Altered Spontaneous Brain Activity in Type 2 Diabetes: A Resting-State Functional MRI Study

Previous research has shown that type 2 diabetes mellitus (T2DM) is associated with an increased risk of cognitive impairment. Patients with impaired cognition often show decreased spontaneous brain activity on resting-state functional magnetic resonance imaging (rs-fMRI). This study used rs-fMRI to investigate changes in spontaneous brain activity among patients with T2DM and to determine the relationship of these changes with cognitive impairment. T2DM patients (n = 29) and age-, sex-, and education-matched healthy control subjects (n = 27) were included in this study. Amplitude of low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) values were calculated to represent spontaneous brain activity. Brain volume and cognition were also evaluated among these participants. Compared with healthy control subjects, patients with T2DM had significantly decreased ALFF and ReHo values in the occipital lobe and postcentral gyrus. Patients performed worse on several cognitive tests; this impaired cognitive performance was correlated with decreased activity in the cuneus and lingual gyrus in the occipital lobe. Brain volume did not differ between the two groups. The abnormalities of spontaneous brain activity reflected by ALFF and ReHo measurements in the absence of structural changes in T2DM patients may provide insights into the neurological pathophysiology underlying diabetes-associated cognitive decline.

Type 2 diabetes mellitus (T2DM) has been shown to be associated with an increased risk of cognitive impairment (1,2), which primarily manifests as declining memory, information processing speed (3), attention, and executive function (4). However, the pathophysiological mechanism of T2DM-induced cognitive impairment is still largely unknown (5).

Neuroimaging has proven to be a useful tool for investigating the diabetic brain. Cerebral atrophy and white matter (WM) lesions, which are commonly reported structural abnormalities in previous studies, are believed to be modestly associated with diabetes-related cognitive dysfunction (6–8). Magnetic resonance (MR) spectroscopy was used to determine the concentration of brain metabolites. A recent MR spectroscopy study in patients with T2DM and major depression revealed that abnormal brain metabolite measurements may be related to mood changes in this population (9). However, little is known about the effects of diabetes on neural activity. Neural activity is a sensitive measurement that has been observed to be acutely altered by brain structural lesions.
Neural abnormalities have been detected in populations at risk for developing cognitive impairment. Based on this evidence, measures of neural activity may be suited to track the early effects of diabetes on brain function.

Resting-state functional MR imaging (rs-fMRI) has been found to be a powerful tool for evaluating spontaneous neural activity. Recently, Musen et al. used rs-fMRI to investigate neural functional connectivity changes in patients with T2DM and found evidence of altered neural networks in non-demented diabetic patients. However, this study focused only on the functional connectivity changes between two distinct brain regions. As the effects of diabetes on the brain may be global, a whole-brain analysis of brain function in patients with T2DM is needed.

Amplitude of low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) analyses are two important methods for depicting the various characteristics of global rs-fMRI signals. ALFF measures the intensity of neural activity at the single-voxel level, whereas ReHo measures the neural synchronization of a given voxel with its neighboring voxels. A previous study indicated that ReHo may be more sensitive than ALFF for detecting regional abnormalities and that ALFF may be complementary to ReHo for measuring global spontaneous activity. Therefore, the combination of these two methods may provide more information about the pathophysiological framework in the human brain than either method alone.

In this study, combined ALFF and ReHo analyses were applied to investigate global spontaneous neural activity in patients with T2DM. We hypothesized that 1) abnormal ALFF and ReHo values would be detected within specific brain regions; and 2) the spontaneous brain activity abnormalities would be related to impaired cognitive performance and T2DM-related biometric measurements.

RESEARCH DESIGN AND METHODS

Subjects
This study was approved by the local institutional review board and conducted between September 2012 and June 2013. Written informed consent was obtained from all subjects. Patients were recruited from the practices of collaborating endocrinologists and from the local community via advertisement. Patients who were between 45 and 75 years of age and who had disease duration of ≥1 year were qualified for this study. T2DM was defined according to the latest criteria published by the American Diabetes Association. All patients were closely self-monitored and were routinely treated with hypoglycemic agents; none had any history of hypoglycemic episodes. Control participants were recruited through advertisements, and were matched with T2DM patients with respect to age, sex, and education. Exclusion criteria for all participants included a history of alcohol or substance abuse; indication of dementia (defined as a Mini-Mental State Examination [MMSE] score of ≤24); history of a brain lesion such as tumor or stroke; psychiatric or neurological disorder unrelated to diabetes; and concomitant medications to MRI. Control participants were excluded if they had a fasting blood glucose level ≥7.0 mmol/L; glucose level ≥7.8 mmol/L after oral glucose tolerance test (OGTT); or a Montreal Cognitive Assessment (MoCA, Beijing edition) score of <26. Participants with vascular risk factors were not excluded (to improve the generalizability of the study results).

