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Observing small fiber dysfunction using nociceptive stimulus detection and evoked potentials

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Explorative investigations with the novel NDT-EP measurement method in a lidocaine model and diabetes mellitus patients

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May 25th, 2020

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Observing small fiber dysfunction using nociceptive stimulus detection and evoked potentials

Explorative investigations with the novel NDT-EP measurement method in a lidocaine model and diabetes mellitus patients

By

Silvano R. Gefferie

May 25th, 2020

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Preface

After one year of hard work at the St. Antonius Hospital, I can finally present this thesis. This report aims to inform the reader about the study that I conducted during my graduation internship, being the concluding piece of seven years in the Technical Medicine program (University of Twente). As of June 2019, I started this graduation internship at the Department of Anesthesiology, Intensive Care and Pain Medicine. The idea for a new study had been thought up, but that was about it. I was given the tasks to figure out a study plan, obtain ethical approval, perform participant recruitment and measurements, determine analysis directions and, finally, present results and conclusions. Occasionally, these responsibilities led to some difficult times, in which I doubted whether I was going to be able to deliver a sound product within the designated time. However, I also believe that these responsibilities have helped me to (further) develop skills relevant to my future professional practice. These include, amongst others, creativity, daring to take initiatives and working together as a team. Especially the latter was a vital factor that contributed to my development as a professional. Therefore, I would like to express gratitude towards those that supported me and that I have worked with during the final year of my studies.

First, Jan. Thank you for all the intellectual ideas and directions that you provided me with during my graduation assignment. Without these, I would not have been able to produce this thesis as it is now. As my technical supervisor, your critical but honest views taught me to be more critical of myself and my work. I am sure that this trait will benefit me in my future academic practices.

Then Imre. You were my medical supervisor during this internship. Thank you for introducing me to clinical practices during my time at the St. Antonius Hospital. It aided me in getting a grasp of the medical context of my graduation assignment. You introduced me to colleagues in the Operation Room, enabling me to attend multiple surgeries. Moreover, I learned a lot from your honest feedback after you had supervised several times at the outpatient pain clinic.

Rian, thank you for having been my process supervisor for two years. I enjoyed the ‘intervision’ appointments, which always felt like a relief during sometimes hectic periods. Furthermore, as we agreed on already, these have genuinely helped me to come closer to and be more honest towards myself. I believe that this development will allow me to better keep track of my progress in the future.

Boudewijn, I would like to express special thanks for all the instances you took the time to help me with the various technical issues that I ran into. Without your quick responses and bright ideas, I would never have been able to rapidly resume my (programming) activities. Thank you for these, and all the appointments you scheduled for electrode sterilizations and your visits to the hospital to help us.

Then, I would like to thank my colleagues and roommates at the St. Antonius Hospital. Tom and Ruben, as my direct colleagues, both of you provided me with valuable contextual input for and different views on my study throughout the year. Yet, you also made sure that we took enough breaks from our work, which included physical activities, and properly celebrated our successes. Of course, I would also like to thank my other roommates for the pleasant working environment. I like how we bonded together and organized fun activities, such as the Christmas brunch.

Additionally, I would like to thank the two Technical Medicine master's students that I collaborated with during this graduation year. Eva, it was a pleasure to perform the lidocaine experiment with you. Your enthusiasm and determination made that we could turn this part of the study into a success. Jelle, I enjoyed supervising your master's internship during rather difficult (COVID-19) times. You showed that the latter was not going to hold you back and made the best out of it. Besides, our running sessions ensured that our physical conditions would not suffer from an increasingly sedentary lifestyle due to the COVID-19 crisis.

Finally, I would like to express my gratitude to my beloved ones. Even though we have been living apart for several years now, I always felt that you, as my family, welcomed me home whenever possible. You helped me to relax and temporarily empty my mind, but also encouraged me to strive for my goals. I would also like to thank my girlfriend for her unwavering support. You made sure that I was not too strict for myself and always knew how to brighten my day, even in my most despairing moments. Thank you for always having believed in me.

Silvano Gefferie

May 25th, 2020

Summary

Introduction. Besides invasive and labor-intensive nerve biopsies, there is a lack of objective measures for small-diameter epidermal nerve fiber function. A novel measurement method ('NDT-EP'), which allows evaluation of tracked responses and evoked potentials (EP) following intraepidermal electrical stimuli, constitutes a potential candidate for this purpose. Therefore, practicality and outcomes of this method were explored in a lidocaine model of small fiber neuropathy (SFN) and diabetes mellitus (DM) patients.

Methods. Three groups of participants were included. The first comprised healthy, pain-free individuals that received 2 hours of lidocaine and placebo patch treatment before measurements ('lidocaine experiment'). The second and the third group involved DM patients with chronic painful diabetic peripheral neuropathy (PDPN) and without pain complaints, respectively. By stimulating dorsa of the hands, stimulus detection probabilities and EPs were obtained. Data from healthy participants in an earlier study, without patches, were included as control data. (Generalized) linear mixed regression was used to compare measurement outcomes between interventions (lidocaine experiment) and between study groups.

Results. 19 healthy participants (average age: 38.9 ± 10.9 years, 12 females), 13 DM patients with chronic PDPN (median age: 68.0 years, two females), and 20 pain-free DM patients (median age: 58.5 years, 11 females) were included. Control data originated from 17 participants in the previous study (average age: 35.9 ± 12.3 years, 14 females). There were no differences in detection probabilities between lidocaine, placebo, and control measurements. Still, EP amplitudes were significantly smaller for lidocaine compared to placebo ($P = 0.049$) and no patch ($P < 0.001$) treatments. DM patients with chronic PDPN demonstrated detection probabilities different from patients without pain ($P < 0.05$), and both groups of DM patients showed different detection probabilities compared to healthy control data ($P < 0.05$). Outcomes for EPs were similar, with lowered amplitudes for PDPN in the DM sample and DM in general ($P < 0.05$). Finally, there were no differences in detection probabilities between lidocaine measurements and pain-free DM patients, nor in EP amplitudes between lidocaine measurements and both groups of DM patients.

Conclusions. The results of this study suggest the general feasibility of NDT-EP measurements in DM patients and that decreased EP amplitudes in these patients resemble experimentally induced small fiber dysfunction. Contrastingly, current evidence that altered detection probabilities mirror the same condition is limited. Differences between DM patients and healthy controls may have first resulted from other group dissimilarities, such as in attentional levels. Continued investigations are advised to further examine demographic influences, experiment with alternative measurement set-ups, and explore the method in other diseases marked by SFN and chronic pain conditions.

Keywords. *Evoked potential, diabetes mellitus, diabetic peripheral neuropathy, lidocaine, linear mixed regression, nociceptive threshold, psychophysics, small fiber neuropathy*.

Table of contents

List of acronyms

BMI	body mass index
CSI	central sensitization inventory
DP	double pulse
DM	diabetes mellitus
EP	evoked potential
EEG	electroencephalography
FBSS	failed back surgery syndrome
GFP	global field power
(G)LMM	(generalized) linear mixed-effects model
(G)LMR	(generalized) linear mixed regression
HC	healthy controls
HP	healthy participants
IES	intraepidermal electrical stimulation
IPI	inter-pulse interval
NCS	nerve conduction study
NDT	nociceptive detection threshold
NDT-EP	nociceptive detection threshold-evoked potential
np	neuropathic pain
NRS	numeric rating scale
(P)DPN	(painful) diabetic peripheral neuropathy
QST	quantitative sensory testing
SFN	small fiber neuropathy
SP	single pulse
TENS	transcutaneous electrical nerve stimulation

1. Introduction

1.1. Assessment of neurological function

The nervous system is an extensive network of cells designed to transmit electrical signals throughout the body. It enables animals to interact with their environment by facilitating reception, transmission, and processing of both internal and external stimuli, and also by facilitating responses to these stimuli. The division of the nervous system occupied with registration and interpretation of physical experiences of the skin, muscles, and joints is the somatosensory system. This system senses, transfers, and interprets stimuli related to body position and movement, temperature, tactile touch, and pain (Kaas, 2012). The latter is registered by a subdivision of the somatosensory system, referred to as the nociceptive system.

Since everyday performance and well-being necessitate a properly working nervous system, investigations into its functional performance have a long history. Archaic forms of the neurological exam were practiced by the Egyptians as early as in the 30th century BC (Patten, 1992). In the following eras, physical examination techniques were advanced by notable innovators such as the Greek physician Hippocrates ("the father of medicine"), the Roman physician Cornelius Celcus and the French scientist Rene Descartes (Patten, 1992; Breitenfeld et al., 2014). However, only with first mass documentation of methodology from halfway the 19th century (Fine and Ziad Darkhabani, 2010; Boes, 2015), knowledge regarding somatosensory examinations in particular increased. This featured development of methods to assess discriminative abilities for two locations of sharp stimuli (Weber, 1846), to grade pressure sensitivity (Von Frey, 1896), to examine vibration sense (Jelliffe and White, 1929) and to test the distinguishment of dull from sharp stimuli (Dejong, 1950).

Parallel to these advances, electrophysiological techniques to assess deeper branches of the peripheral nervous system were established: nerve conduction study (NCS). Their development followed an increased understanding of (bio)electricity (Kazamel and Warren, 2017), but was only catalyzed in the 20th century by enhanced technological abilities. In contemporary practice, NCS serves an indispensable role in the assessment of neurophysiological functioning (Mallik and Weir, 2005).

Previously described assessment methods provide a physical representation of the patient's neuronal status. However, in case of pain, a significant component cannot be defined in this manner. Therefore, to detail pain, unidimensional instruments such as the numeric rating scale (NRS) and the visual analog score are commonly employed (Younger et al., 2009). In case of chronic pain, multidimensional instruments such as the central sensitization inventory (CSI) may clarify whether the patient has developed widespread sensory hypersensitivity (Mayer et al., 2012). By combining neurological examination with NCS, and both uni- and

multidimensional pain characterization instruments (if applicable), physicians attempt to get a firm understanding of their patients' neuronal functioning.

1.2. Problem statement

Although vast in number, most conventional diagnostic tools in neurological practice have a few substantial limitations. Perhaps most burdensome is their subjectivity. Especially during the neurological examination, the attentional levels of both the examinee and the examiner exert a critical influence on the outcomes. Variations in this parameter negatively affect both replicability and reproducibility of diagnostic tests (Patil et al., 2016), limiting translatability into patient-tailored therapies.

Another challenge concerns the investigation of pain chronification on pain perception. Diagnostic instruments such as the CSI address symptoms related to general hypersensitivity that patients may experience unresolved pain complaints. Yet, the questionnaire fails to quantify the effects of chronic pain on nociceptive processing, possibly contributing to the portion of missed- or false diagnoses for central sensitivity syndromes (Neblett et al., 2013, 2015). Thus, the impact of chronic pain on pain sense (nociception) remains a relevant topic in current research.

The diagnostic value of NCS represents a third issue. This method is restricted to the investigation of large-diameter nerves. Nonetheless, small-diameter epidermal nerve fibers (or simply 'small fibers'), responsible for the registration of nociceptive stimuli, are not functionally assessed. This constitutes a considerable drawback, as these aδ- and C-fibers are frequently involved in medical conditions characterized by painful neuropathies, such as sarcoidosis and diabetes mellitus (DM) (Hoitsma et al., 2002; Chao et al., 2010). In case of the latter, somatosensory dysfunction and pain resulting from small fiber neuropathy (SFN) may precede widespread neurological complications. These include physical disability (Gregg et al., 2000), postural instability (Cavanagh et al., 1992), and autonomic dysfunction (Vinik et al., 2003).

The difficulties described above underlined the need for more objective assessment methods. Ideally, these should (also) be capable of quantifying the influence of chronic pain on nociception and including the functional state of small fibers

1.3. Recent developments

Over the past decades, progress has been made in quantitative multimodal assessment of the somatosensory system. One of these advances concerned the development of quantitative sensory testing (QST). The Peripheral Neuropathy Association described QST as "techniques used to measure the intensity of stimuli needed to produce specific sensory perceptions"

(Peripheral Neuropathy Association, 1993). The development of QST saw the introduction of different physical stimulus types to investigate both small and large nerve fiber function. Although more versatile than classic NCS, QST still suffers from considerable dependence on the examinee's mental status (Siao and Cros, 2003).

Even though not included in standardized protocols (Rolke et al., 2006), QST occasionally features electricity. For quantification of cutaneous detection thresholds, generally, three modes of current transmission are possible: via surface electrodes applied over the skin, needle electrodes placed adjacent larger nerves or microneedles inserted into the epidermal layer. In case of the latter, the stimulation technique is called 'intraepidermal electrical stimulation' (IES). IES, when using current intensities well below the tactile activation threshold, is capable of selectively activating superficially located small fibers (Inui et al., 2002b; Mouraux et al., 2010; Inui and Kakigi, 2012). Its discovery and further development paved the way for targeted assessment of the nociceptive system.

Lately, a new approach for tracking detection thresholds for different types of intraepidermal electrical stimuli is being investigated (for details, see 2.2. *The NDT-EP measurement method*). This approach further enables evaluation of how stimulus properties affect these nociceptive detection thresholds (NDT) and simultaneously recorded evoked potentials (EPs). Only recently, such combination, entitled the 'NDT-EP measurement method', was first explored in a clinical setting (Berfelo, 2019). Until then, previous studies had merely considered healthy participants and had been carried out in laboratory environments. This exploratory study in healthy subjects and chronic pain patients indicated that the new measurement approach is (1) replicable in a hospital environment and (2) presumably feasible in chronic pain patients, in whom it suggests altered time-variant behavior of NDTs and EPs. Since the NDT-EP method uses IES, this provoked the thought of whether it could have broader applicability regarding the functional assessment of specifically small fibers in diseased individuals. This led to the following central research question:

Central research question:

Which outcomes does the NDT-EP measurement method yield for dysfunctional small epidermal nerve fibers in a clinical context?

1.4. Approach

To answer this question, a patient population with a disease frequently associated with small fiber dysfunction was needed. For this, DM patients were found most suitable following high disease prevalence and incidence, and painful diabetic peripheral neuropathy (PDPN) occurring as a frequent complication (see 2.3.1. *Diabetes mellitus* and 2.3.2. *(Painful diabetic) peripheral neuropathy*). Two types of DM patients were to be considered: with chronic PDPN,

and presumably progressed small fiber dysfunction, and without pain complaints, possibly showing early signs of functional small fiber deficiency. By performing NDT-EP measurements in these patients, both their applicability in this condition and their ability to reflect potential differences in small fiber functionality could be explored. Regarding the latter, the hypothesis was that NDT-EP measurement measures might demonstrate outcomes characteristic for dysfunctional small fibers. Control data from healthy participants were additionally included to serve as a normative reference for these pathological circumstances.

However, as there was no experience with measurements in a condition of (modeled) small fiber neuropathy, it was unknown to what extent outcomes in patients would truly reflect small fiber dysfunction. This knowledge hiatus motivated the design of another constituent of the study, in which measurements of SFN modeled in healthy individuals were expected to uncover condition-specific outcomes. An appropriate candidate for simulating small fiber dysfunction was found in lidocaine. This local anesthetic specifically blocks small aδ- and C-fibers upon application to the skin (i.e., topical treatment) (Krumova et al., 2012; Kodaira et al., 2014). It was assumed that such a model would provide the opportunity to study the effects of 'isolated' small fiber dysfunction on NDT-EP data – elucidative of the method's construct validity. By adding placebo and, again, normative data from controls without a patch, extents of possible placebo effects could be additionally estimated.

The two parts of the study described above, termed 'DM measurements' and 'lidocaine experiment', respectively, contributed to the following central aim:

Central aim:

Explore the feasibility of the NDT-EP measurement method and its outcomes in (1) simulated small fiber neuropathy and (2) diabetes mellitus patients with and without chronic neuropathic pain.

Two primary objectives and one secondary objective were associated with this aim:

Primary study objectives:

- Describe the outcomes of NDT-EP measurements in a lidocaine model of SFN
- Explore the feasibility and describe the outcomes of NDT-EP measurement in DM patients with chronic neuropathic pain and without pain complaints

Secondary study objective:

- Compare the outcomes from both the lidocaine experiment and in DM patients with healthy controls (without patch), and with each other.

1.5. Thesis outline

This thesis is divided into six chapters. The next chapter, 2. *Background*, elaborates on the scientific principle behind and the essentials of the NDT-EP measurement method. Furthermore, the clinical picture of DM (and its most common complication), topical lidocaine to simulate small fiber dysfunction, preceding work, and clinical significance are addressed in this chapter. Subsequently, chapter 3. *Methods*, depicts the methodological approach adopted in this study. Measurement outcomes for the two separate parts of this study (and their combined results) are described in the following chapter, 4. *Results*. The succeeding chapter, 5. *Discussion*, provides a comprehensive discussion of the results, includes the study's strengths and limitations, and makes recommendations for further research. Conclusions regarding all the work performed for this thesis are finally drawn in the last chapter, 6. *Conclusion*.

2. Background

2.1. Psychophysics

The underlying principle of the NDT-EP measurement method is psychophysics. Its 'founder', Gustav Theodor Fechner, was the first to comprehensively describe this new scientific field incorporating both psychology and physics in his hallmark publication 'Elemente der Psychophysik' (Fechner, 1860). In this book, he advocated that the human mind and physical environment may appear as coming from two different origins, but that, factually, they merely represent two alternative sides of reality. By mathematically defining relationships between conscious events and physical variables, it would be possible to understand how these entities correlate with each other. The general logarithmic function that was put forward by Fechner for this purpose could be described as in eq. 1,

$$\varphi = k \cdot log(\theta) \qquad \text{[eq. 1]}$$

in which ϕ is the psychological correlate of θ, the intensity of a certain physical stimulus, and k represents the physical baseline value needed to instigate a response (Fechner, 1860). This relation stood at the base of one of the most widespread applications of psychophysics: investigations of stimulus detection thresholds.

2.1.1. Psychophysics for stimulus detection threshold experiments

The Cambridge Dictionary defines a stimulus, in a biological sense, as "something that causes part of the body to react" (Cambridge Dictionary, n.d.). In psychophysics, stimuli have characteristic detection thresholds, which mark the weakest physical intensities at which they provoke a psychological response (Engen, 1988). This knowledge promoted experiments in which detection thresholds could be determined by providing the stimuli at discernible intensities first, after which these would be step-wise decreased until the subject would fail to indicate stimulus reception. When stimulus intensity and corresponding responses are thereafter plotted against each other, this results in a

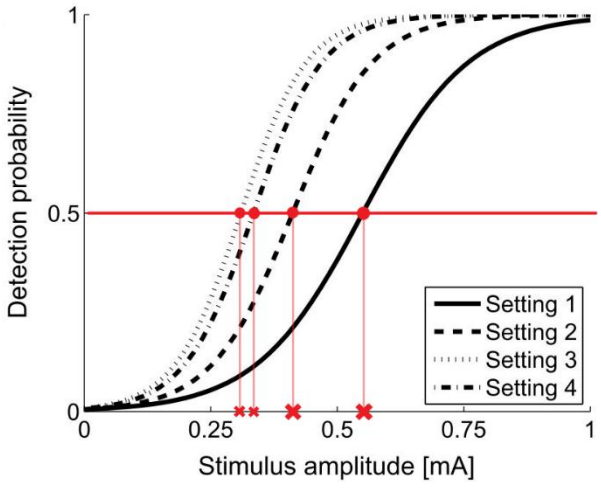


Figure 2.1. Psychophysical curve depicting the relation between detection probability and stimulus amplitudes per stimulus setting. Red marking illustrates that type-specific detection thresholds are defined at stimulus amplitudes resulting in a detection probability of 0.5. *Adapted from Doll et al. 2016.*

psychophysical (or psychometric) curve: the visualization of the relationship between physical intensity and psychological response (Fig. 2.1).

The recipient's psychological response can be interpreted in various ways, e.g., the portion of accurate detections or the response magnitude relative to the assumed maximum. However, to determine detection thresholds, each of the psychological response variables requires an arbitrary cut-off value at which the threshold can be defined (Fig. 2.1).

2.1.2. Non-stationarity

The psychophysical approach provides researchers with the tools to understand the mental associates of external stimuli varying in intensity. However, a critical feature of human stimulus detection is not accounted for by this approach: non-stationarity. A stationary process is a process of which statistical parameters, such as the mean and variance, do not change over time. Yet, when it comes to bodily signals, such as those involved in the neurophysiological processing of received stimuli, these are characterized by high non-stationary (Semmlow, 2018). One could think of several factors making up for this non-stationarity, such as dwindling concentration, increased alertness, or (de)sensitization of neuronal components. Repetition of psychophysiological threshold determination experiments will, therefore, result in time-variant mathematical relations between the response variable and stimulus intensity. This fact motivated the expansion of single-repetition threshold determinations to experiments in which the thresholds are tracked, ideally taking into consideration the influence of different experimental characteristics on threshold variability.

