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ORIGINAL ARTICLE

Type-1 diabetes shapes working memory processing strategies

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Summary
Background. — Type 1 diabetes (T1D) is a metabolic disorder characterized by recurrent hypo- and hyperglycemic episodes, whose clinical development has been associated with cognitive and working memory (WM) deficits.
Objective. — To contrast quantitative electroencephalography (qEEG) measures between young patients with T1D and healthy controls while performing a visuospatial WM task with two memory load levels and facial emotional stimuli.
Methods. — Four or five neutral or happy faces were sequentially and pseudo-randomly presented in different spatial locations, followed by subsequent sequences displaying the reversed spatial order or any other. Participants were instructed to discriminate between these two alternatives during EEG recording.
Results. — A significant increase in the absolute power of the delta and theta bands, distributed mainly over the frontal region was found during task execution, with a slight decrease of alpha band power in both groups but mainly in control individuals. However, these changes were more pronounced in the T1D patients, and reached their maximum level during the WM encoding phase, even on trials with the lower memory load. In contrast, changes seemed to occur more gradually in controls and results differed significantly only on the trials with the higher WM load.
Conclusions. — These results reflect adaptive WM-processing mechanisms in which cognitive strategies have evolved in T1D patients in order to meet task demands.

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Introduction

Type 1 diabetes mellitus (T1D) is a disease in which the beta cells of the pancreatic islets, which produce insulin, are attacked and destroyed by the immune system [2]. Insulin plays an important role in metabolizing glucose and, therefore, has an essential function in regulating the energy supply of the brain, an organ that consumes about 25% of the glucose available in the body [47]. As a result, T1D is characterized by recurrent hypo- and hyperglycemic episodes that have a negative impact on the brain. These glycemic fluctuations have also been related to cognitive deficits that affect executive functions [23], especially working memory [19,59].

Executive functions play a pivotal role in goal-directed behavior. One core executive function is working memory (WM), which operates by holding information in the mind and mentally working with it [4]. WM can be sub-divided into several main processing phases:

- initial encoding of information;
- subsequent maintenance and retrieval of WM items [57].

Encoding and maintaining visuospatial information in WM have been associated with increased recruitment of attention-associated brain areas, including parietal and frontal cortices [20,44]. However, when dealing with different WM load levels, it seems that prefrontal cortex activity varies depending on the task phase [45]. In general, there is agreement on the fact that WM is necessary for language comprehension, translating instructions, incorporating new information into action plans, reasoning, creativity, and making plans and decisions [16]. Despite the growing literature on WM development, its processing mechanisms and primary neurofunctional substrates, the precise mechanisms underlying WM impairments are still poorly understood.

One of the problems linking T1D to WM deficits is that most of the studies approaching this issue have focused on the consequences of glycemic level extremes, often inducing hypoglycemia to evaluate its effects on control participants [1,9,24,59]. As expected, both patients with T1D and control individuals are usually affected by induced hypoglycemia in these studies, though the poorer cognitive performance of T1D patients is emphasized. In contrast, Bolo and colleagues [9] failed to find significant differences between patients and controls on accuracy or reaction times while performing a WM task under euglycemic or hypoglycemic conditions. These authors also reported that brain regions with a supplementary role in WM function, such as the parietal lobe and cerebellum, presented greater fMRI-BOLD activation in patients during hypoglycemia, suggesting that these areas may have been recruited to help maintain cognitive performance.

Other approaches, which did not involve inducing hypoglycemia, have suggested that young adults with T1D—but with average IQ and without comorbidities—already show neural processing that is distinct from that of their peers without diabetes [18,22]. In this regard, Gallardo-Moreno and collaborators [22] argued that the early impact of this disease during neurodevelopment could lead to functional adaptive changes as means to maintain cognitive performance, despite the occurrence of glycemic level extremes. Similar to Bolo and colleagues [9], they found no statistically-significant differences in the behavioral performance between T1D patients and healthy matched controls while performing a visuospatial WM task. However, their patients showed greater fMRI activations in the inferior frontal gyrus, basal ganglia, substantia nigra, and cerebellum. These subcortical activations, in addition to the cortical activations usually required for WM processing, were interpreted as reflecting a compensation mechanism that allowed those patients to maintain a behavioral performance similar to that of controls. In accordance with these results, and applying the same methodology, but in an effective connectivity study, Guàrdia-Olmos and colleagues [28] reported that T1D patients showed a pattern of brain connectivity distinct from that of healthy controls. In the T1D group, fewer brain areas were involved in the WM task and there was a notable lack of involvement of the frontal cortical areas, while the red nucleus and cerebellum showed strong connectivity with the core WM network.