A flowchart of the study design is provided in Supplementary Fig. 1.

Biometric Measurements
Medical history and medication use were recorded according to a standardized questionnaire. Blood pressure levels were measured at three different time points during the interview and then averaged. Hypertension was defined as a systolic blood pressure >160 mmHg, a diastolic blood pressure >95 mmHg, or self-reported use of blood pressure-lowering medication. An OGTT (75 g dextrose monohydrate in 250 mL water) was performed on all subjects except those being currently treated for diabetes. Plasma glucose levels at fasting and 2 h after the OGTT were measured, along with fibrinogen and cholesterol levels. BMI and waist circumference were measured and recorded. Insulin resistance (IR) was determined by homeostasis model assessment (HOMA) of IR for control subjects and patients not being treated with insulin. Diabetic retinopathy was assessed for all participants except for four healthy control subjects who refused the examination. After papillary dilation, stereoscopic color fundus photographs were taken and graded according to the Wisconsin Epidemiologic Study of Diabetic Retinopathy. Peripheral neuropathy, as defined by the Diabetes Control and Complications Trial criteria, was assessed for all participants using a method described previously.

Cognitive Assessment
All participants underwent a battery of neuropsychological tests that covered relevant cognitive domains. Selection of the tests was based on previous literature describing cognitive dysfunction in T2DM patients. The MMSE was used to assess possible dementia. The MoCA was used to screen subjects with mild cognitive impairment and to assess their general cognition. The Auditory Verbal Learning Test and Rey-Osterrieth Complex Figure Test (CFT) (both of which included immediate and delayed recall tasks) were used to assess episodic memory for verbal and visual information. The Trail-Making Test, part A (TMT-A) and part B (TMT-B), was primarily used to evaluate attention and psychomotor speed, and the Clock-Drawing Test was used to address several relevant cognitive
domains including executive function and working memory (24). All of the tests took ~60 min to complete.

MRI
All MR images were acquired at the Radiology Department of Zhongda Hospital via a Siemens (Erlangen, Germany) 3-Tesla Trio scanner. Subjects were instructed to keep their eyes closed but to remain awake, to avoid thinking of anything in particular, and to keep their heads still during the scanning. Functional images were obtained using a gradient-echo planar sequence (36 slices; repetition time, 2,000 ms; echo time, 25 ms; slice thickness, 4 mm; flip angle, 90°; field of view, 240 × 240 mm). Structural images were acquired using a T1-weighted three-dimensional spoiled gradient-recalled sequence (176 slices; repetition time, 1,900 ms; echo time, 2.48 ms; slice thickness, 1.0 mm; flip angle, 9°; inversion time, 900 ms; field of view, 250 × 250 mm; in-plane resolution, 256 × 256). Fluid-attenuated inversion recovery images were also obtained with the following parameters: repetition time, 8,500 ms; echo time, 94 ms; 20 slices; slice thickness, 5 mm; voxel size, 1.3 × 0.9 × 5 mm³.

Structural images (three-dimensional T1-weighted images) were processed using the VBM8 toolbox software (http://dbm.neuro.uni-jena.de/vbm). Images were segmented into gray matter (GM), WM, and cerebrospinal fluid using the unified segmentation model. Statistical parametric mapping between the patient and control groups was generated. The distribution of brain parenchyma volume (sum of the GM and WM volumes) was displayed as a box plot. Subjects with brain parenchyma volume values that were extreme outliers were considered to have abnormal brain volume and were excluded from the study.

Small-Vessel Disease Assessment
WM hyperintensity (WMH) and lacunar infarcts were assessed on fluid-attenuated inversion recovery images with a method described previously (25). Participants with a rating score >1 (confluence of lesions or diffuse involvement of the entire region) were excluded. Two experienced radiologists blinded to the group allocations performed the ratings separately. Consensus was obtained through discussion between the two raters.

