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Peripheral, synaptic and central neuronal transmission is affected in type 1 diabetes☆

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ABSTRACT

Aims: We hypothesized that adults with type 1 diabetes and severe polyneuropathy have alterations in neuronal transmission at different anatomical levels. The aims were to investigate upstream sensory neuronal activation in terms of peripheral, spinal, precortical, and cortical transmission.
Methods: 48 participants with type-1 diabetes and polyneuropathy, and 21 age-matched healthy participants were included. Electrophysiological median nerve recordings were used to analyze peripheral transmission at Erb's point (P9-N11); spinal evoked potentials at Cv7 (P11-N14); subcortical evoked potentials at Oz (N14-P18); early cortical evoked potentials at CP5 (N20-P22); late cortical evoked potentials at C1 (N60-P80) and estimated cortical inter-peak latencies as measures of central conduction time.
Results: In comparison to healthy, the presence of diabetes prolonged peripheral transmission at P9 and N11 (+0.49 ms, $p = .000$; +0.47 ms, $p = .04$, respectively), early cortical evoked potentials at CP5: N20 (+2.41 ms, $p = .003$) and P22 (+5.88 ms, $p = .001$) and cortical potentials at C1: N60 (+39.08 ms, $p = .001$) and P80 (+54.55 ms, $p = .000$) and central conduction time.
Decreased amplitudes were shown peripherally (−2.13 μV, $p = .000$), spinally (−0.57 μV, $p = .005$) and precortically (−0.22 μV, $p = .004$).
In both healthy and people with diabetes increased central conduction time were associated with decreased parasympathetic tone ($\rho = -0.52$, $p = .027$; $\rho = -0.35$, $p = .047$, respectively).
Conclusion: Neuronal afferent transmission and brain responses were significantly impaired in diabetes and the presence of prolonged central conduction time is indicative of severe extensive neuronal damage.
Trial registry number: EUDRA CT: 2013-004375-12; clinicaltrials.gov: NCT02138045.

1. Introduction

An increasing number of people is diagnosed with diabetes mellitus and since treatment has improved, people have longer life-expectancy. Consequently, the presence of diabetes induced macro- and microvascular complications are increasing globally, plausibly due to longer disease duration. Among the microvascular complication, polyneuropathy will affect up to 50% of adults with long-term type 1 diabetes. The pathogenesis is multifactorial and complex and includes vascular, metabolic, immune mediated and inflammatory pathways.[1,2] The clinical manifestations of diabetic symmetrical polyneuropathies (DSPN) include sensory and motor loss of the peripheral nerves to feet and hands, causing painful or painless sensory disturbances.[3] Classically these alterations are measured by nerve conduction studies, which confirm large fiber axonopathy. Autonomic nerves to viscera may also affected,[4–6] and e.g. decreased parasympathetic tone to the heart may lead to arrhythmias and sudden death.[7] In the central nervous system (CNS), alterations of brain responses occur at multiple levels and include transmission alterations in the spinal cord, brainstem and higher cortical structures. Structural changes in the CNS can objectively be assessed

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by MRI that may show morphological alterations as reduced grey and white matter volume, structural changes within nerve tracts and changes in metabolic function.

The use of EEG and somatosensory evoked potentials (SEPs) are advanced methods to evaluate brain responses and the brain integrity from peripheral stimulations.[4,8,9] Compared to MRI, EEG has a better temporal resolution, making it possible to investigate the stimulus specific exogenous response of the ascending sensory pathways.[8] Furthermore, resting state EEG studies have supported diabetes induced neuronal changes in the brain and the brain's sensory processing capabilities.[10] These changes can be interpreted as the net result of many synchronous distorted neuronal processes including transduction, transmission, interneuronal communication, and perception. Median nerve stimulation is a reliable method for eliciting SEP's and the upstream action potentials can be followed from the periphery to the spinal cord, to the brainstem and finally to the cortex.[11] The comprehensive technique provides detailed information on the diabetes induced neuronal alterations on the different level of the nervous system, which is important in understanding the pathogenesis of diabetic neuropathies.

Previous pioneering studies have thoroughly investigated SEP's in type 1 diabetes to characterize the somatosensory pathway from the periphery to the CNS in diabetes.[9,12] The studies concluded that the degree of dysfunction in the somatosensory afferent pathway depends on the stage of peripheral neuropathy and can be characterized mainly by alteration of the cortical sensory complex (reduced amplitude) and peripheral transmission delay. However, due to different patient cohorts, previous hyperglycemic treatment regiments and neurophysiological methods, the existing electrophysiological studies have shown contradictory results in characterizing the central conduction time assessed as interpeak latencies of SEP's.[9,12,13] Therefore, we aimed to provide a contemporary characteristic of the afferent neuronal transmission (periphery to cortex) and central conduction in people with long-term type 1 and severe polyneuropathy.

We hypothesize that adults with type 1 diabetes and verified DSPN have extensive alterations of neuronal transmission evident at multiple anatomical levels in comparison to age-matched healthy participants. The aim of the current study was to compare the upstream sensory neuronal transmission in type 1 diabetes with DSPN and healthy participants, and specifically measure latencies and amplitudes to median nerve stimulations 1) in the peripheral Erb's point, 2) at the Cv7 reflecting the spinal evoked potential, 3) at Oz representing the upstream precortical response and 4) at CP5 and C1 representing cortical processing. Finally, 5) the associations between central conduction time and 1) disease duration, age and height, 2) median nerve conduction velocity, median nerve action potential and, 3) parasympathetic tone were investigated to study whether measures of axonopathy or autonomic involvement is associated to prolonged CNS transmission.

