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Central neuronal transmission in response to tonic cold pain is modulated in people with type 1 diabetes and severe polyneuropathy☆

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ABSTRACT

Aims: This study aimed to investigate cortical source activity and identify source generators in people with type 1 diabetes during rest and tonic cold pain.
Methods: Forty-eight participants with type 1 diabetes and neuropathy, and 21 healthy controls were investigated with electroencephalography (EEG) during 5-minutes resting and 2-minutes tonic cold pain (immersing the hand into water at 2 °C). EEG power was assessed in eight frequency bands, and EEG source generators were analyzed using standardized low-resolution electromagnetic tomography (sLORETA).
Results: Compared to resting EEG, cold pain EEG power differed in all bands in the diabetes group (all $p < 0.001$) and six bands in the controls (all $p < 0.05$). Source generator activity in the diabetes group was increased in delta, beta2, beta3, and gamma bands and decreased in alpha1 (all $p < 0.006$) with changes mainly seen in the frontal and limbic lobe. Compared to controls, people with diabetes had decreased source generator activity during cold pain in the beta2 and beta3 bands (all $p < 0.05$), mainly in the frontal lobe.
Conclusions: Participants with type 1 diabetes had altered EEG power and source generator activity predominantly in the frontal and limbic lobe during tonic cold pain. The results may indicate modulated central transmission and neuronal impairment.

1. Introduction

The prevalence of diabetes mellitus is increasing due to longer life expectancy and increased disease duration, amongst others. Correspondingly, the comorbidities with increased disease duration are reflected in more severe symptom profiles. Polyneuropathy is a common and bothersome microvascular complication of long-term diabetes, and when combined with impaired neuronal perfusion, it may lead to neuronal impairment.[1] Research conducted within the last two decades has documented a variety of alterations in the central nervous system (including the spine), e.g., functional alterations in cortical processing of incoming stimuli and structural changes in specific cortical structures. Mainly, magnetic resonance imaging (MRI) has been used to study specific changes in brain morphology of, e.g., thalamus and the somatosensory cortex, but also brain-metabolites have shown to be affected.[2–4] Furthermore, MRI has been used to study brain connectivity, e.g., communication within specific networks in rest or in response to functional tests.[5,6]

Electrophysiological methods can also be used to assess the function of the nervous system. The electroencephalographic (EEG) signal can be divided into frequency bands for detailed analysis, which is impossible using MRI. Persons with type 1 diabetes have shown that responses to phasic electrical stimuli affect the ability to elicit a withdrawal reflex.[7] Reduced amplitudes and latencies of evoked potentials (mirroring conduction velocity) have consistently been shown in the spine, early brainstem, and brain.[8] Furthermore, inverse modeling of EEG scalp signals can reveal underlying source generators.[9] Experimental assessment of the nervous system's response to pain provides essential information about nerve function and requires reliable test methods. It has been demonstrated that tonic pain mimics chronic pain more than

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phasic stimulations typically used to study evoked potentials.[10,11] In participants with diabetes, there is a knowledge gap in studying the frequency bands and source generators during tonic pain in the brainstem and precortical structures.

Consequently, we hypothesized that adults with long-term type 1 diabetes and polyneuropathy had modulated processing to tonic pain stimulations evident as alterations in frequency bands and underlying cortical source generators. Consequently, the study aimed to characterize differences in the continuous resting-state EEG and tonic cold pain EEG in type 1 diabetes and healthy controls in terms of 1) frequency content of cortical source activity and 2) locations of cortical source generators.

2. Methods

The study was carried out between June 2014 and January 2018 at Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Denmark. The study was approved by the North Denmark Region Committee on Health Research Ethics (N-20130077, N-20090008). All participants gave written informed consent before enrollment. The study was registered at EUDRA CT (reference no. 2013-004375-12) and clinicaltrials.gov (reference no. NCT02138045). All research was carried out in accordance with the Declaration of Helsinki.

2.1. Study population

Forty-eight people with type 1 diabetes and distal symmetrical polyneuropathy (DSPN) were recruited at the Department of Endocrinology, Aalborg University Hospital, Denmark. Potential eligible patients were prescreened based on a recorded vibration perception threshold above 18 V. To diagnose DSPN according to the Toronto criteria, all patients underwent traditional nerve testing not >4 weeks prior to inclusion.[1] Nerve testing was performed and interpreted by a trained neurophysiologist.

Additional inclusion criteria were age >18 years, a verified diagnosis of type 1 diabetes for a minimum of 2 years: HbA1c level >48 mmol/mol [≥6.5 %], stable medication, body mass index >22 kg/m^2, and written consent. Exclusion criteria included type 2 diabetes, other neurological disorders than polyneuropathy, estimated glomerular filtration rate <60 ml/min/1.73 m^2, calcitonin >25 ng/l, HbA1c level <48 mmol/mol (<6.5 %), use of glucagon-like peptide-1 receptor agonists or dipeptidyl peptidase-4 inhibitors.[12] Participants with painful neuropathy were identified using the interview part of the "Doleur neuropathique 4" (DN4) questionnaire where a cutoff value above or equal to 3 was used to identify painful neuropathy.[13]

A sex- and age-matched control group of 21 healthy volunteers were included for comparison.