2.2. The NDT-EP measurement method

2.2.1. Tracking nociceptive detection thresholds

Recently, progress has been made regarding techniques that track detection thresholds for stimuli targeting intraepidermal nociceptors: the free nerve endings that register superficial somatic pain. In a combined computer simulation-human subject study, Doll et al. (2014) investigated which combination of stimulus selection strategy and threshold estimation approach would lead to the highest precision and smallest bias of tracked thresholds. The authors concluded that the combination of a 'random staircase' (or 'adaptive probing') procedure for the selection of stimulus intensities and logistic regression for estimating detection thresholds would yield the most reliable results. In an adaptive probing procedure, stimulus intensities are repeatedly drawn from a small range of intensities, of which all values decrease or increase with a fixed step after a detected or non-detected stimulus, respectively (Doll et al., 2014). The study further featured the introduction of a logistic psychophysical relationship between stimulus intensity x and detection probability p (the continuous analog of the binary participant's response), mathematically visualizable by eq. 2,

$$p(\mathrm{x}) = (1 + \exp(\beta(\alpha - \mathrm{x})))^{-1} \quad \text{[eq. 2]}$$

with β, the slope parameter set at 20 mA^{-1}, and α, the stationary (true) threshold set at 0.3 mA. In a subsequent study (Doll et al., 2015), non-stationarity of involved psycho- and physiological processes were accounted for by an extension of the psychophysical mathematical model (eq. 3)

$$p(\mathrm{x}, \alpha(\mathrm{t}), \beta) = (1 + \exp(\beta(\alpha(t) - \mathrm{x})))^{-1} \quad \text{[eq. 3]}$$

with α now being a function of time. The researchers defined the NDT as the intensity x for which the detection probability p is 0.5. With their results, they advocated the use of psychometric functions assembled from moving windows of stimulus-response pairs. This would enable the most accurate recognition and incorporation of non-stationarity in threshold determination experiments.

2.2.2. Stimulus properties and brain responses

Stimulus detection may be influenced by characteristic features of stimuli other than intensity and endogenous non-stationary processes. In the subsequent study by the same group (Doll et al., 2016b), this was demonstrated for different temporal parameters of stimuli, comprising pulse width, number of pulses, and the interval between pulses. Regression of detection probability with generalized linear mixed-effects models (GLMM), incorporating combinations of temporal parameters as regression variables, showed for the first time that stimulus properties significantly affected nociceptive stimulus processing. This sparked the thought of whether experimental set-ups that enable (concurrent) registration of neurophysiological responses are possible. Such an extension would possibly grant additional, increasingly objective insights into the human nociceptive system.

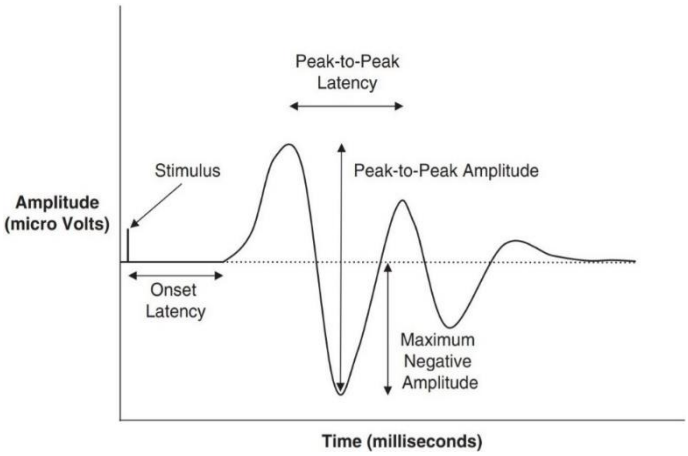


Figure 2.2. The graphed evoked potential and some of its most important properties. In this study, amplitudes of two positive peaks and corresponding latencies in two different EEG derivations were examined. *Reprinted from Lieberman J.A. (n.d.).*

In answer to this, van den Berg and colleagues (2020) conducted a follow-up study combining the threshold estimation experiment (Doll *et al.*, 2016*b*) with concurrent recordings of electroencephalographic (EEG) activity around stimulus application: the 'NDT-EP experiment'. Cortical activity in response to sensory input, referred to as the evoked potential or EP, is observable for stimuli of various origins. EPs may be characterized by peak amplitudes and corresponding latencies (Fig. 2.2). By complementing GLMMs of detection probability with

linear mixed-effects models (LMM) of EP amplitude, the feasibility of combined psychophysical and neurophysiological examination of nociceptive characteristics was demonstrated. Moreover, these examinations revealed that certain stimulus properties significantly modulated the stimulus detection probability (consistent with earlier investigations of the group) and, additionally, amplitudes of EPs.

Results from these studies raised the question of whether this combination may reveal altered nociceptive stimulus processing in diseased conditions. One condition of interest is SFN, which is marked by dysfunctional small fibers and considered to play an essential role in neuropathic pain experienced by part of the DM patients.

2.3. Diabetes mellitus

Diabetes mellitus, or DM, encompasses metabolic disorders characterized by pathologically raised blood sugar concentrations (hyperglycemia). Hyperglycemia may have a variety of causes, including pregnancy (gestational diabetes) or the use of oral corticosteroids. Yet, the most common forms of diabetes are referred to as type 1 and type 2 DM, which concerns DM following auto-immune destruction of insulin-producing beta cells in the pancreas or acquired insulin resistance, respectively. In 2013, estimation of overall DM prevalence in Europe resulted in a percentage of 8.5%, reflecting 56 million cases, a number that is expected to have increased by 10 million cases in 2023 (Tamayo et al., 2014). An extensive review by Saeedi and co-authors (2019) pointed out that, in support of this alarming prognosis, worldwide DM prevalence may experience a dramatic increase of 25% by 2030 and 51% by 2045. Consequently, attention should be directed to the variety of complications that may plague diseased individuals.

In contrast to the risk factors for the two common disease types (Steck and Rewers, 2011; Wu et al., 2014; Bellou et al., 2018), disease consequences are more homogeneous. Some long-term complications concern conditions may arise due to hyperglycemia's effect on the macrovascular system, including ischemic heart disease and cerebrovascular defects (Biessels et al., 1994; Cade, 2008). Other complications can emerge following the microvascular impact of hyperglycemia, most notably retinopathy, nephropathy, and peripheral neuropathy (Chawla et al., 2016). With prevalence estimates ranging between 18% and 35% among DM patients in Europe, the latter is among the most common and invalidating consequences of DM (Tesfaye et al., 2010; Tamayo et al., 2014).

2.3.1. (Painful diabetic) peripheral neuropathy

Peripheral neuropathy is a condition that is marked by damage to peripheral nerves. Such damage can be inflicted by a variety of causes, which include hereditary factors, traumatic insults, use of certain medications (e.g., chemotherapeutic agents), chronic alcohol abuse, and immune systems disorders (Dyck, 1982). Nevertheless, DM is considered the most frequent

cause of peripheral neuropathy (Smith and Singleton, 2006), which is then termed diabetic peripheral neuropathy (DPN).

DPN is a complication of chronic hyperglycemia. The latter is presumed to be the driving force behind several pathological processes affecting neuronal pathways, such as oxidative stress, spontaneous nociceptor firing, and polyol pathway hyperactivity (Veves *et al.*, 2008; Ørstavik and Jørum, 2010; Schreiber *et al.*, 2015) (Fig. 2.3). Resulting nerve damage may manifest itself by negative and positive symptoms (Uceyler *et al.*, 2018). Negative symptoms suggest the loss of neuronal function, e.g., by hypoesthesia or muscle weakness. On the other hand, positive symptoms may give the impression that the nervous system is hyper volatile, reflected by sensations such as paresthesia, fasciculations, or nerve pain (neuropathic pain).

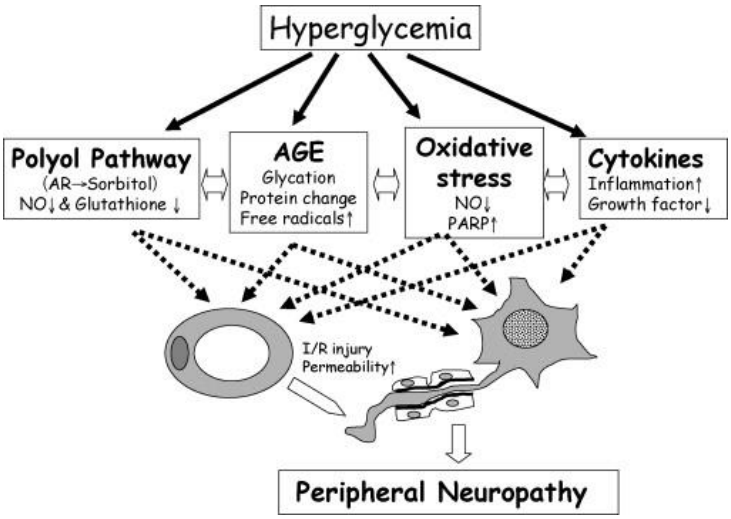


Figure 2.3. Pathological processes by which persistent hyperglycemia leads to diabetic peripheral neuropathy. These processes inflict direct injury on peripheral neurons. Simultaneously, they affect microvascular structures, e.g., via reperfusion (I/R) injury, which causes additional damage to neuronal networks. *Reprinted from Yagihashi et al. (2007).*

According to the International Association for the Study of Pain, neuropathic pain can be defined as "pain that arises as a direct consequence of a lesion or disease affecting the somatosensory system" (Treede et al., 2008). In DM, neuropathic pain is one of the manifestations that causes the biggest reduction in the quality of life among those affected (Van Acker et al., 2009). Estimated prevalence rates have shown that between 10% and 20% of the DM patients suffer from pain, rising to 40%-50% of the DM patients diagnosed with DPN (Veves et al., 2008). DM patients typically first report bilateral pain in lower extremities, in agreement with the characteristic symmetric, length-dependent pattern of DPN. This pattern implicates that the most distally located nerve fibers in the skin, small epidermal nerve fibers, are affected first. Hyperglycemic impact on this type of fibers leads to a subclass of peripheral neuropathy: SFN.

2.3.2. Small fiber neuropathy

Naturally, small epidermal fibers engage in the registration of temperature and noxious stimuli. In several diseases, including DM, these fibers may be damaged and subsequently lost, leading to small fiber neuropathy or 'SFN'. SFN is believed to be a significant source of neuropathic pain in DM (Devigili et al., 2008). However, dysfunction of small fibers may not always lead to pain, but can also manifest in other ways such as subtle loss of thermal sensitivity or can

even be subclinical (Meh and Denišlič, 1998; Lauria et al., 2003; Karsidag et al., 2005; Baron et al., 2010). It may precede generalized diabetic polyneuropathy involving damage to larger nerve fibers (Thomas, 1997; Smith et al., 2001).

The identification of SFN poses some serious diagnostic challenges. NCS, conventionally used to find indications of neuropathy, is insensitive for altered small fiber function (Devigili et al., 2008). Other approaches render either variable diagnostic yield, e.g., QST, or require special skills and cause more patient discomfort due to invasiveness, e.g., skin biopsies (Devigili et al., 2008; Petropoulos et al., 2018). Therefore, (relatively) recent studies mainly focused on the use of different stimulus modalities to estimate nociceptive thresholds and measure EPs in the cerebral cortex (Mueller et al., 2010; Ragé et al., 2011; Suzuki et al., 2016; Omori et al., 2017; Petropoulos et al., 2018). Such novel approaches may help to discriminate between different degrees of functional small fiber impairment and non-diseased conditions.

A promising stimulus modality seems to be IES. Utilization of this technique to elicit and measure EPs is relatively new (Otsuru et al., 2010; Omori et al., 2017; Isose et al., 2018; van den Berg et al., 2020). NDT-EP measurements, which exploit IES, have not yet been attempted in DM patients, who may suffer from different extents of small fiber deterioration. These patients are age-wise above average and occasionally experience disabling pains, which causes some to use analgesic drugs chronically. Therefore, and due to the diagnostic difficulties regarding SFN, it was deemed interesting to explore the feasibility and outcomes of the NDT-EP method in symptomatically heterogeneous DM patients. Such investigation would delineate the method's applicability in these patients and provide first impressions of possible correlates of dysfunctional small fibers.

However, due to the method's novelty, outcomes have not yet been acquired for stimulation of dysfunctional small fibers while minimizing influences of other factors, e.g., demographic or disease-related characteristics. In this respect, the transdermal use of pharmaceuticals may simulate small fiber dysfunction in healthy individuals and provide more information on condition-specific outcomes.

2.4. Lidocaine

2.4.1. Models of human (patho)physiology

For the modeling of human (patho)physiology, several means are available to researchers. For models that resemble normal physiological conditions of the body, one could turn towards animals. Such animal models could be employed in, for example, the several different phases of therapeutic drug development. Moreover, diseases in humans could be further delineated by appropriate selection of animals bearing similar deficiencies, or by experimentally inducing physiological deficits in animals, e.g., by gene knock out or know down techniques. However, with recent advances in (bio)chemistry and growing understanding of human

(patho)physiology, another type of model has gained popularity: those based on pharmaceutical agents. A common application of these models concerns the exploration of experimentally induced pathological conditions and subsequent examinations of drug efficacy in clinical trials. For example, anti-N-methyl-D-aspartate receptor antagonists have been extensively used for simulating psychosis (Rujescu et al., 2006; Corlett et al., 2007), whereas such agents can simultaneously aid the development of psychotropic drugs (Gilles and Luthringer, 2007). Besides, these types of models may assist in the design of medical devices. For instance, therapeutic patches with capsaicin, the irritant in chili peppers, can be used to perturb the nociceptive to study its effects on pain processing (Doll et al., 2016a; Papagianni et al., 2018). A therapeutic drug that may increase the understanding of how the NDT-EP measurement method interacts with peripheral neuropathic conditions is lidocaine.

2.4.2. Topical lidocaine – a model of small fiber neuropathy

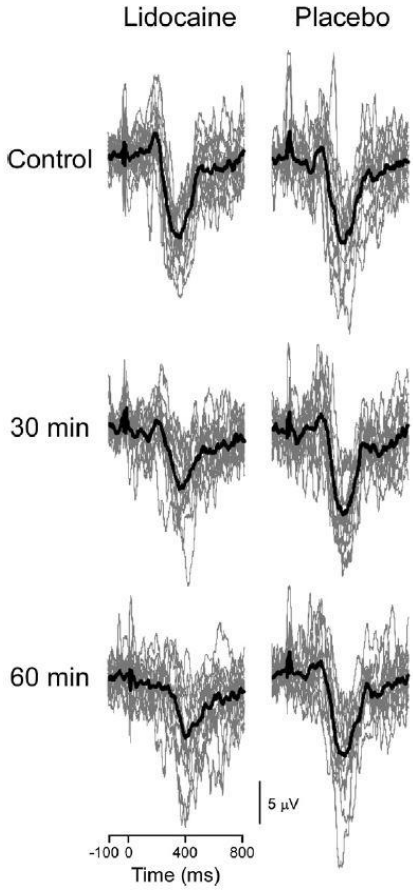


Figure 2.4. Evoked potentials (EP), in microvolt (µV), following electrical stimulation of epidermal aδ-fibers in the foot dorsum. Separate (thin gray lines) and aggregate (thick black lines) EPs are provided after varying durations of lidocaine or placebo tape treatment. *Adapted from Kodaira et al. (2014).*

Lidocaine is a drug frequently used in cardiologic practice, in which it is infused intravenously to exploits its antiarrhythmic properties (Martin et al., 1976). Upon topical application, it acts as a regional anesthetic by numbing underlying cutaneous tissue (Bjerring and Arendt-Nielsen, 1990; Gupta and Sibbald, 1996). This principle has been demonstrated to symptomatically relieve pain in several neuropathic conditions, such as post-herpetic neuralgia (Rowbotham et al., 1996; Garnock-Jones and Keating, 2009; Mick and Correa-Illanes, 2012) and nerve pain after knee surgery (Pickering et al., 2019). Topical lidocaine's mode of action derives from its gradual infiltration of skin layers, resulting in a length-dependent concentration gradient (Singh and Roberts, 1994). Here, the aminoethyl amide extends the refractory period of voltage-gated sodium channels in neuronal membranes (Carterall, 2008). In relatively low concentrations (i.e., 5%), maintained in commercially-sold lidocaine, this leads to selective blockade of small aδ- and C-fibers, without reaching larger tactile aβ-nerve fibers in deeper layers or significant systemic absorption (Gammaitoni et al., 2003; Krumova et al., 2012).

Based on this pharmacodynamic principle, multiple studies investigated whether transdermal lidocaine could simulate neuronal dysfunction in SFN. Indeed,

previous work showed that sensory thresholds and EP amplitudes related to intraepidermal electrical stimuli change after the use of lidocaine tape compared to placebo tape (Otsuru et al., 2010; Kodaira et al., 2014) (Fig. 2.4). It was, therefore, considered interesting to explore the outcomes of the NDT-EP measurement method for this model of SFN. In addition to measurements in DM patients that possibly suffered from SFN (see 4.3. *Part 2 - DM measurements*), this could clarify characteristic outcomes for nociceptive detection probabilities and EPs related to 'isolated' SFN.

2.5. Prior work and preliminary analyses

2.5.1. The NDT-EP method in chronic pain patients

In 2019, the novel NDT-EP measurement approach was explored for the first time in the clinical setting of the St. Antonius Hospital (Nieuwegein, The Netherlands) (Berfelo, 2019). The study comprised measurements in both patients that suffered chronic low back pain because of failed back surgery syndrome (FBSS) and in healthy individuals. One of the main findings was that experimental NDT-EP measurements were replicable in a hospital environment since observed, relevant NDT-EP phenomena for the healthy controls were comparable to those in previous work (van den Berg and Buitenweg, 2018; van den Berg et al., 2020). However, regarding potential future clinical applicability, additional findings from this work were two-fold. First, different initial values and behavior of NDTs tracked over time were found for FBSS patients compared to healthy controls (Fig. 2.5.). Second, particular EP phenomena observed in controls were absent or altered in patients, and linear mixed regression (LMR) with EP data revealed that electrical brain responses were differently modulated by stimulus properties in the latter.

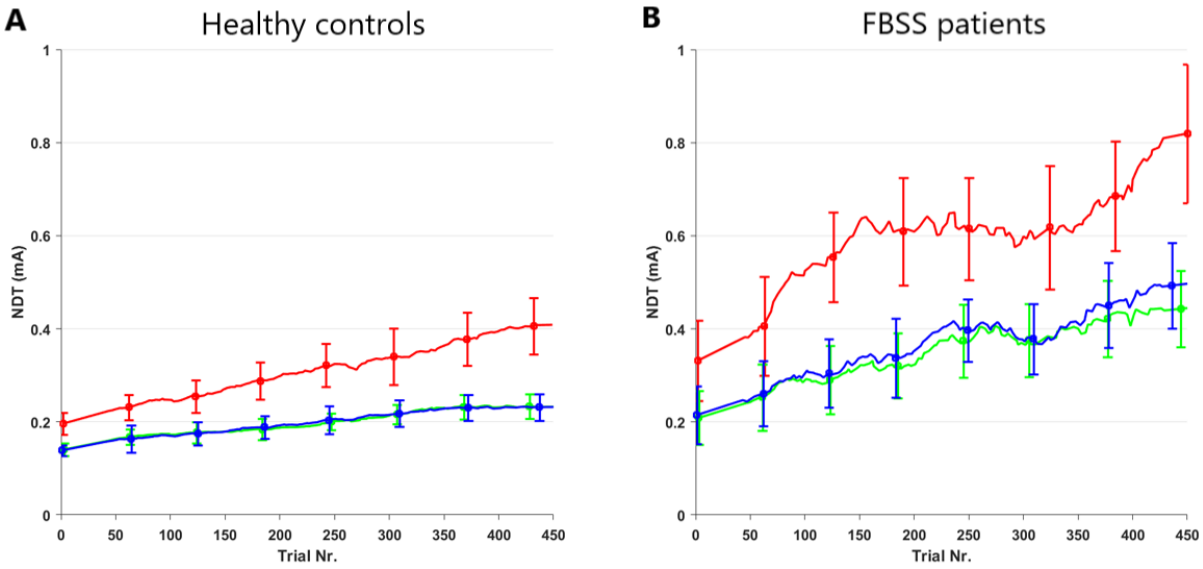


Figure 2.5. Estimated group average nociceptive detection thresholds (NDT) tracked over stimulus number (Trial Nr.) for healthy controls in panel A and failed back surgery syndrome (FBSS) patients in panel B. Values are provided in milliampere (mA). Tracked NDTs for single pulse stimuli are shown in red, for double pulse stimuli with 10ms inter-pulse interval in green and for double pulse stimuli with 40ms inter-pulse interval in blue. Error bars denote standard error of the mean. *Adapted from Berfelo (2019).*

Graphical and statistical differences in tracked NDTs and averaged EPs, respectively, are suggestive of the method's applicability in FBSS patients. This finding raised the question of which outcomes the NDT-EP method produces in other disorders occasionally marked by chronic pain, but due to small fiber dysfunction, such as in complicated DM. Furthermore, the study by Berfelo (2019) revealed that average NDTs, calculated from linear regression coefficients, may be higher in FBSS patients than in healthy controls. This is another captivating outcome, posing the question of whether measurement properties influence the detection probability differently for chronic pain patients and healthy individuals. As such, in the present study, modeled effects of measurement parameters (e.g., number of administered stimuli) on detection probabilities were statistically compared between study groups.

2.5.2. Lidocaine experiment: preliminary considerations

Measurement data from the lidocaine experiment, i.e., obtained after lidocaine and placebo patch treatment, were preliminary analyzed and discussed by a master's student Technical Medicine (Eva Kleinveld). Outcomes are summarized in the following paragraphs.

