Altered Odor-Induced Brain Activity as an Early Manifestation of Cognitive Decline in Patients With Type 2 Diabetes

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Type 2 diabetes is reported to be associated with olfactory dysfunction and cognitive decline. However, whether and how olfactory neural circuit abnormalities involve cognitive impairment in diabetes remains uncovered. This study thus aimed to investigate olfactory network alterations and the associations of odor-induced brain activity with cognitive and metabolic parameters in type 2 diabetes. Participants with normal cognition, including 51 patients with type 2 diabetes and 41 control subjects without diabetes, underwent detailed cognitive assessment, olfactory behavior tests, and odor-induced functional MRI measurements. Olfactory brain regions showing significantly different activation between the two groups were selected for functional connectivity analysis. Compared with the control subjects, patients with diabetes demonstrated significantly lower olfactory threshold score, decreased brain activation, and disrupted functional connectivity in the olfactory network. Positive associations of the disrupted functional connectivity with decreased neuropsychology test scores and reduced pancreatic function were observed in patients with diabetes. Notably, the association between pancreatic function and executive function was mediated by olfactory behavior and olfactory functional connectivity. Our results suggested the alteration of olfactory network is present before clinically measurable cognitive decrements in type 2 diabetes, bridging the gap between the central olfactory system and cognitive decline in diabetes.

Type 2 diabetes is associated with increased risk of cognitive impairment. Individuals with type 2 diabetes have a 1.5–2.5-fold increased risk of dementia compared with those without diabetes (1). With the increasing prevalence of diabetes and growing aging population, dementia attributed to type 2 diabetes represents a major health burden worldwide. Current treatments cannot reverse or delay the dementia progression once clinical symptoms occur (2). Therefore, it is an urgent challenge to identify biomarkers for early diagnosis and prognosis of the cognitive decline that leads to dementia.

Neuroimaging using MRI and functional MRI (fMRI) provides noninvasive options to assess brain structural and neural functional changes to obtain clues of vulnerable regions (3). By detecting blood oxygen level–dependent signals associated with neural activity, fMRI could capture functional abnormities in the brain (4), even before the clinically measurable cognitive impairment (5). Previous MRI studies demonstrated that patients with type 2 diabetes exhibited greater global brain atrophy and vascular lesions with reduced cerebral blood flow than those without diabetes (6). Decreased regional spontaneous neural activation (7) and disrupted functional connectivity (8) in brain regions involving cognitive processing were observed in type 2 diabetes. However, how type 2 diabetes affects olfactory cortex activation and its neural correlates is not well investigated.

Epidemiological investigations showed that olfactory behavior dysfunction, characterized by increased odor thresholds and impaired odor discrimination and recognition, is associated with the transition from normal cognition to mild cognitive impairment and subsequently dementia (9).

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Furthermore, reduced activation in the primary olfactory cortex was observed in patients with Alzheimer disease compared with cognitively normal control subjects and was significantly correlated with poor cognitive performance (10). Therefore, olfactory dysfunction is considered one of the earliest manifestations of neurodegenerative diseases (11) and a potential preclinical marker for future cognitive decline (12). Of note, olfactory-related regions are the brain regions with the highest level of insulin receptors (13), whereas neuronal activity of the olfactory system is modulated by insulin (14). Lower scores in olfactory behavior test were also observed in patients with type 2 diabetes (15,16). However, the neuroimaging changes in the olfactory-related regions of patients with type 2 diabetes and whether diabetes-related clinical variables take a role in such alterations before clinical symptoms of cognitive impairment remain uncovered.

To clarify whether olfactory neural circuit abnormalities involve cognitive impairment in type 2 diabetes, this study evaluated olfactory network alterations and determined the associations of odor-induced brain activation with cognitive function, olfactory behavior, and metabolic parameters in patients with type 2 diabetes, providing an important baseline for future follow-up studies during disease progression and treatments.

RESEARCH DESIGN AND METHODS

Participants

This study was consecutively conducted between February 2016 and August 2017 at the Endocrinology Department of Drum Tower Hospital Affiliated to Nanjing University Medical School. Seventy patients with type 2 diabetes and 60 control subjects without diabetes matched for age, educational level, and sex were enrolled. The flow chart is provided in Supplementary Fig. 1, and a total of 51 patients with type 2 diabetes and 41 control subjects without diabetes were included in the final data analysis. Inclusion criteria for all participants were age ranging from 40–75 years, right-handedness, and $\geq$6 years of education. Type 2 diabetes was defined based on the World Health Organization/International Diabetes Federation criteria (17). Exclusion criteria for all participants were *1*) mild cognitive impairment or probable dementia (Montreal Cognitive Assessment [MoCA] score $<$26); *2*) a history of thyroid dysfunction, cardiovascular or cerebrovascular disease, steroid treatment, or infections; *3*) neurological or psychiatric disorders and depression; *4*) inability to undergo cognitive test or MRI scanning; *5*) alcohol or substance abuse; *6*) nasal pathologies affecting olfactory function such as acute or chronic sinusitis, allergic rhinitis, nasal polyposis, and deviated nasal septum; and *7*) image artifacts and excessive head movement during fMRI scan: $>$2.5-mm shift or $>$2.5° rotation. Patients with type 2 diabetes were excluded if they had a history of frequent hypoglycemic episodes.

This study was approved by the Ethics Committee of the Drum Tower Hospital Affiliated to Nanjing University Medical School in accordance with the Declaration of Helsinki and registered at Clinicaltrials.gov (NCT02738671). All participants provided informed consent before enrollment.

Clinical Data Collection and Biochemical Measurements

Detailed clinical information and anthropometric features of all participants were collected using a standardized questionnaire including medical history, alcohol and smoking habits, family history of diabetes or dementia, and measurement of height, weight, waist and hip circumstance, and resting blood pressure. Control participants without diabetes received a standard 75-g oral glucose tolerance test, whereas patients with type 2 diabetes underwent a standard 100-g steamed-bread meal tolerance test. The streamed bread, a typical Chinese breakfast, was made with quantitative 100-g flour that consists of 75 g carbohydrates. After overnight fasting for 8 h, blood samples were collected at fasting and 2 h after the oral glucose tolerance test or meal tolerance test for the measurement of blood glucose, serum insulin, C-peptide, and HbA_{1c} levels. Fasting total cholesterol (TC), triglyceride (TG), and HDL and LDL cholesterol levels were recorded. Insulin resistance was estimated from fasting C-peptide using the HOMA2 Calculator (HOMA2 v2.2.3; Diabetes Trials Unit, University of Oxford, http://www.dtu.ox.ac.uk/homacalculator/).

Cognitive and Olfactory Behavior Assessment

A comprehensive neuropsychological assessment was performed in all participants on the day of blood sample collection. Global cognitive function was measured by the Mini-Mental State Examination (MMSE) and MoCA (Beijing version) (18). Meanwhile, word and logic memory were respectively measured using the 12-word Chinese version of the Philadelphia Verbal Learning Test and the Wechsler Memory Scale, both of which included immediate and 30-min delayed recall and recognition tests. Visual attention and task switching was assessed by the Trail Making Test A and B. Working memory was evaluated by the Digit Span Test (forward and backward). Word-retrieval performance was assessed by the Boston Naming Test. Word fluency was measured by the Animal Naming Test. Executive function was assessed by the Stroop Color and Word Test (parts I, II, and III). The Hamilton Depression Rating Scale, Hachinski Ischemic Score, and the Clinical Dementia Rating were also used to evaluate the psychological status of each participant. All tests were administered by a trained neuropsychologist and required ~60 min for completion in a fixed order. Results for different cognitive domains were converted to normalized *z* scores.