2. Subjects, materials and methods

2.1. Study population

Forty-eight people with type 1 diabetes were recruited at the Department of Endocrinology, Aalborg University Hospital, Denmark. Potential eligible participants were pre-screened based on a recorded vibration perception thresholds above 18 V. DSPN was verified by nerve conduction tests, according to the Toronto criteria.[2] Additional inclusion criteria were age above 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, and written consent. Exclusion criteria included type 2 diabetes mellitus, other neurological disorders than DSPN, 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. Twenty-one healthy participants were recruited among volunteers from North Jutland and were age-matched to the patient group. Inclusion criteria were age above 18 years, normal peripheral nerve conduction testing. Exclusion criteria included type 1 and type 2 diabetes mellitus, neurological disorders and medication that could alter neuronal function.

Ethical approval was granted by Region Nordjylland, Denmark (N-20130077, N-20090008) and all participants gave written informed consent prior to entry. The study was registered in public databases (EUDRA CT (ref 2013-004375-12) and clinicaltrials.gov (ref NCT02138045)) and performed in accordance with International Council for Harmonization Good Clinical Practice and the Declaration of Helsinki. The experiment was conducted between June 2014 and January 2018 at Aalborg University Hospital.

2.2. Systemic biochemistry

Routine clinical chemistry (HbA1c, cholesterol, and triglycerides) were analyzed at the Department of Clinical Biochemistry, Aalborg University Hospital.

2.3. Peripheral neuronal assessment – peripheral nerve function

All participants underwent a standardized neurophysiological examination of the sensory and motor component of the median, ulnar, sural, radial, tibial, and peroneal nerves, and results were evaluated by a specialist neurophysiologist. In order to avoid the influence of skin temperature on conduction velocities, warming measures were used to ensure that the testing room did not allow skin temperature below 32 °C. For recording of the digital sensory nerve action potentials surface electrode were used. Electrical stimulation was applied through surface plastic bar electrodes.

2.4. Autonomic function

Cardiac vagal tone was recorded in the morning between 09: 00 and 10: 00, with the participant in a comfortable seated position with their legs supported. The participants refrained from nicotine and caffeine ingestion for 2 h prior to recordings being undertaken. Electrocardiographic electrodes (Ambu Blue Sensor P, Denmark) were positioned in left and right subclavicular regions and cardiac apex. The ECG was recorded using a commercially available biosignal acquisition system (Neuroscope, Medifit Instruments, Essex, UK).[14] In brief, the Neuroscope non-invasively measures brainstem parasympathetic efferent activity, known as cardiac vagal tone, in real time using inbuilt 'voltage controlled oscillators' which detect phase shifts in the beat-to-beat RR interval in a process known as 'phase-shift demodulation'.[15] Cardiac vagal tone is measured on a validated linear vagal scale, where 0 represents full atropinisation.[16] Cardiac vagal tone has been demonstrated both a sensitive and specific measure of vagal tone in diabetes which is comparable to other heart rate variability indices.[17]

2.5. Experimental procedure - somatosensory evoked potentials

Data in this study are the baseline data in a clinical study investigating the neuroprotective properties of liraglutide, in a cohort of people with type 1 diabetes and confirmed peripheral neuropathy.[18] The healthy participants were recruited for a single session as a comparative group. In this article, only experimental procedure relevant to this study will be mentioned. Data presented originate from peripheral, spinal, and cerebral neurophysiological recordings following electrical stimulation of the right median nerve. For both groups, the experimental procedure and recording setup are identical and performed in the same lab. The participants were seated comfortably in a supine position, with their eyes open during the entire recording. They were instructed to focus on a fixed point on a wall and try to minimize eye movement during stimulation. Please

see supplementary section for detailed description of electrical stimulation, recording and data analysis.

2.6. Statistical analysis

Descriptive statistics are reported as mean ± standard deviation if not otherwise stated, and differences were assessed by Students *t*-test and Chi-squared tests for categorical variables. To compare latency for the positive and negative component and peak to peak amplitude between healthy participants and people with diabetes, analysis was carried out using one-way multivariate analysis of variance, where age and height were included as covariates. If significant difference was seen, post hoc analyses were carried out to elucidate which component (positive component, negative component, or peak to peak amplitude) gave rise to the differences. The model was applied separately for each of the neurophysiological levels (Erb's point, Cv7, Oz, CP5, C1 and inter-peak latency). Clinical correlation analyses were assessed by Spearman's Rho and Bonferroni corrected for multiple comparisons. *p*-Values < .05 indicate significant differences. Analysis was performed using Stata version 14.2 (StataCore LLC, College Station Texas, US).

3. Results

All participants concluded the study, detailed demographics are shown in Table 1.

As expected, people with diabetes had higher heart rate and systolic blood pressure and accordingly assessment of the parasympathetic tone showed lower cardiac vagal tone reflecting diabetes induced alterations of the autonomic nervous system. Twenty five percent had retinopathy which is another sequela of microvascular complication and none suffered from nephropathy as this was part of the exclusion criteria.