Embury and colleagues [18] also focused on young patients without comorbid conditions and evaluated them while in the normoglycemic range. They argued that this approach would make it possible to reach stronger conclusions concerning the unique impact of diabetes on brain physiology. Interestingly, they reported no between-group differences on accuracy rates on a Sternberg-type verbal WM task performed during a magnetoencephalography (MEG) study. However, they did find that the T1D patients exhibited stronger responses in the superior parietal area during encoding and diminished alpha response in the parieto-occipital cortices during the maintenance phase. These authors suggested that this pattern of neural dynamics in their young patients may reflect a compensatory strategy for decreased neural resources or efficiency by using other network resources, and that this compensation was similar to that observed in an aging population. Another key point for research in this area consists of examining the different phases of WM processing (encoding vs. maintenance) that allows more subtle between-group differences to be identified.

While fMRI studies have provided valuable information on specific brain pattern differences between patients with T1D and healthy controls during WM tasks [9,22], delineating the more active brain areas during each WM processing phase has been more difficult due to the temporal limitations of this technique. Electroencephalography (EEG), given its high accuracy measured in milliseconds, might in contrast reflect differences in both phases of WM processing; that is, encoding and maintenance. Although Embury et al. [18] studied this issue using MEG in T1D patients, to the best of our knowledge, no studies have yet addressed the effects of emotional distractors on WM performance in these patients using an electrophysiological approach.

It is well-known that emotional events influence WM, as occurs in daily life [36,46], and there is compelling evidence that processes such as attention, decision-making, cognitive control, and WM all play central roles in emotion, and that cognition and emotion dynamically influence each other in ways that contribute to adaptive behavior [17,50]. Understanding the mechanisms underlying these

reciprocal interactions is critical for comprehending the fundamentals of healthy functioning. For instance, the stress caused by glycemic dysregulation, daily insulin injections, a special diet and regular medical checkups in T1D patients could interact with cognitive deficits reported in this population, particularly those affecting executive functions, which potentially play a key role in self-management and treatment adherence [11].

Recently, our group explored WM processing in T1D patients while manipulating emotional stimuli through fMRI methods, using a 2-back task detecting facial identity in neutral, happy or fearful faces [25]. Significant differences were found between the neurofunctional activation patterns of T1D patients and healthy controls, particularly characterized by greater prefrontal activation in patients when processing emotional faces, while controls exhibited more activation in parietal regions. The observed group differences were interpreted as the result of a specific interference effect of the emotional content of the stimuli on WM's cognitive control in the patient group. Such interference could elicit attention allocation difficulties and decrease the operational efficiency of WM. In the light of these findings, two additional issues should be further clarified in this clinical population: the effect of varying levels of working memory load, and the sensitivity of distinct stages of WM processing in the time domain.

Jin and colleagues [36] found significant effects of emotional content on WM task performance and ERP correlates with encoding, maintenance and retrieval processes. Specifically, they reported that positive emotions improved WM performance by facilitating maintenance and retrieval processing, while negative content exhibited an enhancement effect only during encoding and maintenance. Guimond and colleagues [29], meanwhile, examined how happy and fearful emotional distractors impact performance on WM tasks by varying the cognitive load using the fMRI technique. They found that patients with schizophrenia had greater difficulty in performing a WM task than healthy controls when regulating emotions, and suggested that the increase in WM load may have placed a high demand on subjects' executive networks and interfered with their ability to regulate emotional distractors. These findings led us to hypothesize that this could also occur in patients with T1D. We agree with Embury and colleagues' hypothesis [18] that the compensatory strategies observed in aging are likely to also be found in T1D patients. Moreover, Hou and colleagues [32] recorded EEGs from healthy adults (young and seniors) under three different conditions: resting state, 0-back task, and 2-back task. They found that age-related disturbances were more prominent during the high-load task, especially in the theta band, and suggested that this band plays an integrative role on WM tasks that could be attributed to compensatory activation in aging. Therefore, it seems that the study of different memory loads could reflect subtle differences in brain dynamics between T1D patients and healthy participants. In our view, the approach used by Guimond and colleagues [29] and Hou and colleagues [32] to explore WM disturbances in this population merits further evaluation. Accordingly, the main aim of the present study was to comparatively explore quantitative EEG measures during performance of a visuospatial WM task in T1D patients and controls, using neutral and happy faces as stimuli, with the intention of maximizing their attractiveness, in the context of two different WM load levels. Based on previous studies, we hypothesize that the encoding stage of WM processing in T1D patients will be more susceptible to the impact of highly attention-appealing stimuli, particularly when WM load increases.

With the above mentioned purpose, we set out to evaluate the effect of WM load and two highly attentional-distracting stimuli on qEEG measures while T1D patients and controls performed visuospatial WM tasks (distinguishing encoding and maintenance/retrieval phases), and compare those results to a task-preceding resting period.

Table 1 Demographic and clinical characteristics of study participants.

	Controls	T1D patients
n	18	18
Age (years)	21.61 (3.94)	22.22 (4.08)
Sex (men/women)	11/7	11/7
Education (years)	12.17 (2.43)	11.53 (2.47)
IQ	111.80 (7.41)	106.06 (10.04)
HbA_{1c} (%)	—	8.64 (1.72)
Last fasting plasma glucose	—	132.72(48.42)
Plasma glucose (before EEG recording)	87.83 (9.31)	237.11 (106.45)

Data are means (standard deviations). *n*: number of cases; HbA_{1c}: glycated hemoglobin.