Subject groupings were blind to the examiner using Olfactory Function Assessment by Computerized Testing (Osmic Enterprises, Inc., Cincinnati, OH; www.osmicenterprises.com). The flow chart is provided in Supplementary Fig. 2. The olfactory threshold test (score range 1–13.5) was determined based on a series of binary dilutions of the *N*-butanol solution in light mineral oil. The higher the score was, the more sensitive the participant was in detecting an odor. Scores of 8–10 were considered normal olfactory

sensitivity, whereas scores of 1–3 signified olfactory dysfunction or anosmia, and scores of ≥10 indicate better olfactory sensitivity. Odor identification and memory tests respectively consisted of two tasks and assessed one's ability to identify and remember odors. In task A (score range 0–10), the participant was presented with 10 odors in sequence and asked to identify each one from four choices. Then, the test broke for 10 min before starting task B (score range 0–20), and the participant was presented with 20 odors, including the 10 old odors from part A and 10 new odors. The participant was asked to identify each odor from four choices (semantic memory) and also indicate whether it was old or new (episodic memory).

Odor-Induced fMRI Paradigm

The odor-induced task fMRI and the olfactory behavior tests were conducted on 2 consecutive days to reduce the interactions of these two measurements in olfactory-related brain regions. The entire paradigm (Fig. 1) consisted of 12 trials, and each trial included 30 s of odorless fresh air and 6 s of lavender odor stimulation. Four gradually increased concentrations of lavender odor, including weakest (0.032%), weak (0.10%), medium (0.32%), and strong (1.0%) concentrations, were provided to counteract brain habituation. Each concentration was repeated three times. The visual cues with a symbol "+" and the word "smell" were used during baseline and odor stimulation. Gray words on black background were set to minimize the effects of visual stimulation. Throughout the scan, the participants were required to maintain normal breathing and press a button using the right-hand thumb once the lavender scent was smelt. All respiratory amplitude and keystrokes were monitored and recorded.

MRI Acquisition

MRI data were acquired on a 3T clinical MR-scanner (Philips Medical Systems, Eindhoven, the Netherlands) using an eight-channel phased array coil. Participants were instructed to remain calm and awake. Structural images were acquired with high-resolution T1-weighted three-dimensional fast field echo structural scan (repetition time 9.7 ms; echo time 4.6 ms; field of view 256 mm × 256 mm × 192 mm; flip angle 8°; and voxel size 1 mm × 1 mm × 1 mm). Resting-state and odor-induced task fMRI were acquired with a gradient-echo planar imaging sequence scan (repetition time 2,000 ms; echo time 30 ms; field of view 192 mm × 192 mm × 140 mm; slice thickness 4 mm; gap: 0 mm; flip angle 90°; and voxel size 3 mm × 3 mm × 4 mm), 230 volumes for resting-state fMRI and 222 volumes for task fMRI.

Data Analysis

Image Preprocessing

Image preprocessing was blind to participant grouping. The preprocessing of task fMRI data was performed using Statistical Parametric Mapping 8 (19), with the following stages: *1*) the first six time points of each scan were excluded from the analysis to remove the initial transit signal fluctuations. *2*) The functional images were corrected for head movement. Six realignment parameters of head motion were also regressed out to control motion effects. *3*) The T1-weighted high-resolution anatomical images were corecorded to the mean functional image, segmented by using a unified

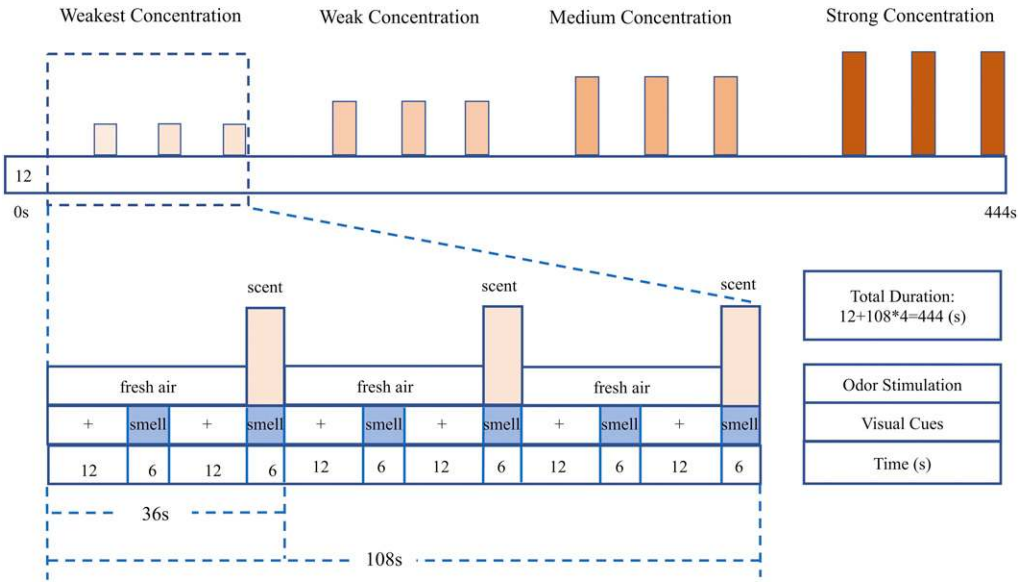

Figure 1—Odor-induced fMRI stimulation paradigm. Each participant underwent a series of scans for 444 s to measure the temporal brain response to a given odor. Four gradually increased concentrations of lavender odor, including weakest (0.032%), weak (0.10%), medium (0.32%), and strong (1.0%) concentrations, were provided to counteract the habituation effect. The visual cues of symbol "+" and the word "smell" were used for baseline and odor stimulation, respectively. The odor of each concentration was assessed three times, with fresh air and scent occurring alternately. Participants were instructed to press a button with the right-hand thumb once the lavender scent was smelt. All respiratory amplitudes and keystrokes were monitored and recorded.

segmentation algorithm, and spatially normalized to the Montreal Neurological Institute space template, in a spatial resolution of 1 × 1 × 1 mm. The time-course images were spatially normalized using the same normalization parameters with a spatial resolution of 3 × 3 × 3 mm. *4*) Spatial smoothing was conducted with a Gaussian kernel of 8-mm full-width at half-maximum. *5*) Low-frequency (0.01–0.08 Hz) fluctuations of the task fMRI signals were assessed to reflect spontaneous neuronal activity.

The preprocessing of resting-state fMRI was performed using Data Processing & Analysis for (Resting-State) Brain Imaging (DPABI_V2.3_170105) (20), with the following stages: *1*) the first 10 time points were automatically deleted to make the signal steady state; *2*) slice-timing correction and motion correction were performed; *3*) resting-state fMRI was registered to the Montreal Neurological Institute space template via each participant's T1-weighted high-resolution anatomical images and spatially normalized using the same normalization parameters with a spatial resolution of 3 × 3 × 3 mm; *4*) spatial smoothing was conducted with full-width at half-maximum; *5*) linear detrending and temporal band-pass filtering (0.01–0.08 Hz) were performed to eliminate high-frequency noise and low-frequency drift; and *6*) simple regression with the residual head motions, white matter signal, and cerebrospinal fluid signal were used as the covariant for temporal nuisance correction.

Brain Activation Analysis

A general linear model was used to estimate brain activation during odor-stimulus tasks. Three conditions, including "fresh air," "scent," and "rest," were extracted separately from the whole sequence. Contrasts between "fresh air > rest" and "scent > rest" for each participant were made for further analysis. According to previous studies (21), several olfactory-related regions were selected, including the bilateral parahippocampus, amygdala, piriform cortex, insula, orbitofrontal cortex, and hippocampus in the Automated Anatomical Labeling templates and Brodmann areas 28 and 34 (entorhinal cortices). These regions were extracted and merged as our olfactory regions of interest (ROIs; total cluster size: 5,029 voxels). Within-group activation and between-group differences were estimated within these olfactory ROIs.