Preliminary results

Neurological examination of the hand dorsum before and after placebo patch treatment did not yield differences for either the soft-cloth or the pin-prick test. However, 7 out of 19 participants had considerable difficulties distinguishing sharp from blunt pinpricks after lidocaine patch treatment compared to no difficulties before. None of them had similar problems detecting subtle tactile stimuli before or after lidocaine treatment.

Linear regression analysis of detection probability demonstrated that several measurement properties (e.g., amplitudes of stimuli with different temporal properties) significantly predicted stimulus detection. However, 'patch type' was not among them, which insinuated that treatment with a lidocaine patch did not result in different detection probabilities compared to the placebo patch (Fig. 2.6).

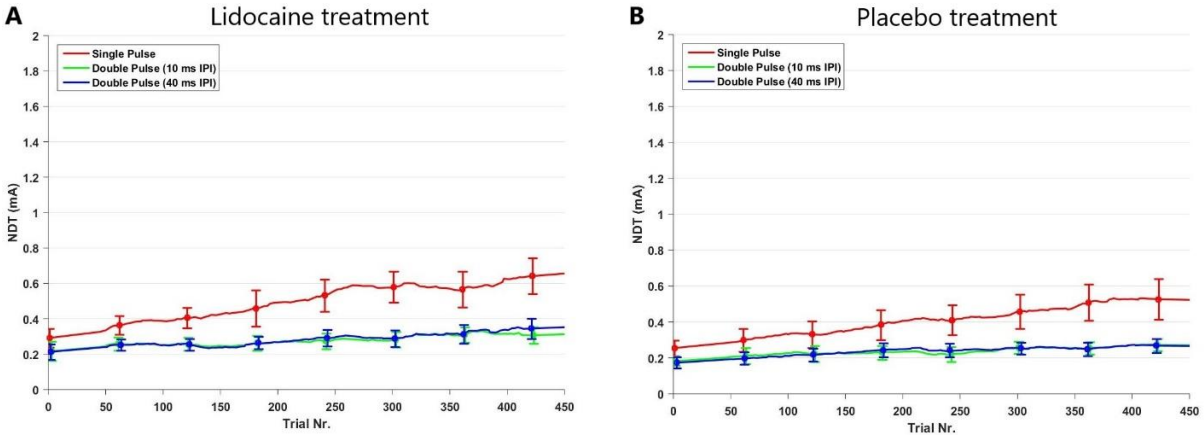


Figure 2.6. Estimated nociceptive detection thresholds (NDTs), in milliampere (mA), tracked over trial number (Trial Nr.) for single pulse stimuli (red), double pulse stimulus with 10ms inter-pulse interval (IPI) (green) and double pulse stimuli with 40ms IPI (blue). Group average trajectories are given for measurements in healthy participants after lidocaine (panel A) and placebo (panel B) patch treatment. Error bars represent standard error of the mean. *Adapted from preliminary, original work by Eva Kleinveld.*

On the contrary, the interaction between patch type and participant's response imposed a significant effect on the grand average EP amplitude in a central EEG derivation (CPz-A1A2). This outcome implied that the EP, assessed at latency 470ms, differed between measurements after lidocaine and after placebo patch treatment for detected stimuli (Fig. 2.7; EPs after detected and undetected stimuli not separated).

Preliminary discussion

The NDT-EP method's outcomes for detection probability after lidocaine patch on hand dorsa are generally in disagreement with similar studies, which tend to produce inconsistent results themselves. Whereas no different detection thresholds for IES after lidocaine tape on the hand dorsum were reported before 3 hours (Otsuru et al., 2010), subsequent investigations of the same group demonstrated significant differences already after 30 minutes when measuring on the food dorsum (Kodaira et al., 2014). Assessment with a broader array of QST modalities showed that measures of small fiber function (e.g., thermal and mechanical pain thresholds) were significantly different after treatment with lidocaine patches, identical to those used in the present study (Krumova et al., 2012). Nonetheless, these tests were carried out on the volar forearm after 6 hours of patch treatment.

Outcomes of preliminary analyses further showed that maximum EP amplitude measured in a central derivation such as CPz-A1A2, regardless of patch type, was reached 470ms after stimulus administration. This latency was in correspondence with investigations in healthy

participants in one preceding study (Berfelo, 2019), but not in another by van den Berg et al. (2020). The discrepancy with the maximum peak in the latter study (P340) may have been due to characteristic demographic differences between study samples. Nevertheless, the significantly smaller EP amplitudes after lidocaine compared to the placebo patch were in line with work by Kodaira et al. (2014), which revealed that cortical responses were altered already after half an hour of topical lidocaine treatment.

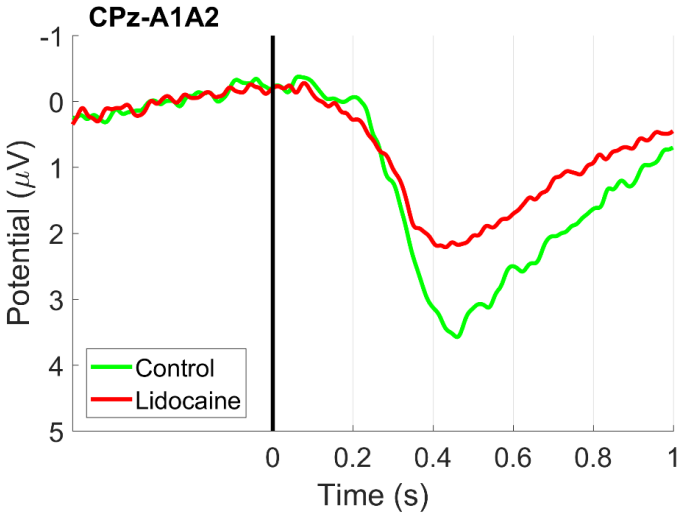


Figure 2.7. Grand average evoked potentials (EP) in EEG derivation CPz-A1A2, elicited by intraepidermal electrical stimulation. Signals, provided in microvolt (μV), were obtained after placebo patch treatment (green) and lidocaine patch treatment (red). *Reprinted from preliminary, original work by Eva Kleinveld.*

The rather unexpected unimportance of patch type for detection probabilities may have had several potential causes. First, partial silencing of nociceptive aδ- and C-fibers by lidocaine could have caused a portion of the fibers to still sense the stimuli (thus, virtually no differences in detection probability), although less sensitively (thus, clear differences in EP amplitude) (Krumova et al., 2012). This fact could also have contributed to observed altered, but not wholly disappeared, pinprick sensation during neurological examinations. Second, the application time in this study may have caused preferential silencing of C-fibers due to their smallest axon diameters, which was suggested by results from comparable experimental set-ups (Sakai et al., 2004; Kodaira et al., 2014). As the NDT-EP measurement method practices IES, which preferably targets aδ – fibers, this may have caused the detection probability to remain unchanged after lidocaine application. However, it may not directly clarify why EP amplitudes did decrease following transdermal lidocaine. Third, IES currents possibly directly reached the epidermal fiber instead of the nociceptor side, even though the impact of lidocaine has been proposed to prevail at the latter (Sakai et al., 2004). Albeit this could be elucidative for obtained results regarding detection probabilities, again, it does not provide a sound explanation for the patch treatment-dependent EP amplitudes.

Strengths of the present study comprised (1) the first use of transdermal lidocaine patches in an NDT-EP measurement setting to study the method's outcomes for simulated SFN, (2) the use of three types of intraepidermal electrical stimuli with different temporal properties and (3) the administration of 150 stimuli per stimulus type, in random order, to additionally observe time-dependent effects on nociceptive stimulus detection. The primary limitation concerned the rather arbitrarily determined patch treatment time (2 hours), which may have been an essential contributor to the study's detection probability results.

Preliminary conclusion and further perspectives

Outcomes from preliminary considerations suggested that EP amplitudes, but not detection probabilities, may have different values for dysfunctional and normally functioning small fibers. Yet, it would be interesting to investigate how these results relate to those obtained from healthy participants without a patch. This would grant insight in the magnitude of potential placebo effects, and thus provide impressions whether (lack of) significant differences were to be expected. The results of these investigations are described in 4.2. *Part 1 - lidocaine experiment*.

2.6. Significance

2.6.1. Clinical relevance

Chronic pain, which is pain persisting longer than a pre-defined period (e.g., 3 months), is a global issue that imposes a significant burden on many aspects of society. It can heavily impact patients' lives by, for example, leading to unemployment, promoting mental disabilities, hampering the preservation of social contacts, and affecting all kinds of daily activities such as exercising and sleeping (Breivik *et al.*, 2006; Reid *et al.*, 2011). An estimated 18% of the Dutch population suffers from moderate to severe chronic pain complaints (Bala *et al.*, 2011), which is a number expected to rise due to an aging population (Fayaz *et al.*, 2016). However, increasing age additionally comes at the cost of increased risk on various diseases, such as (type 2) DM, which in turn could lead to DPN and corresponding chronic pain.

As a rule, small fibers are among the first affected by hyperglycemia in DM (2.3.3. *Small fiber neuropathy*). Damage to these fibers can induce severe pain in some patients, but may only lead to subtle sensory loss in others (Sorensen *et al.*, 2006). These manifestations may anticipate widespread diabetic polyneuropathy, also affecting larger fibers, resulting in further invalidation of patients. Consequently, it is of paramount importance to explore increasingly objective methods for the functional assessment of small fibers and high-order afferents of the nociceptive system. Insights might, in the future, benefit not only DM patients with somatosensory disturbances (such as chronic pain), but also patients with other diseases involving somatosensory dysfunction that have been linked to SFN.

2.6.2. Uniqueness

Investigations of stimulus detection thresholds and EPs have known a long tradition in both experimental models of SFN and DM patients (see 2.4.2. *Topical lidocaine – a model of small fiber neuropathy* and 2.3.3. *Small fiber neuropathy*, respectively). Yet, to the best of my knowledge, previous work has not considered intuitive algorithms for simultaneously tracking detection thresholds for different stimulus types and registering EPs. In this view, the NDT-EP measurement method constitutes a novel and unique approach to potential functional

assessment of small fibers and corresponding higher-order neuronal pathways. By exploring the method's outcomes in healthy conditions, a lidocaine SFN model, and DM patients, an attempt is made to delineate the nociceptive system in both standard and (simulated) pathological circumstances. Findings might contribute to the establishment of new biomarkers for pain processing in patients with persistent (neuropathic) pain or its precursors, one of which could be functional small fiber deterioration.

3. Methods

3.1. Study design

This exploratory, prospective study was performed at the Department of Anesthesiology, IC and Pain Medicine in the St. Antonius Hospital (Nieuwegein, The Netherlands). The study was carried out in agreement with the most-recent Good Clinical Practice guidelines (European Medicines Agency, 2017). Ethical approval was commissioned by the regional medical research ethics committee (Medical Research Ethics Committee United, MEC-U, NL66136.100.18) on 19th August 2019.

This study was separated into two parts: a lidocaine experiment, in which NDT-EP measurements were conducted after patch treatment in healthy individuals, and DM measurements, which featured measurements in DM patients.

3.1.1. Placebo control, randomization and blinding procedures

Both active and placebo patches were employed in the lidocaine experiment. Allocation of patch type to the participant's dominant or non-dominant hand was pre-determined to minimize predictability and ensure equal frequency for all possible combinations (Table 3.1). The experiment was single-blinded. Participants did not know on which side either the active or placebo patch had been applied. However, because of the explorative scope of this experiment and the negligible influence of data preparation on the outcomes, researchers were aware of this.

Table 3.1. Combinations of hand (dominant/non-dominant) and patch type, for the first and second NDT-EP measurement round for each participant in the lidocaine experiment. Every fifth participant, the cycle repeated itself, starting over with the combination for participant 1.

Participant	Cycle	Combination first round	Combination second round
1	1	DH – LID	NDH – PLA
2		DH – PLA	NDH – LID
3		NDH – LID	DH – PLA
4		NDH – PLA	DH – LID
5	2	DH – LID	NDH - PLA
…		…	…

DH = dominant hand, NDH = non-dominant hand, LID = lidocaine (patch), PLA = placebo (patch).

The DM measurements did not concern any interventions, which rendered placebo control and randomization procedures unnecessary. In analogy with the lidocaine experiment, the researcher was not blinded for study groups.

3.1.2. Outcome variables

Primary outcomes variables

Four primary outcome variables were considered in this study (Table 3.2.). Two of them, group average psychophysical NDT and slope, were only calculated following significant interaction between study group and (at least one of the) amplitudes for different stimulus types regarding detection probabilities. These could then further clarify directions of potentially significant group differences.

Table 3.2. Names and descriptions of the four primary outcome variables in this study. Note that 'study group' equals 'patch type' for analyses involving measurements in the lidocaine experiment.

Variable name	Description
Effect(s) of study group on detection probability#	Coefficient estimates and (interaction) effect sizes for 'study group' in a linear regression model of IES detection probability
Effect(s) of study group on EP amplitude#	Coefficient estimates and (interaction) effect sizes for 'study group' in a linear regression model of EP amplitude after IES
Group average NDT*	The stimulus intensity for which the detection probability is 50%, inferred from the psychophysical function mapping stimulus strength to detection probability (Doll *et al.*, 2015)
Group average slope*	The slope of the psychophysical function at group average NDT

= in addition, the main effects of measurement round number were examined.
* = only derived from model coefficients for significant interaction between study group and at least one of the amplitudes of different stimulus types concerning detection probability.

EP = evoked potential, IES = intraepidermal electrical stimulation, NDT = nociceptive detection threshold.

Secondary outcome variables

Secondary outcome variables included general characteristics, applicable to all participants in the present study and healthy controls from a previous study (Berfelo, 2019), and disease-related characteristics, only applicable to DM patients.

General group characteristics comprised age, sex, body mass index (BMI), handedness, medication intake, an indication of current and past pain experience on the NRS, CSI score, self-reported somatosensory abnormalities of hand dorsa (upon availability), soft-cloth test outcomes (upon availability), and pin-prick test outcomes (upon availability).

Disease-related characteristics encompassed type of DM, time since diagnosis of DM, self-assessed somatosensory abnormalities, frequency of anti-DM medication use, intake of other medication, a diagnosis of DPN for experienced pain; and if applicable: time since diagnosis of (P)DPN, duration of neuropathic pain, regions of neuropathic pain, self-assessed somatosensory hypersensitivity and use of analgesics.

3.2. Study population

Generally, participants were recruited through recruitment posters (*Appendix A: Recruitment poster (Dutch)*), and digital announcements on the hospital's social media channels. DM patients, in particular, were also approached via websites of the Dutch Diabetes Association ('Diabetes Vereniging Nederland') and the Dutch Diabetes Fund ('Diabetes Fonds'), and by contacting specialized medical personnel. This led to three groups of participants in the present study, enrolled between September 2019 and February 2020. Additionally, NDT-EP measurement outcomes from healthy participants in a previous study were included as healthy control data (Berfelo, 2019).

General exclusion criteria

General exclusion criteria were refusal to continue participation, communication problems or incapability of following directions, implanted stimulation device, pregnancy, consumption of illicit drugs or alcohol 24 hours before the experiment, skin on at least one of the hand dorsa pathologically affected and central of peripheral nervous system disease (except for DPN in DM patients). Post hoc exclusion was done for unfinished measurement rounds of insufficient data quality, for instance, due to movement artifacts.

3.2.1. Healthy participants (HP group)

Individuals (aged 18 – 65 years) were considered for participation in the lidocaine experiment if they did not have a history of pathological pain and did not experience any pain complaints at the start of the experiment. They could not partake in the study if they used drugs based on or containing amyl nitrite, sodium nitrite, sodium thiosulfate, epinephrine, or prilocaine, or if they were hypersensitive to any component in the lidocaine or placebo patch (e.g., lidocaine or other amide local anesthetics).

In further communications, 'study group' in the context of the lidocaine experiment concerns the collection of data obtained after either lidocaine ('lidocaine measurements') or placebo ('placebo measurements') patch treatment.

3.2.2. DM patients (DM [np] group and DM group)

Individuals (aged 18 – 75 years) were considered for participation when they had been diagnosed with type 1 or type 2 DM. If patients suffered from chronic neuropathic pain (np),

which caused them to be diagnosed with DPN, they were enlisted in the DM [np] group. If there were no pain complaints, patients were placed in the DM group. Nonetheless, if a patient fulfilled any of the following criteria, they were excluded: another cause for painful neuropathy more likely than DM (applicable to the DM [np] group), or severe or chronic non-neuropathic pain complaints.

3.2.3. Healthy controls (HC group)

Seventeen healthy participants without pain complaints were identified from prior NDT-EP measurements between September and November 2018 (Berfelo, 2019). Data from their measurements, performed at the St. Antonius Hospital, were included in this study. For further recruitment and participant (selection) details, refer to chapters 3.1. and 4.1. of this work (Berfelo, 2019). This set of participants are referred to as the (previously assessed) healthy control group (HC group).

Upon interest, individuals received a participant information letter and were given sufficient time to study this. Before the start of measurements, all participants gave verbal and written informed for their wish to participate, which was the final inclusion criterion.

3.3. Materials and procedures

The measurements in this study were conducted with technical equipment, a stimulation and threshold tracking algorithm, and an NDT-EP measurement procedure mostly similar to prior investigations (Doll et al., 2016b; van den Berg and Buitenweg, 2018; Berfelo, 2019; van den Berg et al., 2020).

3.3.1. Technical equipment

Intraepidermal electrical stimuli were applied with a custom-made ‘IES-5’ electrode first manufactured by Steenbergen et al. (2012), but without flat discs. It contained an array of five interconnected microneedles protruding 0.2 mm through the stratum corneum of the skin. This permitted specific activation of mainly nociceptive aδ-fibers in the superficial skin (Inui et al., 2002a; Mouraux et al., 2010; Inui and Kakigi, 2012), which elicited EPs measurable with scalp-EEG. The IES-5 electrodes, servings as cathode during stimulation administration, were sterilized in an autoclave at the University of Twente (Enschede, The Netherlands) before each measurement appointment. In addition, a 90 x 50 mm transcutaneous electrical nerve stimulation (TENS) electrode functioned as the anode.

A NociTRACK AmbuStim stimulator (NociTRACK B.V., University of Twente, Enschede, Netherlands) was connected to the bipolar electrode and used for generation and administration of electrical stimuli upon pressing and holding the stimulator button. The stimulator, in turn, was connected to a laptop (both wired and wirelessly via Bluetooth), which ran a custom

application programmed in LabVIEW 2013 SP1 software (National Instruments Corporation, Austin, TX, USA). This application incorporated a stimulation algorithm that controlled all temporal properties of the stimuli, being (ensembles of) rectangular pulses (see 3.3.2. *IES and threshold tracking*, for details). Furthermore, it registered parameter values related to stimulus detection, i.e., participant's responses (detected or undetected) and response times.

Throughout the measurement, EEG signals were recorded with an EEG cap (Waveguard, ANT Neuro B.V., Hengelo, the Netherlands) containing 64 passive Ag/AgCl electrodes positioned according to the international 10-20 system. The cap was connected to a 72-channel Refa amplifier (TMSi B.V., Oldenzaal, The Netherlands). Three Ag/AgCl ECG electrodes (Kendall, Covidien, Medtronic, Inc., Minneapolis, MN, United States), 24 mm in diameter, were employed as additional leads for bipolar derivations and the ground electrode. Raw EEG data were registered and stored using TMSi Polybench software (TMSi B.V., Oldenzaal, The Netherlands)

3.3.2. IES and threshold tracking

Properties of applied stimuli were selected according to a recently established Multiple Threshold Tracking paradigm (Doll et al., 2016b). The corresponding algorithm randomly determined the set of temporal parameter values, i.e., number of pulses and inter-pulse interval (IPI), for an applied stimulus – referred to as (stimulus) trial. The pulse width was kept at 0.21ms, analogous to earlier studies (Berfelo, 2019; van den Berg et al., 2020). Furthermore, the algorithm automatically adapted the stimulus intensity (step size 0.05 mA) based on the response of the participant, decreasing the current intensity when the stimulus was detected and increasing it when the participant failed to indicate stimulus application. The maximum stimulus intensity was fixed at 1.5 mA for two main reasons: (1) to ensure that results from present investigations could be easily compared to results from previous work, and (2) to minimize coactivation of tactile aβ – nerve fibers, which is expectable at higher current strengths (Mouraux et al., 2010). Variation of temporal parameters and stimulus intensity across trials allowed for simultaneous tracking of stimulus amplitudes and participants' responses for one single pulse (SP) and two double pulse (DP) stimulus types (Table 3.3).

Table 3.3. Values of stimulus properties (NoP, IPI, PW) for each type of intraepidermal electrical stimulus employed in this study.

Stimulus type	Code in figures	NoP	IPI (ms)	PW (ms)
1	SP	1	N.A.	0.21
2	DP10	2	10	0.21
3	DP40	2	40	0.21

DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, IPI = inter-pulse interval, N.A. = not applicable, NoP = number of pulse, PW = pulse width, SP = single pulse.

3.3.3. Local anesthesia

In the lidocaine experiment, lidocaine (5% w/w) hydrogel patches (Versatis, Grünenthal B.V., Breukelen, The Netherlands), with dimensions 100x140 mm and containing 700 mg of active substance, were used to simulate a condition of SFN. Patches identical in appearance but without active ingredients (Versatis, Grünenthal B.V., Breukelen, The Netherlands) were used as placebo. Both active and placebo patches were partitioned in four equal portions to assure that they would fit the dorsa of the participants' hands.

