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Abstract

Introduction

Comprehensive evaluation of the upstream sensory processing in diabetic symmetrical polyneuropathy (DSPN) is sparse. We investigated the spinal nociceptive withdrawal reflex (NWR), and the related elicited somatosensory evoked cortical potentials. We hypothesized that DSPN induces alterations in spinal and supra-spinal sensory-motor processing compared to age- and gender matched healthy controls.

Methods

In this study 48 patients with type-1 diabetes and DSPN were compared to 21 healthy controls. Perception- and reflex-threshold were determined and subjects received electrical stimulations on the plantar site of the foot at three stimulation intensities to evoke a NWR. EMG and EEG were recorded for analysis.

Results

Patients with DSPN had higher perception- ($p<0.001$) and reflex- ($p=0.012$) threshold. Fewer patients completed the recording session compared to healthy (34/48 vs 21/21; $p<0.004$). DSPN reduced the odds ratio (OR) of a successful elicited NWR; (OR=0.045; $p=0.014$). DSPN changed the evoked potentials ($F=2.86$; $p=0.025$), and post hoc test revealed reduction of amplitude (-3.72mV; $p<0.021$) and prolonged latencies (15.1 ms; $p<0.013$) of the N1 peak.

Conclusions

The study revealed that patients with type-1 diabetes and DSPN have significantly changed spinal and supraspinal processing of the somatosensory input. This implies that DSPN induces widespread differences in the central nervous system processing of afferent A-δ and A-β fiber input. These differences in processing may potentially lead to identification of

subgroups with different stages of small fiber neuropathy and ultimately differentiated treatments.

Key words: EEG, EMG, Nociceptive Withdrawal Reflex, diabetes, diabetic symmetrical polyneuropathy

The prevalence of diabetes mellitus (DM) is increasing (1,2). Consequently, microvascular complications accompanying long-term DM have become more common, and among the most serious is diabetic neuropathy (3). Classically, diabetic neuropathy manifests as length dependent diabetic symmetrical polyneuropathy (DSPN), which affects the long nerve fibres in the body and commonly presents symptoms in the feet and hands (4,5). Approximately 30-50% who suffer from type-1 or -2 DM will develop diabetic neuropathy (5–7). These alterations are typically measured by nerve conduction studies (8) which, among other assessments of nerve fibre functionality, are used to diagnose DSPN (8). To obtain a more comprehensive evaluation of the upstream sensory processing in DSPN, somatosensory evoked potentials (SEPs) have been recorded following electrical stimulation of the median and tibial nerves (9,10). Prolonged latencies of the SEPs have been associated to disease duration, motor nerve conduction velocity (11) and peripheral nerve dysfunction (9). Reduced SEP amplitudes were shown in studies based on median nerve stimulation, indicating axonal loss, and consequently affected conduction response in the central as well as peripheral somatosensory pathways (10).

The nociceptive withdrawal reflex (NWR) is a spinal polysynaptic reflex that integrates sensory input to escape a potential harmful stimulation (12). The NWR can be elicited from the plantar side of the foot. In contrast to studies of nerve conduction which measures A-β fiber response, the NWR is primarily mediated by A-δ- and C -fibers (13). In this study, we wanted to investigate how DSPN affects spinal and supra-spinal sensory-motor processing. Therefore, as a proxy of the spinal sensory transmission, we recorded the NWR to electrical stimulation and concomitantly recorded SEPs at the scalp surface, to measure supra-spinal processing of the same stimulus.

We hypothesized that long-term DM induces alterations in spinal and supra-spinal sensory-motor processing compared to age- and gender matched healthy controls. Therefore, the primary objective of the present study was to characterize neuronal response at the spinal and supra-spinal levels in people with long-term DM and verified DSPN compared to healthy controls.

Thus, the aims of the study were to compare: I) ability to elicit an NWR measured as numbers of reflexes, II): quantifications of the NWR using latency and area under the curve (AUC) of the EMG response and III): SEP measured close to the brainstem (Oz), and the vertex (Cz) electrode according to the 10-20 system.

Methods

Study population

Forty-eight patients with DM were recruited at the Department of Endocrinology, Aalborg University Hospital, Denmark. Potential eligible patients 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 (8). Additional inclusion criteria were age above 18 years, a verified diagnosis of type-1 DM for a minimum of 2 years: (hemoglobin A1c (HbA1c) level ≥6.5% [>48 mmol/mol]), stable hyperglycemic medication, and body mass index>22 kg/m^2. Exclusion criteria included type-2 DM, other neurological disorders than DSPN, estimated glomerular filtration rate <60 ml/min/1.73m^2, calcitonin >25 ng/l, HbA1c level <6.5%, use of glucagon-like peptide-1 agonists or dipeptidyl peptidase-4 inhibitors. Twenty-one age-matched healthy volunteers were included for comparison. Inclusion criteria were age above 18 years and normal peripheral nerve conduction. Exclusion criteria included type-1- and 2 DM, 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 entering the study. The study was registered in public databases (EUDRA CT, ref 2013-004375-12) and clinicaltrials.gov (ref NCT02138045) and was 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 2017 at Aalborg University Hospital.
A visual representation of the data analysis steps and the number of subjects who were able to participate in the given parts of the study are shown in Figure *1*.