Functional Connectivity Analysis

Functional connectivity is defined as the correlation coefficients between two different brain regions/voxels. In the current study, brain regions showing significantly different activations between subjects with diabetes and control subjects were selected as seed regions for functional connectivity analyses. For each seed region, the functional connectivity between the seed and each voxel within the olfactory ROIs was assessed voxel by voxel, therefore generating a functional connectivity map.

Statistical Analysis

Demographic information, clinical variables, and cognitive and olfactory behavior assessment scores were reported as mean ± SD and compared between the two groups. Independent-sample *t* test was used for continuous variables, and Pearson χ^2 test was used for categorical variables. A P value <0.05 was considered statistically significant. These analyses were performed with SPSS software (version 20.0; SPSS, Chicago, IL).

To determine the brain functional differences of the two groups, a voxel-based independent-sample *t* test was used with age, sex, education, BMI, and vascular risk factors (including systolic blood pressure [SBP], diastolic blood pressure [DBP], TGs, TC, and LDL cholesterol) as covariates in odor-induced brain activation and every seed region of the resting-state functional connectivity analysis using the DPABI software. Multiple-comparison correction was performed using a threshold ($P < 0.01$) of individual voxel and a cluster size based on the Monte Carlo simulations (22), corresponding to cluster-level $P < 0.05$ by AlphaSim correction. The DPABI software was used for the AlphaSim correction.

To assess whether diabetic parameters were associated with cognition, olfactory behavior, odor-induced brain activation, or functional connectivity, the mean activation (β value) and mean functional connectivity value of the region showing significant differences between the two groups were extracted. Further partial correlation analysis with age, sex, and education controlled was conducted to analyze the correlation of the brain activation and functional connectivity with *z* scores of different cognitive domains (including memory, working memory, word fluency, processing speed, and executive function) and total scores of olfactory behavior tests, whereas partial rank correlation with age, sex, and education controlled was used to analyze those with MMSE, MoCA, olfactory threshold, olfactory identification, and olfactory memory in the group with diabetes and control group separately.

To examine the interrelationship among olfactory system, cognitive function, and diabetic parameters, linear regression models were generated for mediation analysis using bootstrapped mediation procedures included in the PROCESS SPSS macro (23,24). All analyses were estimated 5,000 bias-corrected bootstrap 95% CIs and statistically significant with $P < 0.05$.

RESULTS

Demographics, Clinical Variables, Cognition Status, and Olfactory Behavior

The group with type 2 diabetes and control group without diabetes were matched for age, sex, education, smoking, and alcohol consumption (Table 1). Patients with type 2 diabetes had higher fasting and 2-h plasma glucose, HbA_{1c}, BMI, HOMA2 of insulin resistance, and SBP level and lower HDL level compared with control subjects. There were no differences in DBP and TC, TG, and LDL levels.

No significant differences were observed between the two groups in general cognition status, memory, working memory, visual attention and task switching, word fluency and retrieval performance, and executive function (Table 1), indicating that no measurable impairments presented in all parts of cognition domains in the cohort with diabetes. Although no significant differences were observed in the

Table 1—Demographic and clinical variables, cognitive assessment scores, and olfactory behavior test scores

Index	Control subjects without diabetes (n = 41)	Patients with type 2 diabetes (n = 51)	P value
Demographic factors			
Age (years)	50.6 ± 9.0	51.3 ± 9.8	0.723
Sex (male) [n (%)]#	20 (48.8)	32 (62.7)	0.179
Education (years)	14.1 ± 3.8	14.0 ± 3.1	0.968
Alcohol consumption [n (%)]#	9 (22)	14 (27.5)	0.545
Smoking habit [n (%)]#	10 (24.4)	17 (33.3)	0.349
Diabetes-related characteristics			
Duration of diabetes (years)	—	10.7 ± 6.6	—
HbA_{1c} (%)	5.5 ± 0.3	8.2 ± 1.8	<0.001*
HbA_{1c} (mmol/mol)	37 ± 3.3	66 ± 19.7	—
Fasting glucose (mmol/L)	4.9 ± 0.4	7.9 ± 2.2	<0.001*
2-h postprandial glucose (mmol/L)	5.8 ± 0.9	14.3 ± 4.5	<0.001*
Fasting insulin (mIU/mL)	7.2 ± 3.0	8.6 ± 4.8	0.081
2-h postprandial insulin (mIU/mL)	43.3 ± 22.7	31.7 ± 20.8	0.014*
Fasting C-peptide (pmol/L)	630.0 ± 141.6	641.6 ± 299.9	0.810
2-h postprandial C-peptide (pmol/L)	2,374.9 ± 677.3	1,564.2 ± 687.9	<0.001*
HOMA2-IR	1.2 ± 0.4	1.6 ± 0.8	0.004*
Clinical variables			
BMI (kg/m^2)	23.3 ± 2.8	25.9 ± 3.5	<0.001*
SBP (mmHg)	122.8 ± 16.3	131.4 ± 13.9	0.007*
DBP (mmHg)	79.3 ± 13.1	80.8 ± 11.6	0.576
TG (mmol/L)	1.5 ± 0.7	1.6 ± ± 0.9	0.314
TC (mmol/L)	5.0 ± 0.9	4.6 ± 1.1	0.102
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.1 ± 0.3	<0.001*
LDL cholesterol (mmol/L)	2.9 ± 0.9	2.8 ± 1.0	0.418
Cognitive assessment			
General cognition status			
MMSE	29.2 ± 0.9	29.0 ± 1.0	0.502
MoCA	28.2 ± 1.3	27.8 ± 1.2	0.187
Memory†	0.22 ± 0.14	−0.18 ± 0.15	0.062
AVLT-immediate recall	61.0 ± 8.7	58.0 ± 9.8	0.123
AVLT-delayed recall (30 min)	17.7 ± 3.5	16.8 ± 3.9	0.276
AVLT-recognition	57.6 ± 2.1	56.8 ± 6.9	0.480
WASI-immediate recall	20.9 ± 6.2	18.9 ± 5.6	0.113
WASI-delayed recall (30 min)	17.0 ± 6.6	15.0 ± 5.7	0.125
WASI-recognition	9.9 ± 1.6	9.3 ± 2.0	0.141
Working memory†	0.12 ± 0.15	−0.09 ± 0.15	0.324
DST-forward	8.7 ± 0.6	8.5 ± 0.8	0.151
DST-backward	6.2 ± 1.6	6.0 ± 1.6	0.527
Visual attention and task switching (processing speed)†	−0.06 ± 0.15	0.05 ± 0.14	0.600
TMT-A	10.1 ± 4.3	11.2 ± 6.3	0.335
TMT-B	77.4 ± 31.6	80.2 ± 31.2	0.676
Word fluency and retrieval performance†	0.23 ± 0.15	−0.18 ± 0.14	0.050
ANT	21.3 ± 4.6	20.9 ± 4.4	0.688
BNT	57.6 ± 3.2	56.9 ± 3.1	0.320
Executive functions†	0.10 ± 0.14	0.08 ± 0.15	0.388
SCWT-color	16.6 ± 5.3	17.8 ± 7.1	0.381
SCWT-word	18.5 ± 6.8	20.4 ± 8.7	0.244
SCWT-color word	28.4 ± 9.0	29.3 ± 12.4	0.705
Olfactory behavior test			
Total scores†	0.17 ± 0.12	−0.13 ± 0.16	0.155
Olfactory threshold	10.7 ± 2.5	9.1 ± 3.3	0.010*
Odor identification test†	0.07 ± 0.11	−0.05 ± 0.17	0.548
Task A (10 odors)	8.3 ± 1.2	8.0 ± 1.8	0.356
Task B (20 odors)	15.4 ± 1.9	15.3 ± 2.7	0.816
Odor memory test†	0.02 ± 0.14	−0.01 ± 0.15	0.874
Old 10 odors	8.0 ± 1.6	7.7 ± 1.8	0.457
New 10 odors	7.7 ± 1.5	7.9 ± 1.7	0.597

Data are mean ± SD unless otherwise stated. Independent-sample t test. ANT, Animal Naming Test; AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; DST, Digit Span Task; HOMA2-IR, HOMA2 of insulin resistance; SCWT, Stroop Color and Word Test; TMT, Trail Making Test; WASI, Wechsler Abbreviated Scale of Intelligence. #Pearson χ^2 test. *$P < 0.05$ was considered significant. †Mean standardized z scores ± SE.