3.3.4. Measurement procedures: general

Introduction

Participants were invited to the hospital for one measurement appointment. Measurements were performed in a vacant room at the Intensive Care Unit. After remaining questions and unclarities were dealt with, participants were handed the written informed consent form (*Appendix B: Informed consent form (example), in Dutch*). Mobile devices were removed from the measurement room before further proceedings to reduce potential electromagnetic noise.

Initiation

Following informed consent, a questionnaire inquiring demographic and clinical characteristics was filled in. Part of this was the NRS, for which participants were asked to rate their pain experience, both an average of the past week and at this specific moment, on a scale from 1 (no pain at all) to 10 (most excruciating pain imaginable). In the final section, a basic neurological examination of the dorsa of the hands was performed, which was newly added compared to earlier investigations, e.g. (Berfelo, 2019), for comparison with NDT-EP measurement outcomes. It consisted of three components: (1) a soft-cloth test, which concerned the use of a gauze swab to evaluate the participants' ability to register subtle touch, (2) a pin-prick test, which involved a cotton swab broken in half, leaving a blunt and a sharp end, to test the participants' ability to discriminate sharp from blunt stimuli and (3) a few complimentary questions. Based on the number of false guesses, outcomes of the tests were either marked normal (< 3) or abnormal (3 or more).

Preparation

Subsequently, the EEG cap was fitted to the participants' heads such that electrode Cz was positioned at the intersection of two imaginary, perpendicular lines representing the shortest distances between both mastoids, and between the nasion and the inion. After the skin was prepared with scrub gel (NuPrep EEG and ECG Skin Prep Gel, Weaver and Company, Aurora, CO, USA), ECG electrodes were attached to both ear lobes and the forehead just over the midline between the eyebrows (ground electrode). Impedances of all electrodes were inspected and kept below 5 kΩ throughout the measurements with conductive gel (Electro-Cap Gel,

Electro-Cap Center B.V., Nieuwkoop, The Netherlands). After that, the IES-5 electrode was attached to the skin of the dorsal hand to be stimulated first. The TENS electrode was placed proximally from and directly adjacent the IES-5 electrode, after which both were connected to the NociTRACK Ambustim stimulator.

Familiarization sessions

The first NDT-EP measurement round was preceded by a familiarization session to accustom participants to stimulus sensations and to determine initial detection thresholds. Participants were instructed to press and hold the stimulator button, which caused stimulation device to administer stimuli (1 Hz), slowly increasing (0.05 mA s^{-1}) from 0 mA up to a maximum intensity of 1.5 mA. The latter differed from those maintained in previous investigations (1.0 mA) (Berfelo, 2019), since it was held possible that initial thresholds could exceed the previously operated maximum value. In the lidocaine experiment, this could be due to functional silencing of small fibers (Krumova et al., 2012; Kodaira et al., 2014)), and in DM measurements due to both neuropathic sensory loss and relatively high ages, associated with deterioration of neurophysiological performance (Verdú et al., 2000; Vas and Rayman, 2013)). Participants were instructed to release the button after either the first or third time, dependent on familiarization round, that they ascribed a sensation to a perceived stimulus. If, despite increased maximum stimulus intensity, participants failed to detect the stimulus before the maximum intensity, the initial intensity was set just below this value. Each familiarization session consisted of four rounds (two for stimulus type 1; and one time for stimulus type 2 and 3, each). Subsequently, further instructions for the first NDT-EP measurement round were provided.

NDT-EP measurement rounds

A minimum of 150 stimuli per stimulus type was administered intraepidermally in one NDT-EP measurement round. As described in 3.2.2. *IES and threshold tracking*, stimulus types and amplitudes were selected in a random order (Doll et al., 2014). Additionally, inter-stimulus intervals were randomized with a 1-second uniform distribution. Participants were instructed to hold down the stimulator button continuously, and only quickly release (<1000ms) and re-press the button as soon as they felt a sensation they ascribed to the stimulator. Furthermore, they were told to visually focus on a self-chosen spot in front of them while minimizing face and neck muscle activity to avoid muscular artifacts. If participants needed a short break, they were allowed to release the button for a little longer, which paused registration of electrocortical activity. Throughout the whole measurement round, participants were observed closely to prevent them from falling asleep.

The measurement round ended after the minimum amount of stimuli per type was reached. Participants were asked a fixed set of questions (*Appendix C: NDT-EP measurement experience questions*), surveying their experiences during the measurement. This inquiry was

followed by switching stimulation equipment to the opposite body side. After the EEG registration and the stimulation software had been re-initiated, and electrode impedances had been checked, participants underwent a second NDT-EP measurement round. After this round, the participants were asked the same set of questions, with an additional request to compare their experience with that of the previous NDT-EP round, which was primarily of interest in the lidocaine experiment.

Round-up

At the end of the appointment, all participants were compensated for their participation by a gift voucher.

3.3.5. Measurement procedures: lidocaine experiment

Procedures were slightly different during measurement appointments for the lidocaine experiment. Instead of approximately 3 hours, these lasted roughly 4 hours, including 2 hours of patch treatment time. Additional procedural dissimilarities are schematized in Figure 3.1.

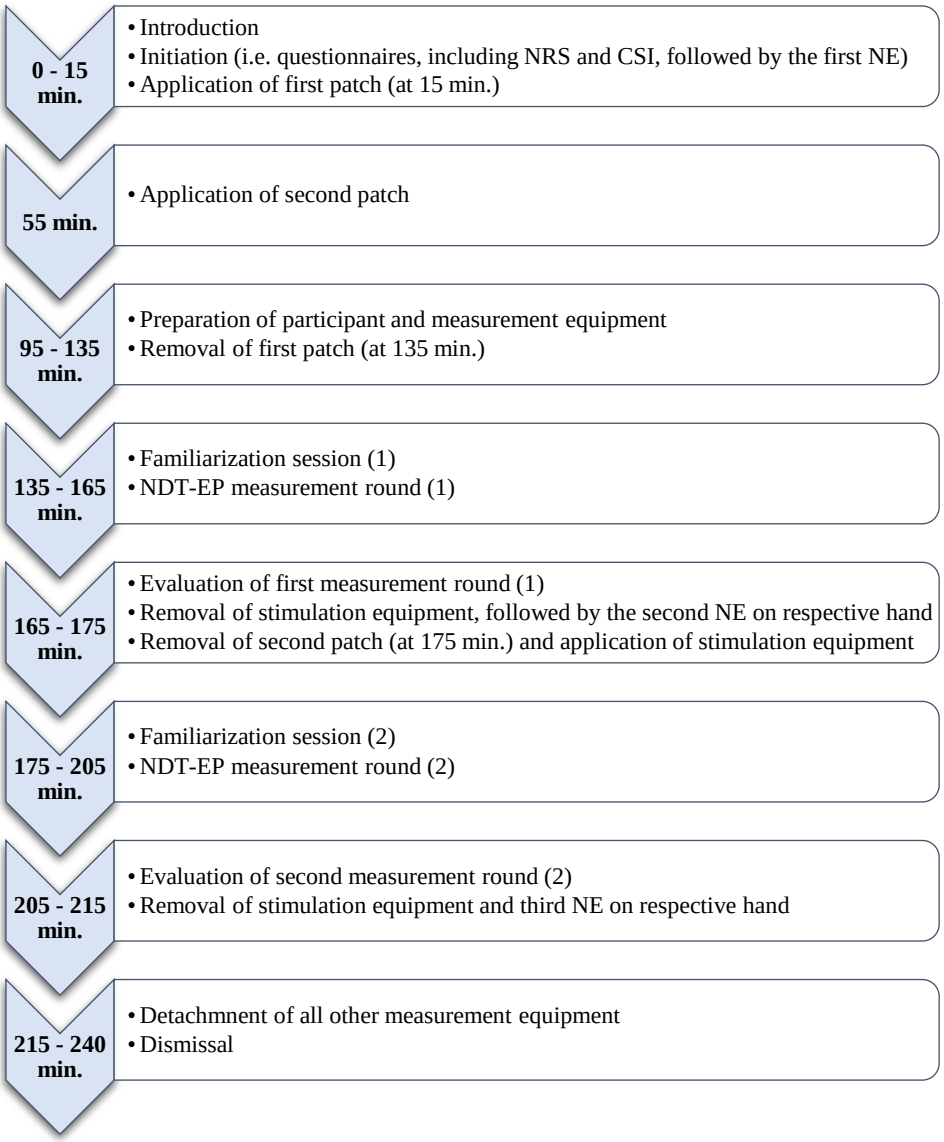


Figure 3.1. Time intervals and corresponding activities performed during one measurement appointment for the lidocaine experiment. CSI = central sensitization inventory, NE = neurological examination, NRS = numeric rating scale.

3.4. Data preparation and visualization

Newly acquired stimulus-detection data and EEG recordings were preprocessed in MATLAB R2018b (The MathWorks, Inc., Natick, MA) using the Fieldtrip toolbox (Oostenveld et al., 2011), based on methods described in related work (Berfelo, 2019; van den Berg et al., 2020). Subsequently, preprocessed measurement data were visualized.

3.4.1. Detection probabilities and NDTs

During measurement rounds, NDTs were real-time estimated from applied intensities per stimulus type, using a moving window of 30 trials and transient psychophysical functions,

which allowed preliminary visualizations. Subsequently, these thresholds were re-estimated with data from multiple study groups using generalized LMR (GLMR), in analogy with previous studies (Doll et al., 2016b; Berfelo, 2019; van den Berg et al., 2020). The corresponding GLMMs, see eq. 4 in Wilkinson notation, described the relation between multiple model factors or parameters (e.g. 'study group') and one dependent variable ('participant's response'). Regarding further descriptions of GLMR analyses, the binary participant's response is referred to by its continuous analog: detection probability. After coefficients for model parameters had been computed, NDTs were trial-wise obtained by linear prediction from these coefficients. Thresholds twice the value in the previous trial were removed. Subsequently, the remainder were displayed, separated by stimulus type and study group, to visually explore their courses.

$$PR \sim 1 + A\_1 * SG + A\_2 * SG + A\_3 * SG + ST * SG + MR + (1 + A\_1 * SG + A\_2 * SG + A\_3 * SG + ST * SG + MR \mid PT) \quad \text{[eq. 4]}$$

GLMM variables

A_1 = *amplitude single pulse*
A_2 = *amplitude double pulse, 10ms inter-pulse interval*
A_3 = *amplitude double pulse, 40ms inter-pulse interval*
MR = *measurement round number*
PR = *participant's response*
PT = *participant number*
SG = *study group (or patch type)*
ST = *stimulus trial number*

3.4.2. Evoked potentials

Simultaneously recorded EEG data were segmented into epochs based on stimulus trial intervals (ranging from 0.5s before to 1s after stimulus administration), resulting in one EP per epoch. Next, these epochs were decomposed into separate signals using an independent component analysis implementation from EEGLAB (infomax/runica) (Bell and Sejnowski, 1995). The 20 independent components (IC) responsible for the biggest variance in the signal were visually inspected for all epochs. ICs representative of non-electrocortical processes (such as eye blinks or signal drift) were manually removed from all epochs. Additionally, epochs (max. 30) that prevented a marginally homogenous distribution of EEG signal variances for all epochs were discarded. The remaining epochs were high- and low-pass filtered at 0.1 Hz and 40 Hz, respectively, after which a baseline correction using the 0.5 to 0.0s pre-stimulus interval was conducted.

Subsequently, butterfly plots with segmented EPs were created for each study group (*Appendix D: Butterfly plots*). Additionally, these showed the global field power (GFP) (Skrandies, 1990), which described the summed EEG activity of all EPs for each instance in time. To examine the effects of study group on cortical responses to IES at maximum amplitude, two latencies were

determined similarly to Berfelo (2019), but based on GFP maxima. Corresponding EP components, peaking in central derivations (in this study: CPz-A1A2) for the relatively longer and contralateral (T7-F4) for the relatively shorter latency, tended to substantially differ between groups. Therefore, final latencies were either inferred from butterfly plots with EP data from all the groups in a single regression analysis, or, in case of computational limitations, averaged between values from separate plots. Finally, for visual explorations, grand averages of EPs were plotted per study group, after which the EP data and latencies were stored for further statistical analysis.

3.5. Statistical analyses

3.5.1. Participant characteristics

Participant characteristics were compared between study groups using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, N.Y., USA). The assumption of normality of sample distributions was visually (quantile-quantile plots) and mathematically (Shapiro-Wilk test) assessed. Differences between groups for categorical variables were investigated with Pearson's chi-squared tests. Two-tailed independent samples *t*-tests were applied to statistically verify differences for normally distributed variables. Non-parametric procedures (Mann-Whitney *U* tests) were employed otherwise.

3.5.2. Detection probabilities and NDTs

Statistical evaluation of modeled effects on detection probability and, if applicable, group average NDTs and slopes was performed in R-3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) using the lme4 library (Bates et al., 2015). Stimulus-detection data were used to fit GLMMs, of the same structure as in preliminary visualizations (eq. 4), coded in R. Computed fixed model effects were statistically tested using a type III ANOVA table. Consequent statistical interpretation of main and interaction effects was limited to those involving study group, in agreement with the objectives of this thesis (1.4. *Approach*). Additionally, the main effect of measurement round (number) was described to evaluate the possible impact on detection probability. Upon significant interaction between study group and amplitude of at least one of the stimulus types, group average NDTs were calculated with the delta method (Doob, 1935), and group average slopes obtained by testing general linear hypotheses. Potentially significant differences were confirmed or rejected by post hoc statistical testing (*Z*-statistics).

3.5.3. Evoked potentials

Analyses of EPs were conducted in MATLAB R2018b (The MathWorks, Inc., Natick, MA, USA). Segmented EEG data were used to fit LMMs, of which the common formula is provided by eq. 5 (Wilkinson notation). The significance of fixed effects on maximum EP amplitudes

was evaluated using *t*-statistics at latencies identified for groups in the respective regression analysis. Again, study group and measurement round were the only model parameters of which statistical effect relevancies were reported and interpreted.

For all statistical test outcomes, the significance threshold was set at $\alpha = 0.05$.

$$EP \sim 1 + A\_1 * SG + A\_2 * SG + A\_3 * SG + ST * PR * SG + MR + (1 + A\_1 * SG + A\_2 * SG + A\_3 * SG + ST * PR * SG + MR \mid PT) \quad \text{[eq. 5]}$$

LMM variables

A_1 = *amplitude single pulse*
A_3 = *amplitude double pulse, 10ms inter-pulse interval*
A_2 = *amplitude double pulse, 40ms inter-pulse interval*
EP = *evoked potential*
MR = *measurement round number*
PR = *participant's response*
PT = *participant number*
SG = *study group (or patch type)*
ST = *stimulus trial number*

4. Results

4.1. Participants

4.1.1. Healthy participants

A total of 33 healthy individuals without pain complaints expressed interest in participating in the lidocaine experiment. Eight individuals were not contactable anymore after first interactions. From the remainder, five participants were a priori excluded based on the following criteria (number of participants between parentheses): age not in desired range (2), pain complaints at the start of the experiment (1), and central of peripheral nervous system disorder (2). One participant was excluded after the measurement appointment as technical difficulties had corrupted the data. This resulted in a group of 19 healthy participants (12 females, age 24 – 57 years, average 38.9 years) in the present lidocaine experiment. The participant selection procedure is illustrated in Figure 4.1.

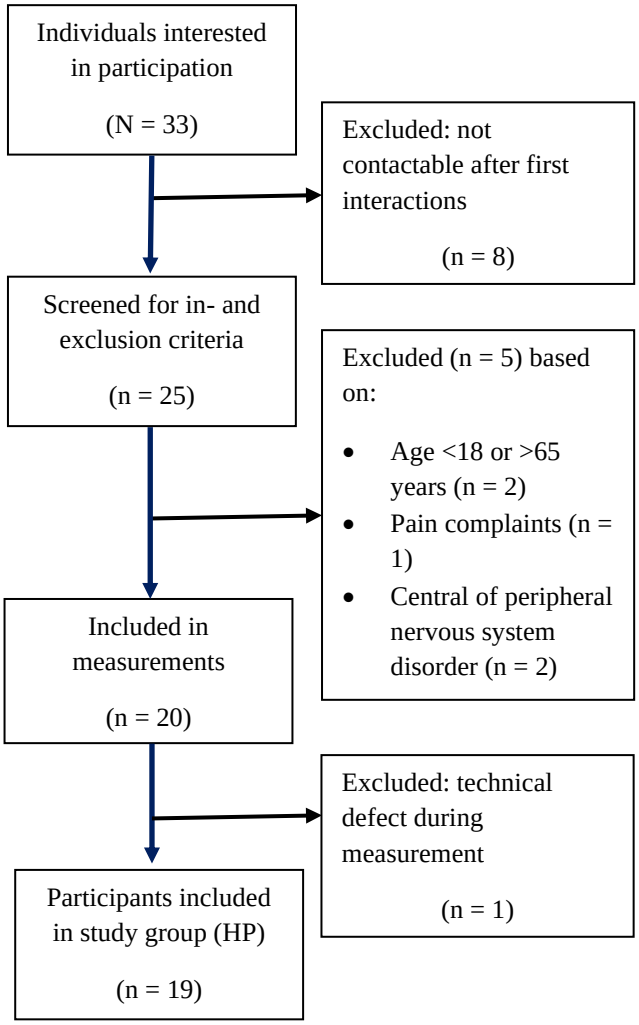


Figure 4.1. Flowchart of the participant selection for the healthy participants (HP) group; the study group for the lidocaine experiment.

4.1.2. DM patients

In total, 90 DM patients responded to recruitment advertisements, which comprised 59 patients with and 31 patients without pain complaints. Eight patients were excluded following the loss of contact or an explicitly stated lack of interest in further participation. Other patients were excluded because of the following reasons (number of

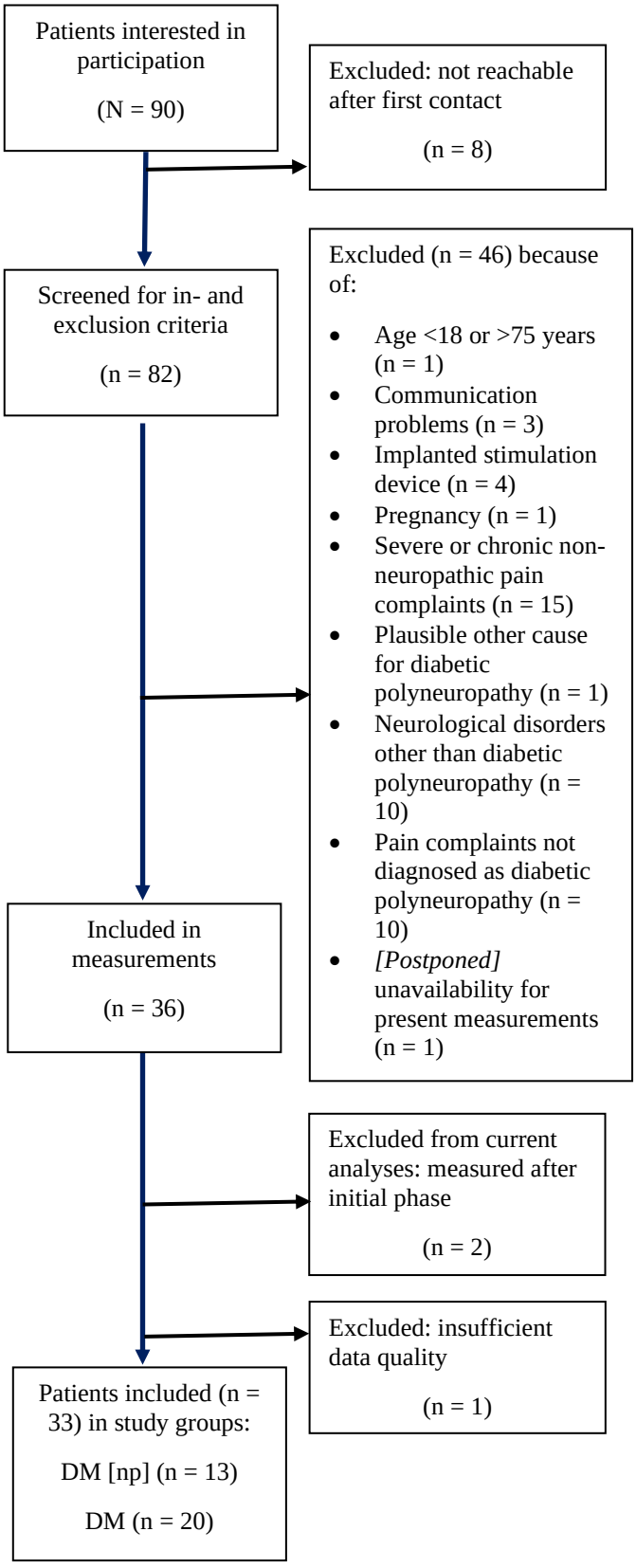


Figure 4.2. Flowchart of the diabetes mellitus (DM) patient selection for the DM [np] group, i.e. patients with chronic neuropathic pain, and the DM group, i.e. patients without pain complaints.

participants between parentheses): age not in the desired range (1), communication problems or incapability of following directions (3), implanted stimulation device (4), pregnancy (1), severe and chronic non-neuropathic pain (15), another cause for painful (poly)neuropathy more likely than DM (1), central or peripheral nervous system disease other than diabetic peripheral neuropathy (10) and pain complaints not related to diabetic peripheral neuropathy (10). The measurement appointment of one included patient was postponed, data from two patients were acquired after the present study phase, and recordings from one participant were not included in the final analyses due to insufficient data quality. The resultant sample of DM patients comprised 13 patients with chronic PDPN (2 females, age 45 – 72 years, average 64.2 years) and 20 patients without pain complaints (11 females, age 20 – 73 years, average 53.4 years). Figure 4.2 schematically demonstrates the in- and exclusion process for DM patients.