Methods

Participants

Individuals diagnosed with T1D were recruited from two associations that specialize in treating diabetes mellitus: the Endocrinology Services of the *Centro Médico Nacional de Occidente* and the *Fray Antonio Alcalde* Hospital. The sample consisted of 18 T1D patients with at least 8 years of disease evolution who reported adequate adherence to treatment and optimal daily glucose control, and 18 healthy control individuals matched according to gender, age and educational level. All participants were right-handed. Their age range was 14—26 years, and they had completed at least 9 years of schooling. The T1D patients (11 males) had a mean age of 21.61 ($\pm$ 3.94) years (mean schooling: 11.53 $\pm$ 2.47 years), while the matched controls had a mean age of 22.22 ($\pm$ 4.08) years (mean schooling: 12.17 $\pm$ 2.43 years). Table 1 shows the demographic characteristics of the study participants.

All participants had an average IQ (90—110; Wechsler Adult Intelligence Scale: WAIS-IV), and had no antecedents of neurodevelopmental, neurological or psychiatric disorders. According to medical records, the T1D patients had no hospital admissions due to diabetes in the 2 years prior to study participation, nor any diabetic complications (retinopathy, nephropathy or neuropathy). Informed written consent was obtained from participants or their parents, in the case of minors. The experimental protocol was approved

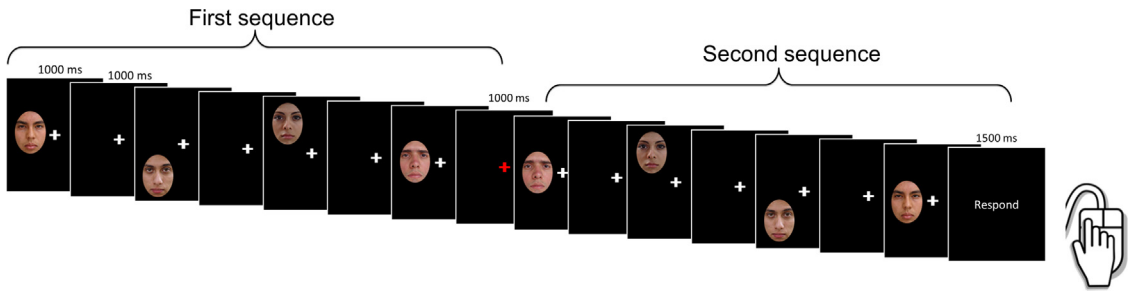

Figure 1 Example of a sequence with a correct reversed order in the spatial location of the stimuli presentation. Each stimulus lasted 1000 ms on the screen with variable inter-stimulus intervals and a response time of 1500 ms.

by the Ethics Committee of the Neuroscience Institute (ET112016-226; *Universidad de Guadalajara*).

Experimental task

Participants performed a visuospatial working memory task with simultaneous EEG recording. Stimulus delivery and response collection were controlled by E-prime Studio 2.0 software. During experimental sessions, which were always scheduled in the morning after having a regular breakfast, participants were seated comfortably in a quiet, dimly-lit room, 60 cm away from a LCD monitor. They were instructed to focus their attention on a fixation point located in the center of the screen in order to minimize head movements. The experimental task used neutral (N) and happy (H) faces as stimuli, which were presented sequentially and pseudo-randomly at eight different screen locations. After a short delay of 1000 ms, a second sequence was presented, and subjects were instructed to press the left button of the mouse if the latter sequence corresponded to the correct inversed spatial order of the first one, but the right button if it did not. Fig. 1 shows the experimental flow chart. The experiment consisted of a total of 120 trials divided into three blocks of 40 sequences each, including 60 trials with four stimuli and the other 60 with five, both counterbalanced across the blocks. Each stimulus lasted 1000 ms with an inter-stimuli interval of 1000 ms. The presentation order of the blocks was pseudo-randomized.

Recognition of the emotional content of the faces used in this task was previously validated in a group of university students with the same age range as the participants.

Electrophysiological recordings

EEGs were recorded from 21 scalp electrodes placed in accordance with the International 10—20 system (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2) and two periocular electrodes (EOG), all referenced to linked earlobes. A 32-channel electroencephalograph (MEDICID-04 system) with low and high cut filters set at 0.3 and 30 Hz, respectively, was used. Interelectrode impedance was kept below 5 kΩ. EEG data were digitized at a sampling rate of 200 Hz.

Artifact-free, 1000-ms EEG epochs were selected by visual inspection in three different main conditions:

- resting EEG, which corresponded to a resting period preceding the task during which subjects simply watched a fixation point displayed in the center of a black background for three minutes;
- visuospatial WM encoding, that is, EEG epochs beginning at the instant when the final stimulus of the first series was presented (fourth in the shorter series, fifth in the longer ones);
- visuospatial WM maintenance, that is, EEG epochs starting at the instant when the final stimulus of the second series was presented (fourth in the shorter series, fifth in the longer ones).