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Experimental procedure

Electrical stimulation

Electrical stimulation of the plantar skin of the foot (innervation site of the medial plantar nerve) was applied through surface electrodes to evoke NWR´s of the foot and elicit SEPs. The cathode was placed in the arch of the sole of the right foot (15X15 mm, Neuroline 700; Ambu A/S, Copenhagen, Denmark). The anode was placed on the foot dorsum (50X90 mm, Synapse; Ambu A/S, Copenhagen, Denmark). The stimulus was delivered by a computer-controlled electrical stimulator (Noxitest IES 230, Aalborg, Denmark) as a constant current burst of five rectangular-wave pulses, with 1 ms duration and 4 ms between pulses. A custom-made LABVIEW software (Center for Sensory-Motor Interaction, Aalborg University, Denmark) was used to control the stimulations. Subjects were positioned in an incline supine position and a pillow was placed under the knee to ensure relaxation in the leg muscles during stimulations. The perception threshold (PT) and NWR threshold (RT) were identified by increasing the stimulus intensity with increments of 1 mA. The PT corresponds to the stimulation intensity at which first sensation was felt. The RT was identified using the staircase method with 3 ascending and 3 descending threshold estimates. The ascending threshold was defined as the first reflex visible on EMG trace of the target muscle, and the descending threshold was 1 mA below the last stimulation to show a reflex visible on EMG. The RT was defined as the average of the 6 staircase end values. Once the RT was found, the subjects were asked to rate the pain intensity of the RT stimulations delivered at threshold (1.0 times RT), medium intensity (1.3 times RT) and high intensity (1.6 times RT) on a visual analogue scale, ranging from 0 to 10, where 0 means no pain and 10 is maximum imaginable

pain. Subsequently, 18 stimuli (six times each of the three intensities) were applied in random order with a varying inter-stimulus interval of 8-12 seconds. For safety reasons a threshold of 50 mA was set as a maximum intensity. If this threshold was crossed, or if pain/unpleasantness became unbearable the recording session was marked as incomplete and the highest stimulation intensity recorded was used to calculate RT.

Nociceptive withdrawal reflex recordings

Two surface electrodes (15X15 mm, Neuroline 700; Ambu A/S, Copenhagen, Denmark) were placed on the belly of the anterior tibial muscle ipsilateral to the site of stimulation, with an inter-electrode distance of 20 mm. The skin was lightly abraded before the electrodes were placed. A ground electrode (50X90 mm, Synapse; Ambu A/S) was placed distally to the patella of the ipsilateral knee. The EMG signals were sampled at 2000 Hz, and recorded 100 ms before and 900 ms after the stimulation for analysis.

Somatosensory evoked potential (SEP) recordings

A 61 surface electrode EEG cap (MEQNordic A/S, Jyllinge, Denmark) was used. Conductive gel was applied to reduce the electrode impedance below 5 kΩ. During NWR stimulations, subjects were asked to relax with their eyes open. The SEP signals were recorded in continuous mode with a sampling rate of 1000 Hz (SynAmp; Neuroscan, El Paso, TX, USA) and stored for analysis.

Data analysis

Electromyography

The EMG data was pre-processed using Matlab (R 2019a Mathworks Inc., Natick, Massachusetts, US.) as follows:

1. Bandpass filtered between 5 and 500 Hz using a zero-phase digital 12^{th} order Butterworth filter

Electroencephalography

The EEG data was pre-processed using Neuroscan software (v 4.5; Neuroscan, El Paso, Texas, USA) as follows:

1. Bandpass filtered between 1 and 30 Hz (zero-phase shift filter with a slope of 24 dB/octave)
2. Epoched from 20 ms before the stimulus to 500 ms after
3. Averaged across the stimulations
4. Manually interpolating bad channels
5. Average referenced

Additionally the latency and peak to peak amplitudes of the highest stimulations was performed on single sweeps of the Oz electrode. In order to improve the signal-to-noise ratio a robust referencing scheme was applied using the early-stage EEG processing pipeline (PREP) (14) This was done within Matlab (R 2019a Mathworks Inc., Natick, Massachusetts, US.) and the EEGLAB toolbox (version 14.1.2, Swartz Center for Computational

Neuroscience, Institute for Neural Computation, University of California, San Diego, US.).