Assessment of the conduction velocity of the median, sural, and peroneal nerves revealed inter-group differences, confirming DSPN in participants with diabetes. The diagnose DSPN was based on clinical examination and several abnormal neurophysiological measures in order to rule out e.g. abnormal median nerve conduction caused by mechanical obstructions such as e.g. carpal tunnel syndrome. In the same line, approximately 50% higher stimulus intensity was needed to elicit a median nerve motor response in diabetes in comparison to healthy (12.1 mA vs. 18.04 mA).

3.1. Evaluation of peripheral, spinal, and cerebral somatosensory evoked potentials

EEG was successfully recorded in 46 participants with diabetes and 20 participants without diabetes. Due to signal quality all EEG component could not always be detected in all recordings. The data analysis is therefore based on all the detected EEG components. Overall there were consistent differences between the two groups in SEP's, measured at multiple anatomical levels (Table 2).

In people with diabetes, post hoc analysis of peripheral transmission (Erb's point) showed increased latencies at P9 (0.49 ms) and N11 (0.47 ms) as well as decreased peak to peak amplitude (−2.13 μV) (Fig. 1 and Table 2). Post hoc analysis of the Cv7-spinal SEP's also showed decreased amplitudes (−0.57 μV). This was again seen at the Oz pre-cortical level (−0.22 μV).

Post hoc analysis of the early cortical processing assessed at CP5 showed increased latencies in people with diabetes at N20 (2.41 ms) and P22 (5.88 ms), but *increased* peak to peak amplitude (2.5 μV). Finally, their late cortical processing also showed increased latencies of the N60 (39.08 ms) and P80 (54.55 ms), with a decrease in amplitude (−0.34 μV). Diabetes prolonged the central conduction time measured between N14 and N60 by 74.4%, from 54.35 ms to 94.77 ms.

Table 1
Demography and clinical characteristics.

	Healthy	Type 1 diabetes	*p*-Value
Age (range), years	51.3 (40–62)	50.0 (33–71)	0.53
Gender	6 female, 15 males	9 female, 39 males	0.48
Height, cm	179.8 ± 9.0	178.4 ± 8.6	0.55
Weight (range), kg	87.3 (62–140)	90.0 (63–132)	0.56
Body mass index, kg/m^2	26.9 ± 5.5	28.5 ± 4.9	0.30
Disease duration, (range), years	–	32.2 ± 9.5 (14–51)	
Pulse rate, beats/min	66 ± 7	73.9 ± 10.5	**0.001**
Systolic BP (mmHg)	129 ± 15	149.9 ± 16	**0.007**
Diastolic BP (mmHg)	76 ± 11	82.3 ± 10.9	0.33
CVT	4.33 (1.7; 1.89–8.1)	3.0 (1.9; 0.63–6.9)	**0.01**
Cholesterol, total, mmol/l	5.40 ± 0.85	4.49 ± 0.80	**0.000**
Triglyceride, mmol/l	1.16 ± 0.55	1.01 ± 0.64	0.35
HDL, mmol/l	1.56 ± 0.43	1.56 ± 0.54	0.97
LDL, mmol/l	3.30 ± 0.87	2.48 ± 0.56	**0.000**
HbA_{1c}, mmol/mol	33.67 ± 3.37	65.96 ± 10.45	**0.000**
HbA_{1c}, %	5.3	8.1 ± 1	
Fasting glucose, mmol/l	5.5 ± 0.80	12.28 ± 5.36	**0.000**
Retinopathy, % of participants	0%	25%	**0.013**
Creatinine, urine	11,019.52 ± 7809	10,905.15 ± 5400	0.94
Albumin, urine	0.0083 ± 0.006	0.074 ± 0.18	0.10
Median nerve stim. intensity, mA	12.1 ± 5.65	18.04 ± 6.93	**0.001**
Median nerve conduction velocity, sensory threshold, m/s	54.7 ± 4.5	43.2 ± 7.2	**0.000**
Median nerve amplitude, sensory threshold, mV	26.6 ± 11.1	13.4 ± 8.0	**0.000**
Median nerve conduction velocity, motor threshold, m/s	55.4 ± 2.5	49.7 ± 4.9	**0.000**
Median nerve amplitude, motor threshold, mV	9.1 ± 1.7	8.2 ± 2.2	0.09
Peroneal nerve conduction velocity, m/s	45.7 ± 2.9	37.3 ± 5.4	**0.000**
Peroneal nerve amplitude, mV	3.7 ± 1.5	1.8 ± 1.3	**0.000**
Sural nerve conduction velocity, m/s	49.7 ± 6.1	42.5 ± 5.8	**0.000**
Sural nerve amplitude, mV	6.6 ± 3.2	3.6 ± 2.3	**0.000**

Data are expressed and mean and SD unless otherwise stated. *p*-Values < .05 indicate significant differences. BP: blood pressure; CVT: cardiac vagal tone; HDL: high density lipoprotein; LDL: low-density lipoprotein (LDL).

Table 2
Assessment of evoked potentials at five different points following median nerve stimulation.

