Resting-State Brain Networks in Type 1 Diabetic Patients With and Without Microangiopathy and Their Relation to Cognitive Functions and Disease Variables

Eelco van Duinkerken,[1,2] Menno M. Schoonheim,[3,4] Ernesto J. Sanz-Arigita,[3,5] Richard G. IJzerman,[1] Annette C. Moll,[6] Frank J. Snoek,[2] Christopher M. Ryan,[7] Martin Klein,[2] Michaela Diamant,[1] and Frederik Barkhof[3]

Cognitive functioning depends on intact brain networks that can be assessed with functional magnetic resonance imaging (fMRI) techniques. We hypothesized that cognitive decrements in type 1 diabetes mellitus (T1DM) are associated with alterations in resting-state neural connectivity and that these changes vary according to the degree of microangiopathy. T1DM patients with (MA^+: $n = 49$) and without (MA^-: $n = 52$) microangiopathy were compared with 48 healthy control subjects. All completed a neuropsychological assessment and resting-state fMRI. Networks were identified using multisubject independent component analysis; specific group differences within each network were analyzed using the dual-regression method, corrected for confounding factors and multiple comparisons. Relative to control subjects, MA^- patients showed increased connectivity in networks involved in motor and visual processes, whereas MA^+ patients showed decreased connectivity in networks involving attention, working memory, auditory and language processing, and motor and visual processes. Better information-processing speed and general cognitive ability were related to increased degree of connectivity. T1DM is associated with a functional reorganization of neural networks that varies, dependent on the presence or absence of microangiopathy.
***Diabetes* 61:1814–1821, 2012**

Type 1 diabetes mellitus (T1DM) increases the risk of cognitive dysfunction, particularly in the domains of verbal intelligence, attention, executive function, and mental speed (1,2). These cognitive decrements have been associated with alterations in brain structure (3–5) and become more prominent as diabetes progresses. The Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) has shown that chronic hyperglycemia, rather than severe hypoglycemia, is the best predictor of cognitive changes (6). Microangiopathy in the eye and kidney has been identified by the DCCT/EDIC and other studies to be the most important biomedical correlate of cognitive decline (7,8), serving perhaps, as microangiopathy is believed to be a generalized condition (9), as a surrogate marker of cerebral microangiopathy. As patients without clinical microangiopathy will inevitably have had hyperglycemic exposure, they may also show (more subtle) cerebral changes (10).

Cognitive performance is known to be mediated by multiple interacting brain circuits and their connections (11). The integrity of this circuitry can be inferred from the degree of "functional connectivity," which is based on the concept that intercorrelations between clusters of neural activity, recorded from different brain regions, reflect exchange of information (i.e., functional connectivity) between these regions (12,13).

In a previous study, we used magnetoencephalography to map functional connectivity and found that in the resting state, functional connectivity was decreased in patients with microangiopathy versus control subjects, whereas it tended to increase in one frequency band in patients without complications (10). That study also showed that increased functional connectivity was correlated with better cognitive performance in domains—information-processing speed, attention, and executive functions—most disrupted in T1DM patients. However, since magnetoencephalography has a low spatial resolution, it is difficult to accurately determine which brain regions are specifically involved in the functional networks that are correlated with cognitive functions.

Functional magnetic resonance imaging (fMRI) is another technique to evaluate functional connectivity. Unlike magnetoencephalography, fMRI has a high spatial resolution and is well suited to identify brain regions with altered functional connectivity. fMRI can be performed during rest or during a task. In the resting-state, spontaneous low-frequency fluctuations in the blood oxygenation level–dependent (BOLD) signal occur in the brain. These fluctuations have been shown to possess strong temporal coherence and have been characterized as neuronal circuits (14). Resting-state fMRI enables differentiation of neuronal circuits independent of specific cognitive functions and task paradigms.

These circuits can be identified using independent-component analysis (ICA). This data-driven, reference-free method decomposes the acquired fMRI time course into different statistically independent time courses and, accordingly, creates spatial maps for these time courses (15). Examples of neuronal networks identified by this technique

From the [1]Diabetes Center/Department of Internal Medicine, VU University Medical Center, Amsterdam, the Netherlands; the [2]Diabetes Psychology Research Group/Department of Medical Psychology, VU University Medical Center, Amsterdam, the Netherlands; the [3]Department of Radiology, VU University Medical Center, Amsterdam, the Netherlands; the [4]Department of Anatomy and Neuroscience, VU University Medical Center, Amsterdam, the Netherlands; the [5]Department of Radiology, Centro de Investigación y Terapias Avanzadas (CITA)-Alzheimer Foundation, San Sebastian, Spain; the [6]Department of Ophthalmology, VU University Medical Center, Amsterdam, the Netherlands; and the [7]Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.
Corresponding author: Eelco van Duinkerken, e.vanduinkerken@vumc.nl.
Received 29 September 2011 and accepted 9 February 2012.
DOI: 10.2337/db11-1358
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-1358/-/DC1.