After applying the PREP pipeline with standard settings the data was processed as follows:

1. Bandpass filtered between 1 and 30 Hz (zero-phase shift filter with a slope of 24 dB/octave)
2. Epoched from 20 ms before the stimulus to 500 ms after
3. Averaged across the high stimulations

Feature extraction

Electromyography

All EMG analysis was performed on single sweeps, and quantified using the interval peak Z score, which reflects the highest peak in the rectified reflex window minus a baseline mean over the standard deviation of the same baseline area (15).

$$Z_{score} = \frac{NWR_{peak} - baseline_{mean}}{baseline_{sd}}$$

The reflex window was between 70 and 160 ms post stimulus and the baseline window was 100 to 10 ms before the stimulation. In all cases a rectified AUC was calculated in the reflex window.

If the Z score was above 12 it was interpreted as an NWR (16), and in the case where no part of the reflex window had a Z score above 12, the stimulation did not elicit an NWR. When successfully elicited, the latency was defined at the first time the rectified EMG trace had a Z-score above 12.

Electroencephalography

Early latency and amplitudes were identified and analysed at the Oz electrode due to its location in proximity to the brainstem. Since the early brainstem evoked potential is

normally referenced to an electrode placed above the jugular notch and the Oz electrode referenced to a cephalic reference the current studies N30 and P40 brainstem potential is an inverted image of the brainstem P30 N40 peak.

Latencies and amplitude of SEP components were identified and analysed at the Cz electrode, because of its central location and maximal EP amplitude due to the electrical stimulation of the foot. The component consisted of the first and second positive peak (P1 and P2) and the largest negative peak (N1).

Statistical analysis

Fisher's exact test was used to test if there was an association between the presence of DM and number of participants that could complete the stimulation session.

A Mann–Whitney U test was used to compare changes between groups of the PT and RT, along with the peak-to-peak amplitude of the early evoked potential.

A Mixed-effects logistic regression model was used to test the binary outcome NWR containing trials (yes/ no) against the group factor (DM vs healthy); categorical stimulation intensities (threshold, medium and high) and the random factor Subject ID.

A mixed linear model was used to test differences in the latencies and AUC of the NWR, with group (DM vs healthy) as a between-subject factor, and the within-subjects factor stimulation intensity (threshold, medium and high).

A Pearson’s correlation analysis was conducted to test the association of disease duration and latency of the P1 peak and amplitude of the N1 peak.

A repeated measures mixed model was used to test the differences in the early latency with group (DM vs healthy) as a between-subject factor, and the within-subject factor peaks

(N30, P40), with subject as a random variable.

Finally, a multivariate analysis of variance (MANOVA) with the independent factors (DM vs. healthy) was used with the Wilkes' lambda distribution to assess the dependent variables (amplitude and latency) of the peaks (P1 N1 P2) in the recorded EEG signals. If significant difference were seen, post hoc analyses was performed to interpret which component (positive or negative peak along with latency) contributed to the differences. The data was tested for normality using the Kolmogorov–Smirnov test for normality.

Analysis was performed using Stata version 14.2 (StataCore LLC, College Station, Texas, US).

Results

In this study 48 participants with DM and DSPN were included and a group of 21 age matched healthy controls were recruited as a comparison; detailed demographics are shown in Table 1.

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Nociceptive withdrawal reflexes

Evaluation of perception and reflex threshold

Compared to healthy subjects, people with DM had significantly increased PT (5 [2, 40] vs. 3[2, 6] mA; $p<0.001$), the presence of DM also significantly increased the RT (22 [5, 50] vs. 15 [6, 37] mA; $p<0.012$). For further analyses, only subjects who were able to complete the reflex recording were included.

Evaluation of the ability to record the nociceptive withdrawal reflexes

Compared to healthy subjects, the presence of DM reduced the numbers of participants (34/48 vs 21/21) in whom an NWR was successfully elicited ($p<0.004$).

Evaluation of the ability to evoke the nociceptive withdrawal reflexes

The presence of DM reduced the odds ratio (0.045 [0.004, 0.54]) of a successful elicited NWR; ($p=0.014$). An increased odds ratio (1.07 [1.018, 1.131]) of eliciting a NWR was shown with increasing stimulus intensities ($p=0.009$).