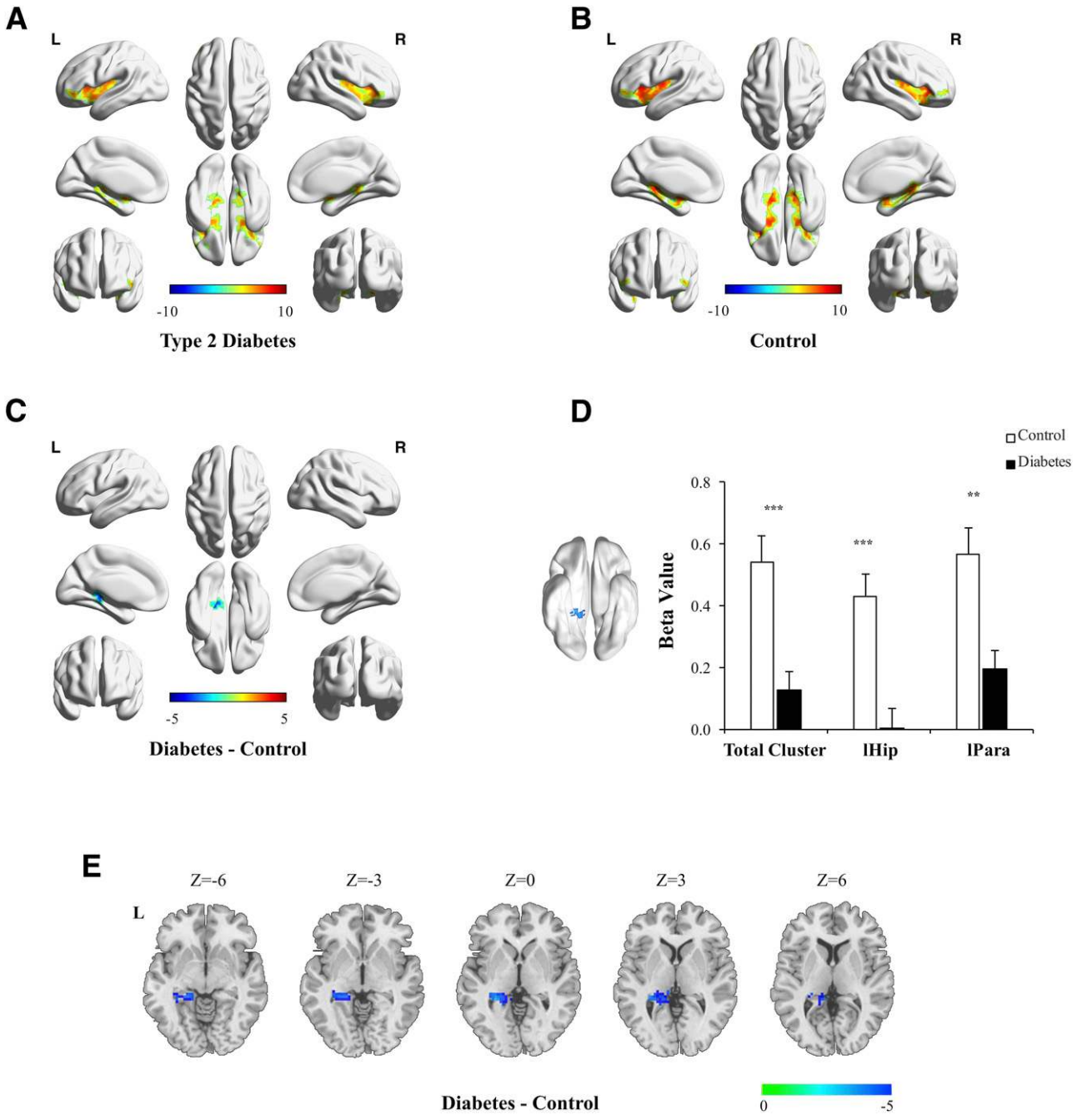

Figure 2—Odor-induced brain responses in patients with type 2 diabetes (*A*) and normal control subjects (*B*) (with AlphaSim correction, voxel level: $P < 0.001$, cluster level: $P < 0.05$, cluster size threshold: 23 voxels). Independent-sample *t* test corrected for age, education, sex, BMI, and vascular risk factors indicated significantly decreased brain activations in patients with type 2 diabetes compared with the control group (with AlphaSim correction, voxel level: $P < 0.01$, cluster level: $P < 0.05$, cluster size threshold: 71 voxels) (*C*). Specifically, the average activation (β values) is decreased in the left hippocampus (lHip) and left parahippocampus (lPara) in diabetes (*D* and *E*). Independent sample *t* test. $^{**}P < 0.01$; $^{***}P < 0.001$. L, left; R, right.

odor identification and olfactory memory test between the two groups, patients with type 2 diabetes had lower olfactory threshold scores (Table 1), indicating that these patients had a weaker ability to detect odors. Meanwhile, no significant differences in both cognitive function and olfactory behavior were observed among patients with different diabetic therapies (Supplementary Table 1).

Odor-Induced Task fMRI

The brain olfactory-related regions of all participants showed bilateral activations in response to odor stimulation, including the primary olfactory cortex (parahippocampus, piriform cortex, amygdala, and entorhinal cortex), insula, orbitofrontal cortex, and hippocampus ($P < 0.01$, with AlphaSim correction) (Fig. 2*A* and *B*). Between-group analysis corrected