Data Preprocessing
FMRI imaging data were preprocessed with the toolbox Data Processing Assistant for Resting-State functional MR imaging (DPARSF; http://www.restfmri.net/forum/DPARSF) through statistical parametric mapping (SPM8; http://www.fil.ion.ucl.ac.uk/spm/) and an rs-fMRI data analysis toolkit (REST1.8; http://www.restfmri.net). Slice timing and realignment for head motion correction were performed. Any subjects with head motion >2.0 mm translation or >2.0° rotation in any direction were excluded. The functional images were then spatially normalized to standard coordinates and resampled to 3 × 3 × 3 mm³.

ALFF and ReHo Analyses
ALFF and ReHo analyses were performed with REST software as described in previous studies (15,16).

For ALFF analysis, the resampled images were first smoothed with a Gaussian kernel of 4 mm. Linear trend and band-pass filtering (0.01–0.08 Hz) were performed to remove the effects of low-frequency drift and high-frequency noise. The time series were transformed to the frequency domain using a fast-Fourier transform. The square root of the power spectrum was then calculated and averaged across 0.01–0.08 Hz within each voxel to obtain the raw ALFF value. Subsequently, the global mean ALFF value was calculated by extracting the raw values from all voxels across the whole brain and averaging them. Finally, the raw ALFF values for each voxel were divided by the global mean ALFF values for standardization. The resulting ALFF value in a given voxel reflects the degree of its raw ALFF value relative to the average ALFF value of the whole brain (26).

ReHo analysis was performed on preprocessed images. After linear trend and band-pass filtering were performed, ReHo maps were generated by calculating the concordance of the Kendall coefficient of the time series of a given voxel with its 26 nearest neighbors (16). The ReHo value of each voxel was then standardized by dividing the raw value by the global mean ReHo value, which was obtained with the same calculation used to determine the global mean ALFF value. Finally, the data were smoothed with a Gaussian kernel of 4 mm for further statistical analysis.

Statistical Analysis
Demographic and clinical variables and cognitive performance scores were compared between the two groups using SPSS software (version 18.0; SPSS, Inc., Chicago, IL). An independent two-sample t test was used for continuous variables, and a χ² test was used for proportions. P values <0.05 were considered to be statistically significant.

Within-Group Analysis
To explore the within-group ALFF and ReHo patterns, one-sample t tests were performed on the individual ALFF and ReHo maps for each group using REST-Statistical Analysis (written by Chaogeng Yan, www.restfmri.net). To display the most significant results and reflect the intrinsic nature of these two algorithms, a conservative statistical significance was set at P < 0.005 and a cluster size of 24 voxels, which corresponded to a corrected P < 0.005 (multiple comparisons with family-wise error using the AFNI AlphaSim program; http://afni.nimh.gov/afni/docpdf/AlphaSim.pdf).
Between-Group Analysis
To investigate the between-group differences of ALFF and ReHo values, two-sample t tests were performed with the REST software (within a GM mask). Age, sex, and education levels were imported as covariates. To exclude the possible effects of structural differences (27), we also stratified the modulated GM maps obtained from VBM analysis. Vascular risk factors (hyperlipidemia, waist circumference, hypertension, and scores of WMH and lacunar infarcts) were also controlled for to exclude possible confounding effects. The statistical threshold was set at $P < 0.01$ and a minimum cluster size of 22 voxels, which corresponded to a corrected $P < 0.01$ (AlphaSim correction).

Correlation Analysis
To identify the association between regional ALFF and ReHo abnormalities, a bivariate correlation was performed between these two measurements. Briefly, the average ALFF and ReHo values of brain regions with significant differences were individually extracted and correlated with one another.

To investigate the relationship among ALFF/ReHo values, cognitive performance, and diabetes-related parameters (fasting plasma glucose and HbA1c levels, HOMA-IR, and disease duration), Pearson correlation analyses were performed in a voxel-wise manner with the RBST software. Analyses were adjusted for the same covariates as those controlled in the two-sample t tests. Because the results could be easily affected by noise, a conservative statistical threshold was set at $P < 0.005$ (after AlphaSim correction) to explore the most significant correlations among MR voxels.