Electrode	Healthy	N	Diabetes	N	Difference	*p*-Value	Post-hoc
Erb's point (peripheral processing)		20		44		**0.000**	
P9 latency, ms	9.45 ± 0.39		9.94 ± 0.69		0.49		$p =$ **.000**
N11 latency, ms	10.98 ± 0.53		11.45 ± 1.10		0.47		$p =$ **.044**
P2P amplitude, μV	4.40 ± 3.26		2.27 ± 1.81		−2.13		$p =$ **.000**
Cv7 (spinal processing)		18		30		**0.032**	
P11 latency, ms	11.31 ± 0.68		11.33 ± 1.70		0.02		$p =$ .96
N14 latency, ms	14.34 ± 0.97		13.59 ± 2.19		−0.75		$p =$ .16
P2P amplitude, μV	1.68 ± 0.84		1.11 ± 0.64		−0.57		$p =$ **.005**
Oz (precordical processing)		20				**0.007**	
N14 latency, ms	16.00 ± 0.97		17.35 ± 3.37	43	1.35		$p =$ .090
P18 latency, ms	19.35 ± 1.87		19.43 ± 3.13	37	0.08		$p =$ .98
P2P amplitude, μV	0.44 ± 0.31		0.22 ± 0.25	37	−0.22		$p =$ **.004**
CP5 (early cortical processing)		20		36		**0.000**	
N20 latency, ms	21.95 ± 1.36		24.36 ± 3.17		2.41		$p =$ **.003**
P22 latency, ms	31.4 ± 3.68		37.28 ± 7.03		5.88		$p =$ **.001**
P2P amplitude, μV	3.95 ± 2.21		6.45 ± 3.08		2.50		$p =$ **.002**
C1 (late cortical processing)		20		46		**0.004**	
N60 latency, ms	70.35 ± 7.43		109.43 ± 50.51		39.08		$p =$ **.001**
P80 latency, ms	107.25 ± 20.98		161.80 ± 64.74		54.55		$p =$ **.000**
P2P amplitude, μV	2.83 ± 1.58		2.49 ± 1.67		−0.34		$p =$ .50
Oz-C1 (central conduction time)		20		43			
N14–N60, ms	54.35 ± 7.18		94.77 ± 50.81		40.42		$p =$ **.001**

Data are expressed and mean and SD unless otherwise stated. *p*-Values < .05 indicate significant differences One-way multivariate analysis of variance (MANOVA) with age and height as covariates is used when comparing healthy controls and people with diabetes. Spearman and Bonferroni corrected.

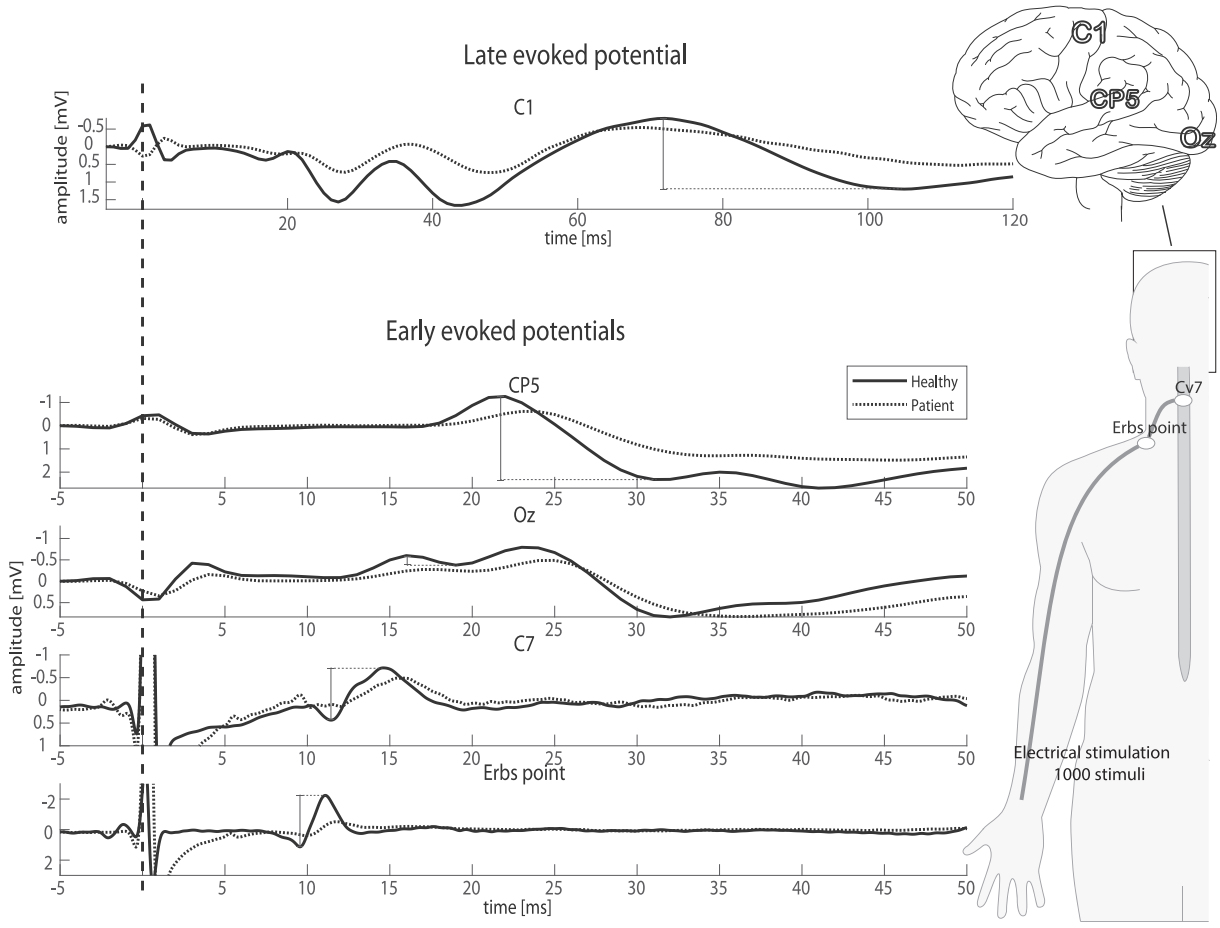

Fig. 1. Graphical presentation of the evoked potentials recorded from different levels in the ascending pathway, following electrical stimulation of the median nerve. Average of all participants with and without diabetes.