Inclusion and exclusion criteria, as well as experimental settings, have previously been listed in the publication of the primary outcome.[12]

2.2. Experimental procedure

Baseline data from the previous study[12] was analyzed. Blood glucose measurements were performed on the morning of the experiment (12.3 ± 5.4 mmol/l in the diabetes group). If the blood glucose measurement was low or the participant experienced symptoms of hypoglycemia, they were offered a glass of juice to prevent hypoglycemic episodes. Experimental procedures were identical for all participants, who were seated comfortably and instructed to focus on a fixed point at the wall and to minimize eye movements and eye blinks during EEG recordings. Each subject underwent two separate EEG recordings: 5 min of EEG recorded at rest and 2 min of EEG recorded during tonic cold pain while they were instructed to position their left hand in a circulated cold-water bath of 2 °C (Grant, Fischer Scientific, Slangerup, Denmark). The total pain experience following immersion of the hand into cold water, was recorded using an 11 (0–10) point Likert VAS scale. EEG data were recorded using a 61-channel prewired cylindrical Ag/AgCl surface electrode cap (MEQNordic A/S, Jyllinge, Denmark). Data were sampled at 10 kHz (SynAmp, Neuroscan, El Paso, TX, USA) and recorded using Neuroscan software (Neuroscan, v. 4.3.1, Compumedics, Charlotte, NC, USA). The impedance between electrodes and the scalp was <5 kΩ when recordings were initiated.

The applied tonic cold pain model and EEG source generator analysis have previously been used and validated in studies of healthy volunteers[14,15] and participants with diabetes.[9]

2.3. Data preprocessing

EEG data were preprocessed offline by using EEGLAB (SCCN, Institute for Neural Computation, University of California San Diego, USA) as follows: 1) zero-phase shift band-pass filtering (1–70 Hz), 2) zero-phase shift notch filtering (49–51 Hz), 3) removing line noise using CleanLine plugin for the EEGLAB MATLAB toolbox (MathWorks Inc., Natick, MA, USA) with frequencies [50 100 150 200 250 300 350 400 450],[16,17] 4) visual inspection of data quality, and electrodes that were flat, noisy/inadequate were interpolated, 5) visual inspection and Artifact Subspace Reconstruction (ASR) algorithm[18] with standard settings selecting and removing bursts contaminated by blink and muscle artifacts, 6) re-referencing to the common average reference. 7) We applied independent component analysis using the logistic infomax ICA algorithm of Bell & Sejnowski on the re-referenced datasets to transform the EEG channel data into temporally independent component signals.[19] The components were first classified using ICLabel function in EEGLAB across seven independent component categories: Brain, Muscle, Eye, Heart, Line Noise, Channel Noise, and Other.[20,21] All components with brain activity higher than 20 % were kept. Components with lower brain activities (<20 %) were classified as either artifact or brain signal by the observer based on scalp topography, the time-series component activity, and the frequency-series component power spectral density.[20,21] The artifactual components were then subtracted from the signals. 8) The cleaned signals were used for following EEG data analysis. This procedure resulted in at least four and a half minutes of resting EEG data and 100 s of tonic cold pain EEG data.

2.4. Spectral analysis of EEG

Spectral analysis of EEG was done in MATLAB version R2019a to calculate the relative power of EEG. The continuous wavelet transform was applied to the EEG signals from all 61 electrodes. The complex Morlet wavelet was used for analysis with a bandwidth of 10 Hz, a center frequency of 1 Hz, and a between-scale frequency resolution of 0.5 Hz. The EEG signal was divided into the following frequency bands: delta (1–4 Hz), theta (4–8 Hz), alpha1 (8–10 Hz), alpha2 (10–12 Hz), beta1 (12–18 Hz), beta2 (18–24 Hz), beta3 (24–32 Hz), and gamma (32–70 Hz). The frequency bands were averaged across time. Then the relative power for each channel was calculated by dividing the absolute power in each frequency band with the total power of all frequency bands and multiplying by 100.

2.5. Source localization

The standardized low-resolution electromagnetic tomography (sLORETA) was used to analyze the three-dimensional current source density distribution (free software package available at: http://www.uzh.ch/keyinst/loreta). It is an inverse modeling method. The result is a three-dimensional distribution of electric current activity at any location in the brain with no error under optimal noise-free conditions. The head model and electrode coordinates used in sLORETA are based on the Montreal Neurological Institute average MRI brain map (MNI152). The sLORETA solution space is limited to include the cortical gray matter, divided into 6239 voxels of a 5-mm cubic spatial resolution. A detailed description of the sLORETA method is explained by Pascual-

Marqui.[22] In this study, the EEG recordings for each subject were divided into 4 second epochs and converted into cross-spectrum files. The sLORETA analysis was performed on a group level in the eight frequency bands listed in the previous section. Neurons in the brain areas identified by sLORETA oscillate more in one condition compared to another, or a higher number of synchronous neurons oscillate in one condition compared to another. The color, yellow or blue, on the sLORETA figures show which condition has brain areas with more or less activated source generator neurons.