4.1.3. Healthy controls

Recordings from seventeen healthy participants (14 females, age 18 – 63 years, average 35.9 years), made in a previous study (Berfelo, 2019), were included in the analyses as healthy control data.

4.1.4. General group characteristics

General characteristics were compared for study groups in the same (G)LMR analysis.

HP group vs. HC group (lidocaine experiment)

Corresponding to expectations, healthy participants in the lidocaine experiment did not significantly differ from healthy controls (without patch) in age, sex, handedness, NRS (past week and current pain experience), and CSI score (Table 4.1). However, healthy participants in the lidocaine experiment had a significantly higher average BMI than the healthy controls without a patch.

Table 4.1. Demographic characteristics and pain evaluation outcomes for healthy participants (HP) in the lidocaine experiment and healthy controls (HC) preliminary measured in an earlier study (Berfelo, 2019), of which data sets were included in this study.

	HP group (n = 19)	**HC group (n = 17)**	***P*-value**
Age [years]	38.9 ± 10.9	35.9 ± 12.3	0.449
Sex [M/F]	7/12	3/14	0.199
BMI [kg m^{-2}]	24.7 ± 3.7	22.2 ± 2.8	0.030*
Handedness [r/l]	15/4	15/2	0.455
NRS score (past week)	1.37 ± 0.83	1.35 ± 0.70	0.925
NRS score (current)	1.0 ± 0.0	1.0 ± 0.0	N.A.
CSI score	14.0 (11)	14.6 (11)	0.975

Numeric variables are expressed as 'average ± standard deviation' for normal or 'median (interquartile range)' for non-normal sample distributions. The asterisk (*) marks a statistically significant difference ($P < 0.05$), determined by a two-tailed independent samples *t*-test.

BMI = body mass index, CSI = central sensitization inventory, N.A. = not applicable, NRS = numeric rating scale.

DM [np] group vs. DM group (DM measurements)

All DM patients with longstanding neuropathic pain reported complaints in lower extremities, whereas 7 out of 13 revealed that they also suffered from (beginning) pain complaints in upper extremities. These patients had significantly higher BMIs, rated pain experiences on the NRS significantly higher, and scored significantly higher on the CSI than DM patients without pain complaints (Table 4.2). Moreover, neurological examinations showed that the former were significantly less capable of distinguishing sharp from blunt stimuli than the latter. Yet, the patient groups were not different for age, sex, handedness, duration since diagnose, DM disease type, and frequency of anti-diabetic medication use.

Table 4.2. Demographic and disease-related characteristics for diabetes mellitus (DM) patients with (DM [np] group) and without (DM group) chronic neuropathic pain.

	DM [np] group (n = 13)	**DM group (n = 20)**	***P*-value**
Age [years]	68.0 (27)	58.5 (31)	0.068
Sex [M/F]	11/2	9/11	0.023***
BMI [kg m^{-2}]	30.6 ± 4.9	26.8 ± 4.1	0.021*
Handedness [r/l]	10/3	16/4	0.833
NRS score (past week)	4.0 (3.0)	1.0 (0.0)	<0.001**
NRS score (current)	4.0 (3.0)	1.0 (0.0)	0.001**
CSI score	33.9 ± 14.4	17.6 ± 8.2	0.002*
NE (soft-cloth) [altered/unaltered]	0/13	0/20	N.A.
NE (pin-prick) [altered/unaltered]	7/6	4/16	0.044***
Duration since diagnosis DM [years]	15.0 (12)	14.5 (11)	0.579
DM disease type [1/2]	2/11	9/11	0.078
Use of anti-DM medication [yes/no]	13/0	18/2	0.239
Duration since diagnosis PDPN [years]	8.5 ± 5.6	N.A.	N.A.
Duration of neuropathic pain [years]	9.5 ± 4.5	N.A.	N.A.
Self-assessed somatosensory hypersensitivity [yes/no]	4/9	N.A.	N.A.
Use of analgesics (24 hours before measurements) [yes/no]	3/10	N.A.	N.A.

Numeric variables are expressed as ‘average ± standard deviation’ for normal or ‘median (interquartile range)’ for non-normal sample distributions. Neurological examination (NE) outcomes are only provided for one hand, as they were the same regardless of hand dominance. * = significantly different by two-tailed independent samples t-test, ** = significantly different by two-tailed Mann-Whitney U test, *** = significantly different by Pearson’s chi-squared test. Significance threshold: a = 0.05.

BMI = body mass index, CSI = central sensitization inventory, N.A. = not applicable, NRS = numeric rating scale, PDPN = painful diabetic peripheral neuropathy.

DM group vs. HC group and DM [np] group vs. HC group (DM measurements)

Pain-free DM patients were on average older (+17.4 ± 5.0 years, difference of averages ± standard error of the difference, $t(35) = -3.520$, $P = 0.001$) and of a higher BMI (+4.6 ± 1.2 kg m^{-2}, $t(35) = -3.890$, $P < 0.001$) than healthy controls, whereas sex ratio ($P = 0.077$), both NRS scores ($P = 0.497$ and $P = 0.619$) and CSI scores ($P = 0.209$) were not significantly different between these groups. However, DM patients with chronic PDPN were, besides older (difference of medians +33.0 years, $U = 8.00$, $P < 0.001$) and higher in BMIs (+8.4 ± 1.4 kg m^{-2}, $t(28) = -5.899$, $P < 0.000$), also different in sex ratio (11/2 for patients vs. 3/14 for healthy controls, male/female ratio, $X^2(1) = 13.274$, $P < 0.001$), past week's pain experience on the NRS (+3 points, $U = 12.500$, $P < 0.001$), current pain experience on the NRS (+3 points, $U = 34.000$, $P = 0.001$), and CSI score (+19.2 ± 4.3, $t(28) = -4.473$, $P < 0.001$), compared to healthy controls. Handedness did not significantly differ among the three study groups.

DM group vs. HP group and DM [np] group vs. HP group (comparison study parts)

From Table 4.3, it appears that pain-free DM patients were, on average, only older than healthy participants in the lidocaine experiment. On the other hand, DM patients with chronic PDPN were substantially different from the latter in age, gender distribution, BMI, NRS scores, and CSI score.

Table 4.3. General characteristics for healthy participants (HP) in the lidocaine experiment, diabetes mellitus (DM) patients with chronic neuropathic pain (np), and DM patients without pain complaints.

	DM [np] (n = 13)	***P*-value**	**HP (n = 19)**	***P*-value**	**DM (n = 20)**
Age [years]	68.0 (27)	< 0.001***	38.9 ± 10.9	0.003*	53.4 ± 16.9
Sex [M/F]	11/2	0.007**	7/12	0.605	9/11
BMI [kg m^{-2}]	30.6 ± 4.9	0.001*	24.7 ± 3.7	0.105	26.8 ± 4.1
Handedness [r/l]	10/3	0.892	15/4	0.935	16/4
NRS score (past week)	4.5 ± 1.7	< 0.001***	0.0 (0.0)	0.550	0.0 (0.0)
NRS score (current)	4.0 (3.0)	0.001***	0.0 ± 0.0	0.607	0.0 (0.0)
NE (soft-cloth) [altered/unaltered]	0/13	N.A.	0/19	N.A.	0/20
NE (pin-prick) [altered/unaltered]	7/6	0.341	7/19	0.243	4/16
CSI score	33.9 ± 14.4	< 0.001*	13.7 ± 7.9	0.149	17.6 ± 8.2

Numeric variables are described by 'average ± standard deviation' for normal and by 'median (interquartile range)' for non-normal sample distributions. Asterisks describe tests used to statistically confirm group differences (blue-shaded columns): * = two-tailed independent samples t-test, ** = Pearson's chi-squared test, *** = two-tailed Mann-Whitney U test. A significance criterion of $P < 0.05$ was maintained.

BMI = body mass index, CSI = central sensitization inventory, N.A. = not applicable, NE = neurological examination, NRS = numeric rating scale.

4.2. Part 1 - lidocaine experiment

4.2.1. Detection probabilities (and NDTs)

Average detection rates for all study groups in this part of the study are provided in Table 4.4.

Table 4.4. Average percentages of detected intraepidermal electrical stimuli for groups with healthy participants after lidocaine patch (HP [lido.]) and placebo patch treatment (HP [plac.]), and with healthy controls without a patch (HC).

Study group	**Detection rate (general) [%]**	**Detection rate (SP stimuli) [%]**	**Detection rate (DP10 stimuli) [%]**	**Detection rate (DP40 stimuli) [%]**
HP [lido.]	46.1	42.7	48.2	47.5
HP [plac.]	47.5	44.1	49.2	49.1
HC	47.6	45.7	48.7	48.5

DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

Effect of patch treatment on detection probability

Preliminary results indicated that lidocaine patch treatment did not modulate detection probabilities differently from placebo patch treatment (2.5.2. *Lidocaine experiment: preliminary considerations*). Likewise, the effects of study group (main and interaction) were not significant for two GLMMs with healthy control and either the lidocaine and placebo measurement data (Table 4.5). Since this suggests that both patch treatments lacked influence on detection probability compared to the absence of a patch, group average NDTs and slopes were not calculated. Furthermore, measurement round number did not influence detection probabilities.

Tracks of estimated NDTs for the three study groups are demonstrated in Figure 4.3. As these were obtained with a different GLMM than the models used for statistical interpretations (Table 4.5.), these are for illustrative purposes only.

Table 4.5. Coefficient estimates and fixed effects for two generalized linear mixed-effects models of detection probability. These were fitted to data from healthy participants (HP) after either lidocaine (lido.) or placebo (plac.) patch treatment, and healthy controls without a patch (reference level of study group).

Fixed model factor	**Coefficient estimate**		**Effect X^2 (df)**		***P*-value**	
	HP [lido.]	*HP [plac.]*	*HP [lido.]*	*HP [plac.]*	*HP [lido.]*	*HP [plac.]*
(Intercept)	-4.27	-4.40	88.70 (1)	79.63 (1)	< 0.001*	< 0.001*
Study group	-0.13	-0.01	0.02 (1)	0.00 (1)	0.875	0.987
Measurement round	0.15	0.62	0.20 (1)	3.56 (1)	0.658	0.059
Amplitude (SP) ∗ Study group	-3.69	-0.78	1.31 (1)	0.04 (1)	0.253	0.841
Study group ∗ Amplitude (DP10)	-0.89	-0.08	0.16 (1)	0.00 (1)	0.687	0.973
Study group ∗ Amplitude (DP40)	-1.44	0.17	0.48 (1)	0.01 (1)	0.488	0.938
Study group ∗ Trial number	0.02	0.14	0.01 (1)	0.73 (1)	0.937	0.394

Convolution symbols (∗) indicate interactions. Type III Wald chi-square tests were performed to assess the significance of fixed effects. Significance, indicated by asterisks (*), was attained for $P < 0.05$.

df = degrees of freedom, DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

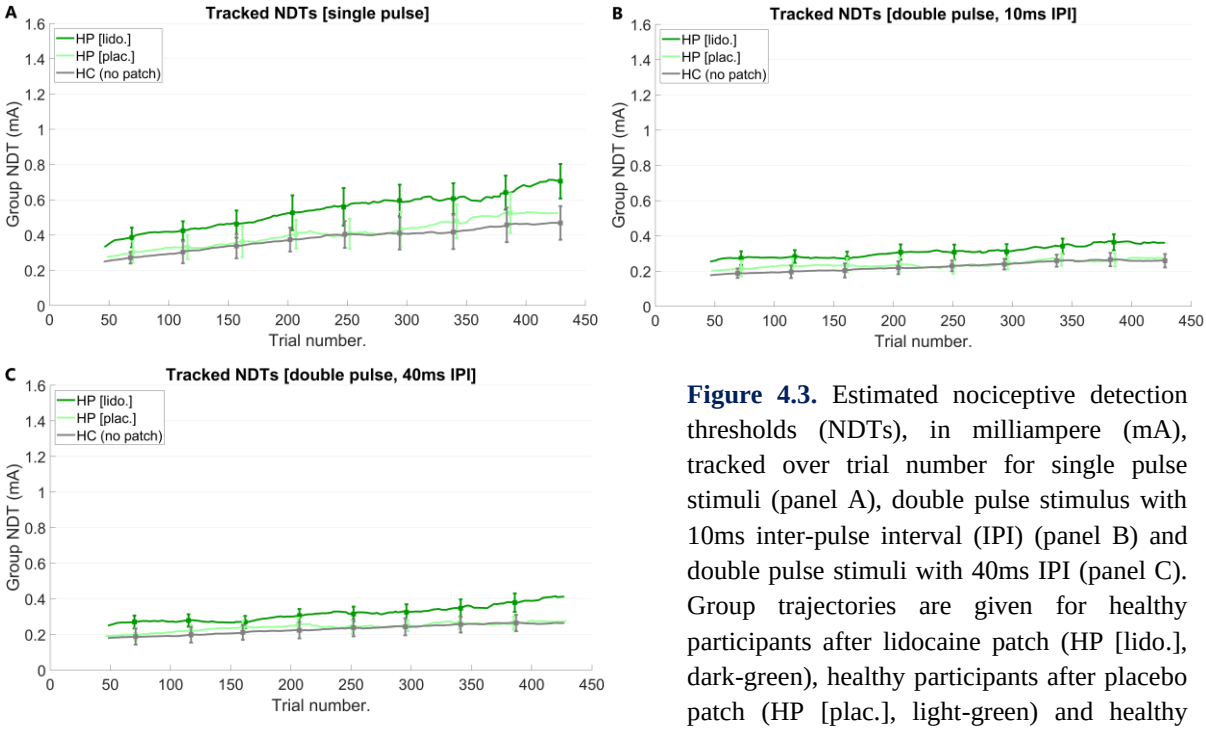


Figure 4.3. Estimated nociceptive detection thresholds (NDTs), in milliampere (mA), tracked over trial number for single pulse stimuli (panel A), double pulse stimulus with 10ms inter-pulse interval (IPI) (panel B) and double pulse stimuli with 40ms IPI (panel C). Group trajectories are given for healthy participants after lidocaine patch (HP [lido.], dark-green), healthy participants after placebo patch (HP [plac.], light-green) and healthy controls without a patch (HC, grey). Error bars represent standard error of the mean. Note that the NDT tracks were estimated by a different model than those used for statistical interpretations, and are therefore for illustrative purposes only.

4.2.2. Evoked potentials

EP latencies for statistical testing of model outcomes

Latencies for statistical investigations of LMM outcomes were substantially different between measurements after lidocaine or placebo patch and for data from healthy controls (*Appendix D: Butterfly plots, Fig. D1 and D2*). Since hardware-related limitations prevented the generation of a collective butterfly plot, definitive latencies were fixed at averages of the three groups: P473 for the central derivation, since P472 did not allow statistical testing of all effects, and P197 for the contralateral derivation.

Effect of patch treatment on EP amplitudes

Lidocaine, compared to placebo, treatment had a significant negative effect on the amplitudes of EPs in the central derivation following detected stimuli (*2.5.2. Lidocaine experiment: preliminary considerations*). In subsequent analysis, additionally involving controls without a patch, interactions between study group (level: healthy participants after lidocaine patch) and amplitudes of both DP stimulus types were significant (Table 4.6, Fig. 4.4). This implicates that topical lidocaine resulted in smaller EP amplitudes for these stimuli compared to the absence of a patch. Measurement round number did not modulate EP amplitudes.

Table 4.6. *T*-statistics for fixed effects of a linear mixed-effects model of evoked potential (EP) amplitude, evaluated at P473. EPs followed from intraepidermal electrical stimulation. The model was fitted to data from healthy participants (HP) obtained after topical lidocaine (lido.) and placebo (plac.) treatment, and from healthy controls without a patch (reference level of study group).

Fixed effect of:	***t*-value**	***P*-value**
Study group		
HP [plac.]	0.67	0.510
HP [lido.]	0.65	0.520
Measurement round	1.39	0.172
Study group ∗ Amplitude (SP)	-	> 0.05
Study group ∗ Participant's response	-	> 0.05
Study group ∗ Trial number	-	> 0.05
Study group ∗ Amplitude (DP10)		
HP [plac.]	-1.40	0.167
HP [lido.]	-4.05	< 0.001*
Study group ∗ Amplitude (DP40)		
HP [plac.]	-0.73	0.469
HP [lido.]	-3.40	< 0.001*
Study group ∗ Participant's response ∗ Trial number	-	> 0.05

Convolution symbols (∗) indicate interactions. The threshold for significance was set at $P < 0.05$. Asterisks (*) mark significant effects. If estimated effects at none of the levels of an interaction were significant, the *t*-values are omitted (-), and the non-significant *P*-values are grouped in '> 0.05'.

DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

For EP amplitudes in contralateral derivation, none of the estimated fixed effects under investigations achieved significance at P197 (data not shown).

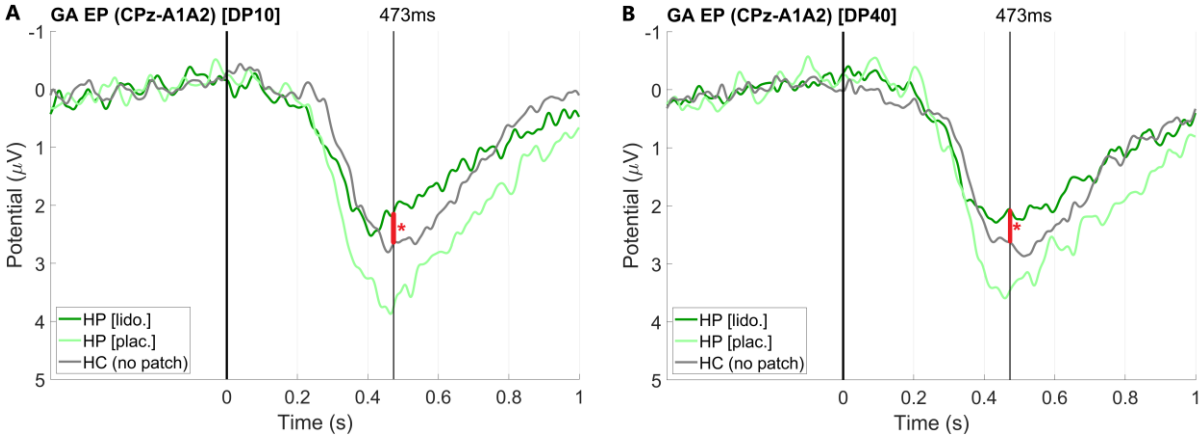


Figure 4.4. Grand average (GA) evoked potentials (EP) in EEG derivation CPz-A1A2, elicited by intraepidermal double pulse stimuli with 10ms inter-pulse interval (IPI) (DP10) in panel A and with 40ms IPI (DP40) in panel B. Signals, provided in microvolt (μV), are plotted for healthy participants after lidocaine patch (HP [lido.], dark-green), healthy participants after placebo patch (HP [plac.], light-green) and healthy controls without a patch (HC, grey) Significant modulations of EP amplitude (*t*-statistic, $P < 0.05$) at P473 by lidocaine measurements compared to healthy controls without patch are illustrated by red connection lines and asterisks (*).

4.3. Part 2 - DM measurements

4.3.1. Detection probabilities (and NDTs)

Average detection rates for all study groups in this part of the study are provided in Table 4.7.

Table 4.7. Average percentages of detected compared to the total number of intraepidermal electrical stimuli for groups with diabetes mellitus (DM) patients with chronic neuropathic pain [np], DM patients without pain complaints, and healthy controls (HC).

Study group	Detection rate (general) [%]	Detection rate (SP stimuli) [%]	Detection rate (DP10 stimuli) [%]	Detection rate (DP40 stimuli) [%]
DM [np]	35.9	28.6	40.6	38.6
DM	44.1	40.3	46.6	45.7
HC	47.6	45.7	48.7	48.5

DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

Effect of chronic PDPN in DM patients on detection probability

DM patients diagnosed with PDPN for more than 3 months demonstrated altered detection probabilities for stimuli of all types compared to pain-free counterparts (Table 4.8). Interestingly, a significant interaction between study group, i.e., a diagnosis of PDPN and trial number, was found. This suggests that DM patients with chronic neuropathic pain seemed to

express a different relation between detection probability and trial number compared to patients without pain. Measurement round was no significant determinant of detection probabilities.

Average psychophysical slopes were significantly steeper in pain-free DM patients compared to patients with chronic PDPN, whereas, surprisingly, average NDTs were not different between the two groups (Table. 4.9).

Table 4.8. Coefficient estimates and fixed effects for generalized linear mixed regression of detection probability with data from the two groups of DM patients: without (reference level of study group) and with chronic neuropathic pain.