Table 3
Assessment of correlation between central conduction time and clinically parameters.

Central conduction time and:	N	Healthy	*p*-Value	N	Diabetes	*p*-Value
Age	20	−0.12	0.62	43	0.03	0.83
Height	20	0.14	0.56	43	0.23	0.13
Disease duration				43	0.02	0.89
Median nerve conduction velocity, motor threshold	20	−0.13	0.60	43	−0.35	**0.023**
Median nerve action potential, motor threshold	20	0.28	0.23	43	0.07	0.68
Cardiac vagal tone	18	−0.52	**0.027**	32	−0.35	**0.047**

Correlations are assessed by Spearman's Rho and corrected by Bonferroni for multiple comparisons. *p*-Values < .05 indicate significant association.

3.2. Associations between central conduction time and age, height, disease duration, median nerve conduction velocity, median nerve amplitude and parasympathetic tone

There were no significant correlations between central conduction time and age, height, disease duration or median nerve action potential for none of the two groups. In diabetes increased central conduction time was correlated to decreased median nerve conduction velocity (Table 3). Increased central conduction time was correlated to decreased parasympathetic tone (CVT) in both healthy and participants with diabetes.

4. Discussion

We have demonstrated that in diabetes and confirmed DSPN, the extensive neuronal changes are manifest as decreased conduction velocities and amplitudes of action potentials at peripheral and central levels. The neuronal transmission was prolonged as the nerve signal travels up through the system and passes an increasing number of synapses, which is reflected in the central conduction time. The findings confirm that neuronal transmission is altered at different anatomical levels of the upstream activation, confirming de-myelinisation, axonal loss, altered central processing and delayed inter-neuronal communication. Finally, associations between increased central conduction time and diminished parasympathetic tone indicate extensive neuronal pathophysiological processes which may progress in parallel.

4.1. Diabetes induced changes at the peripheral level

Nerve conduction studies have classically been used to objectively detect peripheral nerve dysfunctions and have the advantage to e.g. MRI studies, that they assess the functional capacity of the large myelinated peripheral nerves, and thereby serve as a proxy for the severity of DSPN. In the present study, DSPN was diagnosed by assessing motor and sensory nerves of the upper and lower extremities conducted by trained neurophysiologists and our measures of nerve transmission at Erb's point confirmed the diagnosis. However, we did not find an association between median nerve conduction velocity and latencies of SEPs. The median nerve conduction velocity from the clinical neurophysiological investigation is based on electrodes situated proximal to the wrist and distal to the elbow (approximate distance 200 mm). In contrast the conduction velocity (latency) from the evoked potentials calculated from the stimulation point of the median nerve to the Erb's point travel across joint near structures at the elbow and shoulder. Consequently, the two measures support each other, but are not directly comparable. It is established that in response to long-term hyperglycemic exposure both myelin sheaths and the axons itself are affected by changes to the neuronal microenvironment.[19,20] Therefore, the decreased nerve conduction velocity and prolonged latency in the sensory median nerve, serves as a proxy of the health of axons and myelin sheath. Consequently, reduced conduction velocity can arise from degeneration of myelin sheath as well as axonal loss or dysfunction.[21,22] Concomitantly the evidence of reduced amplitudes in the evoked potentials and measurements of the diminished action potential amplitude in peripheral nerves is indicative of axonal atrophy.[23] In conclusion, these findings are in accordance with characteristic evidence in diabetic polyneuropathy.[22,24,25]

4.2. Diabetes induced changes at the spinal and cerebral levels

As the signal travels up-stream through the spinal cord (Cv7), the subcortical level (Oz), and the cortical level (CP5 and C1) an increasing number of synapses and inter-neuronal communications are involved. Hence, group differences at these points are considered the net effect of changes in nerve integrity, synaptic transmission, and cortical processing. Indeed, the prolongation of the central conduction time in diabetes indicates extensive alterations of the central response and it can be speculated that the delayed synaptic transmission contributes proportionally more than reduced conduction velocity to the final latencies of SEP's. In addition, the amplitude of specific electrodes represents central synchronicity of the firing pattern in a number of neurons the so-called central processing of the afferent signal. Furthermore, the amplitudes of the SEPs at spinal, subcortical and late cortical assessment points were consistently reduced indicating extensive axonopathy.