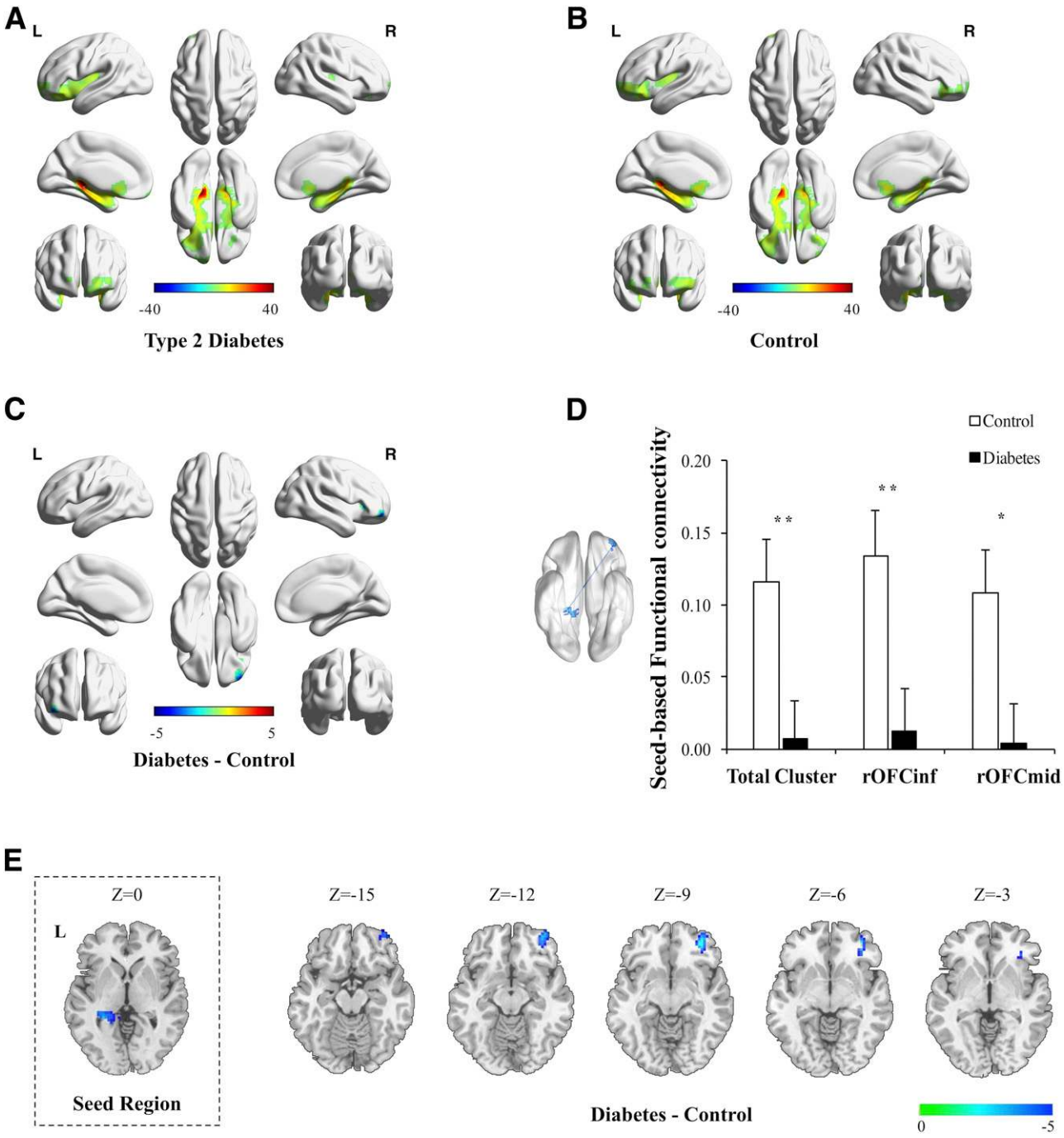

Figure 3—The seed-based functional connectivity in patients with type 2 diabetes (*A*) and normal control subjects (*B*) (with AlphaSim correction, voxel level: $P < 0.001$, cluster level: $P < 0.05$, cluster size threshold: 22 voxels). Independent-sample *t* test corrected for age, education, sex, BMI, and vascular risk factors indicated significantly decreased brain functional connectivity in the subjects with diabetes compared with the control subjects (with AlphaSim correction, voxel level: $P < 0.01$, cluster level: $P < 0.05$, cluster size threshold: 75 voxels) (*C*). Specifically, decreased seed-based functional connectivity with right inferior and middle orbitofrontal cortex was observed in diabetes (*D* and *E*). Independent-sample *t* test. $^{*}P < 0.05$; $^{**}P < 0.01$. L, left; R, right; rOFCinf, right inferior orbitofrontal cortex; rOFCmid, right middle orbitofrontal cortex.

for age, education, sex, BMI, and vascular risk factors revealed the decreased activation in the left hippocampus and the left parahippocampus in patients with type 2 diabetes compared with the control group ($P < 0.05$, with AlphaSim correction) (Fig. 2*C–E* and Supplementary Table 2), whereas no difference was observed when HbA_{1c} was included as a covariate (largest cluster size: 9 voxels; AlphaSim threshold: 48 voxels).

Seed-Based Functional Connectivity Analysis

The brain region showing significantly different activation between subjects with diabetes and control subjects was

selected as the seed region (Fig. 2*C–E*). The general linear model analysis showed significantly decreased seed-based functional connectivity (Fig. 3*C–E* and Supplementary Table 3) with right middle and inferior orbitofrontal cortex in patients with type 2 diabetes compared with the control group after correction of age, sex, education, BMI, and vascular risk factors ($P < 0.05$, with AlphaSim correction).

Associations of Diabetic Parameters With Cognitive Function and Olfactory Behavior

There were significantly positive associations of olfactory behavior test scores with cognitive assessment in the group with diabetes (Table 2), but few in the control group (Supplementary Table 4). No significant correlation between glucose level and cognitive function was observed in patients with type 2 diabetes after correction for age, sex, and education (Table 3). Nevertheless, the fasting glucose level had negative association with the olfactory identification test score ($r = -0.292$; $P = 0.046$) (Table 3). Higher fasting and postprandial C-peptide levels were correlated with reduced time consumption in the processing speed and executive function tests, elevated scores in the olfactory identification and memory tests, and higher total scores in the olfactory behavior tests ($P < 0.05$) (Table 3).

Associations of Olfactory Brain Activation and Functional Connectivity With Neuropsychological Test Scores and Diabetic Parameters

Within the patients with type 2 diabetes, specifically significant positive associations of odor-induced brain activation and functional connectivity were observed with cognitive function and diabetic parameters (Fig. 4). The activation of the left parahippocampus was positively associated with 2-h postprandial C-peptide ($r = 0.288$; $P = 0.047$) (Fig. 4*A*). Seed-based functional connectivity had positive correlation with 2-h postprandial C-peptide ($r = 0.416$; $P = 0.003$), 2-h postprandial insulin ($r = 0.299$; $P = 0.039$), *z* score of memory ($r = 0.323$; $P = 0.025$), and total scores on olfactory behavior tests ($r = 0.371$; $P = 0.010$). Significantly negative associations were observed in the functional connectivity with the time consumption in the processing speed and executive function test, respectively ($r = -0.322$, $P = 0.026$; $r = -0.446$, $P = 0.001$) (Fig. 4*B–G*).

Mediation Models for the Association Among Cognition, Olfactory System, and Diabetic Parameters

Mediation analysis was performed to determine whether the olfactory system acted as a mediating factor between the cognitive function and diabetic parameters. Figure 5 demonstrates that total scores of olfactory behavior tests and seed-based functional connectivity mediated the relationship of pancreatic function and executive function corrected with age, sex, and education ($\beta = -0.1376$, 95% bootstrap CI [−0.3068, −0.0370]; $\beta = -0.1396$, CI [−0.3403, −0.0277], respectively).

DISCUSSION

This study evaluated olfactory behavior and odor-induced brain alteration in patients with type 2 diabetes, providing new experimental data on the olfactory circuit alterations in diabetes. Reduced olfactory threshold scores, brain activation, and functional connectivity of the olfactory circuit were found in patients with type 2 diabetes with apparent normal cognition. Moreover, patients with better pancreatic β-cell function had significantly higher cognitive assessment and olfactory behavior test scores and increased brain activation and functional connectivity. Importantly, the olfactory functional connectivity and olfactory behavior served as mediator factors between pancreatic function and executive function in diabetes.

Cross-sectional studies using olfactory measurements or tools such as the University of Pennsylvania Smell Identification test, Open Essence test, and "Sniffin" Sticks have revealed that patients with diabetes have lower odor threshold, discrimination, and identification scores (16,25–28). Similarly, in this study, patients with type 2 diabetes had significantly reduced ability to detect odors compared with the control, though they were all within normal range of olfactory threshold and general cognitive status (Table 1). Of note, the olfactory test battery in this study was conducted by a computerized instrument that can regulate the duration

Table 2—Associations of olfactory behavior tests with cognitive assessment in patients with type 2 diabetes

	Olfactory behavior test scores							
	Olfactory threshold		Olfactory identification		Olfactory memory		Total scores	
Cognitive assessment	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
MMSE	0.008	0.955	0.132	0.37	0.155	0.292	0.152	0.301
MoCA	0.401**	0.005#	0.372**	0.009	0.307*	0.034	0.452**	0.001#
Memory	0.192	0.191	0.220	0.133	0.217	0.139	0.274	0.059
Working memory	0.219	0.134	0.072	0.629	0.187	0.204	0.223	0.127
Word fluency	0.382**	0.007#	0.372**	0.009	0.409**	0.004#	0.513***	<0.001#
Processing speed	−0.282	0.052	−0.206	0.160	−0.290*	0.046	−0.310*	0.032
Executive function	−0.360*	0.012	−0.371**	0.009	−0.438**	0.002#	−0.487***	<0.001#

Partial rank correlation with age, sex, and education controlled. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. #Statistically significant after Bonferroni correction ($P < 0.0071$).