Fixed model factor	**Coefficient estimate**	**Effect X^2 (df)**	***P*-value**
(Intercept)	-3.12	51.11 (1)	< 0.001*
Study group	1.69	9.42 (1)	0.002*
Measurement round	-0.13	0.36 (1)	0.547
Amplitude (SP) ∗ Study group	-5.05	13.73 (1)	< 0.001*
Study group ∗ Amplitude (DP10)	-3.54	7.75 (1)	0.005*
Study group ∗ Amplitude (DP40)	-3.28	10.13 (1)	0.001*
Study group ∗ Trial number	0.44	10.82 (1)	0.001*

Convolution symbols (∗) indicate interactions. Type III Wald chi-square tests were performed to assess the significance of fixed effects. Significance, indicated by asterisks (*), was attained for $P < 0.05$.

df = degrees of freedom, DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

Table 4.9. Group average nociceptive detection thresholds (NDT) and slopes of psychophysical functions, mapping stimulus intensity to detection probability. The three types of intraepidermal electrical stimuli used to elicit responses are displayed in the top row. Values were derived from a generalized linear mixed-effects model fitted to measurement data from diabetes mellitus (DM) patients with chronic neuropathic pain (np) and without pain complaints. The 95% confidence intervals are provided between parentheses.

	Single pulse		Double pulse, 10ms IPI		Double pulse, 40ms IPI	
	Avg. NDT (mA)	*Avg. slope (mA^{-1})*	*Avg. NDT (mA)*	*Avg. slope (mA^{-1})*	*Avg. NDT (mA)*	*Avg. slope (mA^{-1})*
DM [np]	1.77 (-0.32 – 3.86)	0.56 (-0.28 – 1.40)	0.40 (0.08 – 0.71)	2.48 (0.77 – 4.17)	0.53 (0.13 – 0.94)	1.85 (0.64 – 3.05)
DM	0.56 (0.39 – 0.72)	5.61 (3.07 – 8.15)	0.28 (0.19 – 0.38)	11.07 (6.73 – 15.41)	0.31 (0.21 – 0.41)	10.17 (6.20 – 14.15)
P-value	0.253	< 0.001*	0.475	< 0.001*	0.475	< 0.001*

* = significant group difference ($P < 0.05$), two-tailed independent samples *Z*-test.

IPI = inter-pulse interval, mA = milliampere.

Effect of DM (pain-free and chronic PDPN) on detection probability

A GLMM fitted to data from pain-free DM patients and healthy controls demonstrated that all interaction effects of study group and amplitudes of different stimulus types were significant (Table 4.10). That is, pain-free DM patients expressed detection probabilities different from healthy individuals. Another GLMM, fitted to chronic PDPN instead of pain-free DM data, showed increases of significance for these interactions. This suggested that it was even more certain that detection probabilities were considerably different between DM patients with chronic neuropathic pain and healthy controls. Notably, the interaction between study group and stimulus trial number became significant, implying that the relation between increasing trial number and detection probability was different in DM patients with chronic PDPN compared to healthy controls. The round number of the measurements did not affect detection probabilities for these three groups.

Corresponding to earlier findings, average slopes, but not average NDTs (except those for single pulse stimuli when comparing pain-free DM patients with healthy controls), were different between the groups in the two GLMMs (Table 4.11).

Table 4.10. Coefficient estimates and fixed effects for two generalized linear mixed-effects models of detection probability. The models were fitted to data from either diabetes mellitus (DM) patients without pain complaints or with chronic neuropathic pain (np), and data from healthy controls (reference level of study group).

Fixed model factor	Coefficient estimate		Effect X^2 (df)		*P*-value	
	DM	*DM [np]*	*DM*	*DM [np]*	*DM*	*DM [np]*
(Intercept)	-4.29	-4.31	119.19 (1)	106.72 (1)	< 0.001*	< 0.001*
Study group	1.04	2.73	3.07 (1)	27.70 (1)	0.079	< 0.001*
Measurement round	0.15	0.21	0.30 (1)	0.56 (1)	0.582	0.455
Amplitude (SP) ∗ Study group	-7.57	-12.5	9.57 (1)	29.30 (1)	0.002*	< 0.001*
Study group ∗ Amplitude (DP10)	-3.52	-6.83	3.91 (1)	23.55 (1)	0.047*	< 0.001*
Study group ∗ Amplitude (DP40)	-4.15	-7.27	6.60 (1)	31.75 (1)	0.010*	< 0.001*
Study group ∗ Trial number	0.19	0.68	2.78 (1)	25.88 (1)	0.096	< 0.001*

Convolution symbols (∗) indicate interactions. Type III Wald chi-square tests were performed to assess the significance of fixed effects. The threshold for significance, indicated by asterisks (*), was set at a < 0.05.

df = degrees of freedom, DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

Table 4.11. Group average nociceptive detection thresholds (NDT) and slopes of psychophysical functions, mapping stimulus intensity to detection probability. The three types of intraepidermal electrical stimuli used to elicit responses are displayed in the top row. Values were derived from two generalized linear mixed-effects models: model #1 fitted to data from diabetes mellitus (DM) patients with chronic neuropathic pain and healthy controls (HC), and model #2 fitted to data from pain-free DM patients and HC. The 95% confidence intervals are provided between parentheses.

	Single pulse		Double pulse, 10ms IPI		Double pulse, 40ms IPI	
	Avg. NDT (mA)	*Avg. slope (mA^{-1})*	*Avg. NDT (mA)*	*Avg. slope (mA^{-1})*	*Avg. NDT (mA)*	*Avg. slope (mA^{-1})*
model #1						
DM [np]	1.75 (-0.33 – 3.82)	0.52 (-0.24 – 1.27)	0.36 (0.09 – 0.63)	2.49 (0.92 - 4.05)	0.50 (0.14 – 0.85)	1.83 (0.70 – 2.96)
HC	0.33 (0.26 – 0.40)	13.05 (8.54 – 17.56)	0.20 (0.16 – 0.23)	21.85 (15.18 – 28.52)	0.20 (0.16 – 0.24)	21.64 (15.03 – 28.24)
P-value	0.175	< 0.001*	0.219	< 0.001*	0.219	< 0.001*
model #2						
DM	0.55 (0.37 – 0.73)	5.56 (2.94 – 8.18)	0.28 (0.17 – 0.40)	10.77 (6.00 – 15.55)	0.30 (0.19 – 0.43)	9.93 (5.54 – 14.32)
HC	0.33 (0.26 – 0.39)	13.13 (9.06 – 17.20)	0.20 (0.16 – 0.23)	21.87 (15.94 – 27.79)	0.20 (0.16 – 0.23)	21.64 (15.77 – 27.52)
P-value	0.012*	0.002*	0.116	0.004*	0.116	0.001*

* = significant group difference ($P < 0.05$) according to a two-tailed independent samples *Z*-test.

IPI = inter-pulse interval, mA = milliampere.

Tracks of estimated NDTs for the three study groups in this part of the study are demonstrated in Figure 4.5. As these were obtained with a different GLMM than the models used for statistical interpretations, these are for illustrative purposes only. It can be noticed that for all, but especially SP, stimuli, estimating NDTs for DM patients with chronic PDPN is particularly challenging, reflected by big standard errors.

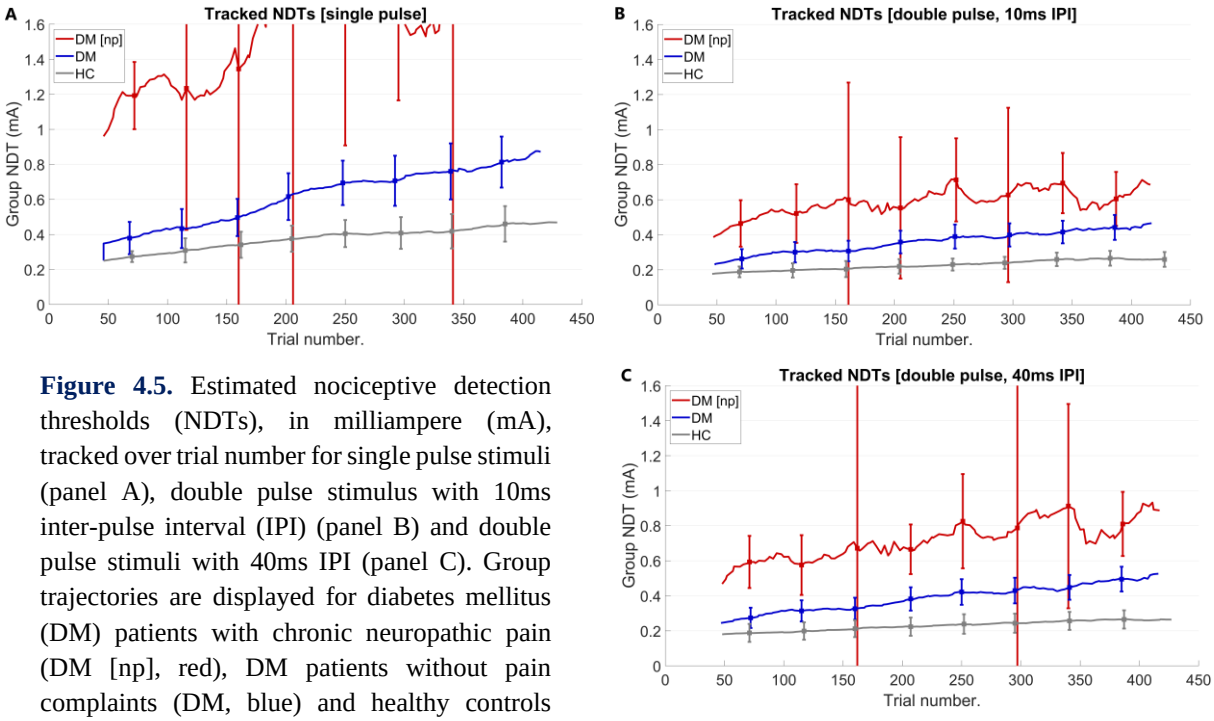


Figure 4.5. Estimated nociceptive detection thresholds (NDTs), in milliampere (mA), tracked over trial number for single pulse stimuli (panel A), double pulse stimulus with 10ms inter-pulse interval (IPI) (panel B) and double pulse stimuli with 40ms IPI (panel C). Group trajectories are displayed for diabetes mellitus (DM) patients with chronic neuropathic pain (DM [np], red), DM patients without pain complaints (DM, blue) and healthy controls (HC, grey). Error bars represent standard error of the mean. Note that NDT tracks were estimated by a different model than those used for statistical interpretations, and are therefore for illustrative purposes only.

4.3.2. Evoked potentials

EP latencies for statistical testing of model outcomes

Latencies for investigations of EP components peaking in the central and contralateral derivations were determined for two LMMs. One was fitted to data from only the DM patients groups, and another was fitted to data from both DM patient groups and healthy controls. For the former model, the butterfly plot indicated latencies of 480ms for the central derivation and 195ms for the contralateral derivation (*Appendix D: Butterfly plots*, Fig. D4). However, because some effects could not be statistically evaluated at P480, this value was replaced by the first adjacent value that allowed statistical testing; P477. For the latter model, due to hardware-related difficulties similar to those in the lidocaine experiment, latencies were eventually based on averages of the three groups (*Appendix D: Butterfly plots*, *Fig. D1 and D3*). This resulted in P480 for the central derivation and P192 (instead of P193; same reasoning as first regression) for the contralateral derivation.

Effect of chronic PDPN in DM patients on EP amplitudes

Statistical analysis of the LMM created with patient EEG data only (derivation CPz-A1A2) produced negative *t*-statistics for interactions between study group and amplitudes of different

stimulus types (Table 4.12). These suggest that DM patients with chronic neuropathic pain generally showed smaller EP amplitudes compared to pain-free patients (Fig. 4.6).

Table 4.12. *T*-statistics for fixed effects of a linear mixed-effects model of evoked potential (EP) amplitude, evaluated at P477. EPs followed from intraepidermal electrical stimulation. The model was fitted to data from diabetes mellitus (DM) patients with longstanding painful diabetic neuropathy and DM patients without pain complaints (reference level of study group).

Fixed effect of:	*t*-value	*P*-value
Study group	-0.30	0.764
Measurement round	-0.04	0.970
Study group ∗ Amplitude (SP)	-2.70	0.011*
Study group ∗ Participant's response	0.18	0.855
Study group ∗ Trial number	0.19	0.851
Study group ∗ Amplitude (DP10)	-2.05	0.046*
Study group ∗ Amplitude (DP40)	-2.98	0.010*
Study group ∗ Participant's response ∗ Trial number	1.00	0.330

Convolution symbols (∗) indicate interactions. The threshold for significance was set at $P < 0.05$. Asterisks (*) mark significant effects.

DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

Neither study group-related effects, nor measurement round, significantly influenced EP amplitudes at P195 in derivation T7-F4 (data now shown).

Effect of DM (chronic PDPN and pain-free) on EP amplitudes

The LMM based on EEG data (derivation CPz-A1A2) from all DM patients and healthy controls demonstrated significant interactions between study group and DP amplitudes (Table 4.13). These suggest that DM patients, both with chronic PDPN and without pain complaints, exhibited smaller EP amplitudes than healthy controls when stimulated with DP stimuli (Fig. 4.6). In analogy with the detection probabilities, trial number had a significantly different effect on EP amplitudes in DM patients with chronic PDPN compare to healthy controls according to a significant interaction. Plots of trial-separated grand average EPs for these groups may be suggestive for this finding (Fig. 4.7).

Again, (interactions with) study group or measurement round did not significantly predict maximum EP amplitudes in the contralateral derivation (data now shown).

Table 4.13. *T*-statistics for fixed effects of a linear mixed-effects model of evoked potential (EP) amplitude, evaluated at P480. EPs followed from intraepidermal electrical stimulation. The model was built with data from two groups of diabetes mellitus (DM) patients, with chronic neuropathic pain (np) and without pain, and from healthy controls (reference level of study group).

Fixed effect of:	***t*-value**	***P*-value**
Study group		
DM [np]	-0.68	0.501
DM	-1.83	0.073
Measurement round	1.49	0.135
Study group ∗ Amplitude (SP)		
DM [np]	-2.14	0.040*
DM	-0.30	0.765
Study group ∗ Participant's response	-	> 0.05
Study group ∗ Trial number		
DM [np]	2.390	0.020*
DM	1.760	0.083
Study group ∗ Amplitude (DP10)		
DM [np]	-4.24	< 0.001*
DM	-3.12	0.002*
Study group ∗ Amplitude (DP40)		
DM [np]	-4.99	< 0.001*
DM	-3.10	0.002*
Study group ∗ Participant's response ∗ Trial number	-	> 0.05

Convolution symbols (∗) indicate interactions. The threshold for significance was set at $P < 0.05$. Asterisks (*) mark significant effects. If estimated effects at none of the levels of an interaction were significant, the *t*-values are omitted (-), and the non-significant *P*-values are grouped in '> 0.05'.

DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

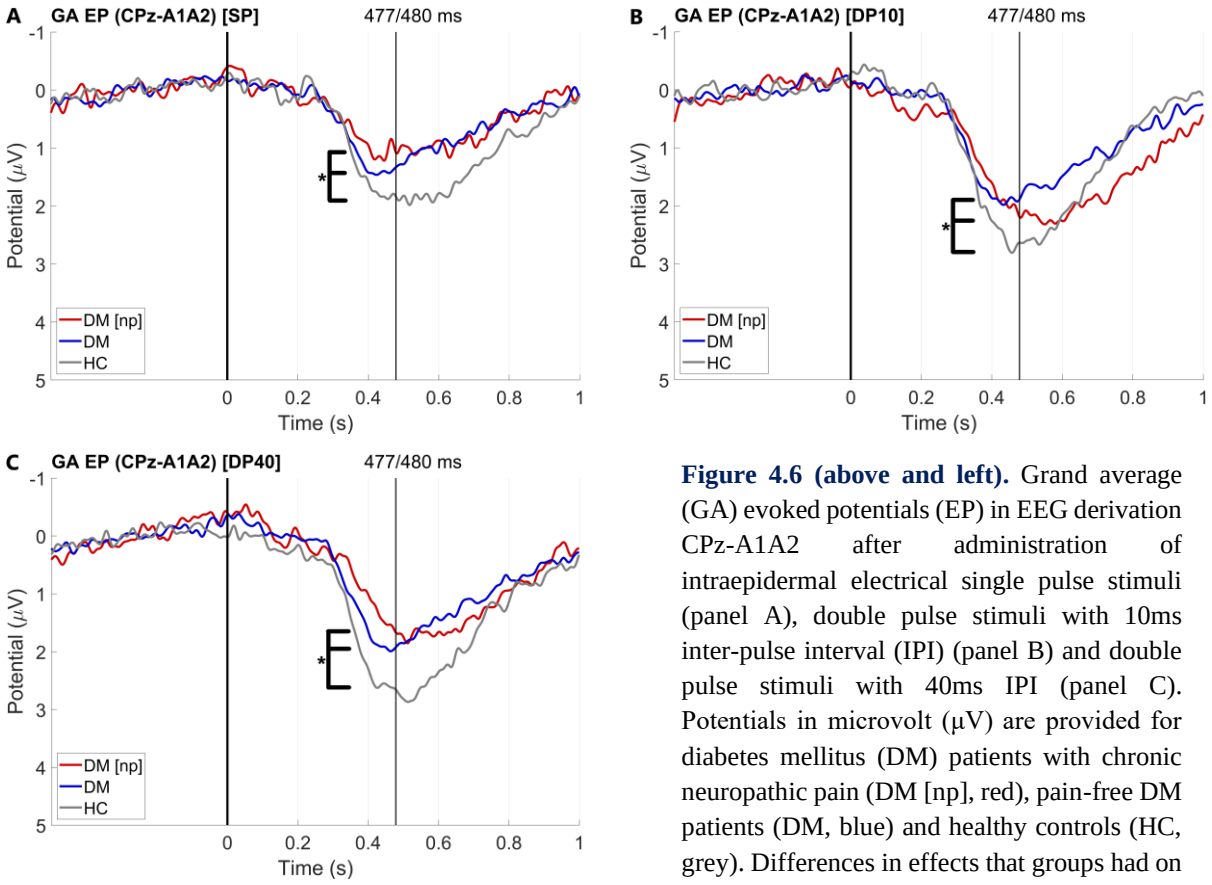
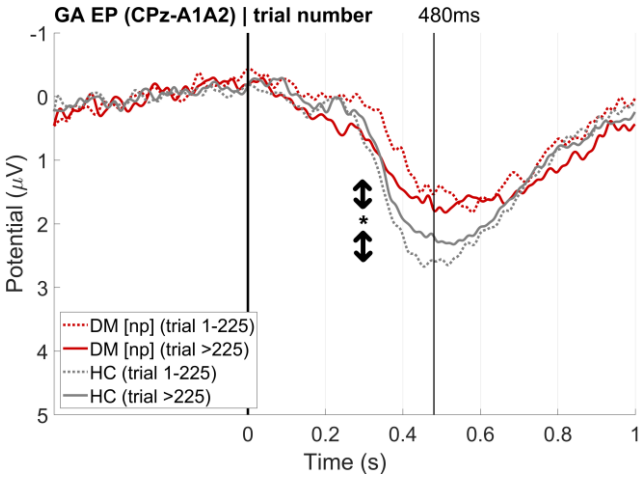


Figure 4.6 (above and left). Grand average (GA) evoked potentials (EP) in EEG derivation CPz-A1A2 after administration of intraepidermal electrical single pulse stimuli (panel A), double pulse stimuli with 10ms inter-pulse interval (IPI) (panel B) and double pulse stimuli with 40ms IPI (panel C). Potentials in microvolt (μV) are provided for diabetes mellitus (DM) patients with chronic neuropathic pain (DM [np], red), pain-free DM patients (DM, blue) and healthy controls (HC, grey). Differences in effects that groups had on EP amplitude, statistically confirmed by *t*-statistics ($P < 0.05$) at P477 or P480 (dependent on groups in respective regression analyses), are illustrated by asterisks (*). However, note that EP amplitudes were not modulated significantly different by DM patients without pain compared to healthy controls for single pulse stimuli (panel A).

Figure 4.7 (right). Grand average (GA) evoked potentials (EP) in EEG derivation CPz-A1A2, sorted by trial number, for diabetes mellitus (DM) patients with chronic neuropathic pain (np) in red and healthy controls (HC) in grey. The narrow, black line depicts the EP latency at which linear mixed-effects model outcomes for EP amplitude (in microvolt (μV)) were statistically evaluated. Black arrows and the asterisk (*) reflect the significant interaction between study group and trial number (*t*-statistic, $P < 0.05$).



4.4. Comparison of the lidocaine SFN model with DM patients

4.4.1. Detection probabilities (and NDTs)

Comparison effects of topical lidocaine and diagnosed DM on detection probability

Data collections, containing lidocaine measurements combined with measurements data from DM patients either with chronic PDPN (model 1) or without pain (model 2), were used to fit two GLMMs. Unfortunately, the statistical computing environment (R) failed to fit model 1, presumably due to overfitting. Contrastingly, model 2 was fitted successfully. The main effect of study group just reached significance (Table 4.14), which implied that pain-free DM patients showed detection probabilities (just) different from healthy controls.

Tracks of estimated NDTs for the groups in this regression analysis are demonstrated in Figure 4.8. As these were obtained with a different GLMM than the model used for statistical interpretations, these are for illustrative purposes only.

Table 4.14. Coefficient estimates and fixed effects for a generalized linear mixed-effects model of detection probability fitted to data from healthy participants after transdermal lidocaine treatment (reference level of study group) and DM patients without pain.