The stimulus intensity to evoke motoric twitch of the thumb, was approximately 50% larger in people with diabetes and consequently the observed decreased amplitudes may be under estimated. However, a reduced amplitude could also be an effect of increased latency jitter[26] and therefore we cannot rule out that this contributes to the reduction in amplitudes. Interestingly, at the early cortical level the amplitude was increased, which hypothetically could be interpreted as hyperexcitability of the thalamus. In addition, the different effect on measured amplitudes at the different cerebral positions could also represent changes in involved pathways, involved networks and integrity of the cortical processing.[27–29]

Our findings are supported by other studies. A previous study in type 2 diabetes, also showed prolongation of latencies and reduced amplitude to electrical median nerve stimulation, indicating a shared pathogenesis of neuropathy in type 1 and type 2 diabetes.[13]

Nakamura et al.[12] investigated neuropathy in a mixed cohort of type 1 and 2 diabetes with an average disease duration of approximately 8 years, which supports our findings of increased latency after median nerve stimulation Furthermore, Ziegler et al.[9] conducted pioneering work where neuropathy in type 1 diabetes was thoroughly investigated and reported increased latency and reduced amplitudes in peripheral, spinal and cortical SEP's following tibial nerve stimulation. However, in contrast to the present study previously studies have not reported alterations in central conduction time.[9,13] Taken together, these studies conclude consistently that the conduction abnormalities in diabetes involves central as well as peripheral somatosensory pathways.[13] Therefore, our study expands the generalizability of their findings, to also include people with long term (32 years) type 1 diabetes, who have received stable hyperglycemic medication including fast and long acting insulin with dosing adjustment according to contemporary regimens, minimizing hypo and hyperglycemic induced neurotoxicity.

Central and peripheral changes of the diabetic neuropathy have been shown by use of different neurophysiological and imaging techniques.[30–32] Studies on resting state EEG have shown altered spontaneous brain oscillations and cortical processing[10] and other EEG studies in people with diabetes have shown altered cerebral processing with functional reorganization.[27–29] EEG changes has previously been observed in people with type 1 diabetes and visceral neuropathy, which steadily have shown increased latency and decreased amplitude of sensory evoked brain potentials following electrical esophageal stimulation.[5] Consequently, these studies support the present findings. In addition, in people with diabetes and upper gastrointestinal

symptoms plasticity within the insula and cingula region of the brain, were involved in symptom generation and maintenance.[4] Furthermore, the involved brain networks encoding the visceral sensation was altered.[28] Finally, in support of our findings which emphasizes extensive multilevel neuronal damage, a study showed association between peripheral nerve conduction deficiencies and acquired cognitive impairment.[34]

4.3. Associations between central conduction time and parasympathetic tone

The study showed an association between prolonged central conduction time and diminished parasympathetic tone, indicating that the pathophysiology leading to neuropathy may be shared in autonomic and central neuropathies. Cardiac vagal tone represents the modulatory efferent vagal nerve originating from the brainstem and influencing the heart. The concomitant presence of abnormal response in the brain and brainstem may represent shared pathophysiological genesis, however since the data is cross sectional it does not allow for a causative interpretation. The association was present in both controls and people with diabetes, indicating that CVT may serve as a proxy of the general integrity of the nervous system, and not only when extensive neuronal alterations are present.

4.4. Methodological considerations and limitations

Firstly, glycemic variability is a risk factor for diabetic polyneuropathy[35] and hypo- and hyperglycemic exposure are known to be neurotoxic.[36] Studies have shown that long-term optimal glycemic control in type 1 diabetes was associated with near-normal nerve function.[37,38]

In the present study, recording of the number of hyper- and hypoglycemic was limited to the preceding 48 h and glycemic control was only measured by HbA1C, reflecting preceding month's glycemic fluctuations and therefore future studies will be improved by assessing continuous glucose monitoring. Secondly, as blood glucose directly influences the neuronal function, it cannot be ruled out that the normoglycemia in participants without diabetes will overestimate the impact of hyperglycemia on nerve function, as the difference in glucose level could have affected not only the nerve conduction – but also the processing in the brain – however results are ambiguous.[39,40] Thirdly, median nerve conduction velocity is only part of the Toronto criteria used to verify DSPN. The clinical diagnose is based on measurements from sensory and motor nerves from upper and lower extremities, and consequently recordings from the median nerve does not necessarily have to be abnormal in these patients even though they were diagnosed with DSPN. From the demographics it is evident, that extensive neuronal changes were present in diabetes.

However, a composite severity score as suggested by Dyck et al.,[41,42] may have been used to assess the severity of neuropathy. Such score has previously shown association to severity of peripheral and central neuronal changes.[9] Fourthly, a comparative group of matched participants with type 1 diabetes but without DSPN or a group of people with the presence of neuropathy for other reasons than diabetes, would have strengthened the study and the present findings do not allow to conclude whether the results of this study are due to peripheral neuropathy alone or neuropathy caused by diabetes. Fifthly, the presence of carpal tunnel syndrome is higher in diabetes and is known to affect nerve transmission passing the carpus. However, to limit this influence the present nerve conduction velocity of the median nerve was assessed from wrist to elbow, minimizing this effect. Finally, the majority of people with diabetes are pre-disposed to co-morbidities and may therefore receive other medication. However, medication which could influence nerve-function was part of the exclusion criteria. Interestingly, our data show that people with diabetes are in tight control of hypercholesterolemia (statins) in accordance with treatment guidelines and consequently had lower levels of cholesterol, which are reflected in their reference values range. (normal value: healthy <5.0; diabetics <4.0).

5. Conclusion

This study provides evidence of changes in the ascending sensory pathway from the periphery and spinal cord to the cortical response in long term type 1 diabetes mellitus and confirmed DSPN. Extensive neuronal impairment manifest as decreased conduction velocities, decreased amplitudes, decreased central conduction time indicate impaired inter-neuronal communication. Furthermore, decreased parasympathetic tone was associated with prolonged central conduction time. These findings confirm that diabetes induces neuronal impairment at multiple anatomical levels– involving peripheral, autonomic and central nerves.