Table 3—Correlation of diabetic parameters with cognitive assessment and olfactory behavior tests in patients with type 2 diabetes

Diabetic parameters	Cognitive assessment						Olfactory behavior tests			
	MMSE	MoCA	Memory	Working memory	Word fluency	Processing speed (time)	Executive function (time)	Olfactory threshold	Olfactory identification	Olfactory memory	Total scores
HbA_{1c}	0.075	−0.098	−0.077	−0.185	−0.283	0.140	0.095	−0.119	−0.203	−0.162	−0.333*
Fasting glucose	0.196	0.111	−0.015	0.253	−0.139	−0.241	−0.052	0.038	−0.292*	−0.127	−0.296*
2-h glucose	0.182	−0.055	0.131	−0.038	−0.029	−0.251	0.087	−0.204	−0.070	−0.077	−0.204
Fasting insulin	−0.024	0.080	0.065	0.011	0.077	−0.205	−0.220	0.030	0.073	0.145	0.041
2-h insulin	0.169	0.154	0.065	0.055	0.035	−0.160	−0.320*	0.012	0.101	0.203	0.059
Fasting C-peptide	0.067	0.209	0.076	0.205	0.217	−0.350*	−0.317*	0.143	0.317*	0.278	0.295*
2-h C-peptide	0.229	0.202	0.227	0.197	0.271	−0.272	−0.434**	0.019	0.402**	0.358*	0.379**
HOMA2-IR	0.041	0.160	0.044	0.200	0.146	−0.341*	−0.336*	0.078	0.023	0.140	0.089

Partial correlation with age, sex, and education controlled was used to analyze the correlation of diabetic parameters with z-scores of cognitive domains including memory, working memory, word fluency, processing speed, and executive function, as well as total scores of olfactory behavior tests, whereas partial rank correlation with age, sex, and education controlled was used to analyze the correlation with scores of MMSE, MoCA, olfactory threshold, olfactory identification, and olfactory memory tests. HOMA2-IR, HOMA2 of insulin resistance. $*P < 0.05$; $**P < 0.01$.

and concentration of odor steadily and thus provide reliable and valid data. Additionally, this study revealed a good consistency of cognitive performance and olfactory behavior specifically in the group with diabetes rather than the control subjects (Table 2 and Supplementary Table 4).

Participants in both groups were all at the normal cognitive range of MMSE and MoCA. They did not exhibit any differences in all parts of cognitive domains either (Table 1). Further brain structural analysis showed no statistically significant differences in gray matter volume nor white matter volume corrected by total intracranial volume between the group with diabetes and the control group (Supplementary Table 5). Importantly, compared with the control group, patients with type 2 diabetes had significantly decreased activation in the hippocampus and parahippocampus of the dominant hemisphere in response to odor stimulation with age, sex, education, and vascular risk factors corrected (Fig. 2 and Supplementary Table 2), whereas such differences disappeared when $HbA_{1c,}$ a major feature differentiating the subjects with diabetes from the control subjects, was included as a covariate. These findings indicated that olfactory function deficits and olfactory circuit alterations may be earlier than brain structural changes or more sensitive than clinical neuropsychological examinations, and such alterations may mainly relate to diabetes. Indeed, olfactory function impairment is considered as a key predictor of several neurodegenerative disorders (29). Patients with Alzheimer disease present neuronal pathology in the olfactory-related regions and the hippocampus (a brain region that is critical for memory) (30). This current study thus innovatively raises the clinical significance that altered odor-induced brain activity could serve as an earlier brain functional feature for cognitive decline in type 2 diabetes.

Previous studies assessing brain network alterations in patients with type 2 diabetes focused on the default mode network, the highly functional connected regions including the posterior cingulate cortex, precuneus, the medial prefrontal cortex, and the lateral parietal area. Patients with type 2 diabetes showed aberrant functional connectivity in the default mode network, which was related to poor cognitive performance (31) and evaluated insulin resistance (32). This study examined the functional connectivity in the olfactory-related regions that have been shown to be an initial region affected in dementing disease, such as Alzheimer and Parkinson disease (30). The declining seed-based functional connectivity was revealed for the first time in patients with type 2 diabetes (Fig. 3 and Supplementary Table 3). Moreover, this decreased connectivity was closely associated with low cognitive performance and olfactory behavior test scores specifically in the group with diabetes but not the control subjects (Fig. 4*B–G*). Importantly, decline in functional connectivity occurs between regions across the two hemispheres. The connectivity between brain regions that are anatomically further apart is more susceptible to the early stage of functional decline because the regions would require synchrony of neuronal activity across multiple synapses. It has been shown that prominent interhemispheric