For practical purposes, from now on we will refer to these phases as ''visuospatial encoding'' and ''visuospatial maintenance''. All EEG epochs selected during these two phases corresponded only to trials with correct responses and the baseline was corrected using each subject's resting state average.

Artifact rejection

In order to achieve the maximum number of artifact-free EEG epochs per condition, the blind source separation (BSS) technique was employed to remove artifacts, which were mainly ocular, according to the methodology presented in Romo-Vazquez and colleagues [56].

First, any trial with EOG voltage deflection greater than 50 μV (relative to baseline) was discarded. Then, an expert reviewed each EEG recording rejecting the epochs with artifacts by visual inspection. Subsequently, EEG segments (20 seconds in length) containing the recordings of interest at the center of the epoch were used for further artifact rejection. Only the source corresponding to the ocular artifact was eliminated. Once it was removed, the entire record was rebuilt. Only 11.75% of the data had to be transformed using the BSS-SOBIRO algorithm [5] due to the presence of ocular artifacts. In addition, 2% of the total EEG channels were lost, so they were removed from the analysis and replaced by the average information for the group involved. Although similar studies have replaced the information from lost channels with their contralateral measures from the same patient [10,34][e.g. 10,34], we did not employ this method because our aim was to analyze EEG changes associated with cognitive performance, so replacing the information on one side with the data recorded in

Table 2 Behavioral results while performing the experimental task.

		Lower WM load		Higher WM load	
		Happy	Neutral	Happy	Neutral
Correct responses (%)	Patients	80.74 (13.9)	81.85 (14.9)	80.37 (13.4)	76.30 (15.0)
	Controls	89.26 (10.6)	91.67 (7.2)	87.96 (8.8)	82.41 (12.4)
Response times (ms)	Patients	541.62 (76.7)	535.52 (86.8)	556.53 (80.9)	550.05 (114.2)
	Controls	466.63 (131.3)	458.36 (130.1)	489.25 (137.0)	470.02 (124.2)

Data are means (standard deviations).

its contralateral homologous side could have affected the interpretation of results.

Data analysis

All analyses were primarily designed to comparatively evaluate two key effects in T1D patients and healthy controls, as follows:

- the impact of task performance on quantitative EEG measures (absolute power in different frequency bands: delta, theta, alpha and beta);
- the impact of memory load while using complex emotional facial stimuli [shorter versus longer sequences; i.e. lower (LL) versus higher (HL) memory load trials].

The analysis of the particular effects of the emotional content of the stimuli lies beyond the scope of the present study; nonetheless their potential impact on behavioral performance was properly tested.

Due to the comprehensive differences in the blood glucose levels between T1D patients and controls prior to the EEG recording, Pearson correlation analyses were performed with the purpose of exploring this effect on qEEG measures in both groups.

Behavioral analysis

Behavioral responses were computed for both groups and analyzed using SPSS (IBM Corp., 2016 release). Analysis of variance (ANOVA) was performed using Group (2: patients, controls) as the between-group factor, with WM load (2: LL: shorter sequences; HL: longer sequences) and Emotion [2: (H: trials with happy faces), (N: trials with neutral faces)] as the within-subject factors, along with the percentage of correct responses and response times as dependent variables.

Quantitative EEGs

Thirty EEG artifact-free epochs corresponding to correct responses were selected from each subject and condition, while balancing their distribution into three identical blocks of the experimental stream of stimuli (40 sequences each) to avoid confounding effects like initial behavioral adjustments, the settlement of cognitive strategies, fatigue, etc. For each EEG epoch selected, the power spectrum was calculated using Fast Fourier Transformation (FFT). The absolute energy of the frequency bands—δ (1—3 Hz), θ (4—7 Hz), α (8—13 Hz) and β (14—30 Hz)—was estimated at each one of the 19 electrode sites. To reduce individual data variability, we analyzed the median of the energy for each frequency band by condition (i.e. resting, visuospatial encoding, visuospatial maintenance, and two WM load levels), in each subject.

For topographic purposes, and due to the important role attributed to the frontal (particularly prefrontal) brain region when processing information in WM, EEG power spectral measures were evaluated in two main extensive regions: frontal (Fp1, Fp2, F3, F4, F7, F8 and Fz), and posterior (O1, O2, P3, P4, Pz, T5 and T6). First, resting qEEG measures were analyzed using a Repeated Measure ANOVA with Frequency Band (4 levels: delta, theta, alpha, beta) and Group (2 levels: T1D, Controls) as factors. Subsequently, RM-ANOVAs were executed to analyze qEEG while performing the task using three within-subject factors: Frequency Band (delta, theta, alpha, beta), Task Phase (Encoding, Maintenance), and WM load (lower: LL, higher: HL), along with Group (T1D, Controls) as the between-subject factor. All calculations were performed considering an alpha value of 0.05. Greenhouse-Geisser corrections to the *df* were applied as needed, with the corrected probabilities reported. Additionally, post hoc tests were calculated to explore any trends in the observed changes using Bonferroni adjustments for multiple comparisons.