To verify and extend the results of voxel-wise analyses, we performed a further correlation analysis based on regions of interest (ROIs). Briefly, regions showing significant differences were specified within an automated anatomical labeling template (http://neuro.imm.dtu.dk/services/brededatabase/index_roi_tourniomazoyer.html), and the mean ALFF and ReHo values for each ROI were

<table>
<thead>
<tr>
<th>Table 1 — Demographic, clinical, and cognitive characteristics of study patients and control subjects</th>
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<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Sex (male/female)*</td>
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<tr>
<td>Education (years)</td>
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<tr>
<td>Disease duration (years)</td>
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<tr>
<td>Fasting glucose (mmol/L)*</td>
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<tr>
<td>HbA1c % (mmol/moL‡)</td>
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<td>HOMA-IR</td>
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<td>Insulin treatments</td>
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<tr>
<td>Retinopathy (background)</td>
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<tr>
<td>Diabetic peripheral neuropathy</td>
</tr>
<tr>
<td>Vascular risk factors</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
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<td>Diastolic BP (mmHg)</td>
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<tr>
<td>Cerebral vessel disease</td>
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<tr>
<td>WMH</td>
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<tr>
<td>Lacunar infarcts</td>
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</tbody>
</table>

Cognitive performance
| MMSE                                                | $28.3 \pm 1.4$           | $29.0 \pm 1.1$               | 0.07      |
| MoCA‡                                                | $23.6 \pm 2.9$           | $27.3 \pm 1.1$               | <0.01     |
| TMT-A (s)‡                                          | $72.8 \pm 24.3$          | $58.9 \pm 14.8$              | 0.01      |
| TMT-B (s)‡                                          | $174.8 \pm 59.1$         | $144.4 \pm 91.3$             | 0.01      |
| AVLT                                                | $6.2 \pm 1.2$            | $6.3 \pm 1.6$                | 0.81      |
| AVLT-delayed recall (20 min)                        | $6.1 \pm 2.5$            | $6.3 \pm 2.5$                | 0.82      |
| CDT                                                 | $3.5 \pm 0.6$            | $3.7 \pm 0.5$                | 0.12      |
| CFT-delayed recall (20 min)‡                         | $13.3 \pm 6.2$           | $19.7 \pm 5.5$               | <0.01     |

Data are mean ± SD, n (%), or median (range) unless otherwise stated. BP, blood pressure; AVLT, Auditory Verbal Learning Test; CDT, Clock-Drawing Test. *The $P$ value for proportions was obtained by $\chi^2$ test. ‡$P < 0.05$. 
extracted. Partial correlations among extracted values, cognition, and diabetes-related variables were calculated using the same covariates as in the voxel-wise analyses.

RESULTS
A total of 58 participants (30 patients and 28 healthy control subjects) were recruited for this study. One patient and one healthy control subject were excluded because of excessive head movement. Thus, a total of 56 subjects were included in the final data analysis.

Demographic and Cognitive Characteristics
Insulin-treated patients comprised 21% of the diabetes group (HbA1c, 8.6 ± 1.5%); the remaining patients were treated with oral antidiabetic agents (58%; HbA1c, 7.9 ± 1.9%) or dietary restriction only (21%; HbA1c, 7.2 ± 1.3%). The patient and control groups did not differ in terms of age, sex, or education (Table 1). No significant differences were observed in total cholesterol level, blood pressure, presence of cerebral small-vessel disease, or MMSE scores between the two groups. Patients with T2DM had significantly higher fasting plasma glucose levels, HOMA-IR, HbA1c levels, and waist circumference than the control group (all \( P < 0.01 \)). Retinopathy was diagnosed in eight patients, but all had background non-proliferative changes only. Peripheral neuropathy was diagnosed in eight patients. In terms of cognitive assessment, patients had a significantly lower MoCA score than control subjects, suggesting that their general cognition was impaired. Patients also performed significantly worse than control subjects on the TMT-A, TMT-B, and CFT-delayed recall tests, indicating that cognitive decrements in these patients are more likely to involve the domains of information processing speed, attention, and visual memory.