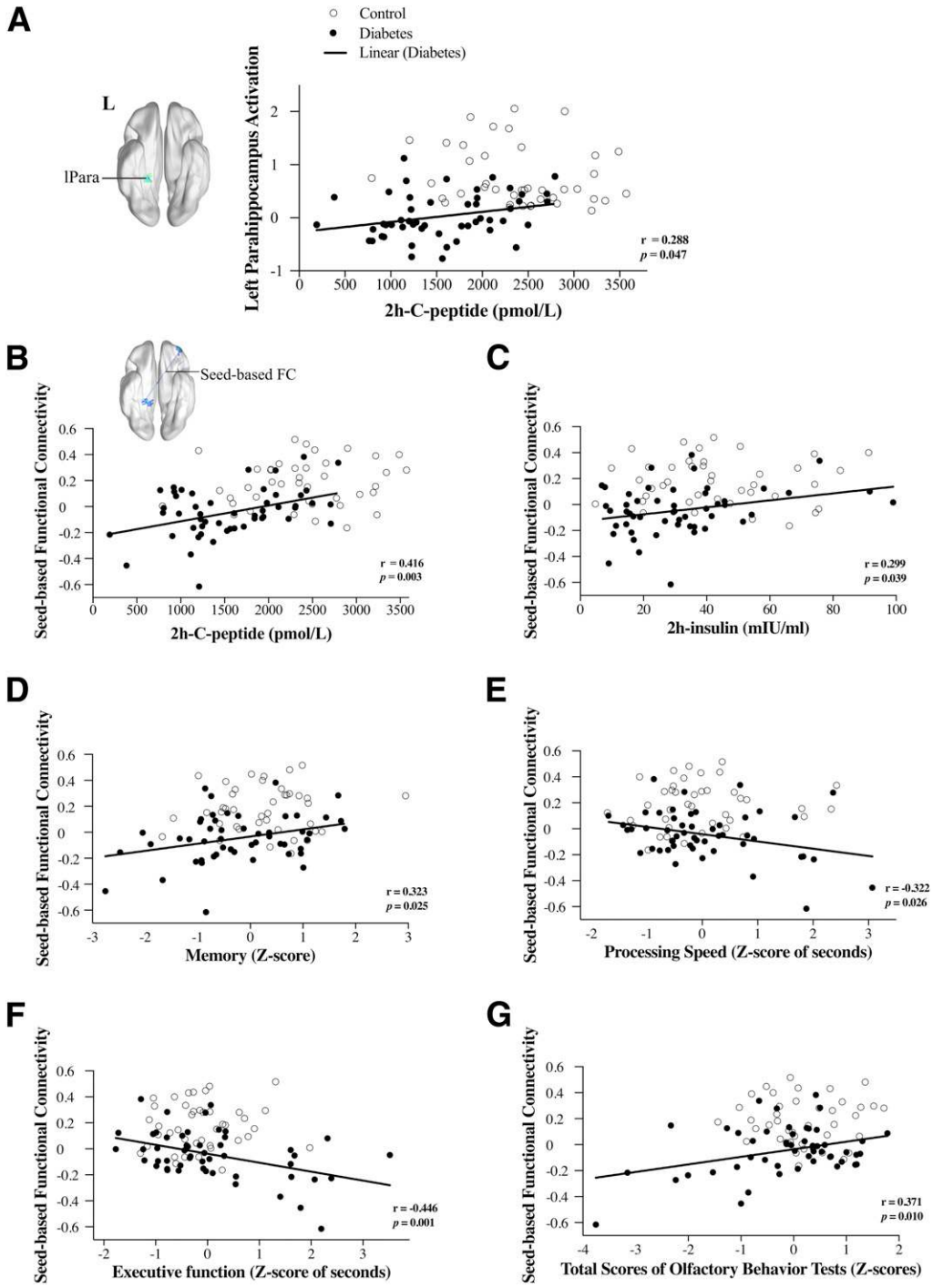

Figure 4—Associations of olfactory brain activation and functional connectivity with neuropsychological test scores and diabetic parameters. *A*: Positive association between the activation of the left parahippocampus (lPara) and 2-h postprandial C-peptide was observed in the group with diabetes (black circles) but not in the control group (white circles). Meanwhile, the seed-based functional connectivity was significantly associated with 2-h postprandial C-peptide (*B*), 2-h postprandial insulin (*C*), *z* scores of cognitive domains including memory (*D*), processing speed (*E*), and executive function speed (*F*), as well as total scores of olfactory behavior tests (*G*). Partial *r* and *P* values were obtained after adjustment for age, sex, and education. $P < 0.05$ was considered significant. FC, functional connectivity; L, left.

connectivity loss is found in patients with early Alzheimer disease (33). Therefore, follow-up investigations are necessary to validate these findings, which may produce potential early functional neuroimaging markers for cognitive decline in type 2 diabetes.

This study found that pancreatic β-cell function was significantly correlated with cognition, olfactory behavior, olfactory brain activation, and functional connectivity in the brain of patients with type 2 diabetes (Table 3 and Fig. 4). Noteworthy, olfactory behavior and olfactory functional

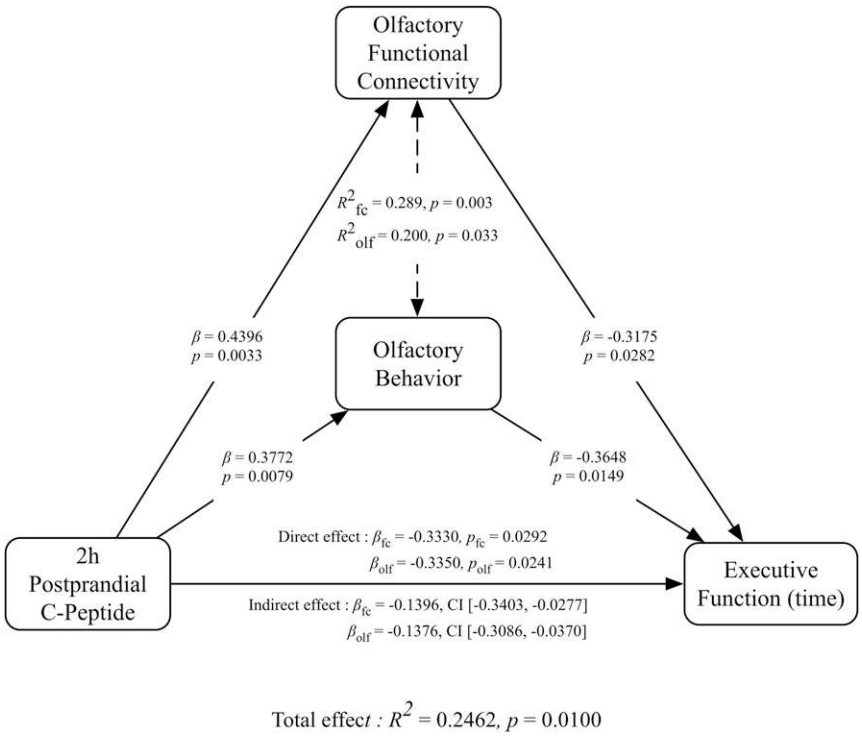

Figure 5— Mediation analysis for the associations among cognition, olfactory system, and diabetic parameters. Standardized β coefficient was derived from the mediation models controlling for age, sex, and education. CI, bootstrap CI; fc, olfactory functional connectivity; olf, olfactory behavior.

connectivity served as mediating factors between pancreatic function and the executive function (Fig. 5), which may emphasize the relationships among insulin pathway, olfaction, and cognition. The brain is considered as an insulin-sensitive organ (34). Insulin receptors are found abundantly expressed throughout the brain, and interestingly, with the highest densities in the olfactory-related regions and hippocampus (35,36). Brain insulin binds to its receptor and modulates glucose and energy metabolism (37). Meanwhile, it is associated with synaptic function and neurotransmitter activity and has neuroprotective effects (38). Therefore, insulin in the brain can directly or indirectly modulate both cognition and olfactory sensory function (39,40). Treatments that enhance central insulin signaling, such as intranasal insulin administration, are protective for neurons and cognition in Alzheimer disease as well as patients with type 2 diabetes (41–43). Future longitudinal follow-up studies are required to assess whether pancreatic β-cell protection delays cognitive decline in patients with type 2 diabetes.

Additionally, this cross-sectional study found few significantly negative associations of HbA_{1c} and glucose level with cognitive function or olfactory behavior. Indeed, the association of average HbA_{1c} with cognitive function in type 2 diabetes was reported to be negative and weak (44). Nevertheless, dysregulation of glucose variability and glucose peaks were shown to be associated with increased risk of dementia in longitudinal studies, and a mutual interaction between hypoglycemia and cognitive impairment was observed in older patients with type 2 diabetes (45–47). Studies yielded inconsistent and insufficient data for long-term effects of glycemic control on cognition in diabetes, and further investigations are warranted.

The results of this study provide a baseline for subsequent longitudinal studies. However, there are several limitations for this study. First, because females generally perform better than males in olfactory behavior tests (48), the difference in the percentage of males between the group with diabetes and control group (62.7 vs. 48.8%), though not significant (P = 0.179), might cause a potential bias influencing the olfactory behavior test results. Therefore, sex was rigorously corrected in data analysis and correlation analysis. Additionally, this study is a cross-sectional study that cannot inform the causality between odor-induced brain activation and pancreatic islet function in patients with diabetes. This study did not include patients with diabetes with cognitive impairment nor provide the cutoff points for odor identification and memory tests. Therefore, larger sex-matched cohorts and follow-up studies are needed to observe the cognitive decline progression and to determine the proper cut points for Chinese subjects and whether these functional changes are specific alterations in the diabetes cohort and would lead to dementia. Finally, the olfactory bulbs were not scanned in this study, as neural activations of the olfactory bulb are difficult to detect in the human brain, and the anatomic relationship between olfactory bulb volume and psychophysical assessment remains unclear (49).

In conclusion, this is the first study to demonstrate significant alterations of odor-induced brain activations

occur before both brain structural changes and clinically measurable cognitive decline in patients with type 2 diabetes with normal cognitive status. Such functional alterations in the olfactory circuit could probably constitute a potential direction for the research on cognitive decline in type 2 diabetes. Remarkably, positive associations of β-cell function with cognition, olfactory brain activation, and functional connectivity were observed. Further randomized controlled trials are required to determine whether pancreatic β-cell function improvement could be beneficial for preserving cognition in diabetes.