Results

Behavioral results

The T1D patients achieved a significantly lower number of correct responses than controls ($F_{(1,34)} = 4.67$; $P = 0.038$) with prolonged reaction times in all conditions ($F_{(1,34)} = 4.47$; $P = 0.042$). The complete behavioral results are summarized in Table 2. In addition, WM load influenced the number of correct responses, as participants had a significantly lower number of correct responses during the trials with the higher WM load ($F_{(1,34)} = 13.52$; $P = 0.001$). In fact, higher WM load level did not affect the number of correct responses when trials with happy facial expressions were evaluated. The statistics on the behavioral results are shown in Table 3.

Electrophysiological results

Quantitative resting phase EEG measures for anterior and posterior brain regions failed to show significant differ-

Table 3 Summary results of the ANOVA for behavioral results.

		F	*df*	*P*	η^2	*1-*β
Correct responses	Load	13.520	1,34	0.001	0.285	0.946
	Emotion	3.420	1,34	0.073	0.091	0.435
	Group	4.667	1,34	0.038	0.121	0.555
	Load*Group	1.066	1,34	0.309	0.030	0.171
	Emotion*Group	0.003	1,34	0.956	0.000	0.050
	Load*Emotion	8.673	1,34	0.006	0.203	0.816
	Load*Emotion*Group	0.387	1,34	0.538	0.011	0.093
Response Times	Load	3.816	1,34	0.059	0.101	0.475
	Emotion	1.839	1,34	0.184	0.051	0.261
	Group	4.466	1,34	0.042	0.116	0.537
	Load*Group	0.022	1,34	0.883	0.001	0.052
	Emotion*Group	0.255	1,34	0.617	0.007	0.078
	Load*Emotion	0.250	1,34	0.621	0.007	0.077
	Load*Emotion*Group	0.218	1,34	0.643	0.006	0.074

F: Snedecor's F statistic; *P*: statistical significance; *df*: degrees of freedom; η^2: effect size; *1-*β: statistical power.

ences between the groups in both the overall power and in the individual frequency bands. Even though we did not observe significant behavioral results for the emotional facial stimuli, we decided to analyze its possible influence on qEEG. However, no significant effects were found. We did observe that the spectral power measurements tended to be lower in T1D than controls, but this tendency reversed during task performing periods, thus probably contributing to the final outcomes.

While performing the task, the results for the anterior brain region revealed significant effects for the factors Frequency Band ($F_{(3,102)} = 24.56$, $P < 0.001$, $\eta^2 = 0.419$, *1-*$\beta = 1.00$) and WM load ($F_{(1,34)} = 15.69$, $P < 0.001$, $\eta^2 = 0.316$, *1-*$\beta = 0.97$). There was a significant interaction between Frequency Band and Group ($F_{(3,102)} = 4.39$, $P = 0.006$, $\eta^2 = 0.114$, *1-*$\beta = 0.861$), indicating that delta and theta powers were significantly higher in T1D patients, while alpha power was significantly lower in controls. Figs. 2 and 3 show the spatial representation of the absolute power for delta and theta frequency bands, respectively. Besides, the significant interaction between Frequency Band and Task Phase ($F_{(3,102)} = 4.07$, $P = 0.009$, $\eta^2 = 0.107$, *1-*$\beta = 0.832$) showed a relevant decrease of beta power from encoding to maintenance in WM. An additional relevant interaction between Frequency Band and WM load ($F_{(3,102)} = 29.06$, $P < 0.001$, $\eta^2 = 0.461$, *1-*$\beta = 1.00$) revealed that delta, theta, and beta powers significantly increased during higher WM load trials, with delta showing the highest increment ($P < 0.001$). The significant interaction between Task Phase and WM load ($F_{(1,34)} = 17.48$, $p < 0.001$, $\eta^2 = 0.340$, *1-*$\beta = 0.982$) denoted that main changes between Task Phases occurred during LL ($P < 0.001$), even though there were also significant changes during HL ($P = 0.04$). Finally, a significant interaction was found between Frequency Band, Task