See accompanying commentary, p. 1653.

include the default mode network and sensorimotor, dorsal and ventral attention, frontal, auditory and language-processing, primary and secondary visual, and left and right frontoparietal networks (16). The existence of these networks has been consistently verified across conditions, including normal aging, mild cognitive impairment, and Alzheimer disease (17–19); multiple sclerosis (20); and depression (21).

At present, resting-state fMRI has not been applied to T1DM patients to assess changes in spatial localization of functional connectivity. To determine whether neuronal circuits are altered by T1DM and, if so, in which anatomical areas, we performed resting-state fMRI in T1DM patients with (MA^+) and without (MA^-) microangiopathy and in comparison with subjects without diabetes. We also assessed the relationship between the integrity of these neuronal circuits and cognition. Based on recent data suggesting that peripheral microangiopathy, as a surrogate marker of chronic hyperglycemia, is associated with cerebral changes, we hypothesized that alterations in fMRI-measured functional connectivity and cognition would be most marked in patients who developed clinically measurable microangiopathy, secondary to chronic hyperglycemia, and to a lesser extent in patients free of microangiopathy, i.e., those with lower glycemic burden.

RESEARCH DESIGN AND METHODS

Fifty-one T1DM MA^+ patients and 53 patients with no clinically significant complications (MA^-) were studied, as were 51 healthy nondiabetic subjects. Because of technical problems, scans were not available for 6 participants, leaving 149 for the actual analysis. Outpatients were recruited from the departments of Internal Medicine and Ophthalmology of the VU University Medical Center and affiliated hospitals and through advertisements in a national newspaper and diabetes patient magazine.

Subjects were eligible if they were 18–56 years of age, right-handed, and proficient in Dutch and, for T1DM patients, had disease duration of at least 10 years. Exclusion criteria were a BMI >35 kg/m^2, history of or current cardio- and cerebrovascular events or epilepsy, pregnancy, contraindication for magnetic resonance imaging (MRI), centrally acting medication, drug or alcohol abuse, psychiatric disorder, thyroid dysfunction, anemia, or insufficient visual acuity to perform the neuropsychological assessment. MA^+ patients were selected based on the presence of proliferative retinopathy, but other microvascular complications, such as microalbuminuria, could also be present. MA^- patients were free of all clinically detectable complications. (See below.) During the study, blood glucose levels of patients had to range between 4 and 15 mmol/L (72 and 270 mg/dL). Glucose levels were checked regularly during the study and corrected if necessary. No subject had glucose levels out of range before fMRI scanning. During neuropsychological testing, one participant experienced mild hypoglycemia; testing was postponed until blood glucose had normalized for 30 min following ingestion of an equivalent of 20 g carbohydrates. This study was conducted in accordance with the Declaration of Helsinki and approved by the medical ethics committee of the VU University Medical Center. Written informed consent was obtained from all participants.

Biomedical, anthropometric, and psychological measures. Anthropometric measures were collected using a standardized questionnaire. Blood and 24-h urine sampling was performed for routine measures (hemoglobin, creatinine, lipid profile, liver enzymes, A1C, thyrotropin, and urine creatinine and albumin) on the study day. Retinopathy was assessed by fundus photography, which was rated according to the EURODIAB classification (22). Only patients with score 0 (no retinopathy) or scores 4 and 5 (proliferative retinopathy or laser coagulation) were included. Microalbuminuria was defined by an albumin-to-creatinine ratio (ACR) >2.5 mg/mmol for men and >3.5 mg/mmol for women. Peripheral neuropathy was based on the results of the annual checkup that patients receive and is incorporated into patients' medical record. This checkup consists of the assessment of vibration perception using a 128-Hz tuning fork and tactile perception with the 10-g Semmes-Weinstein monofilament. These data were used whenever patients were recruited from our and affiliated outpatient clinics (n = 89), whereas patients recruited from other hospitals were asked about the results of the examination (n = 12). Severe hypoglycemic events, defined according to DCCT guidelines (23), were self-reported across the lifetime. Because depressive symptomatology may be elevated in T1DM (10,24) and may confound the study results, the Center of Epidemiological Studies scale for Depression was completed by all participants.

Neuropsychological testing. All participants underwent a detailed neuropsychological assessment covering the domains of general cognitive ability, memory, information-processing speed, executive functions, attention, and motor and psychomotor speed. The tests have previously been described (10). Raw scores were transformed into z scores based on the mean and SD values from control subjects. Higher z scores indicate better performance.