Structural Results
No participants were excluded because of severe atrophy. No significant difference was observed in GM or WM volume between the patients and control subjects;

![ALFF Map](image1)
![ReHo Map](image2)

Figure 1—Representative one-sample t test results of ALFF and ReHo maps \( (P < 0.005 \), AlphaSim corrected). Within each group, standardized ALFF and ReHo values in the PCC, the adjacent PCu, and the medial prefrontal cortex were significantly higher than the global mean values in both groups. Other regions, such as the inferior parietal lobe and bilateral occipital lobes, also had higher spontaneous activity. Note that the brain regions were mainly part of the default-mode network, which demonstrated the correctness of our data analysis. R, right; L, left; Ctrl, control.
overall, however, the GM and WM volumes in the diabetes group were lower than those in the control group (see details in Supplementary Table 1).

**ALFF and ReHo Analyses**

In both groups, standardized ALFF and ReHo values in the posterior cingulate cortex (PCC), precuneus (PCu), and medial prefrontal cortex were significantly higher than the global mean values (Fig. 1).

In T2DM patients, the ALFF and ReHo values were significantly decreased in the occipital lobe (bilateral lingual gyrus, right fusiform, left cuneus, and right calcarine cortex) and parietal regions (left postcentral gyrus [PoCG]) (Fig. 2, Table 2). The ReHo values were also decreased significantly in the thalamus/caudate (Fig. 2B, Table 2).

ALFF and ReHo values in the posterior lobe of cerebellum (PLC) were higher in diabetic patients than in control subjects (Table 2). Increased ALFF and ReHo values were also found in the anterior cingulate cortex and frontal lobe, respectively (Fig. 2).

**Correlation Analysis**

Bivariate correlation analyses indicated that the ALFF and ReHo values extracted from the occipital lobe and left PoCG were significantly correlated with each other (Fig. 3).

Voxel-wise correlation analyses revealed that there were significant correlations between decreased neural activity in the occipital lobe and impaired neurocognitive performance (i.e., CFT-delayed recall test and TMT-B) (corrected P < 0.005) (Fig. 4). For example, ALFF and ReHo values in the cuneus and PCu at the occipital lobe

![ALFF Map](image1)

![ReHo Map](image2)

*Figure 2—ALFF (A) and ReHo (B) differences between T2DM patients and healthy control subjects (P < 0.01, AlphaSim corrected). A: Compared with healthy subjects, patients with T2DM showed significantly decreased ALFF in the PoCG, calcarine cortex, and bilateral lingual gyrus (blue), and increased ALFF in the anterior cingulate cortex (red) and PLC (data not shown). B: Compared with healthy subjects, patients with T2DM showed significantly decreased ReHo in the PoCG, bilateral thalamus/caudate, calcarine cortex, middle temporal gyrus, and bilateral lingual gyrus (blue), and increased ReHo in the medial frontal gyrus (red) and PLC (data not shown). Color scale denotes the t value; x, z, Montreal Neurological Institute coordinates; R, right.*
Table 2—Differences in ALFF and ReHo values between the patient and control groups (P < 0.01, AlphaSim corrected)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>BA</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Voxels</th>
<th>Maximal t value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALFF differences</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>R lingual gyrus/fusiform</td>
<td>18</td>
<td>24</td>
<td>−75</td>
<td>−6</td>
<td>128</td>
<td>−3.81</td>
</tr>
<tr>
<td>L lingual gyrus</td>
<td>18</td>
<td>−6</td>
<td>−75</td>
<td>−3</td>
<td>27</td>
<td>−3.43</td>
</tr>
<tr>
<td>L middle occipital gyrus/cuneus</td>
<td>18/19</td>
<td>18</td>
<td>−84</td>
<td>−15</td>
<td>28</td>
<td>−4.14</td>
</tr>
<tr>
<td>R calcarine cortex</td>
<td>30</td>
<td>18</td>
<td>−63</td>
<td>12</td>
<td>25</td>
<td>−3.53</td>
</tr>
<tr>
<td>L PoCG</td>
<td>3/1</td>
<td>−18</td>
<td>−30</td>
<td>78</td>
<td>34</td>
<td>−4.01</td>
</tr>
<tr>
<td>R ACC</td>
<td>−</td>
<td>9</td>
<td>9</td>
<td>24</td>
<td>41</td>
<td>3.95</td>
</tr>
<tr>
<td>R PLC</td>
<td>−</td>
<td>18</td>
<td>−81</td>
<td>−51</td>
<td>40</td>
<td>3.93</td>
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<tr>
<td>ReHo differences</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>R lingual gyrus/fusiform</td>
<td>18</td>
<td>30</td>
<td>−81</td>
<td>−12</td>
<td>74</td>
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<tr>
<td>L lingual gyrus/cuneus</td>
<td>18/30</td>
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<tr>
<td>R calcarine cortex</td>
<td>30</td>
<td>21</td>
<td>−57</td>
<td>6</td>
<td>25</td>
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</tr>
<tr>
<td>L PoCG</td>
<td>3/4</td>
<td>−15</td>
<td>−30</td>
<td>75</td>
<td>35</td>
<td>−4.25</td>
</tr>
<tr>
<td>R MTG</td>
<td>37</td>
<td>48</td>
<td>−66</td>
<td>0</td>
<td>28</td>
<td>−3.40</td>
</tr>
<tr>
<td>B thalamus/putamen</td>
<td>79</td>
<td>9</td>
<td>−3</td>
<td>12</td>
<td>79</td>
<td>−4.81</td>
</tr>
<tr>
<td>R PLC</td>
<td>−</td>
<td>30</td>
<td>−57</td>
<td>−30</td>
<td>91</td>
<td>3.94</td>
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<td>−12</td>
<td>33</td>
<td>42</td>
<td>32</td>
<td>4.19</td>
</tr>
</tbody>
</table>