2.6. Statistical analyses

Descriptive statistics are reported as mean ± standard deviation (SD) unless otherwise noted. Two-sided paired tests were used to compare spectral content of the pre-defined bands in the two conditions resting state and tonic cold pain state. A two-sided unpaired *t*-test was used to compare the EEG power between the diabetes group and the control group. Statistical analyses of EEG power were conducted using the statistical package SPSS (IBM SPSS Statistics, Version 26, IBM Corp). EEG source localization derived from global cortical activity (whole-brain analysis) was employed for all the subjects: rest versus tonic cold pain and diabetes versus healthy, for each EEG frequency band. EEG source localization was also compared within the diabetes group for people with neuropathic pain versus people without pain in both rest and tonic cold pain conditions. The differences were evaluated using the built-in statistical module in the sLORETA software. A two-sided paired *t*-test on log-transformed data and no normalization methods was used to compare the sLORETA analysis between resting and tonic cold pain states within the two groups. Two-sample *t*-tests with the same settings were utilized when comparing tonic cold pain EEG and resting EEG between the two groups. The same two-sample t-test and settings were likewise used for comparing the diabetes group with pain and the diabetes group without pain both in resting and during tonic cold pain conditions. The default nonparametric randomization method was used to adjust for the multiple comparisons using Fisher's random permutation test. The brain area with max *t*-statistics and the most significant p-value in each frequency band is reported for each sLORETA test. A two-sided paired *t*-test was used to compare pain ratings during the tonic cold pain test between the diabetes and control group. For all statistical tests, $p < 0.05$ was considered significant.

3. Results

We included 48 people with type 1 diabetes and verified polyneuropathy according to the Toronto criteria[1] and 21 age-matched healthy controls who all completed the experimental procedures. Demographics of study population are shown in Table 1. Two of the recordings in the diabetes group and one of the recordings in the healthy group were excluded due to a poor signal-to-noise ratio, thus leaving 46 people with diabetes and 20 healthy controls for further analysis. Eleven of the 46 people with diabetes had painful neuropathy, with a mean-score of 3.8 ± 0.9 on the DN4 questionnaire.

Table 1
Demography and clinical characteristics.

	Type 1 diabetes	Healthy	p-Value
Age (years)	50.0 ± 8.5	49.9 ± 11.9	0.98
Sex (M/F)	38/10	17/11	0.08
Height, cm	178.4 ± 8.6	179.8 ± 9.0	0.55
Weight, kg	90.0 ± 16.0	79.1 ± 12.9	**0.003**
Body mass index, kg/m^2	28.3 ± 4.4	24.9 ± 2.6	**<0.001**
Disease duration (range), years	32.2 ± 9.5 (14–51)	–	
Pulse rate, beats/min	73.9 ± 10.5	66.0 ± 7.0	**0.001**
Systolic BP (mm Hg)	150 ± 16	129 ± 15	**0.007**
Diastolic BP (mm Hg)	82 ± 11	76 ± 11	0.33
CVT	3.0 (1.9; 0.6–6.9)	4.33 (1.7; 1.9–8.1)	**0.01**
Cholesterol, total, mmol/l	4.49 ± 0.80	5.40 ± 0.85	**<0.001**
Triglyceride, mmol/l	1.01 ± 0.64	1.16 ± 0.55	0.35
HDL, mmol/l	1.56 ± 0.54	1.56 ± 0.43	0.97
LDL, mmol/l	2.48 ± 0.56	3.30 ± 0.87	**<0.001**
HbA_{1C}, mmol/mol	65.96 ± 10.45	33.67 ± 3.37	**<0.001**
Fasting glucose, mmol/l	12.3 ± 5.4	5.5 ± 0.8	**<0.001**
Retinopathy, % of participants	25 %	0 %	**0.013**
Creatinine, urine	10,906 ± 5400	11,020 ± 7809	0.94
Albumin, urine	0.074 ± 0.18	0.0083 ± 0.006	0.10
Median nerve stimulation intensity, mA	18.04 ± 6.93	12.10 ± 5.65	**0.001**
Median nerve conduction velocity, sensory threshold, m/s	43.2 ± 7.2	54.7 ± 4.5	**<0.001**
Median nerve amplitude, sensory threshold, mV	13.4 ± 8.0	26.6 ± 11.1	**<0.001**
Median nerve conduction velocity, motor threshold, m/s	49.7 ± 4.9	55.4 ± 2.5	**<0.001**
Median nerve amplitude, motor threshold, mV	8.2 ± 2.2	9.1 ± 1.7	0.09
Peroneal nerve conduction velocity, m/s	37.3 ± 5.4	45.7 ± 2.9	**<0.001**
Peroneal nerve amplitude, mV	1.8 ± 1.3	3.7 ± 1.5	**<0.001**
Sural nerve conduction velocity, m/s	42.5 ± 5.8	49.7 ± 6.1	**<0.001**
Sural nerve amplitude, mV	3.6 ± 2.3	6.6 ± 3.2	**<0.001**

Data are expressed as mean and standard deviations unless otherwise stated. p-Values <0.05 are considered significant and marked in bold font. The demographic data has previous been published.[4,8] Abbreviations: BP, blood pressure; CVT, cardiac vagal tone; HDL, high density lipoprotein; LDL, low-density lipoprotein.