MRI procedure. MRI scanning was performed on a 1.5T whole-body magnetic resonance system (Siemens Sonata, Erlangen, Germany) using an eight-channel phased-array head coil. Scans included a T1-based magnetization-prepared rapid-acquisition gradient echo (T1-MPRAGE) (repetition time 2,700 ms, echo time 5.17 ms, inversion time 950 ms, flip angle 8°, 248 × 330 mm^2 field of view, 1.0 × 1.0 × 1.5 mm voxel size, and 160 contiguous coronal partitions) for registration purposes as well as 10 min of fMRI sequence (10 min, 202 volumes of echo-planar images, repetition time 2,850 ms, echo time 60 ms, flip angle 90°, 384 × 384 mm^2 field of view, isotropic 3.3-mm voxels, and 36 axial slices). Scanning occurred in a darkened room, and subjects were asked to keep their eyes closed and not think of anything particular or fall asleep.

fMRI resting-state analysis. The Software Library (FSL 4.1) of the Functional MRI of the Brain (FMRIB) (http://www.fmrib.ox.ac.uk/fsl) was used for these analyses. The following preprocessing steps were applied to all images. After discarding the first two volumes to allow for occurrence of a steady state, the remaining 200 volumes were motion corrected, brain extracted, and smoothed using a Gaussian kernel of 5 mm. High-pass filtering was applied using a cutoff of 150.0 s. Each scan was first registered to each subject's high-resolution T1-MPRAGE scan using an affine registration (6 df) and afterward nonlinearly registered to standard space (MNI152) using a warp resolution of 10 mm. Finally, the registered fMRI sequences were temporally concatenated into a single four-dimensional dataset. This dataset was then analyzed using ICA (15) to identify large-scale patterns of connectivity across the entire study population. For checking of errors in preprocessing for ICA analysis, all steps were monitored manually; i.e., scan coverage, brain extraction, and excessive motion were checked for. No scans met criteria for excessive motion; all data were used. After this analysis, dual-regression analysis (part of FSL4.1) was performed (14), the aim of which was to create personalized maps of each network for every subject. The first step of the dual-regression is creating the average time course within each network for each subject, which is done using a linear model fit of each group-based network map onto each subject's fMRI dataset (spatial regression). After this, the personalized time course is regressed back onto that subject's fMRI dataset to create personal spatial maps for each network after variance normalization using another linear model fit (temporal regression). A single four-dimensional file of all components was created for each participant. Because of the normalization of the variance of the time series used in the final regression, these spatial maps reflect both amplitude of spontaneous fluctuation in a network and its coherence (correlated BOLD signals) across space. The individual values in these maps, therefore, represent connectivity in a more sophisticated way than just a coherence measure (which implies independence of amplitude).

Group differences were tested using nonparametric permutation testing (5,000 permutations), corrected for age, sex, systolic blood pressure, and depressive symptoms. Data were corrected for multiple comparison using threshold-free cluster enhancement (25), which allows the identification of clusters of significant voxels without having to define them in a binary way, as well as family-wise error, using a final corrected threshold of $P < 0.05$. We investigated four different contrasts: healthy control subjects compared with *1*) all T1DM patients, *2*) only MA^+ patients, *3*) MA^- patients and *4*) MA^+ contrasted with MA^- patients. Each contrast was tested in two directions separately (i.e., increases or decreases), resulting in eight final contrasts.

For each resting-state network, the mean connectivity z value per subject was extracted for further inspection, using a threshold of $z > 3.9$, corresponding to $P < 0.0001$. In networks where group differences were significant, an additional mean z score of significant voxels was calculated per subject.

Statistical analysis. Demographic, medical, and anthropometric measures were analyzed using one-way ANOVA with Bonferroni correction, Student t test, or χ^2, where appropriate. Cognitive domains were compared using a multivariate ANCOVA, corrected for age, sex, systolic blood pressure, and depressive symptoms. In case of a significant multivariate F test, post hoc individual tests were checked for significance. Group differences were Bonferroni corrected.

For determination of associations between demographic, medical, and cognitive variables and changed functional connectivity, regression analyses were performed. For each network, all demographic (age, sex, BMI, depressive symptoms, and systolic blood pressure) and medical (disease duration, onset age, severe hypoglycemic events, microangiopathy, ACR, and A1C) or cognitive domains, corrected for age, sex, systolic blood pressure, and depressive symptoms, were entered in one block as independent variables. A forward

regression was used to determine variables that were significantly associated with resting-state networks. This resulted in 10 regression analyses, with 2 for each network.

All fMRI statistics were performed in FSL4.1. All other statistical analyses were performed in SPSS 15.0 (SPSS, Chicago, IL). A P value <0.05 was considered statistically significant.

RESULTS

MA^+ patients were significantly older and reported the highest number of depressive symptoms compared with both other groups; systolic blood pressure was elevated compared with control subjects only (Table 1). Compared with MA^- patients, MA^+ patients had an earlier onset age and longer disease duration, an increased ACR, and a higher rate of hypertension. Nearly one-half of these subjects had neuropathy, more than one-quarter had microalbuminuria, and twice as many had developed diabetes early in life (<7 years).

