Functional Brain Connectivity and Neurocognitive Functioning in Patients With Long-Standing Type 1 Diabetes With and Without Microvascular Complications

A Magnetoencephalography Study

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OBJECTIVE—Hyperglycemia-associated microvascular disease may underlie changes in cerebral functioning and cognitive performance in patients with type 1 diabetes. Functional connectivity, an indicator of functional interactions and information exchange between brain regions, provides a measure of cerebral functioning. This study addresses functional connectivity and cognition in type 1 diabetic patients with and without proliferative retinopathy, relative to healthy control subjects, using magnetoencephalography.

RESEARCH DESIGN AND METHODS—Fluctuations in magnetic field at scalp for Δ, θ, lower and upper α, β, and lower and upper γ frequency bands were measured using magnetoencephalography. Synchronization likelihood, a measure of functional connectivity, was computed. Using neuropsychological tests, cognitive functioning was assessed and its associations with functional connectivity were determined.

RESULTS—Compared with control subjects, type 1 diabetic patients performed poorer on general cognitive ability, information processing speed, and motor speed, irrespective of their microvascular complication status. Functional connectivity, however, was lowest for type 1 diabetic patients with retinopathy, compared with type 1 diabetic patients without microvascular complications and control subjects, whereas type 1 diabetic patients without microvascular complications showed an increase relative to control subjects. Positive associations were found between functional connectivity and executive functioning, memory, information processing speed, motor speed, and attention.

CONCLUSIONS—Compared with healthy control subjects, functional connectivity and cognition differed in type 1 diabetic patients irrespective of microvascular complication status, indicating that chronic hyperglycemia, among other factors, may negatively affect brain functioning even before microvascular

damage becomes manifest. The association found between synchronization likelihood and cognition suggests functional connectivity plays a significant role in cognitive functioning. *Diabetes* 58:2335–2343, 2009

Mild cognitive deterioration and changes in cerebral anatomy have been demonstrated in patients with long-standing type 1 diabetes. These cognitive disturbances are limited to slowed information processing speed, attentional functioning, and primary motor as well as psychomotor speed (1–3). Using structural magnetic resonance imaging (MRI), reductions in cerebral gray and white matter volume in type 1 diabetes compared with healthy control subjects were reported (4–6). To measure important functional changes, electroencephalography can be used. In children with type 1 diabetes, increases in slow (Δ and θ) activity, decreases in fast (α, β, and γ frequency bands) activity, and a reduction in α peak frequency were found. These functional cerebral changes were correlated with both poor glycemic control and more episodes of severe hypoglycemia (7). Compared with healthy control subjects, there was a loss of fast activity in well-controlled diabetic adults (8). These changes were unrelated to a history of severe hypoglycemic events. Although the underlying neuropathological and biological substrates are undefined, there is evidence that chronic hyperglycemia, leading to microangiopathy in the brain, may be the main cause of these cerebral complications (3,5,6,9).

A relatively novel and more advanced approach to measuring brain activity is magnetoencephalography (MEG). MEG measures fluctuations of magnetic fields of the brain at the scalp. MEG has been widely used in the study of functional changes associated with neurological disorders including Alzheimer's disease (10), Parkinson's disease (11), brain tumors (12), and metabolic disorders such as obesity (13,14) and hepatic encephalopathy (15).

With MEG data, functional connectivity can be calculated. This refers to the assumption that correlations between time series of neural activity recorded from different brain regions reflect functional interactions and information exchange between these regions (16,17). Differences in functional connectivity indicate a different way of communication between brain areas. Functional connectivity has been thought to be a core component of cognitive functioning, as most cognitive functions highly depend on interactions between distinct cerebral regions rather than on single brain regions or structures. There-

fore, functional connectivity may, at least in part, explain cognitive functioning, and changes in functional connectivity might account for cognitive deterioration (18).

In this study, MEG functional connectivity relative to healthy control subjects was investigated in a group of type 1 diabetic patients with proliferative retinopathy as a marker of hyperglycemia and a group of type 1 diabetic patients without microvascular complications. Furthermore, neurocognitive functioning and its association with functional connectivity was assessed. Based on earlier studies (9,19) we expected type 1 diabetic patients with proliferative retinopathy to show cognitive deterioration and lower functional connectivity compared with healthy control subjects and type 1 diabetic patients without microvascular complications.

RESEARCH DESIGN AND METHODS

Fifteen type 1 diabetic patients with proliferative retinopathy, an indicator of microangiopathy resulting from chronic hyperglycemia (type 1 diabetes[+]), 29 type 1 diabetic patients without manifest microvascular complications (type 1 diabetes[−]), and 26 healthy control subjects matched for sex and education level enrolled in this study. Participants were recruited from the departments of Endocrinology and Ophthalmology of the VU University Medical Center, Amsterdam, the Netherlands (50 patients), the department of Internal Medicine, Groene Hart Hospital, Gouda, the Netherlands (10 patients), and by advertisements in diabetes magazines and a national newspaper (10 patients).

Inclusion criteria were age range 18–55 years, right-handedness, for type 1 diabetic patients a disease duration of at least 10 years, proliferative retinopathy as described below, or no signs of microvascular complications. Mastery of the Dutch language was required for all participants. Participants were excluded if they had a BMI above 35 kg/m², current use of drugs affecting cerebral functioning, alcohol abuse (more than 20 g of alcohol per day), psychiatric disorders, anemia, thyroid dysfunction, use of glucocorticoids, hepatitis, stroke, severe head trauma, epilepsy, pregnancy, or poor visual acuity below 0.3. Fundus photography (Topcon NW 100, Capelle aan den IJssel, the Netherlands) was performed to screen for retinopathy. For each eye, one photograph with the macula in the center and one with the optic disc in the center were taken (E.v.D.). Photographs were rated by an ophthalmologist (A.M.) according to the European Diabetes (EURODIAB) classification (20). Only those participants with either an EURODIAB classification score of 0 (no retinopathy) or of 4 or 5 (proliferative retinopathy or lasercoagulation) were included in this study. Twenty-four–hour urine collections were performed to assess albumin-to-creatinine ratio (ACR). The presence of peripheral neuropathy was ascertained by the physician. Type 1 diabetes[−] patients had normoalbuminuria (ACR <2.5 mg/mmol in men and <3.5 mg/mmol in women) and no neuropathy. Type 1 diabetes[+] patients were allowed to have microalbuminuria and/or peripheral neuropathy. Hypertension was defined as a systolic blood pressure of 140 mmHg or above, a diastolic blood pressure of 90 mmHg or above, or the use of antihypertensive drugs. The study protocol was approved by the Medical Ethics Committee of the VU University Medical Center, and written informed consent was obtained from all participants.

Study design. Before eligible participants received written information on the study, their medical records were screened using the aforementioned criteria. Those willing to participate were additionally interviewed over the telephone to collect background information including educational level, using a Dutch scoring system ranging from 1 to 8. One indicates unfinished primary school and 8 indicates a completed university study at masters level. As depression may affect cognitive performance, depressive symptoms were assessed using the Center for Epidemiological Studies scale for Depression (CES-D). Eligible participants were invited for the first visit. During this visit, which took place in the afternoon, fundus photography was performed, blood samples were collected, and MEG registration was performed. Approximately 6 weeks after the first visit participants returned for neuropsychological assessment during a morning visit. All visits took place at the VU University Medical Center.