3.1. Spectral analyses

3.1.1. Type 1 diabetes during tonic cold pain compared to rest

The relative spectral EEG power can be seen in Fig. 1. Compared to rest, tonic cold pain in the diabetes group increased the relative spectral EEG power in delta (13 %, $p < 0.0001$), beta3 (8 %, $p < 0.001$), and gamma (38 %, $p < 0.0001$) bands, while it was decreased in theta (−16 %, $p < 0.0001$), alpha1 (−41 %, $p < 0.0001$), alpha2 (−33 %, $p < 0.0001$), beta1 (−16 %, $p < 0.0001$), and beta2 (−10 %, $p < 0.0001$) bands.

3.1.2. Healthy controls during tonic cold pain compared to rest

Compared to rest, tonic cold pain in the healthy group increased the relative spectral EEG power in the gamma (40 %, $p < 0.0001$) band, while it was decreased in theta (−18 %, $p = 0.003$), alpha1 (−41 %, $p < 0.0001$), alpha2 (−34 %, $p < 0.0001$), beta1 (−13 %, $p < 0.001$), and beta2 (−8 %, $p = 0.004$) bands.

3.1.3. Tonic cold pain in type 1 diabetes compared to healthy controls

Compared to the healthy controls, the diabetes group had decreased power in the beta2 (−9 %, $p = 0.04$) and beta3 (−9 %, $p < 0.001$) bands during tonic cold pain EEG (Fig. A1).

3.1.4. Resting state in type 1 diabetes compared to healthy controls

Compared to the healthy controls, the diabetes group had decreased power in the beta3 (−11 %, $p = 0.02$) band during resting EEG (Fig. A1).

3.2. Source generator analyses

3.2.1. Type 1 diabetes during tonic cold pain compared to rest

Fig. 2 and Table 2 show the differences from the sLORETA tests. In participants with diabetes, exposure to tonic cold pain increased the overall source generator activity in the delta (Frontal Lobe, $p < 0.001$), beta2 (Frontal Lobe, $p = 0.005$), beta3 (Frontal Lobe, $p < 0.001$), and gamma (Limbic Lobe, $p < 0.001$) bands, in comparison to the resting state. In contrast, alpha1 (Limbic Lobe, $p < 0.002$) showed decreased

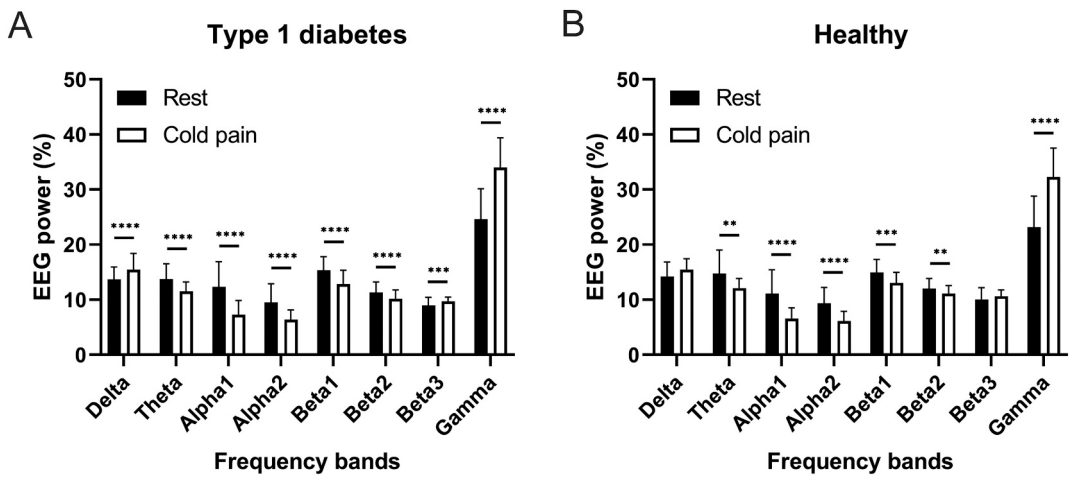

Fig. 1. Changes in spectral EEG between rest and tonic cold pain for type 1 diabetes (A) and healthy controls (B). (**) $p < 0.01$, (***) $p < 0.001$, (****) $p < 0.0001$.