Functional connectivity analysis. The ICA identified 13 components consisting of noise and 10 networks that have previously been described (16,20), which were compared between the three groups. Results are corrected for age, sex, systolic blood pressure, depressive symptoms, and multiple comparisons (threshold-free cluster enhancement and family-wise error), with $P < 0.05$. Group differences are illustrated in Fig. 1 and described below. The networks that did not show significant differences are presented in Fig. 1 of the Supplementary Data. Each voxel represents a connectivity value that can be extracted from the individual fMRI file. For each network, the mean connectivity value of all voxels that differed between patients and control subjects is derived for all participants and is illustrated in Fig. 2.

Sensorimotor network. This network is composed of areas including the motor cortex, pre- and postcentral gyri, and subserves motor and sensory tasks (16). As can be seen in Fig. 1*A*, MA^- patients showed increased connectivity relative to control subjects in the bilateral precentral gyrus and the right postcentral gyrus, cingulate gyrus, superior parietal lobule, and precuneus. By contrast, MA^+ patients demonstrated a decreased connectivity in the right pre- and postcentral gyrus relative to MA^- patients but did not differ significantly from control subjects.

Secondary visual network. This network is thought to represent visual processing and includes portions of the visual cortex (16). Compared with control subjects, MA^- patients were found to have increased connectivity in the bilateral occipital pole, cuneal and lateral occipital cortex, and lingual and occipital fusiform gyrus. Conversely, MA^+ patients showed decreased connectivity in the same areas relative to MA^- patients (Fig. 1*B*).

Ventral attention network. In pooled analysis, T1DM patients, particularly MA^+ and not MA^- patients, showed decreased connectivity relative to control subjects in the left temporal pole and inferior and middle temporal and superior frontal gyrus (Fig. 1*C*). MA^- patients showed values intermediate between the other two groups (linear trend $P < 0.001$, corrected for age, sex, systolic blood pressure, and depressive symptoms) (Fig. 2). Activity in this network includes occipital, temporal, and frontal areas and is thought to reflect attention processes (16).

TABLE 1
Demographic and anthropomorphic variables

	T1DM MA^+	T1DM MA^-	Control subjects	P
N	49	52	48	—
Age (years)	44.5 ± 7.2	37.8 ± 9.3	37.0 ± 11.1	<0.001
Sex (men/women)	20/30	20/32	19/29	0.999
Education level*	5 (1–8)	6 (2–8)	6 (4–8)	0.179
Estimated IQ†	110.0 ± 13.5	107.1 ± 11.2	108.7 ± 11.7	0.488
Depressive symptoms‡	11.4 ± 9.8	7.2 ± 6.7	6.3 ± 6.7	0.003
BMI (kg/m^2)	25.5 ± 4.1	25.0 ± 3.4	24.3 ± 3.6	0.266
Systolic blood pressure (mmHg)	133.3 ± 17.6	129.8 ± 14.3	123.7 ± 11.4	0.006
Diastolic blood pressure (mmHg)	75.7 ± 8.7	78.2 ± 9.1	77.4 ± 7.3	0.331
Total cholesterol (mmol/L)	4.5 ± 0.7	4.7 ± 0.7	4.5 ± 0.9	0.293
A1C (mmol/mol)	64.7 ± 14.2	61.9 ± 9.8	34.3 ± 2.6	<0.001
A1C (%)	8.1 ± 1.3	7.8 ± 0.9	5.3 ± 0.2	<0.001
ACR (mg/mmol)	3.1 ± 5.8	0.6 ± 0.7		0.002
Hypertension (%)§	32 (64.0)	13 (25.0)		<0.001
Blood glucose before MRI (mmol/L)	9.2 ± 3.7	10.6 ± 4.7		0.095
Blood glucose before NPA (mmol/L)	8.7 ± 4.1	8.5 ± 4.0		0.800
Diabetes duration (years)	34.3 ± 7.9	21.4 ± 9.2		<0.001
Diabetes onset age (years)	10.2 ± 7.2	16.4 ± 9.6		<0.001
Diabetes early onset (%)¶	18 (36.0)	9 (17.3)		0.043
Severe hypoglycemic events#	6.1 ± 9.2	5.8 ± 10.2		0.896
Neuropathy (%)**	24 (49.0)			
Microalbuminuria (%)††	13 (26.0)			

Data are means ± SD, median (minimum–maximum values), or absolute numbers (%) unless otherwise indicated. NPA, neuropsychological assessment. *Education level was coded from 1 (unfinished primary school) to 8 (finished university). †Estimated IQ was measured using the Dutch version of the National Adult Reading Test. ‡Depressive symptoms were measured using the Center for Epidemiological Studies scale for depression. §Hypertension was defined as a systolic blood pressure of ≥140 mmHg, a diastolic blood pressure of ≥90 mmHg, or use of antihypertensive drugs. ¶Diabetes early onset was defined as an onset age below the age of 7 years. #Severe hypoglycemic events were self-reported and defined as events for which the patient needs assistance from a third person to recuperate as a result of loss of consciousness or seriously deranged functioning, coma, or seizure owing to low glucose levels. **Neuropathy was based on medical records or, in case they were not available, based on self-report. ††Microalbuminuria was defined as an ACR >2.5 mg/mmol for men and >3.5 mg/mmol for women.