To rule out confounding as a result of extreme blood glucose levels, levels were checked in the type 1 diabetes participants before the start of the neuropsychological assessment and MEG registration and had to be between 4 and 15 mmol/l (72–270 mg/dl). In case of hypoglycemia, participants were instructed to eat 20 g of carbohydrates. In case of hyperglycemia, patients were instructed to inject 2 units of the participants' current rapid-acting insulin analog when blood glucose levels were between 15 and 20 mmol/l

(270–362 mg/dl) and 4 units when glucose levels exceeded 20 mmol/l (362 mg/dl). Glycemic status was evaluated after 30 min. If hyperglycemia (i.e., blood glucose ≥15 mmol/l) would persist, participants were instructed to inject another 2 units of insulin; if hypoglycemia would persist, participants additionally had to eat 20 g of carbohydrates. Two type 1 diabetes[+] participants were hyperglycemic before start of the neuropsychological assessment and one before MEG acquisition. One type 1 diabetes[−] patient indicated symptoms of hypoglycemia during neuropsychological testing. Proper glycemic status was restored for all patients after the first step of the above-mentioned protocol. Data of the one participant who experienced hypoglycemia symptoms during neuropsychological testing were included in all analyses, as this did not change analysis outcomes.

Neuropsychological assessment. Based on earlier type 1 diabetes cognitive research (5,21), as well as clinical neuropsychological practice (22), a battery of cognitive tests was chosen to measure potential differences in five major cognitive domains: memory, information processing speed, executive functions, attention, and motor speed. Domains were based on an earlier principal component analysis using varimax rotation with Kaiser normalization in a large group of healthy subjects and adjusted according to earlier research (5,21,23).

The domain 'memory' was assessed by the Dutch version of the Rey Auditory Verbal Learning Test (RALVT) (24), Wechsler Adult Intelligence Scale–3rd edition revised (WAIS-III-R) Digit Span forward and backward (25), and the WAIS-III-R Symbol Substitution Incidental Learning Test (25). The domain 'information processing speed' was created using the WAIS-III-R Symbol Substitution Test (25), the Stroop Color-Word Test parts 1 and 2 (26), the Concept Shifting Task (CST) parts A and B (27), the Simple Auditory and Visual Reaction Time Tests (28), and the Computerized Visual Searching Task (CVST) (28). Assessment of the domain 'executive functions' was conducted using the Stroop Color-Word Test part 3 (26), the CST part C (27), the D2-test total errors (29), the Wisconsin Cart Sorting Test (30), and the Category Word Fluency Task (31). The domain 'attention' was assessed using the D2-test range, with total correct answers and total span (29). The domain 'motor speed' consisted of the Tapping Test (28) and the CST part 0, administered three times (27). 'General cognitive ability' was constructed by averaging the above mentioned five domains.

To enhance comparability and allow construction of these domains, z-scores for every test were created based on the means ± SD of the healthy control group. Higher z-scores indicate better performance.

MEG protocol. MEG data were obtained using a 151-channel whole-head MEG system (CTF systems; Port Coquitlam, BC, Canada), while participants were in a supine position in a magnetically shielded room (Vacuumschmelze, Hanau, Germany). A third-order software gradient (32) was used with a recording passband of 0.25–125 Hz and a sample frequency of 625 Hz. Participants had to be free of any metal materials. Magnetic fields were recorded for 2 min in an eyes-open, 5 min in an eyes-closed, 10 min in a task, and then 3 min in another eyes-closed condition. Total acquisition time was 20 min. At the beginning, middle, and end of each recording, the head position relative to the coordinate system of the helmet was determined by leading small alternating currents through three head position coils attached to the left and right preauricular points and the nasion on the subjects head. Changes in head position of ~1.5 cm during a recording condition were accepted. If head position changed more than 1.5 cm, recordings had to be repeated. This did not happen in this protocol.

Synchronization likelihood. From the acquired MEG data, information about functional connectivity was calculated by means of a mathematical construct, the synchronization likelihood. For a technical description of synchronization likelihood see Stam and van Dijk (33) and Montez et al. (34). In short, interactions between two neural networks, for instance the frontal (X) and temporal (Y) networks, are of interest. MEG data are recorded for all sensors surrounding the head, including those in the frontal and temporal areas. These signals represent the time series x_i and y_i from the frontal (X) and temporal (Y) areas (Fig. 1). It is assumed that X and Y more strongly interact when x_i and y_i more 'resemble' each other (square with continuous line in Fig. 1). It has been shown, however, that X and Y can also interact when x_i and y_i do not resemble each other (square with the dashed line in Fig. 1). This is called generalized synchronization and can be quantified by computing synchronization likelihood. Parameter settings used for computation of synchronization likelihood are based on the frequency content of the data (for parameter settings see Montez et al. (34)). Synchronization likelihood can range from P_{ref} (low synchronization) to one (complete synchronization). P_{ref} is a value close to zero (zero indicates no synchronization, which is not possible) and was set on 0.01 for all frequency bands.

Data analysis. For this analysis, 141 of the 151 channels were used. Ten channels were deleted as their signals were distorted in some participants. This had no influence on the calculations. Offline, recordings of the first eyes-closed condition were transformed into ASCII-files and imported into the
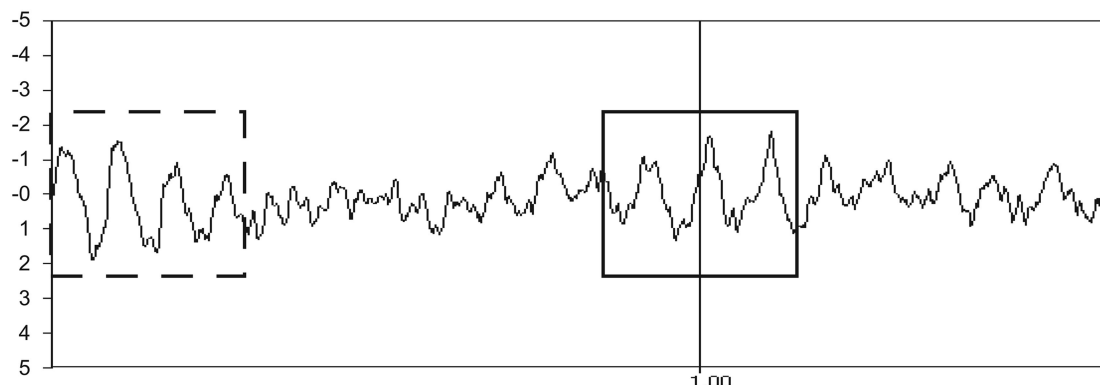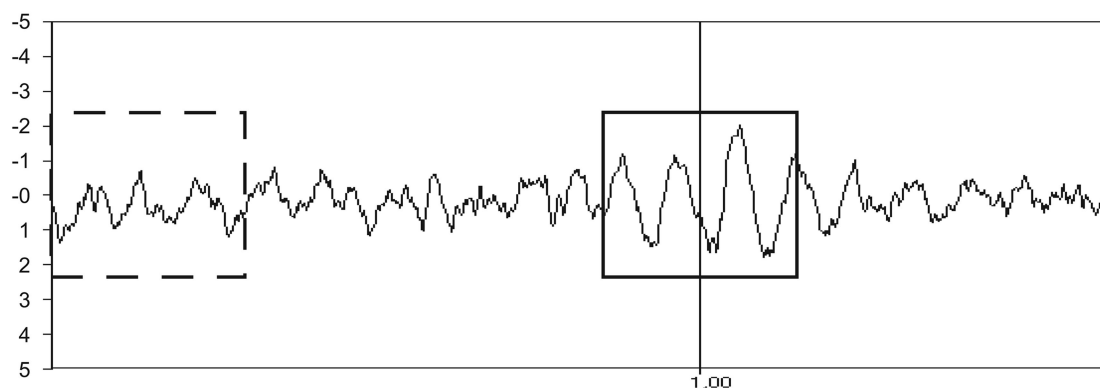
A Network X (frontal)

