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GRAY MATTER ANOMALIES IN ANTERIOR CINGULATE CORTEX AS A CORRELATE OF DEPRESSIVE SYMPTOMS IN DRUG-NAÏVE IDIOPATHIC RESTLESS LEGS SYNDROME

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Abstract—*Background:* Depressive symptoms are frequent in idiopathic restless legs syndrome (RLS). However, little is known, so far, about the neurological basis. The present study aimed to explore the neuroanatomical anomalies in depressed drug-naïve RLS patients using voxel-based morphometry (VBM) analysis.

***Methods:* We recruited 16 drug-naïve idiopathic RLS patients with depressive symptoms (RLS-D), 18 drug-naïve idiopathic RLS patients without depressive symptoms (RLS-ND), and 18 normal controls. All participants underwent structural MRI scans on a 3-T MR system. The differences in regional gray matter (GM) density were determined across groups by VBM8. Additional regression analysis was used to identify any associations between regional GM density and clinical symptoms.**

***Results:* GM density of the bilateral anterior cingulate cortex (ACC) was significantly reduced in RLS-D patients when compared to RLS-ND patients or to the healthy controls. However, there were no significant differences of GM density either when the whole RLS group or the RLS-ND group was compared to healthy controls, respectively. Particularly, we found GM density of right ACC was negatively correlated with the severity and duration of depressive symptoms in RLS-D patients.**

***Conclusions:* Depressive symptoms are associated with GM anomalies in ACC in patients with RLS. We propose that ACC is perhaps an important neuroimaging marker for facilitating treatment strategies in patients with RLS when assessing depressive symptoms.**

Key words: restless legs syndrome, depression, voxel-based morphometry, gray matter density, anterior cingulate cortex.

INTRODUCTION

Restless legs syndrome (RLS) is a common yet underrecognized sleep disorder with an estimated prevalence of 3.9–14.3% in the general adult population (Ohayon et al., 2012). The diagnosis for RLS is based on clinical criteria: (1) an urge to move the legs, usually associated with unpleasant sensations; (2) symptoms occurring during periods of rest, such as sitting or lying down; (3) symptoms relieved by movement; and (4) symptoms worse in the evening or night (Allen et al., 2003). Epidemiologic studies have reported higher depression ratings in patients with RLS than without (Sevim et al., 2004; Winkelmann et al., 2005; Cho et al., 2009; Gupta et al., 2013). In addition, some observational studies demonstrated RLS as a treatment-related adverse event when patients were treated with some antidepressants (Rottach et al., 2008; Hornyak, 2010). The association of RLS with depressive symptoms is of particular interest as comorbid depression may significantly worsen the overall treatment outcome that results in the heavy burden on the individuals and society (Earley and Silber, 2010; Allen et al., 2011). Although some risk factors are associated with the development of RLS with depressive symptoms (RLS-D), the underlying neurobiological abnormalities governing its genesis is largely unknown (Sevim et al., 2004; Picchietti and Winkelman, 2005; Hornyak et al., 2009; Hornyak, 2010; Gupta et al., 2013).

Voxel-based morphometry (VBM) is a powerful method without a priori specification of region of interest that is being increasingly used for the assessment of gray matter (GM) density or volume in healthy subjects and patients *in vivo* (Ashburner and Friston, 2000). To date, structural abnormality in the brain of RLS patients was a controversy. Divergent findings were reported in RLS patients with VBM, such as increased GM in bilateral pulvinar (Etgen et al., 2005), decreased GM volume in the bilateral primary somatosensory cortex and left-sided primary motor areas (Unrath et al., 2007), increased GM density in the bilateral ventral hippocampus and middle orbitofrontal gyrus (Hornyak et al., 2007), decreased white matter (WM) volume in the corpus callosum, anterior cingulum and precentral gyrus (Connor et al., 2011). While four other recent VBM studies yielded no specific

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Abbreviations: ACC, anterior cingulate cortex; ANCOVA, analysis of covariance; GM, gray matter; HDRS, Hamilton Depression Rating Scale; ICV, intracranial volume; IRLS, International RLS Study Group Rating Scale; RLS, restless legs syndrome; VBM, voxel-based morphometry; WM, white matter.

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GM anomalies (Celle et al., 2010; Comley et al., 2012; Margariti et al., 2012; Rizzo et al., 2012). The discrepancy might be attributed to the heterogeneous sample sizes, clinical features and methodologies among the studies (Rizzo et al., 2013). Regarding subjects with depressive symptoms, extensive research demonstrates brain structural abnormalities in the prefrontal cortex and other areas (Hayakawa et al., 2013). To the best of our knowledge, however, no VBM study has yet investigated GM density alterations in drug-naïve patients with idiopathic RLS comorbid depressive symptoms. It is critical as it could help to disentangle in which abnormal brain regions that might be specific to RSL with comorbid depressive symptoms and possibly underlie the worse clinical features and outcome of this comorbidity.