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References

1. Cheng G, Huang C, Deng H, Wang H. Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. Intern Med J 2012;42:484–491

2. Livingston G, Sommerlad A, Orgeta V, et al. Dementia prevention, intervention, and care. Lancet 2017;390:2673–2734

3. Brundel M, Kappelle LJ, Biessels GJ. Brain imaging in type 2 diabetes. Eur Neuropsychopharmacol 2014;24:1967–1981

4. Macpherson H, Formica M, Harris E, Daly RM. Brain functional alterations in type 2 diabetes - a systematic review of fMRI studies. Front Neuroendocrinol 2017;47:34–46

5. van Bussel FCG, Backes WH, van Veenendaal TM, et al. Functional brain networks are altered in type 2 diabetes and prediabetes: signs for compensation of cognitive decrements? The Maastricht Study. Diabetes 2016;65:2404–2413

6. Biessels GJ, Reijmer YD. Brain changes underlying cognitive dysfunction in diabetes: what can we learn from MRI? Diabetes 2014;63:2244–2252

7. Cui Y, Jiao Y, Chen YC, et al. Altered spontaneous brain activity in type 2 diabetes: a resting-state functional MRI study. Diabetes 2014;63:749–760

8. Zhang Y, Lu S, Liu C, et al. Altered brain activation and functional connectivity in working memory related networks in patients with type 2 diabetes: an ICA-based analysis. Sci Rep 2016;6:23767

9. Roberts RO, Christianson TJH, Kremers WK, et al. Association between olfactory dysfunction and amnestic mild cognitive impairment and Alzheimer disease dementia. JAMA Neurol 2016;73:93–101

10. Wang J, Eslinger PJ, Doty RL, et al. Olfactory deficit detected by fMRI in early Alzheimer's disease. Brain Res 2010;1357:184–194

11. Wilson RS, Arnold SE, Schneider JA, Tang Y, Bennett DA. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. J Neurol Neurosurg Psychiatry 2007;78:30–35

12. Lafaille-Magnan ME, Poirier J, Etienne P, et al.; PREVENT-AD Research Group. Odor identification as a biomarker of preclinical AD in older adults at risk. Neurology 2017;89:327–335

13. Havrankova J, Brownstein M, Roth J. Insulin and insulin receptors in rodent brain. Diabetologia 1981;20:268–273

14. Kuczewski N, Fourcaud-Trocmé N, Savigner A, et al. Insulin modulates network activity in olfactory bulb slices: impact on odour processing. J Physiol 2014;592:2751–2769

15. Sanke H, Mita T, Yoshii H, et al. Relationship between olfactory dysfunction and cognitive impairment in elderly patients with type 2 diabetes mellitus. Diabetes Res Clin Pract 2014;106:465–473

16. Gouveri E, Katotomichelakis M, Gouveris H, Danielides V, Maltezos E, Papanas N. Olfactory dysfunction in type 2 diabetes mellitus: an additional manifestation of microvascular disease? Angiology 2014;65:869–876

17. World Health Organization. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia*. Geneva, Switzerland, World Health Organization, 2006

18. Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53:695–699

19. Friston KJ, Holmes AP, Worsley KJ, Poline J-P, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. Hum Brain Mapp 1994;2:189–210

20. Yan CG, Wang XD, Zuo XN, Zang YF. DPABI: data processing & analysis for (resting-state) brain imaging. Neuroinformatics 2016;14:339–351

21. DeVere R. Disorders of taste and smell. Continuum (Minneap Minn) 2017;23(2, Selected Topics in Outpatient Neurology):421–446

22. Ledberg A, Åkerman S, Roland PE. Estimation of the probabilities of 3D clusters in functional brain images. 1998;8:113–128

23. Hayes AF. *PROCESS: A Versatile Computational Tool for Observed Variable Mediation, Moderation, and Conditional Process Modeling*. Columbus, OH, The Ohio State University, 2012

24. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods 2008;40:879–891

25. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania smell identification test: a standardized microencapsulated test of olfactory function. Physiol Behav 1984;32:489–502

26. Wolfensberger M, Schnieper I, Welge-Lüssen A. Sniffin'sticks: a new olfactory test battery. Acta Otolaryngol 2000;120:303–306

27. Le Floch JP, Le Lièvre G, Labroue M, Paul M, Peynegre R, Perlemuter L. Smell dysfunction and related factors in diabetic patients. Diabetes Care 1993;16:934–937

28. Zaghloul H, Pallayova M, Al-Nuaimi O, Hovis KR, Taheri S. Association between diabetes mellitus and olfactory dysfunction: current perspectives and future directions. Diabet Med 2018;35:41–52

29. Doty RL. Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? Lancet Neurol 2017;16:478–488

30. Daulatzai MA. Olfactory dysfunction: its early temporal relationship and neural correlates in the pathogenesis of Alzheimer's disease. J Neural Transm (Vienna) 2015;122:1475–1497

31. Cui Y, Li S-F, Gu H, et al. Disrupted brain connectivity patterns in patients with type 2 diabetes. AJNR Am J Neuroradiol 2016;37:2115–2122

32. Chen Y-C, Jiao Y, Cui Y, et al. Aberrant brain functional connectivity related to insulin resistance in type 2 diabetes: a resting-state fMRI study. Diabetes Care 2014;37:1689–1696

33. Wang Z, Wang J, Zhang H, et al. Interhemispheric functional and structural disconnection in Alzheimer's disease: a combined resting-state fMRI and DTI study. PLoS One 2015;10:e0126310
34. Deem JD, Muta K, Scarlett JM, Morton GJ, Schwartz MW. How should we think about the role of the brain in glucose homeostasis and diabetes? Diabetes 2017;66:1758–1765
35. Plum L, Schubert M, Brüning JC. The role of insulin receptor signaling in the brain. Trends Endocrinol Metab 2005;16:59–65
36. Kullmann S, Heni M, Fritsche A, Preissl H. Insulin action in the human brain: evidence from neuroimaging studies. J Neuroendocrinol 2015;27:419–423
37. Heni M, Kullmann S, Preissl H, Fritsche A, Häring H-U. Impaired insulin action in the human brain: causes and metabolic consequences. Nat Rev Endocrinol 2015;11:701–711
38. Kleinridders A, Ferris HA, Cai W, Kahn CR. Insulin action in brain regulates systemic metabolism and brain function. Diabetes 2014;63:2232–2243
39. Cholerton B, Baker LD, Craft S. Insulin, cognition, and dementia. Eur J Pharmacol 2013;719:170–179
40. Palouzier-Paulignan B, Lacroix MC, Aimé P, et al. Olfaction under metabolic influences. Chem Senses 2012;37:769–797
41. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Häring H-U. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. Physiol Rev 2016;96:1169–1209
42. Schiöth HB, Craft S, Brooks SJ, Frey WH II, Benedict C. Brain insulin signaling and Alzheimer's disease: current evidence and future directions. Mol Neurobiol 2012;46:4–10
43. Novak V, Milberg W, Hao Y, et al. Enhancement of vasoreactivity and cognition by intranasal insulin in type 2 diabetes. Diabetes Care 2014;37:751–759
44. Geijselaers SLC, Sep SJS, Stehouwer CDA, Biessels GJ. Glucose regulation, cognition, and brain MRI in type 2 diabetes: a systematic review. Lancet Diabetes Endocrinol 2015;3:75–89
45. Rizzo MR, Marfella R, Barbieri M, et al. Relationships between daily acute glucose fluctuations and cognitive performance among aged type 2 diabetic patients. Diabetes Care 2010;33:2169–2174
46. Rawlings AM, Sharrett AR, Mosley TH, Ballew SH, Deal JA, Selvin E. Glucose peaks and the risk of dementia and 20-year cognitive decline. Diabetes Care 2017;40:879–886
47. Mattishent K, Loke YK. Bi-directional interaction between hypoglycaemia and cognitive impairment in elderly patients treated with glucose-lowering agents: a systematic review and meta-analysis. Diabetes Obes Metab 2016;18:135–141
48. Doty RL, Cameron EL. Sex differences and reproductive hormone influences on human odor perception. Physiol Behav 2009;97:213–228
49. Mazal PP, Haehner A, Hummel T. Relation of the volume of the olfactory bulb to psychophysical measures of olfactory function. Eur Arch Otorhinolaryngol 2016;273:1–7