Fixed model factor	Coefficient estimate	Effect X^2 (df)	*P*-value
(Intercept)	-4.65	53.88	< 0.001*
Study group	1.53	3.86	0.049*
Measurement round	-0.18	0.51	0.474
Amplitude (SP) ∗ Study group	-4.51	2.71	0.100
Study group ∗ Amplitude (DP10)	-2.37	1.06	0.302
Study group ∗ Amplitude (DP40)	-2.72	1.67	0.196
Study group ∗ Trial number	0.20	0.93	0.336

Convolution symbols (∗) indicate interactions. Type III Wald chi-square tests were performed to assess the significance of fixed effects. Significance, indicated by asterisks (*), was attained for $P < 0.05$.

df = degrees of freedom, DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

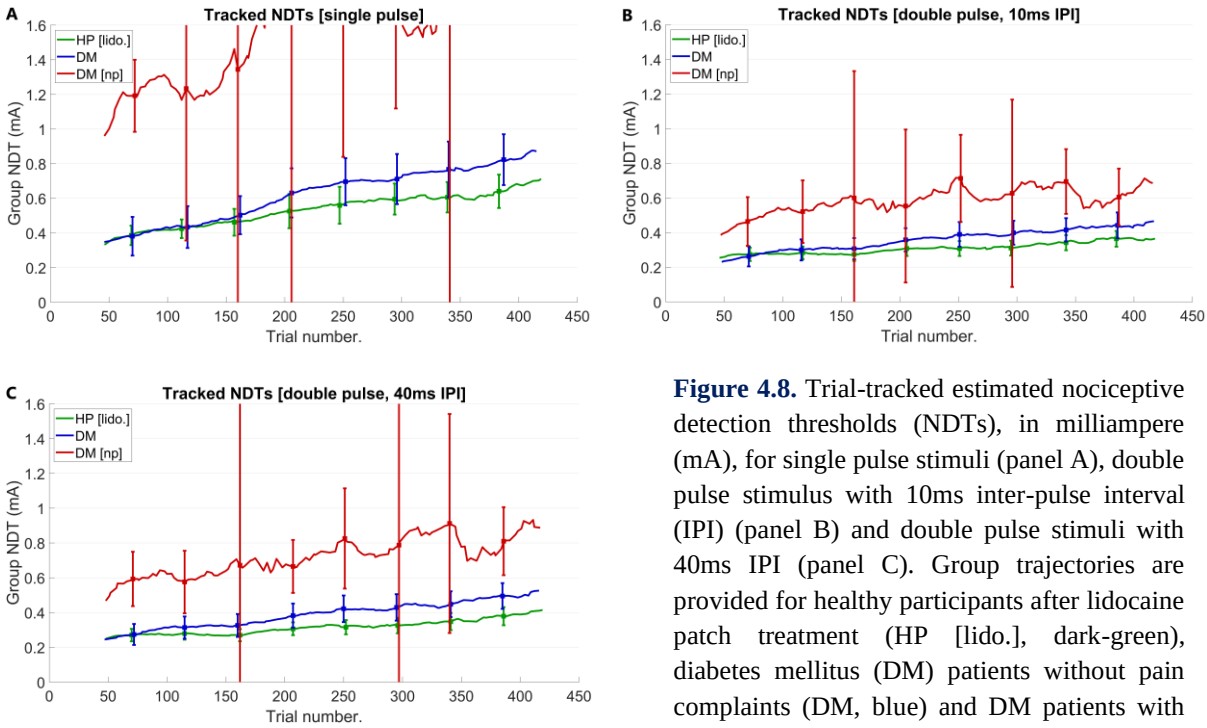


Figure 4.8. Trial-tracked estimated nociceptive detection thresholds (NDTs), in milliampere (mA), for single pulse stimuli (panel A), double pulse stimulus with 10ms inter-pulse interval (IPI) (panel B) and double pulse stimuli with 40ms IPI (panel C). Group trajectories are provided for healthy participants after lidocaine patch treatment (HP [lido.], dark-green), diabetes mellitus (DM) patients without pain complaints (DM, blue) and DM patients with chronic neuropathic pain (DM [np], red). Error bars represent standard error of the mean. Note that NDT tracks in these plots were estimated by a different model than those used for statistical interpretations, and are therefore for illustrative purposes only.

4.4.2. Evoked potentials

EP latencies for statistical testing of model outcomes

Latencies for statistical investigation at EP components peaking in derivations CPz-A1A2 and T7-F4 were obtained by averaging latencies determined for the three separate groups (*Appendix D: Butterfly plots*, Fig. D2 and D3). This prompted the use of P456 and P208 for the central and contralateral derivation, respectively. Yet, similar to previous events, some effects were not statistically analyzable at P208. Consequently, this point on the EPs was replaced by P207.

Effects of topical lidocaine and diagnosed DM on EP amplitudes

Neither the main, nor the interaction effects of study group on EP amplitudes in derivation CPz-A1A2 were significant (Table 4.15). This indicated that maximum EP amplitudes after topical lidocaine treatment and in DM patients, with chronic PDPN or without pain, do not seem to differ, as suggested by Fig. 4.9.

Following statistical testing of LMM outcomes at P207, the interaction between DM patients without pain and trial number reached significance (t = -2.15, P = 0.033).

Table 4.15. *T*-statistics for fixed effects of a linear mixed-effects model of evoked potential (EP) amplitude, evaluated at P456. EPs followed from intraepidermal electrical stimulation. The model was generated for data

from healthy participants after topical lidocaine treatment (reference level of study group), diabetes mellitus (DM) patients with chronic neuropathic pain, and patients without pain complaints.

Fixed effect of:	***t*-value**	***P*-value**
Study group		
DM [np]	-0.68	0.500
DM	-0.85	0.400
Measurement round	0.04	0.967
Study group ∗ Amplitude (SP)	-	> 0.05
Study group ∗ Participant's response	-	> 0.05
Study group ∗ Trial number	-	> 0.05
Study group ∗ Amplitude (DP10)	-	> 0.05
Study group ∗ Amplitude (DP40)	-	> 0.05
Study group ∗ Participant's response ∗ Trial number	-	> 0.05

Convolution symbols (∗) indicate interactions. The threshold for significance was set at $P < 0.05$. Asterisks (*) mark significant effects. If estimated effects at none of the levels of an interaction were significant, the *t*-values are omitted (-), and the non-significant *P*-values are grouped in '> 0.05'.

DP10 = double pulse with 10ms inter-pulse interval, DP40 = double pulse with 40ms inter-pulse interval, SP = single pulse.

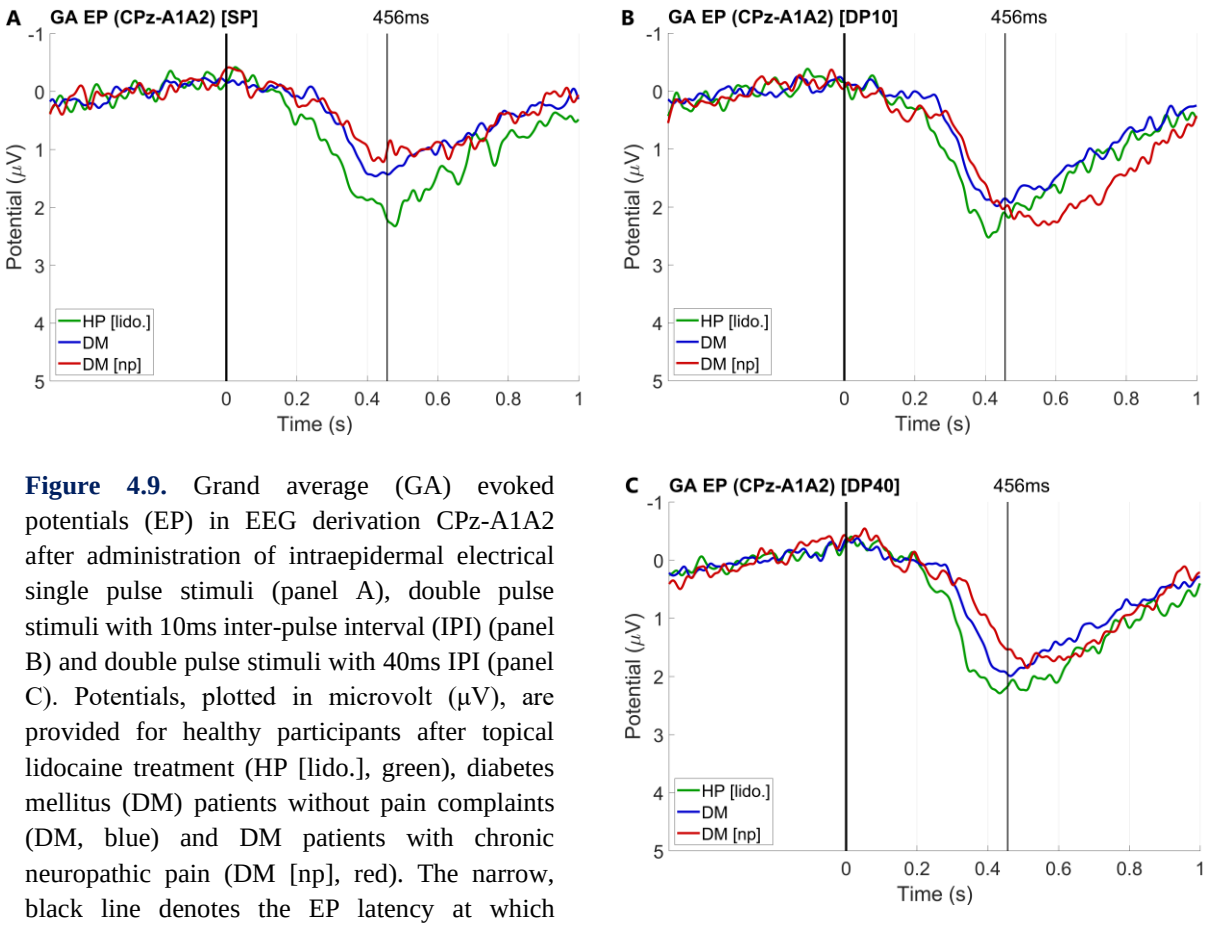


Figure 4.9. Grand average (GA) evoked potentials (EP) in EEG derivation CPz-A1A2 after administration of intraepidermal electrical single pulse stimuli (panel A), double pulse stimuli with 10ms inter-pulse interval (IPI) (panel B) and double pulse stimuli with 40ms IPI (panel C). Potentials, plotted in microvolt (μV), are provided for healthy participants after topical lidocaine treatment (HP [lido.], green), diabetes mellitus (DM) patients without pain complaints (DM, blue) and DM patients with chronic neuropathic pain (DM [np], red). The narrow, black line denotes the EP latency at which estimated effects of study group(-related interactions) on EP amplitude were statistically evaluated.

5. Discussion

This thesis addressed the question of which outcomes the NDT-EP measurement method generates for stimulation of dysfunctional small fibers in a clinical context. In that sense, an explorative, prospective study consisting of two parts was conducted. The first part demonstrated that topical lidocaine treatment, simulating small fiber dysfunction, does not seem to statistically affect detection probabilities, whereas it does lower maximum EP amplitudes (EEG derivation CPz-A1A2). The second part revealed different detection probabilities and EP amplitudes (CPz-A1A2) for DM patients with chronic PDPN compared to pain-free counterparts, and for DM patients in general compared to healthy controls. Furthermore, stimulus detection probabilities were different between pain-free DM patients and healthy participants with a lidocaine SFN model. Yet, amplitudes of EPs in both EEG derivations did not differ between the latter and both groups of DM patients. These findings suggest (1) general applicability of the method in DM patients and (2) that, in partial accordance with the prior hypothesis, decreases in their EP amplitudes, but not necessarily in detection probabilities, approach measurement outcomes for experimentally induced small fiber dysfunction.

5.1. Part 1 - lidocaine experiment

Both preliminary and present analyses indicated that experimental condition is not a significant determinant of detection probabilities (2.5.2. *Lidocaine experiment: preliminary considerations*, Table 4.5). In line with this, a recent study of somatosensory performances elucidated that detection chances were unaltered following lidocaine patch application (Costa et al., 2017). However, the researchers applied the patch 45 minutes instead of 120 minutes, treated the face instead of hand dorsum, and used pinprick stimulators and pressure algometers instead of IES. Hoberg and co-investigators (2019), in contrast, showed that detection thresholds for intracutaneous electrical stimulation could mirror modification of small fiber membrane properties after 2-hour lidocaine mixture treatment. Yet, essential methodological discrepancies include application and measurements on the volar forearm, and a considerably different ‘adaptive probing’ method.

Opposite to the detection probabilities, topical lidocaine treatment did significantly mitigate the EPs at maximum amplitude (Table 4.6). Such findings concur with the discovery that prolonged topical lidocaine treatment could eliminate EPs in almost all (after 3 hours) or even all (after 5 hours) participants in comparable set-up (Otsuru et al., 2010). In a clinical sense, this complete disappearance of EPs, presumably following complete small fiber dysfunction, corresponds to difficulties to elicit cortical responses from lower extremities in DM patients with (advanced) neuropathic symptoms (Mueller et al., 2010; Siedler et al., 2020).

5.1.1. Interpretation of the results

Besides possible explanations provided in the preliminary discussion, two essential methodological dissimilarities between this and comparable work could have contributed to the detection probability results. In this study, the participant's response was the correlate of nociceptive stimulus detection fed to GLMMs as an independent variable. Conversely, in comparable studies (Otsuru et al., 2010; Krumova et al., 2012; Kodaira et al., 2014), the correlates of small fiber sensitivity were stimulus intensities at which participants indicated appearance and loss of pinprick or noxious thermal sensations. Second, participants' responses obtained for a vast series of trials were used to estimate detection probabilities in the present study. In contrast, researchers from previously mentioned studies averaged sensory thresholds across relatively few repetitions. These disagreements, besides other procedural differences such as patch treatment time and time intervals between treatments, may have contributed to unexpected results from this study compared to similar work.

The results of this study render the influence of placebo effects unlikely. Regarding the detection probabilities, this is supported by a lack of significant differences after the placebo compared to the absence of patch application. Likewise, regarding EPs at maximum amplitudes, similar support for the absence of placebo effects follows from the LMM fitted to EEG data from the lidocaine experiment (Table 4.6). Considering the effect of topical lidocaine on EPs, significance shifted from interaction with participant's responses in preliminary analyses to interactions with amplitudes of DP stimulus types. It may be reasoned that supplying more data to the respective LMM resulted in a better fit. Speaking for this could be already marginally significant (P = 0.064 and P = 0.051) effects of interactions between lidocaine patch and DP stimulus amplitudes (10ms and 40ms IPI, respectively) in preliminary analyses.

5.1.2. Strengths and limitations

In addition to previously described strengths (2.5.2. *Lidocaine experiment: preliminary considerations*), the abundance of supplied stimuli presumably facilitated identification and minimization of random influences on (G)LMR outcomes. Another strength worth mentioning is the placebo-controlled design, permitting the detection of possible placebo effects. Besides, by also conducting a standard neurological examination, it was possible to observe how the method under investigation compared to established diagnostic instruments.

Besides previously reported limitations, the possibility for participants to guess sides of patch types was another disadvantage. Factors such as cutaneous flushing, the lidocaine patch perceived as colder, and neurological examination outcomes could have been indicative of patch type. Furthermore, the approach for three rounds of neurological examination (one before both measurement rounds, and two after each respective round) may have constituted another limitation. Whereas the first neurological examination was done on both hands, the second and

third examinations were carried out on one hand at the time, resulting in the lack of an extra reference site. Lastly, this study did not control for possible epidermal disparities, such as skin thickness or regional blood flow, even though these have been suggested to considerably influence neurophysiological outcomes (Kanai et al., 2010).

5.1.3. Recommendations for further research

The main suggestion following this part was the exploration of the NDT-EP method's applicability and outcomes in a human model of dysfunctional small fibers. This was dealt with in the second part of the study. In a second step, it may be worthwhile to investigate the results of modified stimulation settings, e.g., C-fiber targeting, and an adapted definition of nociceptive stimulus detection. Such exploration would further clarify the dependence of the method's measurement outcomes on the mechanistic approach.

5.2. Part 2 - DM measurements

The majority of NDT-EP measurements in DM patients produced analyzable outcomes, which, at first glance, supports practicality in this patient population. Moreover, detection probabilities were different for chronic PDPN in DM (Table 4.8), corresponding to significantly altered pin-prick sensitivities for patients with this complication, and for DM in general (Table 4.10). Such outcomes broadly concur with higher electrical pain detection thresholds in DM patients without neuropathic signs than in healthy controls (Telli and Cavlak, 2006; Suzuki et al., 2016; Sasaki et al., 2019), and in neuropathic compared to non-neuropathic DM patients (Kukidome et al., 2016; Suzuki et al., 2016). However, methodological discrepancies, such as other modes of stimulation and measurements restricted to lower extremities, should be taken into account.

Surprisingly, post hoc analyses (mainly) did not confirm alterations in detection probabilities by group differences in average NDTs, whereas average slopes were consistently different between groups (Table 4.9, Table 4.11). Comparable statistical investigations into these specific outcome variables, involving both healthy individuals and (pain) patients, have not been conducted thus far. This limits speculations about the possible origins of this remarkable outcome. Whether slopes may be more suitable than NDTs for indicating directions of altered detection probabilities may be a topic of further research.

Analogous to detection probabilities, chronic PDPN in DM patients and a diagnosis of DM in the general study population resulted in significantly lower EP amplitudes (Table 4.12 and Table 4.13). These results are in line with observed smaller EP amplitudes in patients with neuropathic manifestations compared to healthy individuals (Chao et al., 2010; Mueller et al., 2010; Hansen et al., 2015; Omori et al., 2017). Moreover, they agree with the notion that the degree of EP amplitude reduction reflects the extent of neuropathic deficits in DM (Ziegler et al., 1993; Mueller et al., 2010; Omori et al., 2017). Concerning EPs in non-neuropathic DM patients, various investigations discovered differences compared to healthy controls, but

primarily for the stimulation of lower extremities (Pozzessere et al., 2002; Ragé et al., 2011; Suzuki et al., 2016). Up until now, EPs elicited from upper extremities of asymptomatic DM patients remain under-investigated. Hence, this study may provide novel insights regarding the suitability of the NDT-EP method for describing small nerve fiber-mediated anomalies in these regions.

5.2.1. Interpretation of the results

Demographic characteristics are essential modulators of pain sensitivity (Kemp et al., 2014; El-Tumi et al., 2018). Thus, reasoning whether dissimilarities in group characteristics could have (partially) driven the results may further argue for or against the method's applicability in this context. In a literature review, Lautenbacher and colleagues (2017) concluded that detection thresholds for electrical stimuli were not subject to age in most cases. Recently, this was reinforced by a study that demonstrated that age does not influence detection thresholds for IES (Suzuki et al., 2020), rendering significant confounding by age-related neurophysiological differences in the present study unlikely. On the contrary, aging is generally associated with decreased amplitudes of somatosensory EPs (Desmedt and Cheron, 1980). As this has also been discovered for cutaneous electrical stimulation (Kemp et al., 2014), age-related central nervous system decline may have impacted EP recordings, which should be accounted for during interpretation.

Electrical hypoalgesia has been proposed as a nociceptive trait of obese individuals (Maffiuletti et al., 2011; Dodet et al., 2013). To the best of my knowledge, no investigations probed IES detection thresholds for stratified BMIs yet. Nonetheless, thermal pain thresholds were not altered in obese compared to non-obese individuals after stimulating regions without excessive adiposity (Price et al., 2013), which may render considerable influence of BMI on nociceptive sensitivity in this study unlikely. Similarly, although under-investigated for somatosensory EPs, related types of evoked electrocortical responses have not been associated with the subjects' weights (Solanki et al., 2012, 2013; Ghugare et al., 2016).

Sex has been recognized as a vital determinant of pain sensitivity to electrical stimuli, although outcomes tend to differ (Walker and Carmody, 1998; Maffiuletti et al., 2011; Rocha et al., 2011; Seno et al., 2019). In analogy with BMI, studies that have investigated the relationship between IES detection thresholds and sex were not found. Nevertheless, a recent study by Averbeck et al. (2017) revealed that females seem more thermosensitive on the hands than males, which suggests that small fiber-related thresholds are sex-dependent. Conversely, electrical stimulation of the median nerve in an investigation by Zumsteg and Wieser (2002) did not yield sex-specific EP parameters. However, as the researchers used superficial disk electrodes and evaluated EPs at smaller latencies, comparisons should be cautious, and the influence of sex on the measurement outcomes cannot be ruled out completely.

In addition to potential demographic influences, LMR outcomes pointed to distinct trial-dependency of EPs in DM patients with chronic PDPN compared to healthy controls (Table

4.13, Fig. 4.7), which was also discovered for detection probabilities (Table 4.10). Such observations agree with preceding work, which also found that EP amplitudes in FBSS patients were probably not coded by the number of administered stimuli (Berfelo, 2019). Pathological impairment of short-term central and peripheral modulatory mechanisms might be a common underlying cause of these observations in chronic pain patients. Subsequent investigations in other patients might further elucidate possible similarities.

5.2.2. Strengths and limitations

The main strength of the DM measurements was the inclusion of pain-free DM patients, in addition to patients with chronic PDPN and healthy controls. This enabled exploration of possibly (subclinically) altered small fiber function in patients with the identical underlying disorder as the chronic pain patients, but without overt neuropathic (pain) manifestations.