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Description of the nociceptive withdrawal reflex

When the NWR could be elicited, no significant differences between the two groups (DM, Healthy) for latency (DM; p=0.168; 95% CI [-3.94, 22.7]) and AUC (DM; p=0.081; 95% CI [-14.38, 0.84]) were shown. There was, however, a significant longer latency for lowest and medium stimulation intensities compared to the high intensity (threshold; p<0.001 95% CI [5, 12], medium; p<0.001 95% CI [3, 9]) and a significant lower AUC for the lowest and medium stimulation intensities compared to the high intensity (threshold; p<0.00 95% CI [-9.64, -5.84] medium; p<0.00 95% CI [-5.8, -2]) A grand average EMG for healthy controls and patients with diabetes can be seen in

Figure 2.

EEG

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One healthy control, and two patients with DM were excluded from the EEG analysis due to poor data quality. A grand average EEG for healthy controls and patients with diabetes can be seen in Figure 3

Effect of disease duration on severity of diabetic symmetrical polyneuropathy (DSPN)

There is no significant correlation between disease duration of the people with DM and latency of the P1 peak ($p > 0.32$) and the N1 amplitude ($p > 0.63$).

Somatosensory Evoked Potentials (SEPs)

In comparison to the healthy controls, the presence of DM did not significantly change the latency of the SEP at Oz ($p = 0.746$, 95% CI [-5.3, 7.3]). The peak to peak amplitude also did not change between DM (median 1.91) vs. healthy (median 2.24) ($p > 0.95$)

In comparison to healthy controls, the presence of DM significantly changed the SEP at Cz ($F=2.86$ $p<0.025$). Post-hoc test revealed changes of the N1 peak where amplitudes were reduced by -3.72 mV ($p<0.021$) and latencies were increased by +15.1 ms ($p<0.013$), detailed results are shown in Table 2.

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Discussion:

To our knowledge this paper is the first study which investigates both spinal and supra-spinal responses to nociceptive withdrawal reflex stimulation in people with type-1 DM and DSPN and compare them to healthy controls. In the DM group the number of participants in whom NWR could be evoked was significantly smaller and the odds ratio of eliciting a NWR was reduced. In DM, the N1 peak measured at the vertex by EEG had decreased amplitude and prolonged latencies. The findings imply that DM induces widespread changes of both spinal and supra-spinal somatosensory signal processing.

Diabetes induced changes at the peripheral and spinal level

The differences in the elicited NWR in patients with DM could indicate demyelination of the A-δ fibers, since these are necessary for the activation of the NWR (17). DSPN typically affects the nerves in a length-dependent manner and therefore affects the feet and hands first (8), by demyelinating the nerve fibers, and consequently decreases the conduction velocity (18–20). The nerve conduction test mainly measures the thickly myelinated A-β fibres. Therefore, the current study, which measures the smaller A-δ fibres from the NWR (12,13) is complementary to classical nerve testing in understanding the widespread effects of DSPN. Small fibre neuropathy affects the thinner myelinated A-δ and unmyelinated C fibres without affecting the thicker myelinated fibres, and are therefore not diagnosed when using traditional conduction velocity tests (21). It should be stressed, however, that the reported changes of latency and AUC in this paper may be not be entirely representative for the DSPN population as it was not possible to elicit an NWR in all subjects.

The EMG recordings of the anterior tibial muscle in response to an electrical stimulation investigates the input-output relationship of the stimulation, and the time it takes from the

stimulus to an NWR is evoked (i.e. from the sole of the foot through the spinal reflex pathway to activation of the muscle), and the size of the response in the muscle. These measures characterize the nature of the NWR arch and thus also a part of the spinal pathway.

The difficulties in recording the NWR in DM are plausibly due to DM induced neuropathy, which is supported by a previous study in which the NWR could only be recorded in three out of seven (43%) of the included diabetes patients with DSPN (22). Recently, the method used to elicit the NWR of the anterior tibial muscle compared to the traditionally used biceps femoris has been found equally reliable at measuring the NWR and the choice of measuring reflexes at anterior tibial muscle is recommended in clinical trials due to the lower perception of pain (23).

Diabetes induced changes at the supra-spinal level

In order to investigate the responses at the supra-spinal level we aimed at estimating the afferent input in order to rule out any differences of the upstream activation, as this could explain any changes in the supra-spinal recordings. However, in this study we found no significant differences in the early evoked potentials recorded at Oz close to the brainstem, indicating that the afferent input was not significantly different between groups. At the Cz electrode (at the vertex), in contrast, DM did cause a significant decrease of the N1 amplitude and increase of latency in the recorded EEG signal. The P1 component at the vertex reflects exogenous processing of the primary somatosensory cortex activity and thus is insensitive to stimulus intensity and perceived pain. The endogenous components at the vertex appears 100 ms post-stimulus (N1 and P2), and increase with pain intensity (24). The

differences in the N1 component between groups indicate altered responses of the later cortical signal.