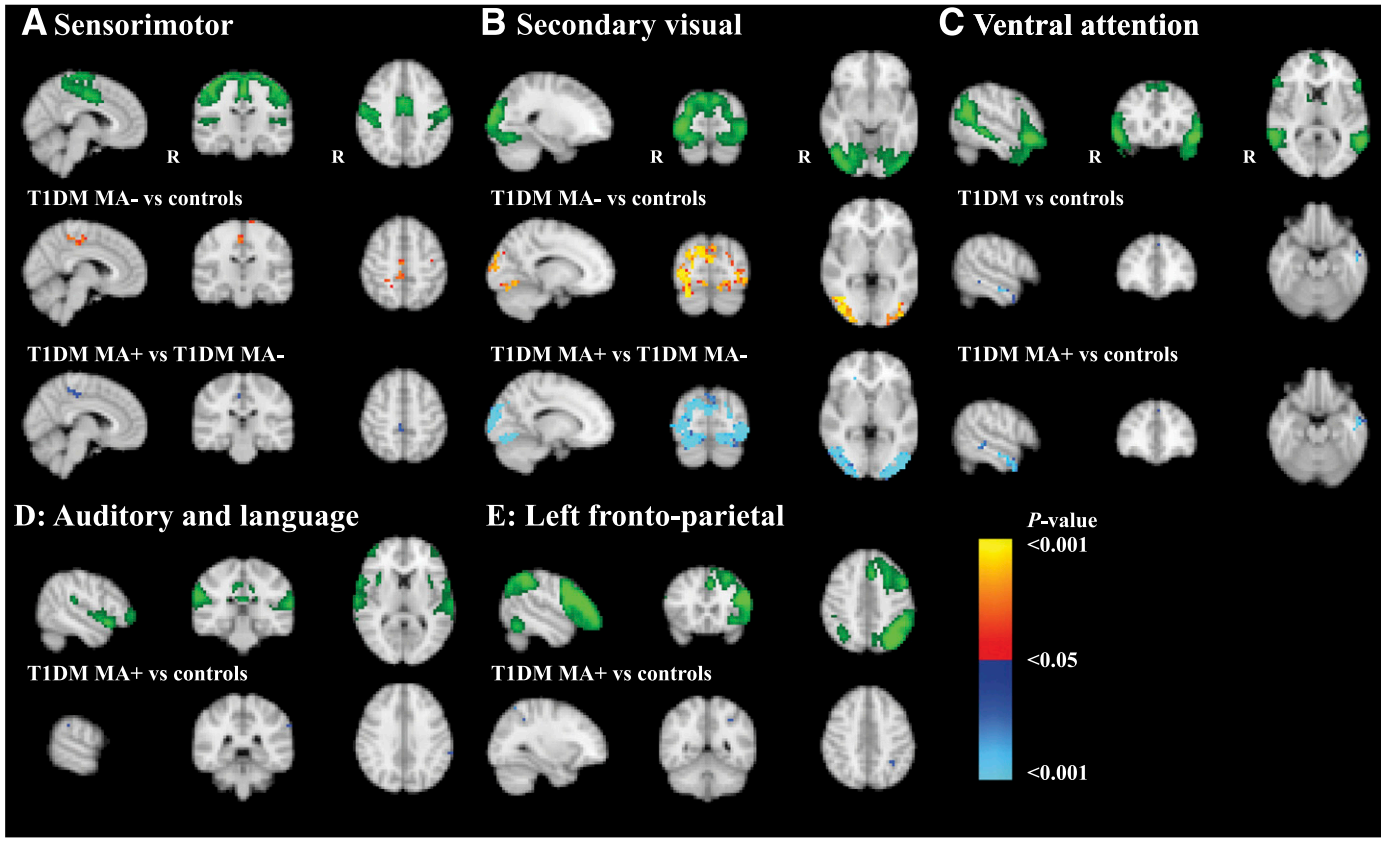

FIG. 1. Presented are the networks that show significant differences in the dual-regression analysis. For each network, denoted with a letter, only voxels exceeding $z = 3.9$ ($P < 0.0001$) are presented in green. The other rows are significantly differing contrasts found by the dual-regression method. The more yellow or light blue, the lower the *P* value. On the images, left is right and right is left. *A*: Sensorimotor network. *B*: Secondary visual network. *C*: Ventral attention network. *D*: Auditory and language-processing network. *E*: Left frontoparietal network. (A high-quality digital representation of this figure is available in the online issue.)

Auditory and language-processing network. This network is thought to be related to processing of auditory and language information and includes connectivity within the superior temporal, insular, and postcentral cortex (16). MA^+ patients showed decreased connectivity compared with control subjects in the left supramarginal gyrus (Fig. 1*D*). A linear trend across the three groups was evident ($P < 0.001$, corrected for confounding) (Fig. 2).