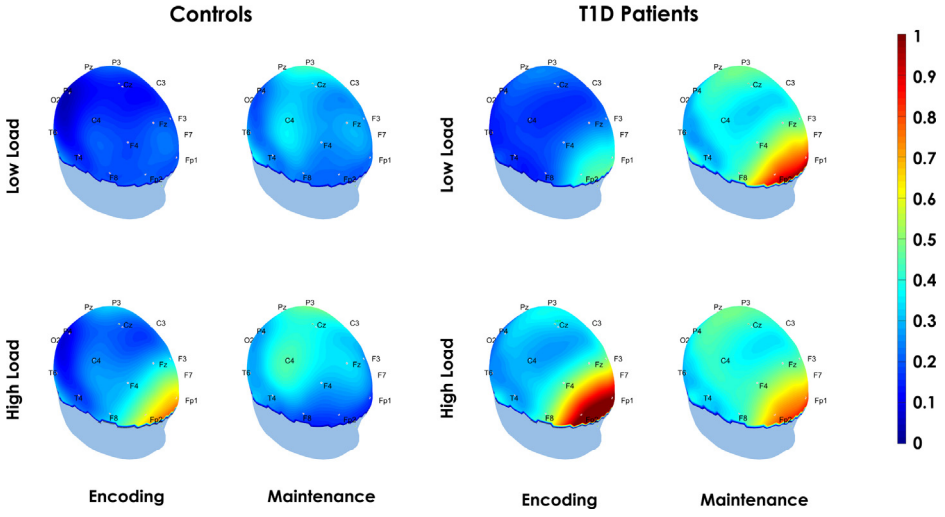

Figure 2 Spatial representation of the absolute power of the delta band—normalized with respect to the energy measured during resting in the same group—in the two main experimental visuospatial WM phases and WM load levels, for both T1D patients and controls.

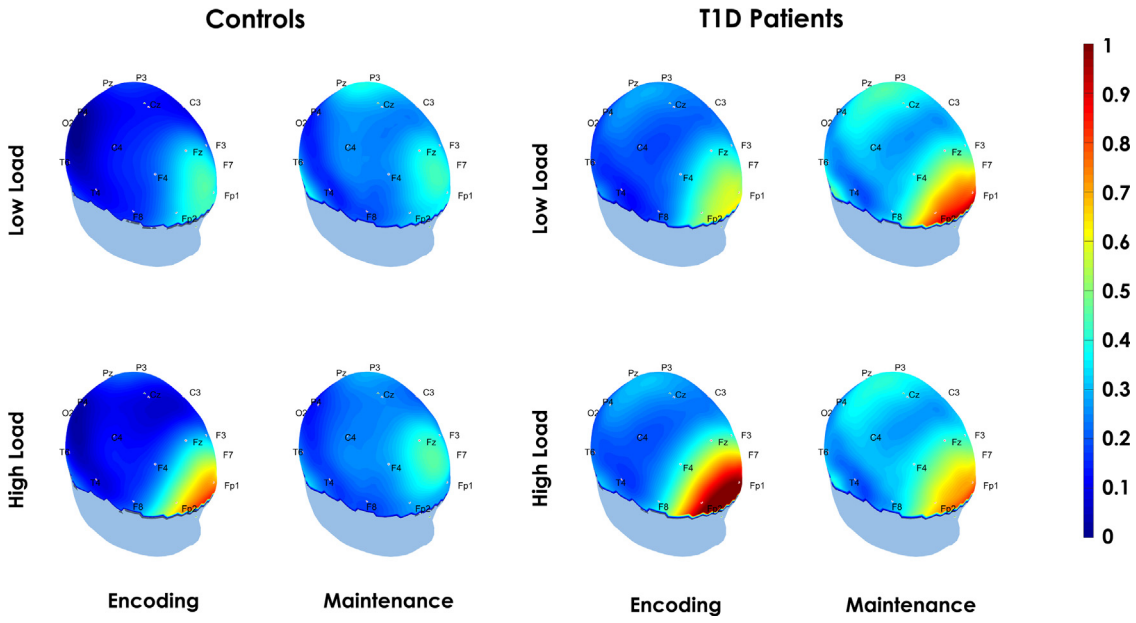

Figure 3 Spatial representation of the absolute power of the theta band—normalized with respect to the energy measured during resting in the same group—in the two main experimental visuospatial WM phases and WM load levels, for both T1D patients and controls.

Phase and WM load ($F_{(3,102)} = 14.81$, $P < 0.001$, $\eta^2 = 0.303$, *1-*$\beta = 1.00$), reinforcing the notion that delta and theta powers significantly varied during LL in both Task Phases ($P < 0.001$, and $P = 0.04$, respectively), while significant variations on alpha and beta powers were restricted to HL ($P = 0.011$, and $P = 0.003$, respectively).

On the other hand, statistical analysis of the posterior region showed significant effects for Frequency Band ($F_{(3,102)} = 34.31$, $P < 0.001$, $\eta^2 = 0.502$, *1-*$\beta = 1.00$) and Task Phase ($F_{(1,34)} = 25.43$, $p < 0.001$, $\eta^2 = 0.428$, *1-*$\beta = 0.998$), with significant interactions between Frequency Band and Task Phase ($F_{(3,102)} = 47.71$, $P < 0.001$, $\eta^2 = 0.584$, *1-*$\beta = 1.00$), exposing the significant increase of delta power during the *visuospatial WM maintenance* (see Fig. 1). Another significant interaction between Frequency Band and WM load ($F_{(3,102)} = 16.54$, $P < 0.001$, $\eta^2 = 0.327$, *1-*$\beta = 1.00$) described how HL condition determined a relevant increment for delta power and a slight decrement in alpha power. Fig. 4 depicts the spatial distribution of the absolute power of the alpha frequency band. Ultimately, the interaction between Task Phase and WM load ($F_{(1,34)} = 4.21$, $P = 0.04$, $\eta^2 = 0.110$, *1-*$\beta = 0.513$) showed that greater changes between the