It is plausible that the evoked potentials are associated to disease duration and thereby hyperglycemic exposure to the axons. However, our findings indicate that no such association is found within this group of subjects. This may be explained by several factors: Firstly, it is generally accepted that disease duration in itself does not explain the progression of neuropathy, it is most likely doe to the number of events of hypo- and hyperglycemia that induces neurotoxicity, especially in well regulated cases (25). Secondly, this cohort may be vulnerable to selection bias, since they all had long disease duration and verified distal symmetrical polyneuropathy, and therefore represent a homogeneous group, which may hamper a true association between disease duration and EP´s.

In contrast to MRI studies which can detect both structural and functional neuronal changes in response to DM with/without painful DSPN (26–30). This electrophysiological study does not allow such interpretation, as pain is the conscious interpretation of the nociceptive input, which is continuously influenced by multiple cortical regions (31). The alterations of the neuronal responses in DM in the current study reflects altered activation of somatosensory processing, it is not possible to determine if there are any changes to the pain matrix given the current experimental setup (32).

EEG studies have found significant changes in the integrity of the processing of resting EEG and somatic and visceral elicited EP´s, (33–36), and taken together the complementary techniques mentioned above supports the current findings.

One study has shown that NWR´s elicited with intensities below RT (sub-RT) lead to increased amplitude of the SEP associated to the stimulation intensity, however amplitudes

were not significant different when stimulation intensities were chosen at or above RT (37). Consequently, the concomitantly recorded EEG during NWR in each subject were averaged across stimulation intensities to optimize signal-to-noise ratios.

Limitations

This study was not conducted without limitations. Firstly, the recruitment was vulnerable to selection bias as in particularity individuals with a surplus of resources, might be overrepresented. Nevertheless, all patients had verified type-1 DM and DSPN. Recordings were however only completed in a sub-group which increases the risk of a type-II error and limits the external validity, and thus our findings should be interpreted cautiously. Furthermore, the results within this paper do not relate to all people with DM, since no subjects with DM, but without DSPN, were included.

Secondly, the use of a calculated Z-score produces a binary outcome of the reflex. The Z-score is susceptible to poor signal to noise ratio, and thus true NWR may have been discarded due to high level of biological/electrical noise. The choice of a threshold of 12 yields a high true positive rate, but also carries the risk of false negatives (15). However, it is an objective and reliable method of detecting the presence of a successful elicitation of an NWR, and as such not hampered by subjective interpretation. Thirdly, the use of interpreting EP´s at the Oz electrode reveals that no differences occur at the spinal/brainstem level, however we cannot determine the central alterations based on interpretation of the Cz alone.

In conclusion the study revealed that patients with type-1 diabetes and DSPN have significantly changed spinal and supraspinal processing of the somatosensory input. This implies that DSPN induces widespread differences in the central nervous system processing

of afferent A-δ and A-β fiber input. These differences in processing may potentially lead to identification of subgroups with different stages of small fiber neuropathy and ultimately differentiated treatments.