B Network Y (temporal)

FIG. 1. Example of time series of the frontal and temporal network. The square with the continuous line indicates a time series showing high resemblance between both networks. The square with the dashed line is an example of a time series showing low resemblance. *A*: Network X (frontal). *B*: Network Y (temporal).

DIGEEGXP software (C.J. Stam, VU University Medical Center, Amsterdam, the Netherlands). For each participant five artifact-free epochs (6.25 s) of 4,096 samples were selected by two authors (E.v.D. and N.S.). Total data used were 31.25 s. With DIGEEGXP synchronization likelihood was calculated (33). Synchronization likelihood was calculated between signals recorded at all possible sensor pairs for Δ (0.5–4 Hz), θ (4–8 Hz), lower α (8–10 Hz), upper α (10–13 Hz), β (13–30 Hz), lower γ (30–45 Hz), and upper γ (55–80 Hz) frequency bands. Subsequently, MEG sensors were clustered according to their anatomical location, respectively, right and left frontal, central, parietal, temporal, and occipital. Average synchronization likelihood values were then obtained for short-distance, intrahemispheric long-distance, and interhemispheric long-distance groups. Short-distance group consisted of 10 areas, left and right hemisphere central, frontal, parietal, occipital,

and temporal regions. For each of these 10 areas, the synchronization likelihood between all possible pairs of sensors belonging to that area was averaged. This resulted in ten, local short-distance synchronization likelihood values. Average synchronization likelihood for long distances was obtained by averaging all pair wise synchronization likelihood values of sensors belonging to two different areas. Intrahemispheric long-distance group consisted of eight pairs, left and right hemisphere frontoparietal, frontotemporal, parietooccipital, and temporooccipital. Interhemispheric long-distance group consisted of five pairs, left to right hemisphere central, parietal, temporal, occipital, and frontal (12,13). See Fig. 2 for anatomical locations. Now, synchronization likelihood resembles the likelihood that the data obtained from all sensors in the above-defined short-distance and long-distance groups are synchronized.
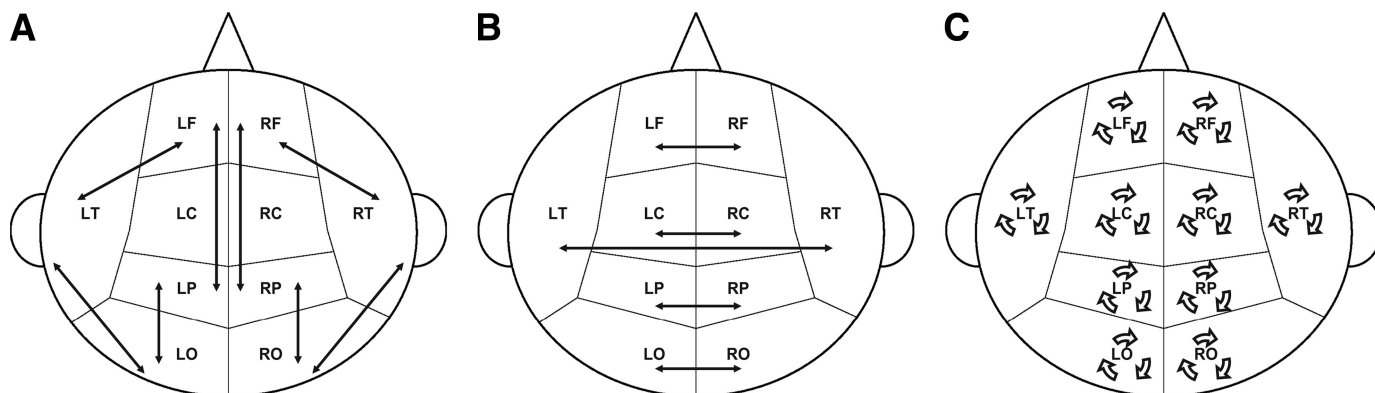
FIG. 2. Schematic brain, with left and right frontal (LF and RF, respectively), central (LC and RC, respectively), parietal (RP and LP, respectively), occipital (LO and RO, respectively), and temporal (LT and RT, respectively) areas indicated as abbreviations. *A* and *B*: Long-distance intrahemispheric pathways. Eight long-distance intrahemispheric pathways (*A*) and five long-distance interhemispheric pathways (*B*). *C*: Ten short-distance local hemispheric pathways.

TABLE 1
Patient characteristics

	Type 1 diabetes[+] patients ($n = 15$)	Type 1 diabetes[−] patients ($n = 29$)	Control subjects ($n = 26$)	P
Age (years)	44.2 ± 6.74	39.0 ± 8.76	36.7 ± 10.5	0.046
Sex (men/women)	6/9	16/13	16/10	0.950
Education level*	5.5 ± 2.1	5.7 ± 1.85	5.6 ± 1.45	0.953
Estimated IQ†	110.7 ± 10.44	105.3 ± 12.57	107.4 ± 11.67	0.366
A1C	7.7 ± 0.81	7.8 ± 0.91	5.4 ± 0.24	<0.001
Depressive symptoms‡	11.3 ± 9.04	7.5 ± 6.46	5.4 ± 0.96	0.027
Risk of major depression§ (%)	3 (20)	2 (7)	1 (4)	0.203
BMI (kg/m^2)	25.6 ± 5.89	24.8 ± 3.27	25.2 ± 3.95	0.853
Hypertension‖ (%)	10 (67)	9 (31)	—	0.030
Type 1 diabetes onset age (years)	9.7 ± 4.62	19.2 ± 10.03	—	<0.001
Type 1 diabetes disease duration (years)	34.5 ± 6.75	19.8 ± 8.86	—	<0.001
Type 1 diabetes early onset¶ (%)	3 (20)	3 (10)	—	0.394
Severe hypoglycemic events**	7.0 ± 7.76	4.8 ± 10.73	—	0.478
Blood glucose level before MEG (mmol/l)	9.9 ± 3.81	9.0 ± 4.74	—	0.549
Blood glucose level before NPA†† (mmol/l)	9.7 ± 4.41	9.0 ± 4.23	—	0.656
Daily insulin units injected (units per day)	50.3 ± 20.12	56.3 ± 20.01	—	0.361
Neuropathy‡‡ (%)	8 (53)	—	—	—
Nephropathy§§ (%)	2 (13)	—	—	—