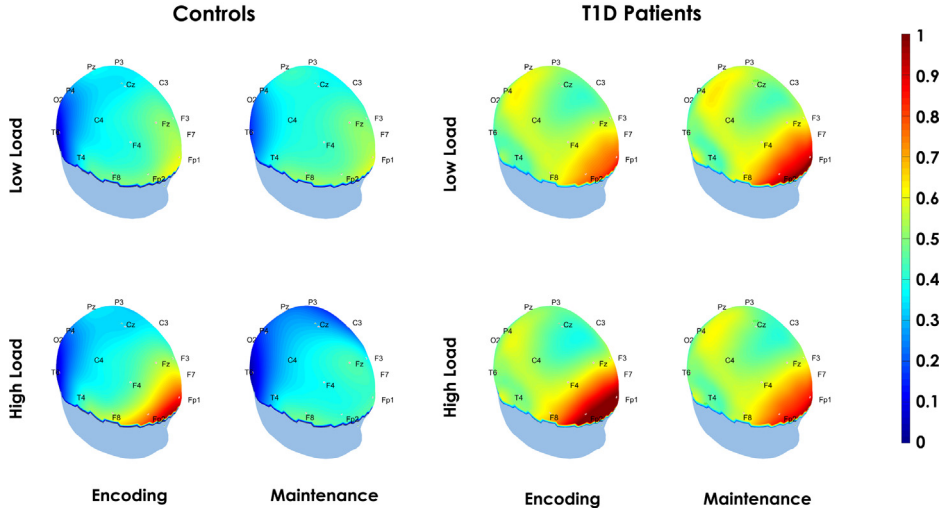

Figure 4 Spatial representation of the absolute power of the alpha band—normalized with respect to the energy measured during resting in the same group—in the two main experimental visuospatial WM phases and WM load levels, for both T1D patients and controls.

WM load levels occurred during the encoding phase, while greater changes between Task Phases occurred during LL trials.

Correlation analyses

Correlation analyses between blood glucose levels and EEG quantitative measures failed to show significant effects. However, delta power at frontal regions, during both encoding ($r = 0.465$, $P = 0.05$) and maintenance ($r = 0.441$, $P = 0.06$) stages, reached an achievable tendency exclusively in healthy individuals while processing the lower level WM load.

Discussion

The main aim of the present work was to characterize electrophysiologically the dynamic brain activity of young people with T1D and healthy controls during WM encoding and maintenance associated with the execution of a cognitive task, while evaluating the effect of the WM load level using faces as stimuli. This was done by adding a differential emotional content, irrelevant to task fulfilment, in order to enhance attentional distraction.

Although neuropsychological evaluation did not discern signs of cognitive impairment, the behavioral results for the experimental task showed significant between-group differences, as the T1D patients showed a lower number of correct responses with prolonged reaction times. Though not statistically significant, similar behavioural trends have been observed in T1D patients with good glycemic control [22,27].

Despite the observed behavioral differences, an important and necessary distinction between the clinical characteristics of the current group of T1D patients and those regularly reported in the literature must be made. This is because all our experimental subjects had regular medical follow-ups and no hospital admissions due to diabetes in the 2 years prior to their participation in this study. Also, they had a higher mean educational level than that corresponding to the Mexican population as a whole, coupled with optimal daily glucose control. In this context, differences in cognitive processing between the groups may be interpreted as being caused directly by the impact of the illness on central nervous system development and current operation.

Behavioral responses showed two clear additional findings in both groups. First, an increase in WM load generated a significant reduction in the number of correct responses, though this effect was not seen when happy faces were used as stimuli. This finding is consistent with previous reports which postulate that emotional information can enhance and facilitate episodic memory consolidation [43,48], WM capacity [51], n-back task performance [15], and visuospatial WM abilities [26]; thus facilitating WM processing on more difficult trials probably via greater cortex activation associated with what has been defined as ''emotional attention'' [52,53]. Stated briefly, under certain circumstances—such as in the present experiment—the emotional nature of stimuli can attract additional attentional processing resources and so facilitate task execution, even though this emotional content is not relevant to task completion.

Second, although there is at yet no general consensus on this topic, the role of delta waves during mental tasks has been associated with cortical deafferentation or the inhibition of the sensory afferences that interfere with internal concentration [30,31]. In fact, interference control is considered an executive-attention function of the prefrontal cortex [21] in which attention is expedited by suppressing distraction; thus, the presence of EEG delta oscillations may signal the inhibition of other processes that could interfere with solving the mental task (see [30], for a comprehensive review]. In the present experiment, patients showed significant frontal increase of delta energy during encoding that was only seen in controls on the trials with the higher WM load. This result reinforces the notion that even lower WM load levels might demand more processing resources in T1D patients. Moreover, these additional efforts seem to persist over frontal areas in patients during WM maintenance, also involving posterior locations, as observed in the control group.