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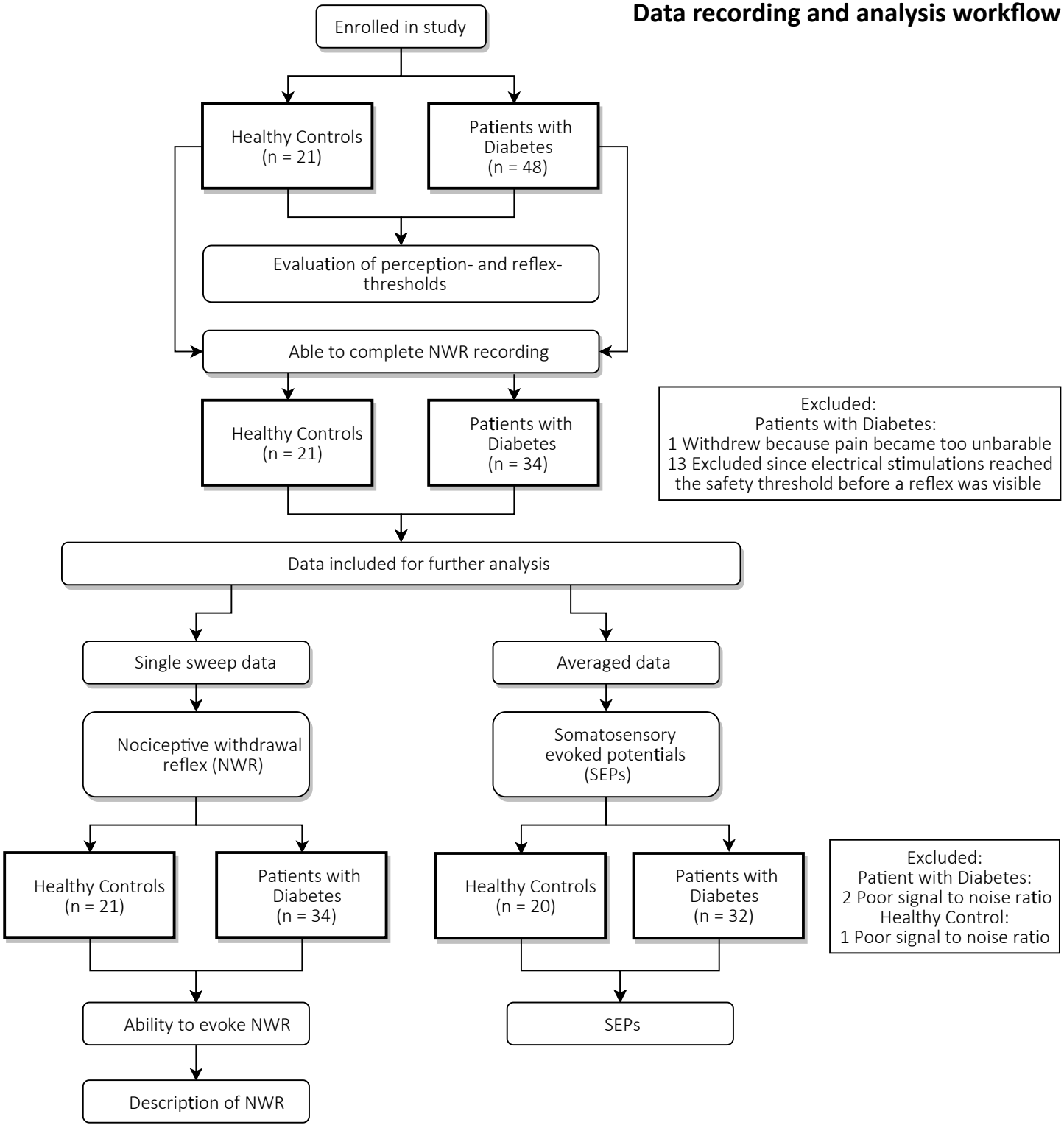
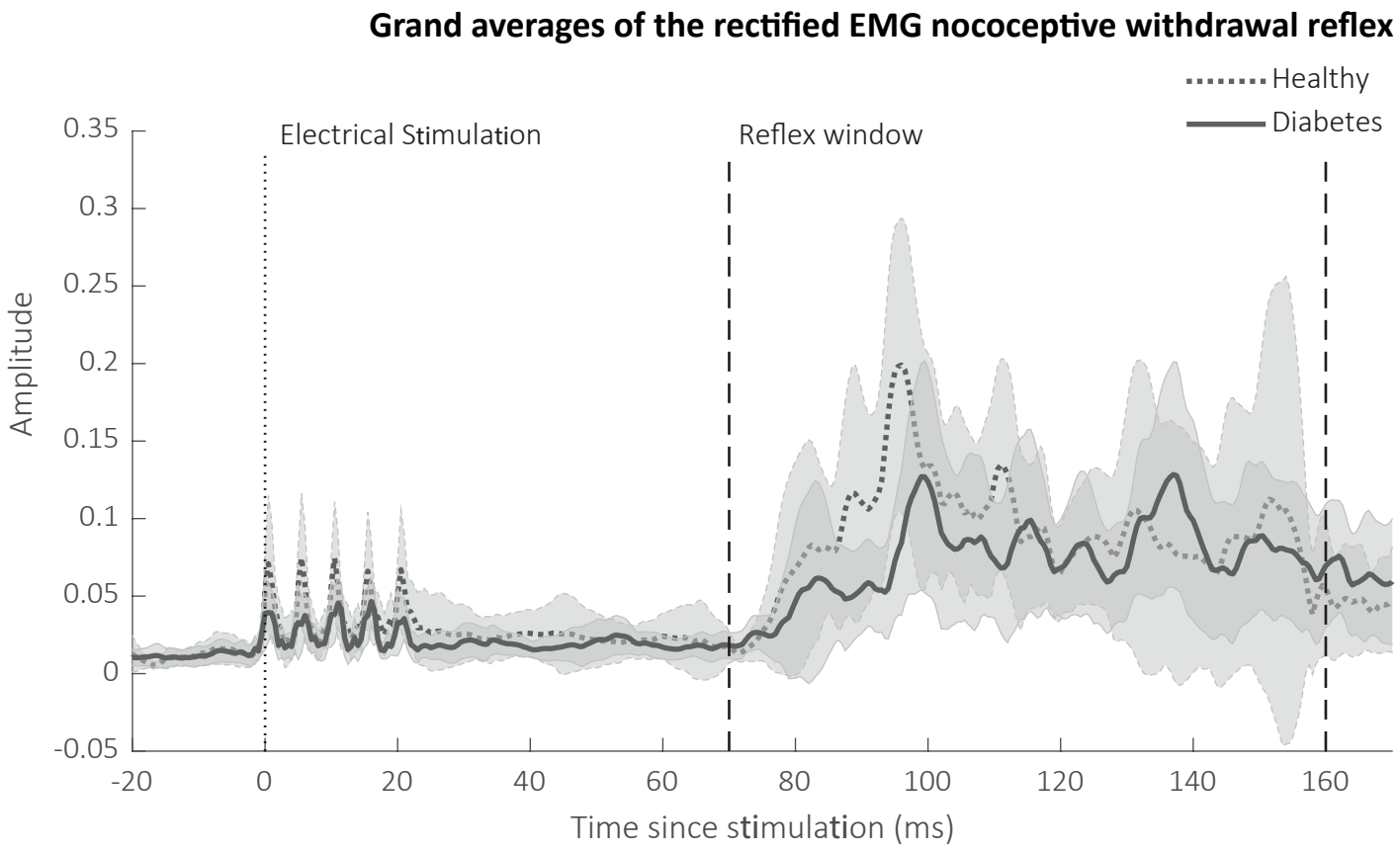
Figure 1 Visual representation of the data analysed within this study. Not all patients were able to complete the experimental procedure. Two patients with diabetes and one healthy control was excluded from the somatosensory evoked potential due to poor signal to noise ratio.

