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Alterations in the limbic/paralimbic cortices of Parkinson's disease patients with hyposmia under resting-state functional MRI by regional homogeneity and functional connectivity analysis

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ABSTRACT

Background: Hyposmia is a cardinal early symptom of Parkinson's disease (PD), but the pathophysiological mechanisms underlying it remain unclear. Resting-state functional MRI (RS-fMRI) demonstrates spontaneous neuronal activity. We hypothesized that there would be alterations in the olfaction-related regions of the limbic/paralimbic cortices in PD patients with obvious hyposmia by RS-fMRI.

Methods: We used the "Five Odors for Olfactory Detection Arrays" to test the threshold of olfactory detection (TOD) for 54 PD patients and 22 age-matched controls. Using the mean TOD of the control group, patients were subdivided into two groups: PD with obvious hyposmia (OH-PD, n = 38) and PD with none/less obvious hyposmia (NOH-PD, n = 16). The regional brain activity of all subjects was investigated using RS-fMRI, in combination with regional homogeneity (ReHo) and functional connectivity (FC) analysis.

Results: There were different ReHo values in the limbic/paralimbic cortices between the OH-PD and NOH-PD groups. ReHo was significantly decreased in OH-PD patients in parts of the traditional olfactory regions (e.g. the amygdala, olfactory gyrus, orbital frontal cortex, parahippocampal gyrus and insula) and some non-traditional olfactory centers (e.g. the rectal gyrus and superior temporal pole), while increased in the left anterior/posterior cingulate cortex. FC analysis revealed decreased functional connectivity within the limbic/paralimbic cortices, especially in regions with reduced ReHo in the OH-PD group.

Conclusions: PD with hyposmia is related to altered functional activity not only in the traditional olfactory center, but also in some non-traditional olfactory regions of the limbic/paralimbic cortices.

1. Introduction

Olfactory disorder occurs frequently in patients with Parkinson's disease (PD). More than 50% of PD patients have anosmia, 14% experience moderate hyposmia and 35% experience severe hyposmia [1]. However, the exact pathophysiological mechanism of olfactory damage is unclear.

Several studies have emphasized neuroimaging and pathological changes in olfactory dysfunction in PD. Results from voxel-based morphometry (VBM) suggested that olfactory impairment in PD correlated with atrophy of the piriform cortex and amygdala [2], or the parahippocampal gyrus (PHG) and orbitofrontal cortex (OFC) [3], or the olfactory bulb (OB) and entorhinal cortex [4]. In addition to these regions, further functional magnetic resonance imaging (fMRI) studies employing olfactory stimuli [5] suggested that the inferior frontal gyrus (IFG), insula and cingulate cortex were also involved in olfactory dysfunction in PD. Autopsy studies [6,7] in PD patients have also documented that Lewy bodies are located not only in the OB, but also in the amygdala, hippocampal gyrus, anterior olfactory nucleus, entorhinal cortex and piriform cortex. These data suggest that olfactory dysfunction in PD may be associated with structural and pathological abnormalities in the limbic/paralimbic cortices.

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Resting-state functional MRI (RS-fMRI) is a non-invasive imaging method that can be used to investigate functional changes in the brain without the need for deliberate stimulation or intentional movement: It has mostly been used to investigate the neurodegenerative diseases. Regional homogeneity (ReHo) [8] is a RS-fMRI analysis method to detect the temporal similarity between one voxel and its nearest neighbors within a functional cluster. Similar to the ReHo, functional connectivity (FC) [9] is a method of studying brain networks and examining the temporal correlation of neuronal activity between spatially independent brain areas [10]. To date, there have been few studies investigating the functional alterations in the olfaction-related regions under resting-state in PD.

In this study, we tested the hypothesis that PD patients with hyposmia have decreased ReHo and/or disrupted FC in the olfaction-related regions of the limbic/paralimbic cortices of the brain.

2. Materials and methods

2.1. Subjects

PD patients (age ≤75 years, disease duration ≤15 years) were recruited from the First Affiliated Hospital of Chongqing Medical University, following diagnosis by neurologists according to the United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria [11]. All subjects underwent Mini-Mental State Exam (MMSE), Hoehn and Yahr staging (H&Y stage), and Unified Parkinson's Disease Rating Scale (UPDRS). Inclusion criteria were an MMSE score ≥24, H&Y stage 1–3, and UPDRS score ≤100. The healthy controls (MMSE score ≥ 24) were age-matched to patients with PD and recruited from volunteers including the patients' spouse and/or friends. Additionally, all patients were under their recommended PD medication during the "on" stage of the olfactory test, and they were required to stop taking PD medication for >12 h prior to the MRI data acquisition to minimize the impact of drugs.

All subjects gave their written informed consent and the study was approved by the Ethics Committee of the First Affiliated Hospital, Chongqing Medical University in China.

2.2. Exclusion criteria

Exclusion criteria for all subjects included: cognitive disorders diagnosed by a neurologist on the basis of the Movement Disorder Society Diagnostic Criteria [12]; a history of heavy smoking, history of neurological or psychiatric diseases or nasal diseases; white matter lesions due to small vessel ischemic changes on T2-weighted MRI; and any history of coryzal illness 2 weeks prior to evaluation.