Values are means ± SD or absolute numbers (%). *Education level measured with a Dutch scoring system from 1 to 8, with 1 representing unfinished primary school and 8 representing university at masters level. †Measured with the Dutch version of the National Adult Reading Test. ‡Depressive symptoms measured using the CES-D. §Risk of major depression was defined as a CES-D score of 16 or above. ‖Hypertension was defined as a systolic blood pressure of 140 mmHg or above, a diastolic blood pressure of 90 mmHg or above, or use of antihypertensive drugs. ¶Early disease onset is defined as onset below 7 years of age. **Averaged amount of self-reported severe hypoglycemic events per individual. Severe hypoglycemic events are those events for which the participants need others' assistance to cope with the effects of low blood glucose, loss of consciousness, or seizure. ††Neuropsychological assessment. ‡‡Neuropathy was based on physicians report. §§Nephropathy was based on a 24-h urine sample and defined as an ACR of 2.5 or above for men and 3.5 for women.

Statistical analysis. Differences between groups for demographical and medical variables were calculated using Student's t test for independent samples or one-way ANOVA with Tukey post hoc test (for continuous variables) and χ^2 test (for categorical variables).

To determine group differences in neuropsychological performance MANCOVA was used with all cognitive domains as dependent variables and group as independent variable. In case of a group difference on a domain, post hoc MANCOVA was used to determine which groups differed.

Synchronization likelihood data were normalized by means of a transformation LN10(x/[1-x]) (35) to allow the use of parametric statistical tests. To minimize statistical tests, for each MEG frequency band ANCOVA with repeated measures was used to determine group differences. For each frequency band, three ANCOVAs with repeated measures were performed for intra-, inter-, and local hemispheric connections. Repeated measures for intrahemispheric connections consisted of the 8 above-mentioned levels, interhemispheric connections of 5 levels, and local hemispheric connections of 10 levels. In case of significant interaction (P values of Greenhouse-Geisser correction for degrees of freedom) or main effects of group with frequency band, post hoc MANCOVA analysis was used to determine effects of group and spatial location. To determine associations between cognitive functioning and synchronization likelihood, a procedure proposed by Stoffers et al. and used in other MEG research was implemented (11,12). To reduce multiple comparisons, cognitive domains were added as covariates to the above-mentioned ANCOVA with repeated measures method. Effects of group on changing cognitive domains and MEG regions were determined using MANCOVA tests. Positive or negative associations were further calculated using regression analysis. To correct for possible confounding influences, all statistical tests were corrected for age, sex, hypertension, neuropathy, BMI, education level, and depressive symptoms.

Partial η^2 is reported as a proportion of the total variance explained of the determinant by the independent factor corrected for the used covariates. All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS, Chicago, IL).

RESULTS

Patient characteristics. Table 1 summarizes group characteristics. The control group was significantly younger than the type 1 diabetes[+] patients and had a significantly lower mean score of depressive symptoms. As expected, type 1 diabetes participants had a higher A1C than control subjects. Type 1 diabetes[+] patients had a significantly lower age of disease onset and longer disease duration.

Neuropsychological assessment. Compared with control subjects, both patient groups had a significantly lower information processing speed (type 1 diabetes[+]: $P = 0.003$, $\eta^2 = 0.242$; type 1 diabetes[−]: $P = 0.009$, $\eta^2 = 0.135$) and motor speed (type 1 diabetes[+]: $P < 0.001$, $\eta^2 = 0.311$; type 1 diabetes[−]: $P = 0.014$, $\eta^2 = 0.122$) as well as general cognitive ability (type 1 diabetes[+]: $P = 0.007$, $\eta^2 = 0.198$; type 1 diabetes[−]: $P = 0.004$, $\eta^2 = 0.165$). There were no statistically significant differences between the patient groups. The z scores are displayed in Fig. 3.

MEG measurements. MEG results are shown in Table 2 (with P values and η^2) and visually represented in Fig. 4. First, repeated-measures ANCOVA was performed for all frequency bands. Significant interaction effects with group were found for the θ band local ($P = 0.001$), lower α band local ($P = 0.004$), upper α band local ($P = 0.008$), β band interhemispheric ($P = 0.043$), and β band local ($P = 0.020$). Significant main effects of group were found for the lower α band intrahemispheric ($P = 0.028$), lower α band local hemispheric ($P = 0.005$), and upper α band local ($P = 0.042$). Post hoc MANCOVA analysis revealed significantly higher synchronization likelihood in the lower α right parietooccipital pathway for the type 1 diabetes[−] compared with the healthy control subjects. Comparing type 1 diabetes[+] patients with control subjects revealed significantly lower synchronization likelihood in the θ and lower α left and right central and parietal areas, in the upper α left frontal and right frontal, central, and parietal areas, and in the β interparietal, left parietal and right central, and parietal areas. Comparisons of type 1 diabetes[+] with type 1 diabetes[−] patients yielded lower synchro-
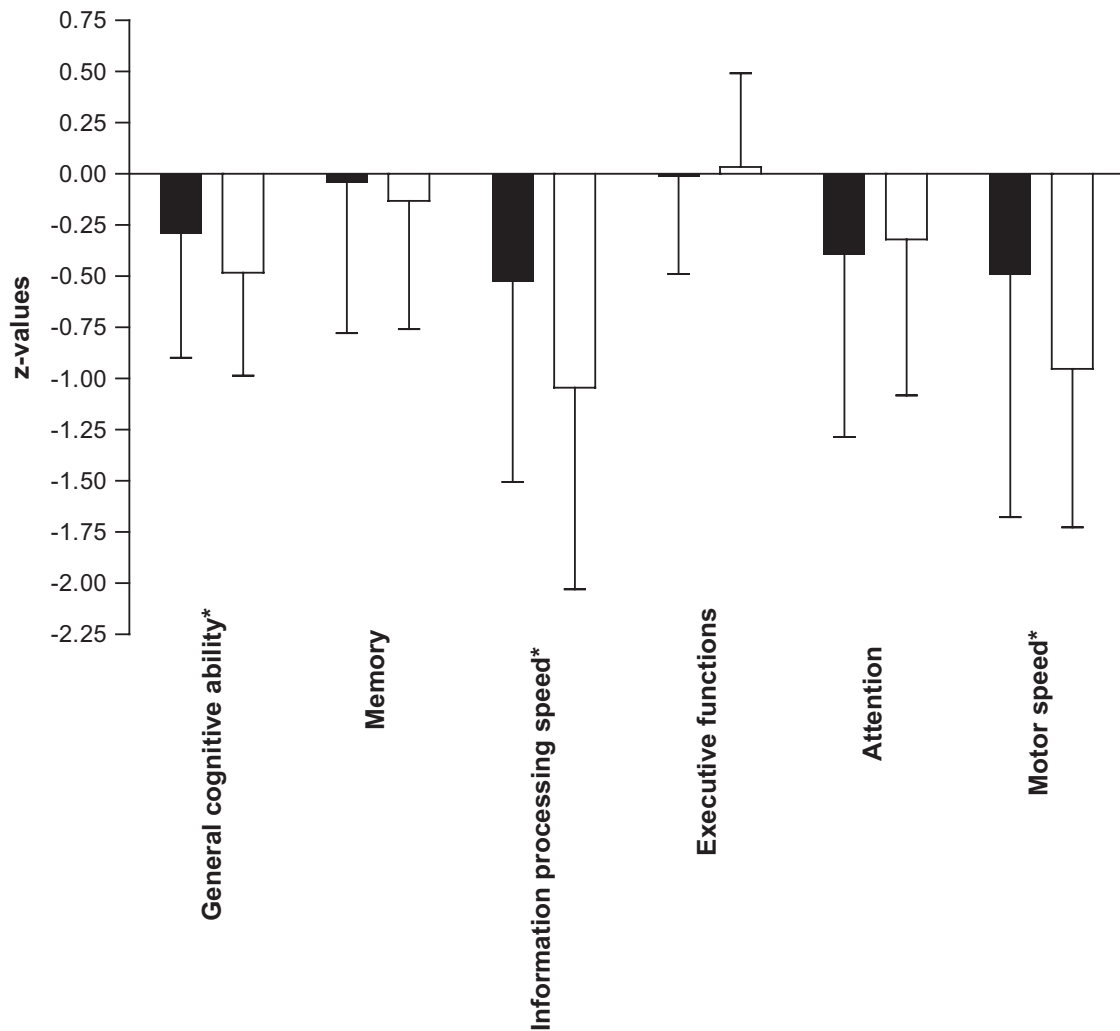
FIG. 3. Bar chart of all cognitive domains. The *x*-axis represents healthy control subjects. Black bars indicate type 1 diabetes[−] and white bars indicate type 1 diabetes[+] patients. *Z* values were created based on the means ± SD of the healthy control subjects. Asterisk indicates significant decline for type 1 diabetes[−] and type 1 diabetes[+] patients compared with healthy control subjects.