Figure 2. Grand average of all data of rectified EMG traces of the nociceptive withdrawal reflex with a z-score above 12, the shaded area is the 95% confidence interval. The reflex window (70-160 ms. after the electrical stimulation) is the area in which the area under the curve (AUC) of the single sweep reflex is calculated. Time 0 is when the electrical stimulation was applied to the skin of the plantar side of the foot.

Figure 3 Grand average of healthy controls and patients with diabetes at the Cz electrode location, the shaded area is the 95% confidence interval. Time 0 is when the electrical stimulation was applied to the skin of the plantar side of the foot.


Data recording and analysis workflow
Enrolled in study
Healthy Controls (n = 21)
Patients with Diabetes (n = 48)
Evaluation of perception- and reflex-thresholds
Able to complete NWR recording
Healthy Controls (n = 21)
Patients with Diabetes (n = 34)
Excluded:
Patients with Diabetes:
1 Withdrew because pain became too unbarable
13 Excluded since electrical stimulations reached the safety threshold before a reflex was visible
Data included for further analysis
Single sweep data
Averaged data
Nociceptive withdrawal reflex (NWR)
Somatosensory evoked potentials (SEPs)
Healthy Controls (n = 21)
Patients with Diabetes (n = 34)
Healthy Controls (n = 20)
Patients with Diabetes (n = 32)
Excluded:
Patient with Diabetes:
2 Poor signal to noise ratio
Healthy Control:
1 Poor signal to noise ratio
Ability to evoke NWR
SEPs
Description of NWR


Grand averages of the rectified EMG nococeptive withdrawal reflex
Healthy
Diabetes
Electrical Stimulation
Reflex window
Amplitude
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-20
0
20
40
60
80
100
120
140
160
Time since stimulation (ms)