2.3. Olfactory function tests

"Five Odors for Olfactory Detection Arrays" provided by the Chinese Academy of Sciences [13] were used to determine the threshold of olfactory detection (TOD) and threshold of olfactory identification (TOI) for all subjects. The five odors including valeric acid, peppermint, phenylethanol, acetic acid and 3-methyl indole were test reagents, and water was the control reagent. Each test reagent had six grades, each representing ten times the concentration of the adjacent scale (10^{-2}–10^{3}). Test score was given the logarithmic value of the corresponding density of each odor. Each test was carried out under conditions of ventilation and without foreign odors. All subjects were told to sit comfortably with eyes closed and breathing normally. Water was always firstly smelled before each odor test. Each odor was presented to the participants at approximately 1 cm from each participant's nose, from low to high intensity with minimum interval of 45 s before the next test to avoid olfactory fatigue. When the participant was able to detect but might not be able to distinguish the actual odor, the corresponding score was defined as the TOD. When the subject was able to name the odor, the score was described as TOI. Failure to detect an odor gave the score 3.5. The mean value of the five odors was the final TOD and TOI score for each subject.

2.4. Subject grouping

The mean TOD score range in healthy controls was obtained from mean ± standard deviation. In PD patients, a TOD score less than the upper limit value was considered as no obvious dysosmia; a higher TOD score (than the upper limit) was classed as significant hyposmia. This allowed us to divide the subjects into three groups: PD with obvious hyposmia (OH-PD), PD with none/less obvious hyposmia (NOH-PD) and healthy controls (HC).

2.5. MRI data acquisition

All MR images were obtained with a GE Signa Hdxt 3.0T scanner (General Electric Medical Systems, USA) with a normal eight-channel phased-array head coil. All subjects were asked to relax with eyes closed but to remain awake. Functional images were gained using an echo-planar image pulse sequence sensitive to blood oxygen level-dependent (BOLD) contrast, 33 axial slices with thickness of 4.0 mm, repetition time (TR) of 2000 ms, echo time (TE) of 40 ms, flip angle of 90°, matrix size of 64 × 64, field of view (FOV) of 240 × 240 mm^2. The 240 time points were obtained in 8 min. Meanwhile, high-resolution 3D-T1 (with slice thickness 1.0 mm, TR 8.3 ms, TE 3.3 ms, flip angle 15°, matrix size 256 × 192, FOV 240 × 240 mm^2) images were also obtained.

2.6. Data preprocessing

MR data preprocessing was performed using the toolboxes of Data Processing Assistant for Resting-state fMRI (DPARSF version 2.1), Statistical Parametric Mapping (SPM version 8) and Resting-state fMRI Data Analysis Toolkit (REST version 1.8) by Matlab version 7.9.0.529 (R2009b).

The first 10 volumes of the functional images were abandoned. The remaining 230 time points were retained for slice timing correction, head-motion correction (with no more than 1.5 mm of maximum translation or 1.5° of maximum rotation in the x, y and z directions), unified segmentation using T1 images, spatial normalization (with voxels resampling at 3 × 3 × 3 mm^3), time course de-trending, and band-pass filtering (0.01–0.08 Hz). A whole-brain mask provided by DPARSF was chosen as the target regions of the brain during the data preprocessing.

2.7. ReHo analysis

ReHo analysis, by calculation of Kendall's coefficient of concordance (KCC) [14], was performed for each subject using DPARSF software to measure the similarity of the time series of one voxel to its neighbor voxels within the whole-brain mask. Subsequently, spatial standardization and smoothing with 4 × 4 × 4 mm^3 voxels were performed for all images.

2.8. FC analysis

FC analysis, depending on the BOLD contrast mechanism [15], was performed by the software DPARSF. Some brain areas with significant ReHo differences generated in two-sample t-tests between the OH-PD and NOH-PD groups and related to olfaction, were defined as regions of interest (ROIs) and used as the seeds for FC analysis. Thus eight ROIs including the left rectal gyrus, OFC, superior temporal pole (STP), posterior cingulate cortex (PCC); the right insula, amygdala, PHG and IFG were obtained to investigate the connectivity between each ROI and the whole brain for all subjects. The global mean signal, cerebrospinal fluid signal and six head movement parameters were considered as covariates and regressed out before the FC analysis.

2.9. VBM analysis

The 3D-T1 images were used to examine volume alterations in the gray/white matter in the three groups with VBM analysis by the software SPM. Briefly, all T1 images were segmented into the gray matter, white matter and cerebrospinal fluid, then normalized to the Montreal Neurological Institute (MNI) template and smoothed with a Gaussian kernel filter of 8 × 8 × 8 mm^3.

2.10. Statistics

All statistical analyses for clinical data were performed by the software SPSS version 17.0. Statistical differences were determined by using a multiple linear regression, or two-sample t-test, or Pearson chi-square test, or one-way analysis of variance (ANOVA) followed by Bonferroni's or Dunnett's post-hoc test at a significance level of $p < 0.05$.

Statistical analyses for RS-fMRI data were performed by the software REST, and $p < 0.05$ described statistical significance. Two-sample two-tailed t-test with Monte Carlo simulation correction (AlphaSim correction, $p < 0.05$ and cluster size >54 voxels or 1458 mm^3) was used to detect ReHo differences and FC differences among the three groups. The brain areas with significant ReHo differences in the comparison between OH-PD and NOH-PD groups were combined to form a new mask for the later correlation analysis and VBM analysis.

We carried out correlation analyses with AlphaSim correction ($p < 0.05$ and cluster size >13 voxels or 351 mm^3) in order to investigate the relationship between ReHo values and TOD scores in patients with PD, within the new mask via the software REST, while other variables such as TOI scores were considered as covariates and regressed out. Additionally, we performed another two-sample t-test with false discovery rate (FDR) correction with the software SPM to detect VBM differences in the new mask among the three groups.