nization likelihood scores for type 1 diabetes[+] patients in the θ left and right central and parietal areas, in the lower α band left frontotemporal, left parietooccipital, right parietooccipital, and right temporooccipital pathways, in the left frontal and left and right central and parietal areas, upper α left parietal and right central and parietal areas and in the β-right parietal area. Anatomical locations are illustrated in Fig. 2.

To summarize, type 1 diabetes[+] patients showed lower synchronization likelihood in both higher and lower frequency bands compared with both type 1 diabetes[−] patients and control subjects. Type 1 diabetes[−] patients showed increased synchronization likelihood in the lower α band compared with control subjects. No statistically significant differences were found when the combined patient groups were compared with healthy control subjects. On the contrary, a significant difference in the Δ intrahemispheric region was observed (supplementary Table 1*A* and *B*, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0425/DC1).

Correlation between MEG and cognitive performance. To test for associations between functional connectivity in different MEG frequency bands and cognition,

MEG statistics were repeated with cognitive domains added as additional covariates for both type 1 diabetes groups. In type 1 diabetes[+] patients significant associations were found between the domain of executive functions and Δ intrahemispheric ($P = 0.041$), Δ interhemispheric ($P = 0.008$), and Δ local ($P = 0.043$) frequency band. Furthermore, Δ interhemispheric was related to the domains of memory ($P = 0.030$), information processing speed ($P = 0.040$), and motor speed ($P = 0.014$). The lower γ local frequency band was related to attention ($P = 0.047$). For executive functions only, significant relations with anatomical areas were found. In the type 1 diabetes[−] patients significant relations were found between attention and Δ intrahemispheric ($P = 0.048$), upper γ intrahemispheric ($P = 0.013$), and interhemispheric ($P = 0.043$). Motor speed was associated with Δ local ($P = 0.028$) and θ local ($P = 0.036$). Lastly, β intrahemispheric was associated with information processing speed ($P = 0.040$). Subsequently, regression analysis was used to determine positive or negative relations between synchronization likelihood and cognition. In both groups, an increase in synchronization likelihood was associated with better

TABLE 2
Raw synchronization likelihood values for significant group differences

	Type 1 diabetes[+] patients ($n = 15$)	Type 1 diabetes[−] patients ($n = 29$)	Control subjects ($n = 26$)	P	η^2
θ band (4–8 Hz)					
Left central	0.100 ± 0.016	0.113 ± 0.013		0.010	0.171
	0.100 ± 0.016		0.114 ± 0.017	0.003	0.237
Left parietal	0.182 ± 0.026	0.215 ± 0.034		0.008	0.182
	0.182 ± 0.026		0.214 ± 0.032	0.001	0.270
Right central	0.088 ± 0.009	0.100 ± 0.013		0.014	0.158
	0.088 ± 0.009		0.105 ± 0.018	0.003	0.243
Right parietal	0.147 ± 0.018	0.167 ± 0.022		0.015	0.153
	0.147 ± 0.018		0.169 ± 0.025	0.001	0.273
Lower α band (8–10 Hz)					
Left frontotemporal	0.031 ± 0.008	0.033 ± 0.010		0.042	0.110
Left parietooccipital	0.041 ± 0.010	0.047 ± 0.013		0.018	0.147
Left frontal	0.109 ± 0.026	0.118 ± 0.020		0.039	0.113
Left central	0.104 ± 0.019	0.124 ± 0.023		0.001	0.249
	0.104 ± 0.019		0.118 ± 0.020	0.027	0.140
Left parietal	0.192 ± 0.040	0.234 ± 0.045	0.226 ± 0.040	0.003	0.218
	0.192 ± 0.040			0.031	0.133
Right parietooccipital	0.037 ± 0.006	0.048 ± 0.018		0.004	0.209
		0.048 ± 0.018	0.040 ± 0.018	0.034	0.093
Right temporooccipital	0.028 ± 0.007	0.029 ± 0.005		0.050	0.102
Right central	0.091 ± 0.013	0.108 ± 0.018		0.005	0.196
	0.091 ± 0.013		0.109 ± 0.019	0.005	0.211
Right parietal	0.152 ± 0.026	0.187 ± 0.036		0.002	0.230
	0.152 ± 0.026		0.180 ± 0.033	0.005	0.212
Upper α band (10–13 Hz)					
Left frontal	0.100 ± 0.164		0.112 ± 0.025	0.034	0.129
Left parietal	0.192 ± 0.048	0.218 ± 0.038		0.038	0.114
Right frontal	0.117 ± 0.017		0.133 ± 0.020	0.011	0.179
Right central	0.089 ± 0.012	0.099 ± 0.013		0.035	0.118
	0.089 ± 0.012		0.102 ± 0.016	0.023	0.179
Right parietal	0.153 ± 0.027	0.177 ± 0.026		0.012	0.162
	0.153 ± 0.027		0.176 ± 0.020	0.004	0.227
β band (13–30 Hz)					
Interparietal	0.031 ± 0.012		0.036 ± 0.009	0.025	0.025
Left parietal	0.174 ± 0.034		0.199 ± 0.030	0.027	0.139
Right central	0.082 ± 0.011		0.090 ± 0.011	0.016	0.165
Right parietal	0.142 ± 0.025	0.157 ± 0.024		0.043	0.109
	0.142 ± 0.025		0.158 ± 0.021	0.016	0.163

Data are given as mean synchronization likelihood values per group ± SD. P value and η^2 (percentage of total variance explained by the determinant) are given.

performance on the related domains. Table 3 summarizes MANCOVA and regression tests.