Comparisons were performed at P < 0.01, corrected for multiple comparisons. BA, Bredmann area; MNI, Montreal Neurological Institute; x, y, z, coordinates of primary peak locations in the MNI space; ACC, anterior cingulate cortex; MTG, middle temporal gyrus; MFG, medial frontal gyrus. *Negative t values: patients with T2DM < control subjects; positive t values: patients with T2DM > control subjects.

were positively correlated with CFT-delayed score and negatively correlated with time spent on TMT-B. HOMA-IR in the diabetes group was found to be negatively correlated with neural activity in the parietal, frontal, and temporal lobes, and the lingual gyrus. However, no such correlations were detected in the control group. Detailed coordinates of these correlations are provided in Supplementary Table 2.

ROI-based correlation analyses indicated that spontaneous neural activity in the cuneus and lingual gyrus had significant correlations with cognitive performance (Fig. 5). Higher ALFF and ReHo values in the cuneus were related to less time on the TMT-B (ALFF, R = −0.451, P = 0.035; ReHo, R = −0.535, P = 0.010). Both values in the lingual gyrus were also negatively correlated with TMT-B (ALFF, R = −0.435, P = 0.043; ReHo, R = −0.500, P = 0.018). CFT-delay scores were correlated with ReHo values in the cuneus (R = 0.403, P = 0.041). Among diabetic patients, HOMA-IR was found to be significantly correlated with neural activity in the cuneus (R = −0.469, P = 0.037).

Effects of Retinopathy and Neuropathy

We performed an analysis to explore the possible effects of retinopathy on neural activity in the visual cortex and the effects of neuropathy on neural activity in the PoCG. First, we divided the patients into the following two groups: patients with and patients without retinopathy. ALFF and ReHo values were extracted from three brain regions in the visual cortex and compared between these two groups and the control subjects. Subsequently, patients were divided into another two groups: patients with and patients without neuropathy. ALFF and ReHo values were extracted from the PoCG and compared between these two groups and the control subjects. Results showed that the mean ALFF and ReHo values at the occipital lobe in the control group were significantly higher than the values in the diabetes groups among patients with and without retinopathy. No such difference was observed between the two diabetes groups (Fig. 6A and B). ALFF and ReHo values extracted from the PoCG also did not differ between patients with and without neuropathy (Fig. 6C and D).

DISCUSSION

This study demonstrated decreased neural activity in several specific brain regions in T2DM patients versus control subjects. These neural abnormalities were primarily found in the occipital lobe and PoCG, and were related to the impaired cognitive performance seen in patients.

ALFF and ReHo analyses have been used to investigate the intrinsic neuropathology of various mental disorders (17,26,28,29). These two methods are based on different neurophysiology mechanisms, with ALFF analysis demonstrating neural intensity and ReHo analysis demonstrating neural coherence. In this study, abnormal neural activity was detected by both methods in several brain regions. The coexisting functional intensity and coherence abnormalities in these regions may represent
more severe functional changes than those reflected by a single method. Therefore, we believe our results represent reliable information that is necessary for understanding cognitive decline in T2DM patients.

