of the disease, deficits in acquisition of learning will appear because both sides of the dorsal rostral putamen will be affected.

Our results also showed that both retrieval and generalization function were normal in PD. It has been reported that PD might exhibit impaired retrieval function, which could benefit substantially from cueing.[46] Normal retrieval function in our study might be due to the fact that these early-stage PD patients had relatively normal executive function at that point, or because the faces shown on the computer screen acted as efficient cueing to help patients recall what they had learned. Normal generalization in our study indicated that in the early stage of PD, asymmetric dopamine depletion didn't affect MTL function and patients had normal cognitive flexibility to use learned knowledge in a new context, which is consistent with the previous studies.[10, 11, 47]

Limitations and Conclusions

Although the relationship between asymmetric motor symptoms and laterality of dopaminergic depletion is well founded,[3, 48] there are a considerable proportion of PD patients who have ipsilateral or bilateral deficits of dopaminergic function. Erro R et al. analyzed a dataset of 46 [^{123}I] FP-CIT scans of PD patients and reported a prevalence of 4.3% of scans with predominant ipsilateral dopaminergic deficit.[49] The limitation in this study is that severity of motor symptoms instead of neuroimaging was used as the

indicator for the extent of relative dopamine depletion between the hemispheres. In a study by Kaasinen V, motor asymmetry index and DAT binding asymmetry index showed good accordance with each other in both right-handed and left-handed PD patients, which provided evidence that motor asymmetry index could be used as an indicator for asymmetry evaluation.[50] Future studies should apply neuroimaging to directly measure dopaminergic asymmetry and analyze its associations with associative learning.

In conclusion, our study showed that motor-symptom laterality could affect feedback-based associative learning in L-naïve PD patients. We conjecture that dysfunction of the right dorsal rostral putamen in L-naïve but not in R-naïve patients might be a rationale to explain why L-naïve patients performed worse than R-naïve patients in acquisition. Left and right dorsal rostral putamen might function differently or respond differently to dopamine depletion, which needs further exploration.

Authors' Roles:

Dr. Huang, Dr. Tan and Dr. Chen had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. PH: provision of study material, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. YYT: conception and design, subjects' recruitment and execution, administrative support, data analysis and interpretation, manuscript writing, final approval of manuscript. DL, YZ: fMRI data

analysis and interpretation, revised manuscript writing, final approval of manuscript. MMH, EL, MAG: learning data analysis and interpretation, manuscript editing, final approval of manuscript. YW: subjects' recruitment and execution, final approval of manuscript. SC: conception and design, subjects' recruitment and execution, administrative support, manuscript writing, final approval of manuscript.

Acknowledgements

The authors also thank Dr. Na-Ying He, Dr. Ke-Min Chen, Dr. Fu-Hua Yan and Dr. Hua-Wei Ling at Department of radiology, Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine for the contribution of the MRI scanner. We thank all the subjects who participated in the study. Financial support was generously provided by grants from the National Natural Science Foundation of China (30872729, 30971031, 81100952, 81201083, 81520108016), Program for Outstanding Medical Academic Leader (LJ 06003), and the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health (1 P50 NS 071675-02).

Conflict of interests: None.

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Figure legends

Fig.1 Learning performance on the Acquired Equivalence Task.

(A) L-naïve patients made significantly more errors than R-naïve patients and NC in the acquisition phase. R-naïve patients performed as well as NC in acquisition. No significant difference in accuracy was found either in the retention phase or in the generalization phase among three groups.

(B) There was no significant difference in response time in the acquisition phase, retention phase and generalization phase among L-naïve, R-naïve and NC groups.

L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls.

Fig. 2 Difference in neural activity asymmetry and its correlation with learning.

(A) The ANCOVA analysis among L-naïve, R-naïve and NC groups in the ReHo lateralization within the striatum (the left striatum was shown). A significant difference was found in the dorsal rostral putamen.

(B) Post-hoc analysis between L-naïve and NC group. Increased ReHo lateralization was detected in the dorsal rostral putamen in L-naïve group.

(C) Post-hoc analysis between L-naïve and R-naïve group. Increased ReHo lateralization was detected in the dorsal rostral putamen in L-naïve group compared to R-naïve group.

(D) Scatter plot of ReHo activity in bilateral dorsal rostral putamen among groups. The

horizontal line represents the mean level of ReHo. Group differences in ReHo lateralization were due to the decrease of ReHo in the right dorsal rostral putamen of L-naïve group.

(E) The mean number of errors in acquisition was inversely correlated with the ReHo activity both of the right dorsal rostral putamen and the left dorsal rostral putamen in L-naïve group.

L-naïve = left-onset medication-naïve patients with Parkinson's disease; R-naïve = right-onset medication-naïve patients with Parkinson's disease; NC = normal controls; ReHo = regional homogeneity.

Fig. 3 Correlation between ReHo activity of the dorsal rostral putamen and UPDRS-III score. No significant correlation was found between UPDRS-III score and neural activity of either the right or the left dorsal rostral putamen in L-naïve patients. L-naïve = left-onset medication-naïve patients with Parkinson's disease; ReHo = regional homogeneity.