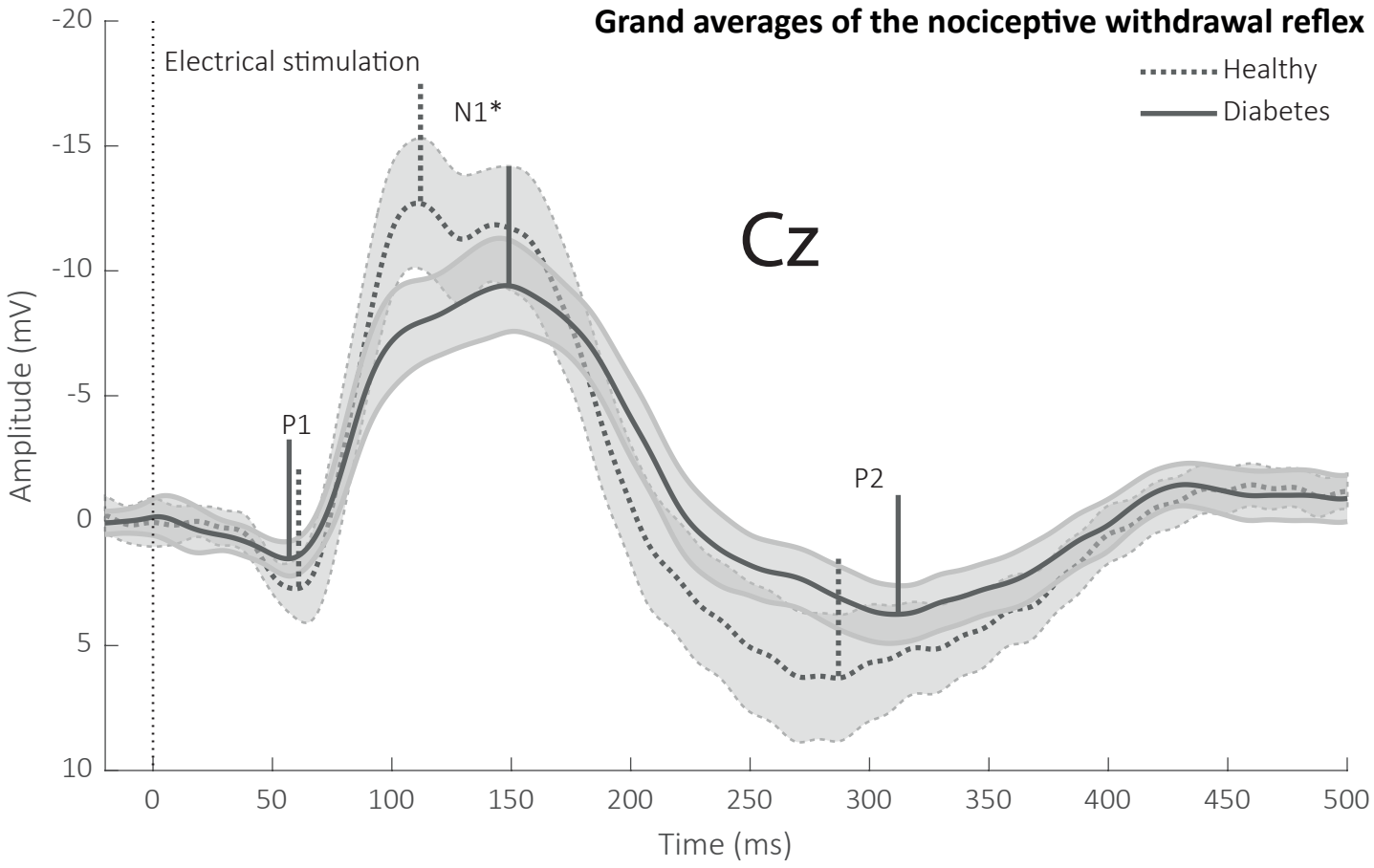

Grand averages of the nociceptive withdrawal reflex
Healthy
Diabetes
Electrical stimulation
N1*
P1
P2
Cz
Amplitude (mV)
-20
-15
-10
-5
0
5
10
0
50
100
150
200
250
300
350
400
450
500
Time (ms)

Table 1: Demography

	Healthy (n = 21)	**Diabetes (n = 48)**	**p-value**
Age (years)	51.3 (6.4; 40-62)	50.0 (8.5; 33-71)	0.53
Gender	6 female. 15 males	9 female. 39 males	0.48
Height (cm)	179.8 (9.0; 158-192)	178.4 (8.6; 158-192)	0.55
Weight (kg)	87.3 (20.5; 62-140)	90.0 (16.0; 63-132)	0.56
BMI (kg/m^2)	26.9 (5.5; 21-40)	28.5 (4.9; 22-43)	0.30
Conduction velocity sural nerve (m/s)	49.67 (6.06; 40-66)	42.17 (5.84; 32-60)	**<0.001**
Disease duration (years)	--	32.2 (9.5; 14-51)	

All statistical analysis is performed using t-test. Average (SD; range). P-values<0.05 indicate significant differences

Table 2: Assessment of evoked potentials at three different points

Electrode	Healthy (n = 20)	Diabetes (n = 32)	Difference	p-value	Post-hoc
Cz				**0.024**	
P1 latency (ms)	61.5 (9.27)	58.21 (14.45)	3.29		p=0.240
N1 amplitude (μV)	15.59 (4.82)	11.87 (5.83)	3.72		p=**0.021**
N1 latency (ms)	146.15 (16.43)	161.25 (22.81)	-15.1		p=**0.013**
P2 amplitude (μV)	7.4 (5.52)	6.28 (4.84)	1,12		p=0.739
P2 latency (ms)	280.5(36.7)	284.85(38.7)	-4,35		p=0.448

The outcome of the MANOVA and subsequent post hoc test. Numbers are reported in average (SD)