The design of this study may have led to two limitations. First, DM patients with all severities of neuropathic pain were allowed to participate. This may have caused a bias towards patients suffering from less severe forms, as patients in much pain could have been less inclined towards participation. Second, due to the explorative scope of the study, a diagnose for SFN was not incorporated in the inclusion criteria. As a result, it was not entirely clear in which portion of the patients and which bodily extremities functional small fiber abnormalities were to be expected. Hence, especially for detection probabilities, considerable uncertainty remains regarding to what extent group effects reflected dysfunctional small fibers or other factors.

Because of the measurement round lengths, relatively older participants generally experienced more difficulties maintaining a sufficient concentration level. A substantial portion of somatosensory sensitivity is attributed to attentional factors (Arntz et al., 1991). Therefore, part of the abundantly missed stimuli by older patients could have been due to worse focus. This may have lowered their detection probabilities beyond the influence of pathological factors, increasing the chances of erroneously finding significant group effects. Another limitation could have been that the IES approach preferentially targeted aδ-fibers. Yet, the other type of small nociceptive nerves, C-fibers, has been suggested to be the first affected in the earliest (subclinical) stages of DPN (Kukidome et al., 2016). Accordingly, attempts to discriminate between study groups may have been hampered by this discrepancy.

An additional limitation may have been that, statistically, a substantially larger part of EPs in DM patients with chronic neuropathic pain compared to other participants resulted from undetected stimuli. In LMMs, this may have promoted differences between these groups of participants regarding the modulation of EP amplitudes. In further investigations, regarding the regression models, the participant's response could be added to the interactions between study group and stimulus amplitudes to account for possible influences of stimulus detection on EP amplitudes.

Lastly, drug intake may have impacted nociceptive processing in DM patients with chronic PDPN. As there were no restrictions on medication use, 3 out of 13 participants in this group reported intake of either pregabalin (anticonvulsant), duloxetine (antidepressant), or amitriptyline (antidepressant) less than 24 hours before measurements. Since these drugs have been ascribed analgesic properties (McQuay et al., 1993; Perahia et al., 2006; Taylor, 2009), potential influence on outcomes for these patients, i.e., lower detection probability and smaller EP amplitude, cannot be excluded.

5.2.3. Recommendations for further research

To verify initial impressions regarding the method's feasibility in DM patients, a first suggestion would be to control for potential confounding by demographic characteristics (e.g., via matched-control design or covariate analyses). Moreover, it may be interesting to use pain complaints as a discriminant between groups of neuropathic DM patients, as recent research postulated that pain experience correlates with IES thresholds (Sasaki et al., 2019). This would ideally be accompanied by C-fiber stimulation to observe whether this would yield a better indication of early-phase asymptomatic small fiber dysfunction. Furthermore, it could be interesting to incorporate a diagnosis, or strong clinical indications, of SFN as an inclusion criterion and perform stimulation in lower extremities. Such modification places more emphasis on the peripheral component of abnormal nociception in DM patients, potentially resulting in measurement outcomes mirroring hyper- rather than hypoalgesia (Campbell and Meyer, 2006). Lastly, future studies should preferably also consider DM patients with acute instead of chronic neuropathic pain and employ follow-up measurements. By excluding the possible influence of (central) neuroplastic changes due to chronic pain, it may be clarified how recently developed pain complaints are reflected by the method's outcomes. Besides, follow-up measurements could elucidate whether the temporal progression of these outcomes is associated with clinical deterioration or improvement.

5.3. General limitations

A general limitation may have been the subjectivity of neurological examinations. Firstly, the varying amounts of applied pressure and alternating degrees of sharpness/bluntness between separate pin-prick tests decreased intra-observer reliability. Besides, somatosensory sensitivity was not quantified in neurological examinations, but qualitatively described (normal/altered) based on an arbitrarily selected cut-off value of three misguesses.

Comparisons between DM patients and healthy participants with a lidocaine SFN model were, in analogy with the DM measurements, limited by dissimilarities in group characteristics (Table 4.3). Such influences may have been complemented by rather transient factors, as measurements constitute highly momentary evaluations. Sleep quality of the night before, menstruation cycle, and emotional status are examples of possible determinants of pain

sensitivity (Onen et al., 2001; de Brito Barbosa et al., 2013; Peters, 2015), which this study did not control for. Additionally, statistical testing of LMM outcomes for EP amplitudes may have been restricted by the latency determination approach (3.4.2. Evoked potentials). As group sizes were inconsistent, combinations of channel-averaged EPs from multiple groups in one butterfly plot probably led to skewed data ratios, resulting in latencies most representative of the largest group.

Efforts to fit GLMMs of detection probability to stimulus-detection participant data were not always successful. On the one hand, an attempt to fit a model to data after lidocaine treatment and DM patients with chronic PDPN led to a computational error. This error presumably resulted from model overfitting (Hawkins, 2004), caused by the relatively small target data set compared to the parametric complexity of the GLMM. Consequently, it was not possible to statistically investigate whether detection probabilities were significantly different between these groups, even though visual representation suggested profound discrepancies (Fig. 4.8). On the other hand, hardware-related constraints necessitated the construction of two distinct GLMMs for the regression analysis of data from three study groups at the same time. As a consequence, the separate comparisons of two groups to another group may not have resulted in differences as precisely approached as when one model was fitted to data from all three groups.

5.4. General recommendations for further research

In subsequent investigations, neurological examinations may be standardized. This could be achieved by utilizing calibrated pin-prick stimulators instead of cotton swabs, which would lower both intra- and inter-observer variability and provide quantitative correlates of pin-prick sensitivity. To further expand quantitative reference measures of small nerve fiber functioning, an extra QST procedure such as thermal detection or pain threshold determination could be introduced (Rolke et al., 2006). Additionally, shorter measurement round lengths might be experimented with. Now lasting approximately 30 minutes, the duration imposed serious strains on some participants' concentration, which possibly restricts the method's suitability in these individuals. Shortened measurement rounds could decrease these strains, but experiments should first demonstrate whether these would still yield sufficient amounts of data for the (G)LMMs. A possible compromise could be the withdrawal of DP with 40ms IPI from the set of stimulus types, since outcomes suggest that detection probabilities and EP amplitudes are not differently affected by the two different IPIs in this study. Alternatively, brief interruptions of the measurement rounds could be experimented with.

Further, EP components peaking in the contralateral EEG derivation in this study (T7-F4) were generally not modulated by study group. This corresponded to prior work showing that such 'P2' peaks were only predicted by stimulus detection (Berfelo, 2019), possibly explainable by

their mere dependence on attention and unconscious stimulus matching to memory-stored information (Miltner et al., 1989; Evans and Federmeier, 2007). Thus, as these contralateral components do not seem to reveal additional group-related information, further studies may choose to only consider a central derivation, corresponding to recent work (van den Berg et al., 2020).

Ultimately, the NDT-EP measurement method may be evaluated in patients suffering from SFN in other diseases. These could include individuals with small fiber-related neuropathic pain due to sarcoidosis (Hoitsma et al., 2002), Fabry disease (Dütsch et al., 2002) or hereditary gene mutations (Axelrod and Hilz, 2003). Such an extension would grant insight into how (dynamical) psychophysical and neurophysiological behavior in response to IES compares between SFN conditions of different disease origins. Moreover, it could further elucidate the method's potential regarding the delineation of altered nociceptive processing resulting from varying neuropathic pain conditions.

6. Conclusion

This thesis demonstrates that measurements performed according to the NDT-EP method are generally properly executed by and thus seem feasible in DM patients with different neuropathic manifestations. They provide evidence that patient outcomes share characteristic similarities with an experimental small fiber neuropathy model by EP amplitude reductions compared to healthy controls, being proportional to a diagnosis of PDPN. On the contrary, altered stimulus detection probabilities expressed by these patients may less directly reflect small fiber dysfunction. It is believed that they rather describe other differences between groups, such as in attentional levels, central dysregulation due to persistent nociceptive input or demographic features. As a whole, these results suggest that (part of) this method could be of value in future searches for quantitative diagnostic markers of small fiber dysfunction. To further explore initial findings and applicability in similar (clinical) context, subsequent investigations that outline influences of potential (demographic) confounders, consider alternative measurement strategies and mechanistic approaches, and in a last step, include patient groups with different (SFN-related) chronic pain syndromes, are recommended.

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Appendix A: Recruitment poster (Dutch)

Deelnemers gezocht voor studie naar gevoelsklachten bij suikerziekte

In het St. Antonius Ziekenhuis onderzoeken we een nieuwe manier om de verwerking van (pijn)prikkels te meten. Zo hopen we gevoelsklachten bij suikerziekte, zoals gevoelsverlies en chronische pijn, beter te kunnen voorspellen en in kaart te brengen. We zoeken voor dit onderzoek deelnemers voor de volgende drie groepen:

Groep 1: gezonde personen tussen de 18 en 65 jaar, zonder pijnklachten.
Groep 2: diabetespatiënten tussen de 18 en 75 jaar, zonder zenuwpijn.
Groep 3: diabetespatiënten tussen de 18 en 75 jaar, met diabetische polyneuropathie (zenuwaandoening door suikerziekte) en langdurige pijnklachten.

Wat houdt het onderzoek in?
Als deelnemer in groep 1 komt u voor een afspraak van ca. 4,5 uur naar het St. Antonius Ziekenhuis (Nieuwegein). De eerste 2 uur hiervan zijn wachttijd, waarin u een pleister met lidocaïne (een licht, lokaal verdovingsmiddel) opkrijgt. Tijdens de wachttijd mag u uw eigen werkzaamheden uitvoeren. Als deelnemer in groep 2 of 3 komt u voor een afspraak van ca. 3 uur naar het St. Antonius Ziekenhuis (Nieuwegein).

Vervolgens krijgt u op uw hand milde elektrische prikkels toegediend. Deze zult u licht voelen. Tijdens het toedienen van deze prikkels meten we tegelijk uw gevoelsdrempels en de signalen in uw hersenen. Na uw afspraak kunt u gewoon naar huis.

Vergoeding
Voor deelname ontvangt u een Bol.com-cadeaubon van €50,- en eventuele reiskostenvergoeding.

Valt u binnen een van deze groepen? En bent u geïnteresseerd of wilt u meedoen?
Bel dan naar 088 320 8809 (werkdagen, 09.00-17.00 uur) of stuur een e-mail naar s.gefferie@antoniusziekenhuis.nl voor meer informatie of om u aan te melden.

ST ANTONIUS
een santeon ziekenhuis

UNIVERSITY OF TWENTE.

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Appendix B: Informed consent form (example), in Dutch

KENMERK
TV

VERSIE
V4.0

ABR NUMMER
NL66136.100.18

ONDERWERP
Toestemmingsverklaring

TITEL ONDERZOEK
Elektrische Hersensignalen Tijdens de Verwerking van Nociceptieve Stimuli Rond de Detectiedrempel: een Exploratieve Studie in Pijn Patiënten

Ik bevestig dat ik de informatiebrief voor de proefpersoon, kenmerk “PIF-DM-np” versie ‘V4.0’, heb gelezen. Ik bevestig dat ik de informatie heb begrepen. Ik heb voldoende tijd gehad om over mijn deelname na te denken en ben in de gelegenheid geweest om vragen te stellen. Deze vragen zijn naar tevredenheid beantwoord.

Ik geef toestemming om mijn behandelend arts eenmalig te benaderen voor vastgestelde datum/data van diagnose/diagnosen en huidig gebruik van medicatie.

Ik geef toestemming voor deelname aan het bovengenoemd medisch-wetenschappelijk onderzoek.

Ik weet dat mijn deelname geheel vrijwillig is en dat ik mijn toestemming op ieder moment kan intrekken zonder dat ik daarvoor een reden hoef op te geven.

Ik weet dat voor de controle van het onderzoek sommige mensen toegang tot al mijn gegevens kunnen krijgen. Die mensen staan vermeld in deze informatiebrief. Ik geef toestemming voor die inzage door deze personen.
Ik geef toestemming om de gegevens te verwerken voor de doeleinden zoals beschreven in de informatiebrief met kenmerk “PIF-DM-np” versie ‘V4.0’.

Ik geef toestemming om mijn onderzoeksgegevens 15 jaar na afloop van dit onderzoek te bewaren.

Ik wil meedoen aan dit onderzoek.

Naam proefpersoon:
Handtekening: Datum:

Ik verklaar hierbij dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

Naam onderzoeker:
Handtekening: Datum:

Appendix C: NDT-EP measurement experience questions

Participants were asked the following questions following an NDT-EP measurement round to evaluate their experiences (in Dutch).

Both parts of the study:

1. Hoe vond u het zelf gaan?
2. Hoe zou u het gevoel omschrijven dat hoorde bij een gedetecteerde prikkel?
3. Hoe was uw concentratie over het beloop van de meting?
4. Merkte u een verschil in prikkels?
5. Waren er afleidende factoren en zo ja, welke?

Only after the second measurement round:

6. Hoe vond u het detecteren en het gevoel van de prikkels ten opzichte van de eerste meetronde?

Only in the lidocaine experiment:

Only after the second measurement round:

1. Heeft u een vermoeden op welke hand de lidocaïne- en op welke hand de placebopleister was geplakt? Zo ja, op welk moment tijdens deze meetafspraak begon dat vermoeden?

Appendix D: Butterfly plots

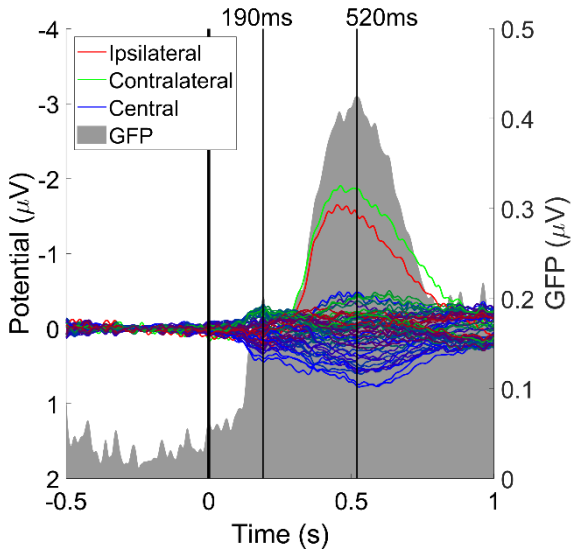

Figure D1. Butterfly plot of epoched potentials, averaged across channel type and displayed in microvolt (μV), after intraepidermal electrical stimulation of healthy participants, without a patch, in an earlier study (Berfelo, 2019). Narrow, vertical black lines denote latencies of evoked potential maxima, determined at global field power (GFP) peaks, in the central derivation CPz-A1A2 (520ms) and contralateral derivation T7-F4 (190ms) used in this study.

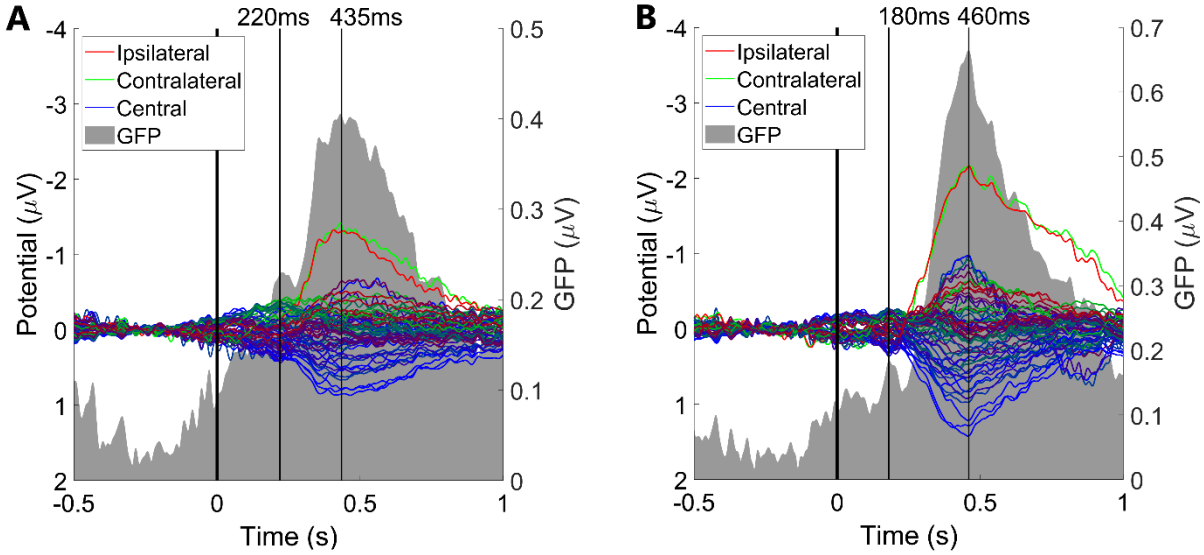

Figure D2. Butterfly plot of epoched potentials, averaged across channel type and displayed in microvolt (μV), after intraepidermal electrical stimulation of healthy participants after transdermal lidocaine (panel A) and placebo (panel B) treatment. Narrow, vertical black lines denote latencies of evoked potential maxima, determined at global field power (GFP) peaks, in the central derivation CPz-A1A2 (435ms and 460ms) and contralateral derivation T7-F4 (220ms and 180ms) used in this study.

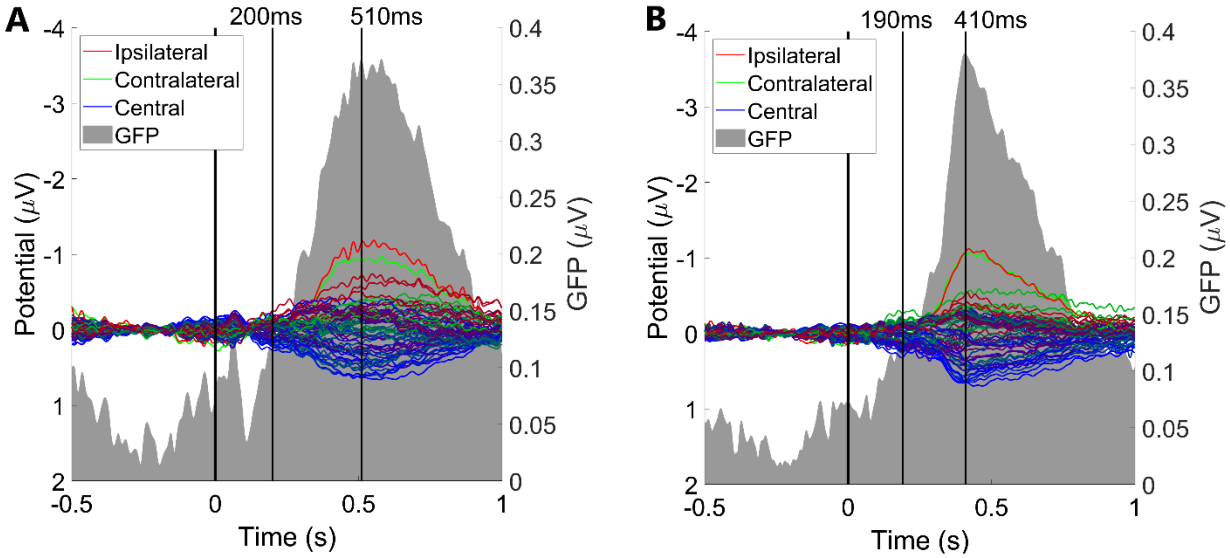

Figure D3. Butterfly plot of epoched potentials, averaged across channel type and displayed in microvolt (μV), after intraepidermal electrical stimulation of diabetes mellitus patients with chronic neuropathic pain (panel A) and without pain complaints (panel B). Narrow, vertical black lines denote latencies of evoked potential maxima, determined at global field power (GFP) peaks, in the central derivation CPz-A1A2 (510 and 410ms) and contralateral derivation T7-F4 (200ms and 190ms) used in this study.

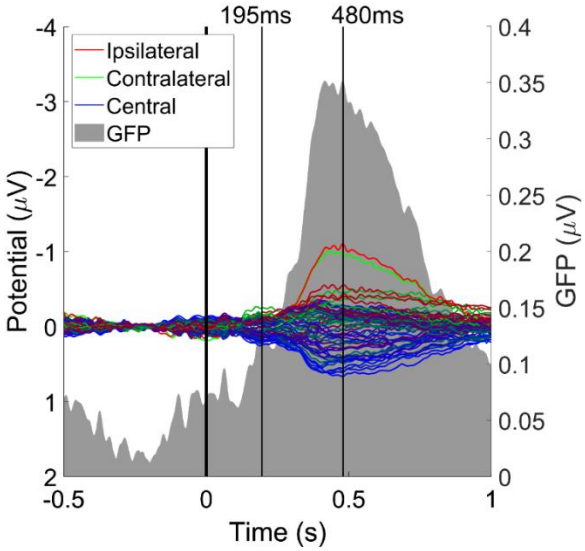

Figure D4. Butterfly plot of epoched potentials, averaged across channel type and displayed in microvolt (μV), after intraepidermal electrical stimulation of participants in both DM patient groups. Narrow, vertical black lines denote latencies of evoked potential maxima, determined at global field power (GFP) peaks, in the central derivation CPz-A1A2 (480ms) and contralateral derivation T7-F4 (195ms) used in this study.