Left frontoparietal network. This network is thought to be involved in working-memory processes (16). Figure 1*E* shows lower connectivity in the left lateral occipital cortex and superior parietal lobule for MA^+ patients versus control subjects. Again, a linear trend toward lower connectivity in both patient groups compared with control subjects was found ($P < 0.001$, corrected for confounding) (Fig. 2).

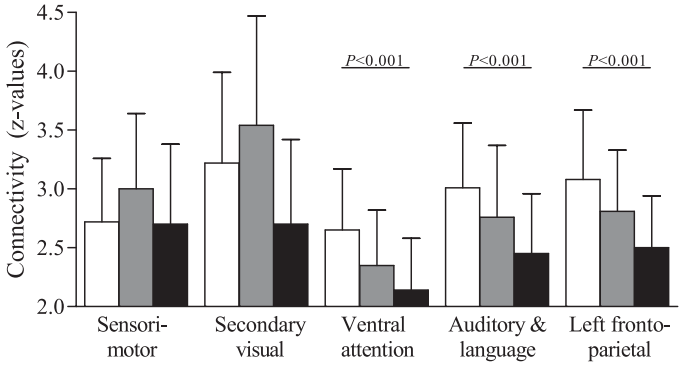

FIG. 2. Bar graph with SDs of connectivity *z* values of each network showing altered connectivity in T1DM patients. White bars, control subjects; gray bars, MA^- patients; black bars, MA^+ patients. The *P* values represent those of the linear trend analyses.

Matched dual-regression analysis. To limit the effect of disease duration and onset age on the results of the dual-regression analysis, we matched patients for these variables. Twenty-seven MA^+ patients (mean disease duration 28.7 ± 5.7 years, onset age 13.2 ± 7.6 years, and age 41.9 ± 7.8 years), 28 MA^- patients (disease duration 27.4 ± 8.3 years, onset age 12.9 ± 8.4 years, and age 40.4 ± 9.0 years) and all ($n = 48$) control subjects (age 37.0 ± 11.1 years) were included in these analyses. The matched analysis, albeit with a smaller sample size, shows a similar pattern of changed connectivity in the sensorimotor and secondary visual networks in essentially the same brain regions (Supplementary Fig. 2). The results in the other networks were not statistically significant, although for the ventral attention network there was a trend ($P = 0.09$). Effect sizes of the different contrasts were similar in both analyses (Supplementary Table 1) but failed to reach statistical significance because of the reduction in sample size.

Cognitive functioning. The *F* test for both the original and matched analyses was significant, allowing post hoc testing (all $P < 0.02$). As seen in Fig. 3, MA^+ patients performed more poorly than control subjects on measures of general cognitive ability, information-processing speed, and attention. Compared with their MA^- counterparts, they

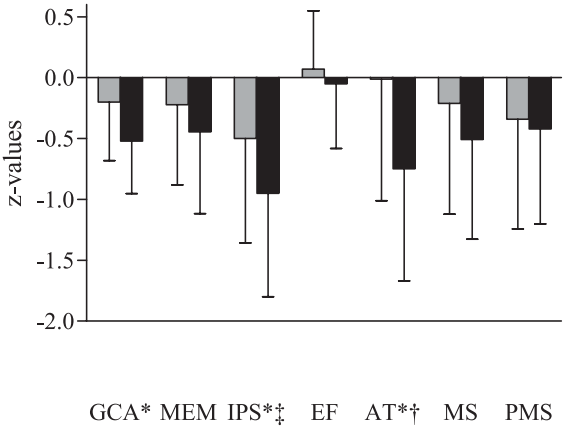

FIG. 3. *z* value bar graph with SDs of changes in cognitive functions in MA$^+$ patients (black bars) and MA$^-$ patients (gray bars) compared with healthy control subjects. Control subjects are represented as the *x*-axis, as *z* values were created on the basis of means and SDs of control subjects. AT, attention; EF, executive functions; GCA, general cognitive ability; IPS, information-processing speed; MEM, memory; MS, motor speed; PMS, psychomotor speed. *Lower performance in MA$^+$ patients compared with control subjects. †Lower performance in MA$^+$ patients compared with their MA$^-$ counterparts. ‡Lower performance in MA$^-$ patients compared with control subjects.

performed worse on attention. MA$^-$ patients also were slower on information-processing tasks than control subjects. After matching patient groups for diabetes duration and onset age, differences between MA$^+$ patients and control subjects on general cognitive ability and information-processing speed remained significant ($P < 0.05$). As can be seen in Supplementary Fig. 3*B*, effect sizes remained similar in both analyses.

Relationships between functional connectivity, demographic/disease variables, and cognition. These correlations were calculated in T1DM patients only and only for the voxels that showed differences between MA$^+$ or MA$^-$ T1DM patients versus control subjects (Table 2). Across different networks, decreased connectivity was independently related to a subset of variables including older age (sensorimotor and ventral attention networks), female sex (sensorimotor and auditory and language-processing networks), higher BMI (ventral attention and left frontoparietal networks), and presence of microangiopathy (secondary visual, auditory and language-processing, and left frontoparietal networks). There were no correlations with depressive symptoms, A1C, or other factors. Increased connectivity in the secondary visual network correlated with increased general cognitive ability scores and increased information-processing speed (Table 2).