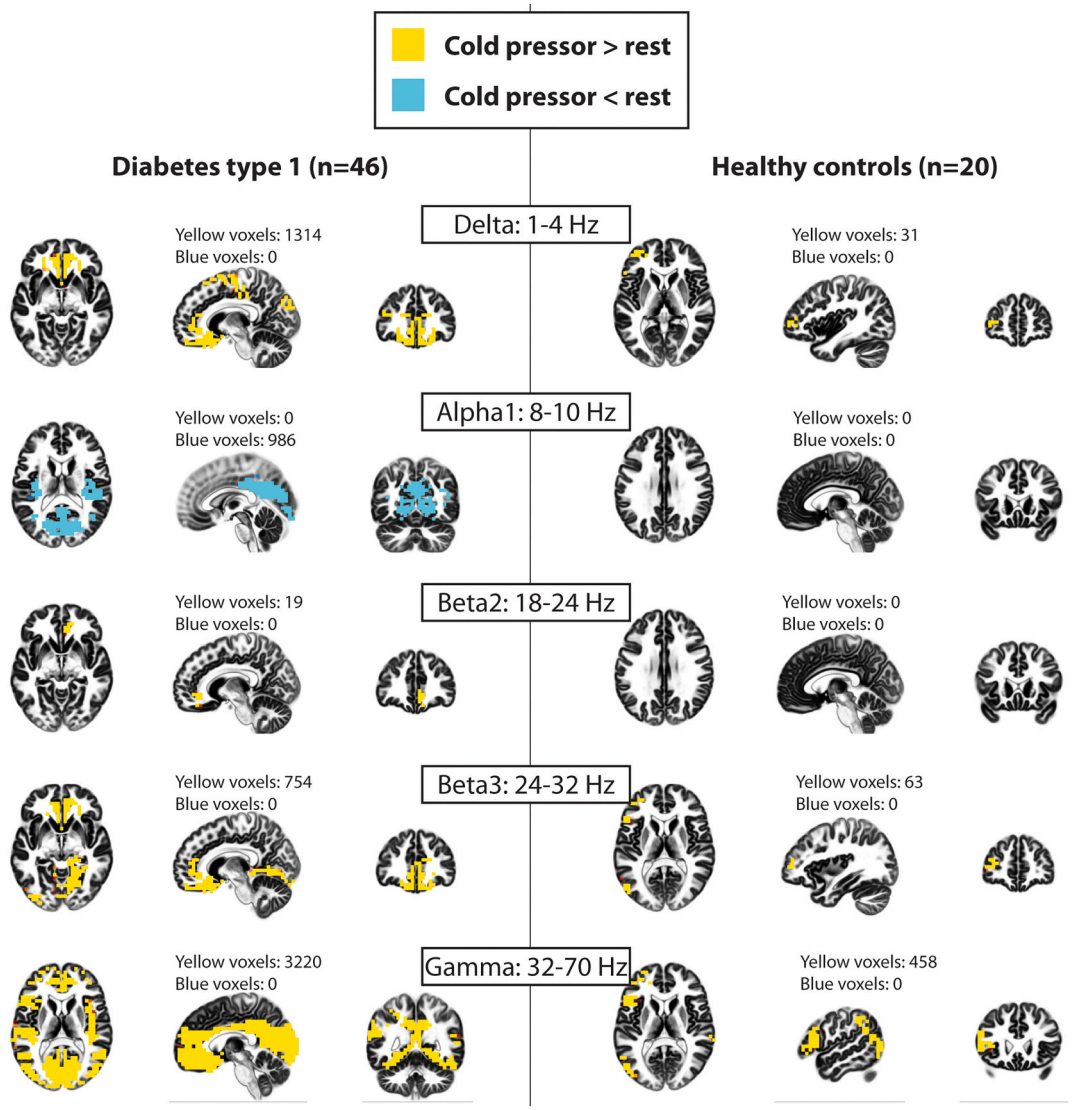

Fig. 2. Changes in cortical source generators between rest and tonic cold pain in type 1 diabetes (left) and healthy controls (right). Yellow indicates increased activity from rest to tonic cold pain; blue indicates decreased activity. Total number of significant voxels at the $p < 0.05$ level is shown at each frequency band and group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Brain areas with max *t*-statistics for each test and frequency band.

Band	Brain region with max *t* statistics	MNI coordinates (X, Y, Z)	Change	p-Value
Type 1 diabetes: Cold pain vs. rest				
Delta	Frontal lobe, medial frontal gyrus	10, 40, −5	↑	<0.001
Alpha1	limbic lobe, posterior cingulate	0, −65, 15	↓	<0.002
Beta2	frontal lobe, medial frontal gyrus	10, 40, −5	↑	0.005
Beta3	frontal lobe, medial frontal gyrus	10, 40, −5	↑	<0.001
Gamma	limbic lobe, posterior cingulate	5, −50, 10	↑	<0.001
Healthy control: Cold pain vs. rest				
Delta	Frontal lobe, inferior frontal gyrus	-40, 55, 5	↑	0.005
Beta3	Frontal lobe, middle frontal gyrus	−35, 50, 10	↑	0.01
Gamma	Frontal lobe, inferior frontal gyrus	−55, 25, 10	↑	0.002
Cold pain: Type 1 diabetes vs. healthy controls				
Beta2	Frontal lobe, precentral gyrus	45, −20, 45	↓	0.04
Beta3	Frontal lobe, precentral gyrus	45, −20, 45	↓	0.01
Rest: Type 1 diabetes vs. healthy controls				
Theta	Parietal lobe, postcentral gyrus	−65, −20, 35	↓	0.03
Alpha2	Parietal lobe, postcentral gyrus	−60, −25, 40	↓	0.03
Beta1	Parietal lobe, postcentral gyrus	−65, −20, 35	↓	0.03
Beta2	Parietal lobe, postcentral gyrus	−60, −20, 35	↓	0.02
Beta3	Frontal lobe, middle frontal gyrus	−35, −10, 50	↓	0.01

source generator activity during tonic cold pain compared to resting state.

3.2.2. Healthy controls during tonic cold pain compared to rest

For healthy controls, exposure to tonic cold pain increased source generator activity in the delta (Frontal Lobe, $p = 0.005$), beta3 (Frontal Lobe, $p = 0.01$), and gamma (Frontal Lobe, $p = 0.002$) bands in comparison to the resting state.