DISCUSSION

This is, to the best of our knowledge, the first study using MEG to assess functional brain connectivity in type 1 diabetes. We showed that, compared with sex- and education-matched type 1 diabetes[−] patients and control subjects, type 1 diabetes[+] had decreased functional connectivity. This decrease was most profound in the left and right central and parietal areas in the θ, lower and upper α, and β frequency bands. Moreover, some intra- and interhemispheric differences were found in the lower α and β bands. In contrast, an increase in functional connectivity was found in the lower α band when comparing the type 1 diabetes[−] patients with control subjects. Several frequency bands were positively related to cognitive domains, indicating these domains to be dependent of functional connectivity. On cognitive assessment, type 1 diabetic patients with and those without proliferative retinopathy performed significantly poorer on information

processing speed, motor speed, and general cognitive ability. These deficits are in line with previous research (1,3,36). Results are in line with the hypothesis that type 1 diabetes[+] patients show cognitive and cerebral changes. Changes found in type 1 diabetes[−] patients were, however, not hypothesized. When both patient groups were pooled and compared with control subjects, cognitive results did not change; however, all differences in MEG measurements, but those in the Δ intrahemispheric band, lost statistical significance (supplementary Table 1*A* and *B*, available in an online appendix). This indicates that type 1 diabetes[−] patients largely resemble the control subjects regarding functional connectivity.

Given the novelty of the technique used, caution is required when it comes to the interpretation of our connectivity findings. As there is currently only one study available that uses our method in metabolic disease, it may be difficult to put the current findings into a broader perspective. In female obese adolescents, significant increases in functional connectivity in Δ and β frequency bands were found (13), possibly because of an increase in
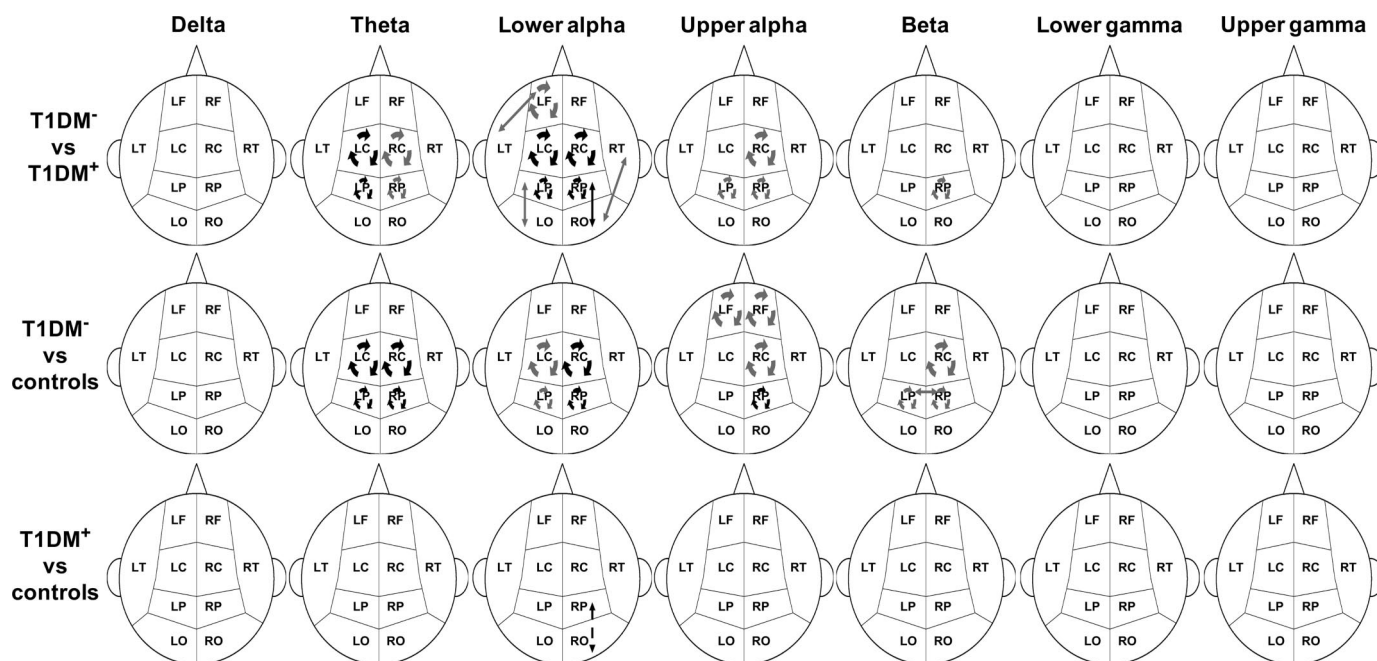
FIG. 4. Schematic brain with between-group differences in synchronization likelihood per frequency band. Black and gray indicate lower synchronization likelihood; black dashed arrows indicate higher synchronization likelihood. Gray circles indicate lower short-distance local hemispheric differences with $P < 0.05$; black circles indicate lower short-distance local hemispheric differences with $P < 0.01$; gray arrows indicate either lower long-distance intra- or interhemispheric differences with $P < 0.05$; black arrows indicate either lower long-distance intra- or interhemispheric differences with $P < 0.01$; black dashed arrow indicates higher long-distance intrahemispheric differences with $P < 0.05$. T1DM, type 1 diabetes.

white matter, which has been reported in MRI studies of obese adolescents. This is contrary to the decrease we found in our group. In contrast, in multiple sclerosis a decrease in functional connectivity was reported (37). This might be related to a loss of white matter, one of the core features of the disease. Following this line of reasoning, our results for the type 1 diabetes$^+$ patients could be a consequence of white matter loss, as has been found

earlier in type 1 diabetes$^+$ patients than type 1 diabetes$^-$ patients and healthy control subjects (5). Conversely, the increase in functional connectivity in type 1 diabetes$^-$ patients might reflect a compensatory mechanism, which fails in type 1 diabetes$^+$ patients. Because of large inter-individual differences in cognitive performance, this compensation was not observed in cognitive functioning. Therefore, larger samples are needed to enable the detec-

TABLE 3
Associations between cognition and MEG frequency bands for type 1 diabetes$^+$ and type 1 diabetes$^-$ patients