Significantly decreased ALFF and ReHo values in the occipital lobe and the PoCG in T2DM patients are the major findings in this study. Previous fMRI studies indicated that decreased neural activity in the occipital area and PoCG were related to visual impairment (30) and sensory loss (31), respectively. Diabetes is known to be associated with retinopathy and neuropathy that can lead to visual and sensory impairment. In this study, most of the patients did not have clinical visual or sensory changes, suggesting that decreased neural activity may be an early change that occurs before the appearance of clinically measurable symptoms. It will be interesting to follow these patients over time to determine the clinical significance of these findings.

In the correlation analysis, neural abnormalities in the cuneus and lingual gyrus were found to be related to impaired cognitive performance on TMT-B and CFT-delayed tests in the diabetes group. The cuneus is the center of inhibitory control (32), whereas the lingual gyrus is responsible for visual memory (33). Decreased neuronal activity in the cuneus and lingual gyrus may therefore have contributed to patients’ poor performance on related cognitive tests such as the TMT-B and CFT-delayed tests. These region-specific neural cognition relationships support our hypothesis that neural activity abnormalities play an important role in diabetes-related cognitive dysfunction.

In this study, HOMA-IR was negatively correlated with neural activity in the frontal, temporal, and parietal regions in T2DM patients, but not in the control group. These results are compatible with those from a positron emission tomography study in which IR was associated with reduced brain metabolism in T2DM patients (34). Interestingly, a similar pattern of hypometabolism has been observed in patients with early Alzheimer’s disease (AD) (35). These results suggest that increased IR may be a risk for the development of AD, which is associated with reduced neural activity in diabetic subjects. Our results showed no correlation between blood glucose and neural activity changes, suggesting that blood glucose was not a main contributor to the neural activity difference, at least during the period of the current study.

A previous rs-fMRI study suggested that reduced neural activity in AD patients was predominantly located in the PCC, medial temporal lobe, and several other regions (26). In our study with T2DM patients,
significant hypoactivity was found mainly in the occipital lobe and PoCG. Although T2DM is a known risk factor for dementia (36), the relationship between T2DM and AD is still under debate. The Rotterdam study suggested that there is an increased risk for the development of AD in patients with T2DM (37). However, a population-based study failed to find such a risk in elderly patients with T2DM; this study did demonstrate a twofold higher risk of vascular dementia among diabetic patients (38). A recent systematic review of 14 longitudinal studies indicated that the risks for both AD and vascular dementia were increased in diabetic patients (39). Our study suggests that increased IR might be a potential risk factor for the development of AD; however, further studies are needed to test this relationship.

The current study has several limitations. First, the diabetic subjects were receiving various medications that may have effects on neural activities. Further studies should include medication-naive subjects to rule out this possible bias. Second, the small sample size in the current study may have reduced our ability to detect more neural activity changes. Third, there are no diagnostic criteria for diabetes-related cognitive dysfunction, and this lack of objective and specific neurocognitive assessment limited our interpretation of the results. Should such criteria become available, they would help to identify patients who are at risk for the development of dementia and
would allow researchers to explore their brain patterns. Finally, our study did not measure cerebrovascular reactivity because of technical limitations. Combining CO₂ stimulation with blood oxygenation level-dependent MR mapping has been proved to be a potential tool for detecting cerebrovascular reactivity changes (40) and may further assist our understanding of the cognitive changes that occur in diabetic patients.

In conclusion, our combined ALFF and ReHo analyses demonstrated a significant decrease in spontaneous brain activity in various brain regions prior to structural changes in patients with T2DM. These decreased neural activities were mainly located in the occipital lobe and PoCG, and were correlated with impaired cognitive functioning. This study provides a new approach to investigating brain function abnormalities in T2DM patients, and enhances our understanding of the
Figure 6—Differences in ALFF and ReHo values among the three groups (i.e., patients with retinopathy/neuropathy, patients without retinopathy/neuropathy, and control group). Low mean ALFF and ReHo values indicate decreased neural activity. A and B: The mean ALFF and ReHo values extracted from three brain regions of the occipital lobe among control subjects were all significantly higher than values in both of the diabetes groups. However, the two diabetes groups (with and without retinopathy) did not differ from each other. C and D: The mean ALFF and ReHo values extracted from the left PFC also did not differ between patients with and patients without peripheral neuropathy. *P < 0.05. A.U., arbitrary units.

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