3.2.3. Tonic cold pain in type 1 diabetes compared to healthy controls

During tonic cold pain EEG, the diabetes group had less source generator activity than the healthy controls in beta2 (Frontal Lobe, $p = 0.04$) and beta3 (Frontal Lobe, $p = 0.01$) bands. The source generator localizations are visualized in Fig. A2.

3.2.4. Resting state in type 1 diabetes compared to healthy controls

During resting EEG, the diabetes group had less source generator activity compared to the healthy controls in theta (Parietal Lobe, $p = 0.03$), alpha2 (Parietal Lobe, $p = 0.03$), beta1 (Parietal Lobe, $p = 0.03$), beta2 (Parietal Lobe, $p = 0.02$), and beta3 (Frontal Lobe, $p = 0.01$) bands. The source generator localizations are visualized in Fig. A2.

3.2.5. Type 1 diabetes with painful neuropathy compared to no pain

No differences in source generator activity were observed between the diabetes group with painful neuropathy ($n = 11$) compared to the diabetes group without pain ($n = 36$) during rest (all ps > 0.4) or during tonic cold pain (all ps > 0.4).

3.3. Pain ratings during tonic cold pain

No difference in reported pain ratings during the tonic cold pain was observed between the diabetes group compared to the controls (7.7 ± 1.7 vs. 7.6 ± 1.6, $p = 0.8$). Likewise, no difference in pain ratings during the tonic cold pain was observed between the diabetes subgroup with neuropathic pain and the subgroup without neuropathic pain (7.5 ± 2.3 vs. 7.8 ± 1.5, $p = 0.5$).

4. Discussion

The main finding of this study was that participants with type 1 diabetes and confirmed polyneuropathy regardless of painful neuropathy, had increased EEG power in the delta band during tonic pain in comparison to rest. This was not observed in the healthy controls.

Additionally, source localization analysis showed that people with diabetes and DSPN had widespread activation of brain areas during tonic pain, predominantly in the pain-processing areas, known as the pain matrix, including the frontal and limbic lobes. This was not observed in the healthy controls.

Diminished source generator activity during both tonic cold pain and rest, were shown in in people with diabetes and DSPN in comparison to healthy controls, which support previous findings of modulated central transmission and neuronal impairment in the same cohort.[7,8]

The presence of neuropathic pain did not change the activity or localizations of the source generators neither during rest nor during tonic cold pain in the subset analysis. Likewise, neuropathic pain did not affect pain ratings. These findings should be interpreted with caution since only 25 % had neuropathic pain, and therefore delicate source activity or localization may not be revealed by use of these methods (falsely negative) or in case they are negative and source activity and localization are unaltered, the finding points towards a predominant peripheral change.

Frequency analyses of brain oscillations have been widely used to describe alterations of brain function in response to diabetes.[23,24] For instance, it has previously been shown that fast brain oscillations, especially in the beta-band of the temporal lobe, are slowed in young individuals with diabetes, indicating that it is an alteration in response to relatively short disease duration, and thus; may be caused by diabetes per se, and to a lesser extent, due to neuropathic processes.[23] This interpretation is supported by data from people with diabetes and a history of recurrent severe hypoglycemia, who showed decreased beta activity.[25] Despite small sample size, their results indicate that the faster frequency bands (alpha and beta bands) seems to be diminished by diabetes, and poorly regulated blood glucose may play a key role.[25] In our previous study of source localization in individuals with diabetes and unknown neuropathic phenotype, we observed decreased source activity in the fast gamma band (left parietal lobe) when compared to healthy individuals in rest,[9] much in line with our current findings.

In our cohort consisting of individuals with longstanding diabetes and severe neuropathy, we replicate the findings, but the dataset does not allow to interpret the causative path. Interestingly, we found no differences in beta-activity between the phenotypical representation pain/no pain, which again add valuable information to the existing discussion of the pain component, as decreased beta activity was reported in a group of people with chronic painful neuropathy including painful diabetic neuropathy.[26]

Individuals with type 1 diabetes and severe polyneuropathy responded to tonic cold pain, assessed as decreased alpha source generator activity and increased beta source generator activity compared to rest. These findings are supported in the literature during cold- or heat-induced stimuli, indicating robust recordings.[27,28] Interestingly, the individuals with diabetes and polyneuropathy showed widespread activation of brain areas during tonic pain in comparison to focal activation in the control group. It is plausible, however not directly shown, that the widespread activation is a consequence of brain

microstructural abnormalities or impaired tracts of white matter, which previously have been assessed with diffusion tensor imaging methods.[29,30] Similar alterations could be present in this cohort, as we have previously shown affection of peripheral, synaptic, and central neuronal transmission, e.g. evident as increased central conduction velocity and significantly changed spinal and supraspinal processing compared to healthy controls.[8]

Cortical brain networks used in processing somatic and visceral pain are altered in participants with diabetes compared to healthy controls.[31,32] In addition, the localization and activity of specific brain source generators are typically used for connectivity analysis or spectral analysis. The latter has shown day-to-day reproducibility at rest and during experimental tonic cold pain.[14,33] In our previous eLORETA study in healthy subjects, we showed that the day-to-day reproducibility of the pain response to the cold pressor test was high.[14] In healthy volunteers, we showed increased source activity during tonic cold pain in the slower delta band and the faster beta3, and gamma bands, which is different to earlier findings where increased activity in the theta, beta1, and beta2 bands were reported.[14] However, the minor differences between affected bands may be caused by relatively low sample size of healthy controls or minor differences in the preprocessing pipeline. Taken together, the decreased brain source activity supports previous reported modulated peripheral and central transmission and may explain less synchronicity and widespread neuronal activation.