Frequency band	Cognitive domain	P	η^2	β
Type 1 diabetes$^+$				
Δ				
Right frontotemporal	Executive functions	0.039	0.478	0.205
Right temporooccipital	Executive functions	0.048	0.450	0.384
Inter frontal	Executive functions	0.037	0.486	0.421
Right frontal	Executive functions	0.029	0.559	0.498
Right occipital	Executive functions	0.034	0.498	0.550
Right temporal	Executive functions	0.020	0.560	0.453
Type 1 diabetes$^-$				
Δ				
Left central	Motor speed	<0.001	0.502	0.662
Left parietal	Motor speed	0.005	0.338	0.517
θ				
Left central	Motor speed	0.001	0.413	0.596
Left frontal	Motor speed	<0.001	0.502	0.736
Left parietal	Motor speed	0.038	0.198	0.391
Right parietal	Motor speed	0.030	0.215	0.414
β				
Left frontotemporal	Information processing speed	0.016	0.257	0.504
Upper γ				
Right frontotemoporal	Attention	0.036	0.202	0.398
Right parietooccipital	Attention	0.034	0.205	0.412
Right temporooccipital	Attention	0.048	0.182	0.393

tion of significant differences between type 1 diabetes^{+} and type 1 diabetes^{-} patients.

Importantly, we found a significant positive relationship between cognitive functioning and functional connectivity, suggesting changes in functional connectivity are involved in cognitive dysfunction in type 1 diabetic patients.

Our data support the hypothesis that cerebral changes are related to the presence of microvascular complications. However, we also found indications that cerebral and cognitive changes may be present before microvascular complications become apparent. It could be hypothesized that chronic hyperglycemia, even in the absence of clinically detectable microvascular complications, can negatively affect cognitive functioning and cerebral communication. It is known that hyperglycemia as such leads to a cascade of changes, including increases in formation of reactive oxygen species and advanced glycation end products (38,39), as well as activation of the hypothalamic-pituitary-adrenal axis (40), which could have an effect on the brain. Effects of hypoglycemia, particularly of asymptomatic hypoglycemic episodes because of hypoglycemia unawareness, cannot be completely ruled out as a contributor. Subsequently, we corrected for severe hypoglycemic events. This did not change the results (supplementary Table 2A–C, available in an online appendix).

Another possible contributor to the altered functional connectivity and cognitive functions observed in type 1 diabetic patients may be cerebrovascular, that is, macrovascular disease, the subclinical form of which can be estimated by ultrasound measurements of the carotid intima media thickness, which is known to be increased in asymptomatic type 1 diabetic patients relative to their nondiabetic peers (41). Furthermore, early diabetes onset, defined as disease onset before 7 years of age, has been shown previously to negatively affect intellectual and cognitive performance and cerebral structure (42,43).

Some limitations of this study should be mentioned. First, MEG provides a high temporal resolution. Unfortunately, the spatial resolution is lower. For a higher spatial resolution fMRI is currently the method of choice. Furthermore, the cognitive tests chosen had the highest sensitivity to detect cognitive changes. However, these tests may not be sufficiently sensitive to detect very subtle changes in type 1 diabetes–associated cognitive decline. Second, age of disease onset and subsequently disease duration differ between the patient groups. Although the percentages of early disease onset do not statistically differ between groups, for the type 1 diabetes^{+} patients age of onset reflects childhood and early adolescence, whereas for the type 1 diabetes^{-} patients it reflects late adolescence and early adulthood. In a subsequent analysis we compared both patient groups, additionally correcting for age of diabetes onset and diabetes duration (see supplementary Table 3A–C, available in an online appendix). Taking into account the risk of emerging power problems when adding two more covariates to this small sample, these adjustments did not essentially change the conclusions regarding cognitive functioning and functional connectivity. Disease duration might be less of a confounder here as the type 1 diabetes^{-} patients had an average disease duration of almost 20 years, which is sufficient for complications to develop. As stated before, an increase in functional connectivity was found for the lower α band in type 1 diabetes^{-} patients. Although the possibility of a statistical aberration cannot be completely ruled out, synchroniza-

tion likelihood tended to be higher for 20 of the 23 anatomical locations of the lower α band in type 1 diabetes^{-} patients than control subjects (supplementary Table 4, available in an online appendix). Moreover, we additionally repeated the post hoc MANCOVA for the lower α band correcting for multiple comparisons. This did not change the statistical significance of the parietooccipital pathway. Thus, these findings suggest that the significant increase detected is valid. As both age and depressive symptoms differed between groups, we statistically corrected for both variables in all analyses. Supplementary Table 5, available in an online appendix, summarizes the relation between age and depressive symptoms and cognitive and MEG variables. Third, A1C values over the time period preceding MEG recording and neuropsychological assessment unfortunately were not available.

In conclusion, this study showed that changes in functional brain connectivity and cognitive function exist in type 1 diabetic patients with and without microvascular complications. Furthermore, interactions between functional connectivity and cognitive domains have been demonstrated. Whether these abnormalities have clinical implications remains to be determined. Longitudinal research in larger samples is required to determine the predictive clinical value of our findings for the progression of cognitive deterioration in type 1 diabetic patients as well as its potential to serve as clinical outcome parameter.

REFERENCES

1. Brands AMA, Biessels GJ, de Haan EHF, Kappelle LJ, Kessels RPC. The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diabetes Care 2005;28:726–735
2. The Diabetes Control and Complications Trial Research Group. Effects of intensive diabetes therapy on neuropsychological function in adults in the diabetes control and complications trial. Ann Intern Med 1996;124:379–388
3. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group. Long-term effect of diabetes and its treatment on cognitive function. N Engl J Med 2007;356:1842–1852
4. Musen G, Lyoo IK, Sparks CR, Weinger K, Hwang J, Ryan CM, Jimerson DC, Hennen J, Renshaw PF, Jacobson AM. Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. Diabetes 2006;55:326–333
5. Wessels A, Rombouts S, Remijnse P, Boom Y, Scheltens P, Barkhof F, Heine R, Snoek F. Cognitive performance in type 1 diabetes patients is associated with cerebral white matter volume. Diabetologia 2007;50:1763–1769
6. Wessels A, Simsek S, Remijnse P, Veltman D, Biessels G, Barkhof F, Scheltens P, Snoek F, Heine R, Rombouts S. Voxel-based morphometry demonstrates reduced grey matter density on brain MRI in patients with diabetic retinopathy. Diabetologia 2006;49:2474–2480
7. Hyllienmark L, Maltez J, Dandenell A, Ludvigsson J, Brismar T. EEG abnormalities with and without relation to severe hypoglycaemia in adolescents with type 1 diabetes. Diabetologia 2005;48:412–419
8. Brismar T, Hyllienmark L, Ekberg K, Johansson BL. Loss of temporal lobe β power in young adults with type 1 diabetes mellitus. NeuroReport 2002;13:2469–2473
9. Wessels AM, Scheltens P, Barkhof F, Heine RJ. Hyperglycaemia as a determinant of cognitive decline in patients with type 1 diabetes. Eur J Pharmacol 2008;585:88–96
10. Stam CJ, Jones BF, Manshanden I, van Cappellen van Walsum AM, Montez T, Verbunt JPA, de Munck JC, van Dijk BW, Berendse HW, Scheltens P.