Herein, in this investigation we compared drug-naïve idiopathic RLS patients with depressive symptoms, and patients without depressive symptoms, and healthy controls by VBM. We hypothesize the following: (1) no GM abnormalities were detected in the non-depressed RLS patients; (2) some brain regions in the prefrontal-limbic systems (Bora et al., 2012) detected are correlated with the severity and duration of depressive symptoms in RLS patients.

EXPERIMENTAL PROCEDURES

Subject selection

Diagnosis of idiopathic RLS was made according to the criteria by the International Restless Legs Syndrome Study Group (IRLSSG) revised in 2003 (Allen et al., 2003). It was translated and back-translated by a senior neurologist and a professional translator to ensure accuracy. The patients underwent a detailed history survey, physical examination (conducted by two trained and senior neurologists (HC.S. and JG.Z.)), laboratory tests, conventional MRI and electromyography to exclude secondary forms of RLS due to iron deficiency anemia, pregnancy, chronic renal disease, diabetes, peripheral neuropathy, radiculopathy, Parkinson's disease, psychiatric illness, head injury, serious medical condition, or history of drug or alcohol addiction. All the patients had never been treated with dopaminergic agents, antidepressants, neuroleptics, or hypnotics. The severity of RLS was assessed by the International RLS Study Group Rating Scale (IRLS; Walters et al., 2003). RLS patients were divided into two subgroups according to the 17-item Hamilton Depression Rating Scale (HDRS) scores (cut off = 7). Other clinical data such as gender, age, duration of RLS, and duration of depressive symptoms in RLS were evaluated. Patients finally recruited in the present study included 18 idiopathic RLS patients without depressive symptoms (RLS-ND, 6 men and 12 women; average age 47.8 ± 11.6 years; disease duration 6.7 ± 3.2 years), and 16 idiopathic RLS patients with depressive symptoms (RLS-D, 5 men and 11 women; average age 49.2 ± 9.7 years; disease duration 7.2 ± 3.0 years). The control group comprised 18 age- and gender-matched healthy subjects (HC) with no history of neurologic or psychiatric diseases (6 men and 12 women; average age 48.4 ± 10.2 years). This study was approved by the local ethics committee, and all participants signed informed consent forms.

MRI scans and VBM

High resolution T1-weighted images were acquired via a 3.0-Telsa MRI system (SIGNA EXCITE, GE Healthcare, Milwaukee, WI, USA), with a volumetric three-dimensional spoiled gradient recall sequence using an eight-channel phase array head coil (TR = 8.5 ms, TE = 3.4 ms, flip angle = 12°, slice thickness = 1 mm, gap = 0, matrix size = 256 × 256, field of view = 240 × 240 mm^2, in-plane resolution = 0.47 × 0.47 mm^2) that produced 156 contiguous coronal slices. The scan protocol was identical for all subjects.

Data processing and analysis were performed with VBM8 by the Statistical Parametric Mapping 8 package (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) running under Matlab R2010b (The Mathworks, Natick, MA, USA). Each image was inspected for reconstruction artifacts. All imaging analysis processes were conducted as described in the VBM Tutorial (http://www.fil.ion.ucl.ac.uk/~john/misc/VBMclass10.pdf). The process is briefly summarized here. The T1-weighted images were normalized to the same stereotaxic space generated from the complete data set using the DARTEL algorithm that significantly reduces the imprecision of inter subject registration. Then the images were segmented into WM and GM and non-brain voxels (CSF, skull). Subsequently, all images were "modulated" to correct volume changes by the Jacobian determinants. Following this, images were smoothed by convolution with an isotropic Gaussian kernel of 8-mm full-width at half maximum for statistical analyses.

Statistical analyses

Statistical analyses of demographical and neuropsychological data were performed with SPSS 17.0 software for Windows (SPSS, Chicago, IL, USA). Demographic and clinical variables were compared across groups using an analysis of covariance (ANCOVA) or independent-sample t test for continuous variables and χ^2 tests for dichotomous variables.

Voxel-wise comparisons of GM density were performed between groups (RLS vs. HC and RLS-D vs. RLS-ND) using an ANCOVA with SPM8 by a general linear model. Age, gender, years of education, and total intracranial volume (ICV) were included as nuisance covariates. The significance of group differences was estimated by the theory of random Gaussian fields, and significance levels were set at $P < 0.05$ (whole brain FDR corrected). Two linear contrasts (1, −1) were made for positive and negative correlations, respectively. The relationship between GM density and HDRS scores and duration of depressive symptoms in the RLS-D group was examined using multiple regression analyses with age and ICV as confounding factors. Correlation analyses between brain regions and IRLS scores, and disease duration were also performed in all RLS subjects ($P < 0.05$ corrected).