4.1. Clinical relevance

Source localization analysis of pain stimulus in people with diabetic neuropathy may serve as a proxy for the central neurodegeneration of the brain's pain matrix. The current study shows that compared to controls, people with diabetes had less source generator activity in the pain centers, especially in the frontal and limbic lobes, indicating decreased synchronicity of the response to external stimuli. These findings may partly explain the typical symptoms of symmetrical polyneuropathy, including e.g. reduced ability to feel pain, sharp pains, extreme sensitivity to touch, tingling or a burning feeling. However, larger studies need to be performed to investigate the relation between altered source generator activity and symptoms of peripheral neuropathy.

4.2. Limitations

We believe that a strength of the study is that data were preprocessed using a standardized objective approach that includes independent component analysis. However, comparison with previous results of similar pain stimuli is challenging when methods are improved. The 61-channel recordings may limit the spatial resolution and thus the accuracy of source localization,[34] however, previous reports have used <32 channels. Therefore, we believe that the spatial resolution in specific brain regions is trustworthy.

However, the study was not conducted without limitations. Firstly, the included control group was healthy volunteers, which may limit our interpretation of our findings, as we cannot distinguish between central neuronal alterations caused by microvascular dysfunction in diabetes per se,[35] or presence of central neuropathy. Such discrepancy would have been more evident if the control group consisted of a matched (disease duration, gender) cohort of people with type 1 diabetes and no polyneuropathy (ruled out by conventional nerve testing). Secondly, it is also known that muscle artifacts or eye movements during, e.g., the tonic cold pain affects the sLORETA source generator analysis. For instance, eye blinks are known to superimpose especially delta and theta frequencies, while muscle activity is shown in the gamma band. The utilized preprocessing pipeline with independent component analysis has, however, the advantage of removing most of these artifacts and the influence is thought to be similar in healthy as well as in people with diabetes. Furthermore, our current results show significant differences in the alpha and beta frequency bands, and thus we believe that the external artifacts have influenced our results minimally. Thirdly, hypoglycemia has been shown to increase overall cortical power and slow brain waves.[36] Therefore, on the morning of the experiment, the blood glucose level of participants with diabetes was measured. Study personnel offered a glass of juice if the blood glucose level was low, however no other steps to avoid hypoglycemia were performed.

5. Conclusion

Participants with type 1 diabetes and severe polyneuropathy, regardless of pain, had increased EEG power in the slow delta band during tonic cold pain in comparison to rest, while this was not seen in healthy participants. Source localization analysis showed that people with diabetes and DSPN had widespread activation of the pain-matrix during tonic pain, while this was not observed in the healthy controls. Finally, the dynamic changes of source generator activity during tonic cold pain compared to rest were more pronounced in participants with type 1 diabetes and DSPN than in healthy controls. The diminished source generator activity both during tonic cold pain and rest in comparison to healthy, may be explained by less neuronal synchronicity, modulated central transmission and widespread neuronal impairment in this specific cohort with diabetes and multilevel polyneuropathy.

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CRediT authorship contribution statement

Esben Bolvig Mark: Writing - Original draft preparation, Conceptualization, Data curation, Writing - Reviewing and editing, Methodology. **Donghua Liao:** Data curation, Writing - Reviewing and editing, Methodology. **Rasmus Bach Nedergaard:** Writing - Reviewing and editing, Data curation. **Tine Maria Hansen:** Writing - Reviewing and editing, Methodology. **Asbjørn Mohr Drewes:** Conceptualization, Supervision, Writing - Reviewing and editing. **Christina Brock:** Conceptualization, Investigation, Supervision, Writing - Reviewing and editing.