Magnetoencephalographic evaluation of resting-state functional connectivity in Alzheimer's disease. NeuroImage 2006;32:1335–1344

11. Stoffers D, Bosboom JLW, Deijen JB, Wolters EC, Stam CJ, Berendse HW. Increased cortico-cortical functional connectivity in early-stage Parkinson's disease: an MEG study. NeuroImage 2008;41:212–222

12. Bosma I, Douw L, Bartolomei F, Heimans JJ, van Dijk BW, Postma TJ, Stam CJ, Reijneveld JC, Klein M. Synchronized brain activity and neurocognitive function in patients with low-grade glioma: a magnetoencephalography study. Neuro-Oncology 10.1215/15228517-2008-034

13. Olde Dubbelink KTE, Felius A, Verbunt JPA, van Dijk BW, Berendse HW, Stam CJ, Delemarre-van de Waal HA. Increased resting-state functional connectivity in obese adolescents: a magnetoencephalographic pilot study. PLoS ONE 3:10.1371/journal.pone. 0002827

14. Tschritter O, Hennige A, Preissl H, Grichisch Y, Kirchhoff K, Kantartzis K, Machicao F, Fritsche A, Häring HU. Insulin effects on β and θ activity in the human brain are differentially affected by ageing. Diabetologia 2008;52:169–171

15. Timmermann L, Butz M, Gross J, Ploner M, Südmeyer M, Kircheis G, Häussinger D, Schnitzler A. Impaired cerebral oscillatory processing in hepatic encephalopathy. Clin Neurophysiol 2008;119:265–272

16. Aertsen AMHJ, Gerstein GL, Habib MK, Palm G. Dynamics of neuronal firing correlation: modulation of 'effective connectivity'. J Neurophysiol 1989;61:900–917

17. Lee L, Harrison LM, Mechelli A. A report of the functional connectivity workshop, Dusseldorf 2002. NeuroImage 2003;19:457–465

18. Bressler SL. Understanding cognition through large-scale cortical networks. Curr Dir Psychol Sci 2002;11:58–61

19. Ryan CM, Geckle MO, Orchard TJ. Cognitive efficiency declines over time in adults with Type 1 diabetes: effects of micro- and macrovascular complications. Diabetologia 2003;46:940–948

20. Aldington SJ, Kohner EM, Meuer S, Klein R, Sjølie AK, Group Eurodiab Iddm CS. Methodology for retinal photography and assessment of diabetic retinopathy: the EURODIAB IDDM Complications Study. Diabetologia 1995;38:437–444

21. Brands AMA, Kessels RPC, Hoogma RPLM, Henselmans JML, van der Beek Boter JW, Kappelle LJ, de Haan EHF, Biessels GJ. Cognitive performance, psychological well-being, and brain magnetic resonance imaging in older patients with type 1 diabetes. Diabetes 2006;55:1800–1806

22. Lezak M. *Neuropsychological Assessment*. Oxford, Oxford University Press, 2004

23. Klein M, Postma TJ, Taphoorn MJB, Aaronson NK, Vandertop WP, Muller M, van der Ploeg HM, Heimans JJ. The prognostic value of cognitive functioning in the survival of patients with high-grade glioma. Neurology 2003;61:1796–1798

24. Saan RJ, Deelman BG. New version of 15 words test (15WTA and 15WTB). In *Neuropsychological Diagnostics Handbook*. Bauma AMJ, Lindeboom J, Eds. Amsterdam: Swets & Zeitlinger, 1986

25. Wechsler D. *Wechsler Adult Intelligence Scale Manual*. New York, The Psychological Cooperation, 1955

26. Golden CJ. Stroop Colour Word Test. In *A Manual for Clinical End Experimental Uses*. Golden CJE, Ed. Chicago, Stoelting, 1978

27. Van der Elst W, Van Boxtel MPJ, Van Breukelen GJP, Jolles J. The concept shifting test: adult normative data. Psychol Assess 2006;18:424–432

28. Alpherts WCJ, Aldenkamp AP. Computerized neuropsychological assessment of cognitive functioning in children with epilepsy. Epilepsia 1990;31:S35–S40

29. Brickenkamp R. *Aufmerksamkeits-Belastungs-Test (Test D2). [The d2 test of Attention.]*. Göttingen, Hogrefe, 1962

30. Heaton RK, Chelune GJ, Talley JL, Kay GG, Curtiss G. *Wisconsin Cart Sorting Test Manual: revised and expanded (WCTS)*. Odessa, FL, Psychological Assessment Resources (PAR), 1993

31. Thurstone LL, Thurstone TG. *Primary Mental Abilities*. Chicago, University of Chicago Press, 1962

32. Vrba J, Anderson G, Betts K. 151-Channel whole-cortex MEG system for seated or supine positions. In *Recent Advances in Biomagnetism*. Yoshimoto T, Kotani M, Kuriki S, et al., Ed. Sendai, Japan, Tohoku University Press, 1999

33. Stam CJ, van Dijk BW. Synchronization likelihood: an unbiased measure of generalized synchronization in multivariate data sets. Physica, D 2002;163:236–241

34. Montez T, Linkenkaer-Hansen K, van Dijk BW, Stam CJ. Synchronization likelihood with explicit time-frequency priors. NeuroImage 2006;33:1117–1125

35. Gasser T, Bächer P, Möcks J. Transformations towards the normal distribution of broad band spectral parameters of the EEG. Electroencephal Clin Neurophysiol 1982;53:119–124

36. Biessels GJ, Deary IJ, Ryan CM. Cognition and diabetes: a lifespan perspective. The Lancet Neurology 2008;7:184–190

37. Cover KS, Vrenken H, Geurts JJG, van Oosten BW, Jelles B, Polman CH, Stam CJ, van Dijk BW. Multiple sclerosis patients show a highly significant decrease in α band interhemispheric synchronization measured using MEG. NeuroImage 2006;29:783–788

38. Beisswenger PJ, Makita Z, Curphey TJ, Moore LL, Jean S, Brinck-Johnsen T, Bucala R, Vlassara H. Formation of immunochemical advanced glycosylation end products precedes and correlates with early manifestations of renal and retinal disease in diabetes. Diabetes 1995;44:824–829

39. Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. Endocr Rev 10.1210/er.2007-0034

40. Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. Nat Neurosci 2008;11:309–317

41. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. N Engl J Med 2003;348:2294–2303

42. Ferguson SC, Blane A, Wardlaw J, Frier BM, Perros P, McCrimmon RJ, Deary IJ. Influence of an early-onset age of type 1 diabetes on cerebral structure and cognitive function. Diabetes Care 2005;28:1431–1437

43. Perantie DC, Lim A, Wu J, Weaver P, Warren SL, Sadler M, White NH, Hershey T. Effects of prior hypoglycemia and hyperglycemia on cognition in children with type 1 diabetes mellitus. Pediatric Diabetes 2008;9:87–95