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Appendix A. Supplementary data

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References

1. Callaghan BC, Cheng H, Stables CL, Smith AL, F EL. Diabetic. neuropathy: clinical manifestations and current treatments. Lancet Neurol 2014;11:521-34. https://doi.org/10.1016/S1474-4422(12)70065-0.Diabetic.
2. Tesfaye S, Boulton AJM, Dyck PJ, et al. Diabetic neuropa. thies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33:2285-93. https://doi.org/10.2337/dc10-1303.
3. Pop-Busui R, Boulton AJM, Feldman EL, et al. Diabetic neuro. pathy: a position statement by the American Diabetes Association. Diabetes Care 2017;40:136-54. https://doi.org/10.2337/dc16-2042.
4. Brock C, Søfteland E, Gunterberg V, et al. Diabetic autonomic neu. ropathy affects symptom generation and brain-gut axis. Diabetes Care 2013;36:3698-705. https://doi.org/10.2337/dc13-0347.
5. Frøkjær JB, Egsgaard LL, Graversen C, et al. Gastrointesti. nal symptoms in type-1 diabetes: is it all about brain plasticity? Eur J Pain 2011;15:249-57. https://doi.org/10.1016/j.ejpain.2010.08.004.
6. Farmer AD, Pedersen AG, Brock B, et al. Type 1 diabetic. patients with peripheral neuropathy have pan-enteric prolongation of gastrointestinal transit times and an altered caecal pH profile. Diabetologia 2017;60:709-18. https://doi.org/10.1007/s00125-016-4199-6.
7. Wheeler SG, Ahroni JH, Boyko EJ. Prospective study of autonomic neuropathy as a predictor of mortality in patients with diabetes. Diabetes Res Clin Pract 2002;58:131-8, http://www.ncbi.nlm.nih.gov/pubmed/12213355. Accessed November 15, 2018.
8. Kupers R, Kehlet H. Brain imaging of clinical pain states: a critical review and strategies for future studies. Lancet Neurol 2006;5:1033-44. https://doi.org/10.1016/S1474-4422(06)70624-X.
9. Ziegler D, Muhlen H, Dannehl K, Gries FA. Tibial nerve somatosensory evoked potentials at various stages of peripheral neuropathy in insulin dependent diabetic patients. . J Neurol Neurosurg Psychiatry 1993;56:58-64. https://doi.org/10.1136/jnnp.56.1.58.
10. Frøkjær JB, Graversen C, Brock C, et al. Integrity of central nervous . function in diabetes mellitus assessed by resting state EEG frequency analysis and source localization. J Diabetes Complications 2017;31:400-6. https://doi.org/10.1016/j.jdiacomp.2016.11.003.
11. Lelic D, Valeriani M, Fischer IWD, Dahan A, Drewes AM. Venlafaxine and oxycodone have different effects on spinal and supraspinal activity in man: a somatosensory evoked potential study. . Br J Clin Pharmacol 2017;83:764-76. https://doi.org/10.1111/bcp.13177.
12. Nakamura Y, Takahashi M, Kitaguchi M, et al. Clinical utility of somatosensory evoked potentials in diabetes mellitus. Diabetes Res Clin Pract 1989;7:17-23. https://doi.org/10.1016/0168-8227(89)90039-9.