DISCUSSION

This is the first study to show that in T1DM patients, resting-state cerebral connectivity networks differ from those in healthy comparison subjects and that in patients, the direction of the effects vary according to complication status. The dual-regression analysis identified five networks that were affected in T1DM. MA$^+$ patients showed decreased connectivity in all five networks. For MA$^-$ patients versus control subjects, increased connectivity was found in the sensorimotor and secondary visual networks. In the other three networks (ventral attention, auditory and language-processing, and left frontoparietal networks), a significant dose-response relationship was evident, indicating that connectivity is lowest in MA$^+$ patients, intermediate in MA$^-$ patients, and highest in control subjects (Fig. 2). Changes in network integrity were associated not only with microangiopathy but also with age, sex, and BMI. Moreover, in at least one network (secondary visual), increased connectivity was associated with higher general cognitive ability scores and faster information-processing speed. The observed connectivity results in these patients are consistent with our earlier magnetoencephalography findings (10).

TABLE 2
Associations of demographic, disease, and cognitive variables and the mean *z* value of the significantly differing voxels of functional connectivity in all T1DM patients

	Sensorimotor	Secondary visual	Ventral attention	Auditory and language processing	Left frontoparietal
Age	−0.308**		−0.322***		
Sex	−0.218*			−0.191*	
BMI			−0.336***		−0.388***
Hypertension					
Diabetes duration					
Diabetes onset age					
Severe hypoglycemia					
Microangiopathy		−0.456***		−0.274**	−0.292***
ACR					
A1C					
General cognitive ability		0.214*			
Memory					
Information-processing speed		0.217*			
Executive functions					
Attention					
Motor speed					
Psychomotor speed					

All correlations between secondary visual and cognitive domains were corrected for age, sex, systolic blood pressure, and depressive symptoms. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

At first glance, increased connectivity seems counterintuitive, but it is also found in early multiple sclerosis and mild cognitive impairment (20,26,27). This phenomenon may be explained by loss of local inhibitory (inter)neurons that results in increased firing of long-distance neurons and that in turn leads to increased connectivity (28). This increase could also be a reaction to loss of connectivity in other networks that inhibit connectivity in visual and sensorimotor networks (29). Another explanation is that increased connectivity is a sign of functional reorganization (30), occurring in response to early subtle brain damage. Early cerebral compromise has been demonstrated in T1DM youth and adults with no or only minimal microvascular complications (3,31,32). It has been shown in multiple sclerosis that with increasing disease severity, connectivity decreases, possibly as a consequence of failing functional reorganization (27,30). This may also explain the decreased connectivity in our MA^+ patients. Why increased connectivity in our MA^- patients is limited to the primary and secondary visual and sensorimotor networks is unknown (Figs. 2 and 4). It might be that increased connectivity can be longer retained in networks comprising brain regions situated close together. Alternately, these three networks receive direct neuronal input from retinal and peripheral nerve fibers. Already in MA^- patients, subclinical retinal and peripheral nerve changes have been found (33,34), which have been related to increased brain activity measured with electroencephalography (35).

We dichotomized patients with respect to microangiopathy presence as a proxy for overall glycemic burden. Our fMRI and cognitive results, however, suggest more of a dose-response relationship of glycemic burden. Accumulating hyperglycemia, in MA^- patients, may lead to increased connectivity but decreased cognitive performance. With the presence of clinical microangiopathy, i.e., increased glycemic burden, connectivity decreased and cognitive functions further decreased. We did not observe an effect of severe hypoglycemic events on our results. Although this may have been due to the difficulty in accurately determining these events, our results are in line with other studies in adults (6,23). In addition, neuropathy and nephropathy may contribute to the results. Given the study design, we were not able to tease out their relative contribution, which should be determined in future studies.

The connectivity results may be confounded by several factors. For factors like age, sex, BMI, IQ, blood pressure, depressive symptoms, and severe hypoglycemic events, groups were matched or we statistically corrected for those variables. Disease duration and onset age may also be important confounders. However, results remained similar when we analyzed groups matched for these variables. This may indicate that microangiopathy, as a surrogate marker of hyperglycemic burden, may be the most prominent diabetes-related variable associated with connectivity and cognition changes. Besides these demographic and medical factors, technical factors may also influence BOLD signal and thereby our results. First, BOLD signal is influenced by the cardiac and respiratory systems, cerebrospinal fluid, blood flow, and head movement. Given the strong temporal coherence of these systems, ICA analysis will identify these as independent components (15). Second, cerebral microvascular changes may lead to altered hemodynamic responses, as well as to changes in neurovascular coupling (i.e., process by which neuronal activity influences the surrounding vasculature), which may affect BOLD signal. Third, hypoperfusion can also lead to changes in BOLD signal. Perfusion has been mostly studied in relation to hypoglycemia and found to increase (36,37). One study found disturbed increased perfusion after a dilatory stimulus in T1DM versus control subjects, related to the presence of microangiopathy (38). Although it is difficult to exclude the influence of these factors, as we used ICA and given our magnetoencephalography data, which are less influenced by these technical factors, we may conclude that our results, at least in part, represent true connectivity changes.