Appendix A

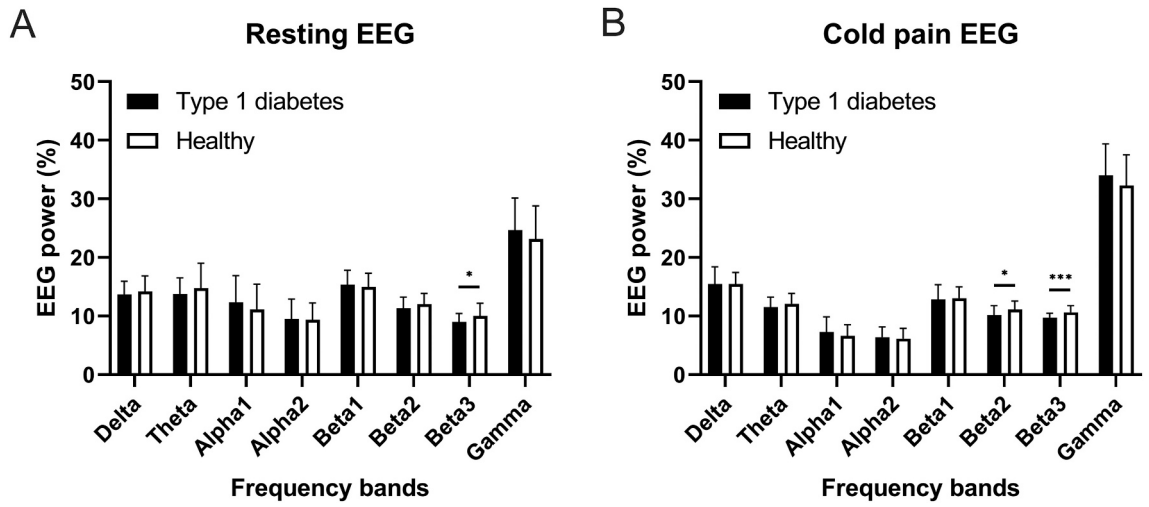

Fig. A1. The averaged relative EEG activity in participants with type 1 diabetes and healthy controls during resting state (A) and tonic cold pain (B). Data are expressed as means and standard deviations, (*) $p < 0.05$, (***) $p < 0.001$.

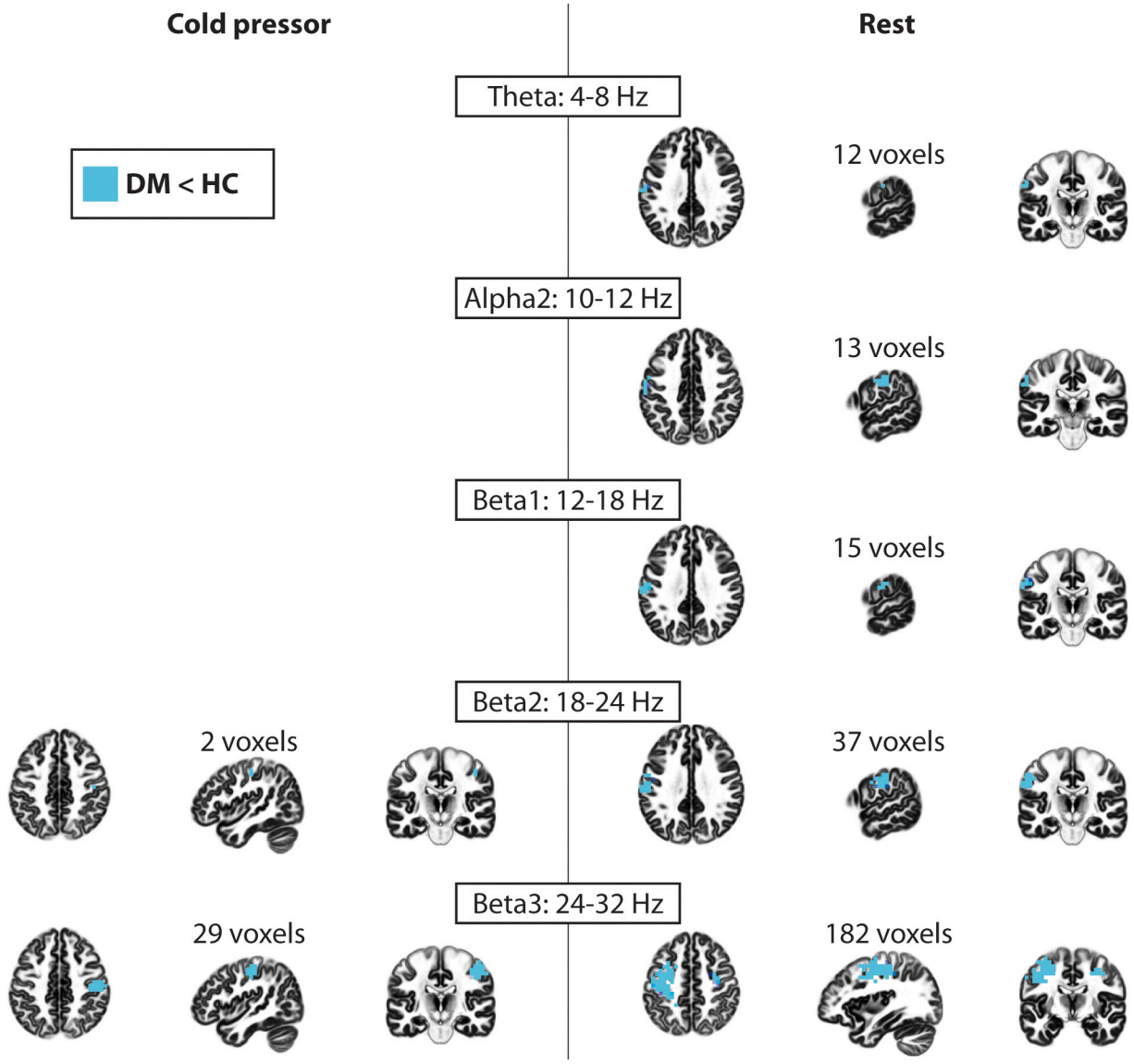

Fig. A2. The difference in cortical source generator activities between participants with type 1 diabetes (DM) and healthy controls (HC) during tonic cold pain (left) and resting (right). Blue represents cortical areas with decreased activity in type 1 diabetes compared to healthy controls. Total number of significant voxels at the $p < 0.05$ level is shown at each frequency band and group.

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