13. Suzuki C, Ozaki I, Tanosaki M, Suda T, Baba M, Matsunaga M. Peripheral and central conduction abnormalities in diabetes mellitus. Neurology 2000;54. :1932-7. https://doi.org/10.1212/WNL.54.10.1932.
14. Botha C, Farmer AD, Nilsson M, et al. Preliminary report: modulation of parasympathetic nervous system tone influences oesophageal pain hypersensitivity. Gut 20. 15;64:611-7. https://doi.org/10.1136/gutjnl-2013-306698.
15. Farmer AD, Coen SJ, Kano M, et al. Normal values and reproducibility of the real-time index of vagal tone in healthy humans: a multi-center study. Ann Gastroenterol 2014;27:362-8, http://www.ncbi.nlm.nih.gov/pubmed/25330916. Accessed March 17, 2019.
16. Julu PO. A linear scale for measuring vagal tone in man. J Auton Pharmacol 1992;12: 109-15, http://www.ncbi.nlm.nih.gov/pubmed/1583025. Accessed March 17, 2019.
17. Brock C, Jessen N, Brock B, et al. Cardiac vagal tone,. a non-invasive measure of parasympathetic tone, is a clinically relevant tool in type 1 diabetes mellitus. Diabet Med 2017;34:1428-34. https://doi.org/10.1111/dme.13421.
18. Brock C, Hansen CS, Karmisholt J, et al. Liraglutide treatment redu. ced interleukin-6 in adults with type 1 diabetes but did not improve established autonomic or polyneuropathy. Br J Clin Pharmacol 2019;85:2512-23. https://doi.org/10.1111/bcp.14063.
19. Meldgaard T, Olesen SS, Farmer AD, et al. Diabetic enteropathy. : from molecule to mechanism-based treatment. J Diabetes Res 2018;2018:3827301. https://doi.org/10.1155/2018/3827301.
20. Dyck PJ, Zimmerman BR, Vilen TH, et al. Nerve glucose, fructose, sorbitol, *myo*. -inositol, and fiber degeneration and regeneration in diabetic neuropathy. N Engl J Med 1988;319:542-8. https://doi.org/10.1056/NEJM198809013190904.
21. Waxman S. Determinants of conduction velocity in myelinated nerve fibers. Muscle Nerve 1980;3:141-50. https://doi.org/10.1002/mus.880030207.
22. Said G, Goulon-Goeau C, Slama G, Tchobroutsky G. Severe early-onset p. olyneuropathy in insulin-dependent diabetes mellitus. N Engl J Med 1992;326: 1257-63. https://doi.org/10.1056/NEJM199205073261905.
23. Rivner MH, Swift TR, Malik K. Influence of age and height on nerve conduction. Muscle Nerve 2001;24:1134-41. https://doi.org/10.1002/mus.1124.
24. Said G, Baudoin D, Toyooka K. Sensory loss, pain. s, motor deficit and axonal regeneration in length-dependent diabetic polyneuropathy. J Neurol 2008;255:1693-702. https://doi.org/10.1007/s00415-008-0999-z.
25. Said G. Focal and multifocal diabetic neuropathies. Arq Neuropsiquiatr 2007;65: 1272-8, http://www.ncbi.nlm.nih.gov/pubmed/18345446. Accessed December 11, 2018.
26. Gram M, Graversen C, Nielsen AK, et al. A novel approach to phar. maco-EEG for investigating analgesics: assessment of spectral indices in single-sweep evoked brain potentials. Br J Clin Pharmacol 2013;76:951-63. https://doi.org/10.1111/bcp.12120.
27. Cooray G, Nilsson E, Wahlin Å, Laukka EJ, Brismar K, Brismar T. Effects of intensifie. d metabolic control on CNS function in type 2 diabetes. Psychoneuroendocrinology 2011;36:77-86. https://doi.org/10.1016/j.psyneuen.2010.06.009.
28. Lelic D, Brock C, Simrén M, et al. The brain networks encoding v. isceral sensation in patients with gastrointestinal symptoms due to diabetic neuropathy. Neurogastroenterol Motil 2014;26:46-58. https://doi.org/10.1111/nmo.12222.
29. Brismar T, Hyllienmark L, Ekberg K, Johansson B-L. Loss of temporal lobe beta power in young adults with type 1 diabetes mellitus. Neuroreport 2. 002;13:2469-73. https://doi.org/10.1097/01.wnr.0000047688.08940.ab.
30. Tesfaye S, Selvarajah D, Gandhi R, et al. Diabetic peripheral neuropathy may not be as its name suggests. Pain 2016. ;157:S72-80. https://doi.org/10.1097/j.pain.0000000000000465.
31. Franc DT, Kodl CT, Mueller Ba, Muetzel RL, Lim KO, Seaquist ER. . High connectivity between reduced cortical thickness type 1 diabetes. Diabetes 2011;60:315-9. https://doi.org/10.2337/db10-0598.
32. Frøkjær J, Brock C, Søfteland E, et al. Macrostructural brain changes in pat. ients with longstanding type 1 diabetes mellitus - a cortical thickness analysis study. Exp Clin Endocrinol Diabetes 2013;121:354-60. https://doi.org/10.1055/s-0033-1345120.
33. Brismar T, Maurex L, Cooray G, et al. Predictors of cognitive impairment in type 1 diabetes. Psychoneuroendocrinology 2007;32:10. 41-51. https://doi.org/10.1016/j.psyneuen.2007.08.002.
34. Akaza M, Akaza I, Kanouchi T, Sasano T, Sumi Y, Yokota T. Nerve conduction study of the association between glycemic variability and diabetes neuropathy. . Diabetol Metab Syndr 2018;10:69. https://doi.org/10.1186/s13098-018-0371-0.
35. Boulton AJ, Knight G, Drury J, Ward JD. The prevalence of symptomatic, diabetic neuropathy in an insulin-treated population. Diabetes Care. 8(2):125–128, http://www.ncbi.nlm.nih.gov/pubmed/3996169. (Accessed November 19, 2018).
36. Ziegler D, Behler M, Schroers-Teuber M, Roden M. Near-normogly. caemia and development of neuropathy: a 24-year prospective study from diagnosis of type 1 diabetes. BMJ Open 2015;5, e006559. https://doi.org/10.1136/bmjopen-2014-006559.
37. Larsen JR, Sjoholm H, Hanssen KF, Sandvik L, Berg TJ, Dahl-Jorgensen K. Optimal blood glucose control during 18 years preserves peripheral nerve function in patients with 30 years' duration of type 1 diabetes. . Diabetes Care 2003;26:2400-4. https://doi.org/10.2337/diacare.26.8.2400.
38. Morley G, Mooradian A, Levine A, Morley J. Mechanism of pain in diabetic peripheral neuropathy. Effect of glucose on pain perception in humans. Am J Med 1984;77: 79-82. https://doi.org/10.1016/0002-9343(84)90439-X.
39. Frøkjær JB, Søfteland E, Graversen C, Dimcevski G, Drewes AM. Effect of acute hyperglycaemia on sensory processing in diabetic autonomic neuropathy. . Eur J Clin Invest 2010;40:883-6. https://doi.org/10.1111/j.1365-2362.2010.02335.x.
40. Dyck PJ, Litchy WJ, Daube JR, et al. Individual a. ttributes versus composite scores of nerve conduction abnormality: sensitivity, reproducibility, and concordance with impairment. Muscle Nerve 2003;27:202-10. https://doi.org/10.1002/mus.10320.
41. Dyck PJ, Carter RE, Litchy WJ. Modeling nerve conduction criteria for diagnosis of diabetic polyneuropathy. . Muscle Nerve 2011;44:340-5. https://doi.org/10.1002/mus.22074.