Although one might expect to find a strong relationship between functional connectivity and cognition (11), there have been many negative findings (20), including results from the present set of analyses. The absence of an association between cognition and functional circuitry could be explained in several ways. On the one hand, it could be

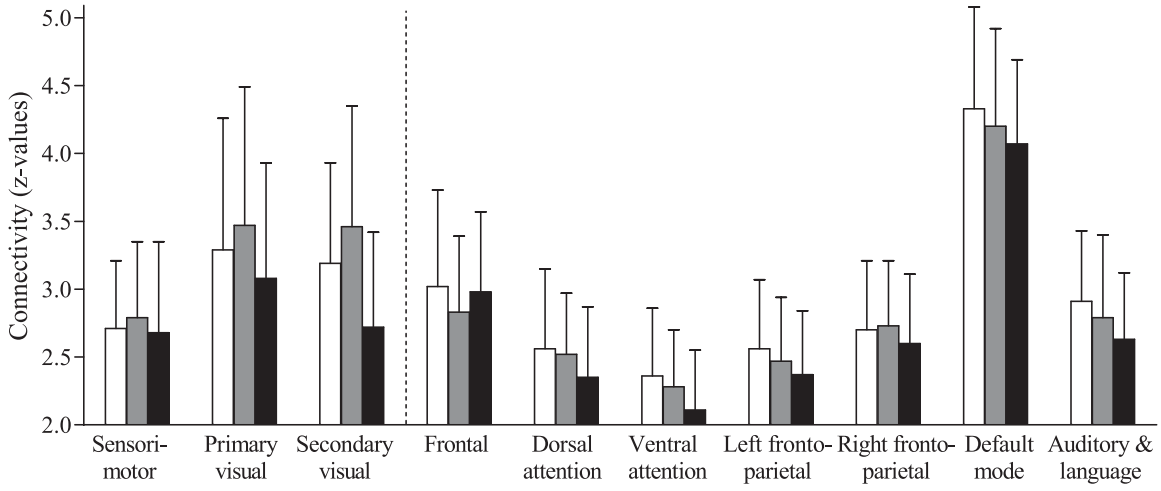

FIG. 4. Bar graph with SDs of the mean connectivity value of each network resulting from the ICA. Networks left of the dotted line are composed of brain areas relatively close to each other and are thought to involve lower-order cognitive functions, whereas networks right of the dotted line represent networks with a more complex topography and involvement in higher-order cognitive functions. White bars, control subjects; gray bars, MA^- patients; black bars, MA^+ patients.

a limitation of the methods used. We correlated a single functional connectivity value (comprised of multiple values summed across multiple brain regions) with each cognitive domain (comprised of multiple cognitive test scores). A voxel-wise correlation analysis between specific cognitive test scores and the individual voxels of a particular network may be more sensitive and more strongly correlated to cognition. On the other hand, there is growing evidence to suggest that complex cognitive processes are mediated by multiple interacting neural networks (39,40) and may not be limited to activity in the discrete networks identified by resting-state fMRI.

One potential limitation is the large number of statistical comparisons performed in the dual-regression analysis. Although all components are statistically independent, and therefore the sets of statistical tests are also independent, many statistical comparisons were performed, and there is a possibility that our results capitalized on chance associations. However, given the novelty of the analysis and the thought that cognition is mediated by multiple neuronal networks, we chose this approach.

In conclusion, we showed that functional connectivity of neuronal circuits is compromised in MA^+ patients, whereas increased resting-state connectivity was observed to some extent in MA^- patients. This altered resting-state connectivity may, in part, contribute to the impairments in cognitive performance observed in T1DM patients, particularly in those with microangiopathy.

ACKNOWLEDGMENTS

This study was supported by Grant 2005.00.006 from the Dutch Diabetes Research Foundation.

No potential conflicts of interest relevant to this article were reported.

E.v.D. participated in the design of the study, performed the study and the statistical analyses, wrote the manuscript, and made crucial revisions to the manuscript. M.M.S. and E.J.S.-A. supervised the MRI analyses, drafted the manuscript, and made crucial revisions to the manuscript. R.G.IJ. supervised the statistical analyses, drafted the manuscript, and made crucial revisions to the manuscript. A.C.M. rated all fundus photographs, drafted the manuscript, and made crucial revisions to the manuscript. F.J.S. participated in the design of the study and made crucial revisions to the manuscript. C.M.R. critically interpreted the results, drafted the manuscript, and made crucial revisions to the manuscript. M.K., M.D., and F.B. participated in the design of the study and made crucial revisions to the manuscript. M.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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