Furthermore, our T1D patients showed a substantial increase of the absolute power of the theta band in frontal areas during the WM encoding phase, which was only seen in controls while performing the higher WM load level. Increased frontal theta activity has been shown to correlate positively with visual working memory capacity [38], increased WM load [49], and coding of new information [41]. This suggests that frontal theta activity is associated with enhanced motivation and attention [38]. Recently, it has been proposed that during stimulus encoding, the theta frequency band produces a positive subsequent memory effect and that the magnitude of frontal post-encoding-related theta activity correlates with the discrimination accuracy of the test stimulus [55]. Moreover, recent evidence suggests that oscillations in frontal theta power reflect specific control adjustments of behavior [14]; thus, they are considered a neural marker of mid-prefrontal cortex engagement to support goal-directed control [12,13]. All these findings suggest that the differences observed in the absolute theta power in our groups could also reinforce the idea that they represent part of the additional cognitive efforts required by T1D patients to fulfill task demands. In addition, they support previous hypotheses assuming that T1D patients need to deploy greater processing resources in order to match the cognitive processing level shown by controls [9,22].

No significant differences were found between T1D patients and controls for the absolute powers of delta and theta bands during resting. In contrast, slight decreases in delta and theta activity were previously described at rest for T1D patients without antecedents of early severe hypoglycemia [3]. Several other studies, however, have reported an increase in the energy of the slower frequency bands in patients with diabetes during rest, but this has been attributed mainly to a history of severe hypoglycemia [7,8,10,33] or to the hypoglycemic state itself [54,60,61].

Another relevant result, which is worth pointing out, is that controls showed widespread significantly lower alpha activity while performing the task with respect to T1D patients. This difference may be related to an effect on

the implementation of inhibitory processes [35,58] that has been associated with the alpha band [6,42]. In fact, alpha band frequencies have been largely associated with spatial attention [37,40] even though their actual functional role is still under debate. Recently, Keitel and colleagues [39] postulated that the attentional modulation of dynamic visual stimulation relies on cortical mechanisms, including retinotopic alpha suppression. In this context, the dissimilarity between T1D and controls on task-related alpha band activity might imply that T1D patients fail to dynamically control their spatial attention, at least with similar efficiency to controls.

One remarkable point was the lack of significant correlation between the blood glucose level at the beginning of the experiment and qEEG measures. This result emphasizes the notion that spectral parameters of the EEG are relatively stable, thus increasing its usefulness to study pathological conditions. In this regard, the tendency observed in healthy individuals to relate glycemia and delta power could represent a physiological adaptive response to preserve homeostasis; such a mechanism would be probably affected by the progress of a metabolic disease as T1D.

Taken together, the effects found on quantitative EEG measures from the earlier steps of WM processing (encoding), particularly those on the delta and theta frequency bands, can be explained as an expression of a ''switch-all-on'' strategy adopted by T1D patients. Several plausible explanations for this finding could be the early recruitment of an ''excessive'' amount of cognitive resources in anticipation of subsequently higher task demands, difficulties in gradually adapting the recruitment process to increasing task demands, difficulties with the control of spatial attention or all of the above. Regardless of which may be the best explanation, they all suggest an executive failure in the ''on-line'' evaluation of cognitive demands, and/or the existence of an adaptive mechanism to fulfill cognitive demands that mainly affects the earlier steps of WM processing.

Conclusions

Our results suggest that patients with T1D exhibit a distinctive behavioral and quantitative EEG pattern compared to that observed in healthy controls during performance of a visuospatial WM task. These group differences are observable even when certain favorable patient characteristics, such as proper educational level, good social integration, optimal metabolic control, and good clinical development without recent hypo- or hyperglycemic events, were present.

Interestingly, qEEG changes reflect how patients deploy additional resources at early processing stages -the WM encoding phase- even in the lower level memory load condition, whereas it mainly occurs in the healthy controls in the higher memory load condition. Indeed, these effects differentially affect electrophysiological processing in frontal and posterior brain areas, thus evidencing how the metabolic restrictions induced by diabetes can drive processing alternatives, and probably, the cognitive strategies employed.

Despite limitations due primarily to the relatively small sample size, this study found specific interactions between WM load and task performance in a context in which the stimuli presented were emotional faces, and was able to discern, electrophysiologically, between T1D patients and healthy controls during the different steps of WM processing. These findings suggest the need to continue analyzing these effects using other types of cognitive tasks, and to evaluate the potential specific contributions of other variables, such as sex, age, and years of disease evolution, among others.

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Disclosure of interest

The authors declare that they have no competing interest.

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