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Characterization of chemotherapy and sarcoidosis induced peripheral neuropathy through combined nociceptive detection thresholds and brain responses

An explorative study in mamma carcinoma, sarcoidosis patients and healthy, pain-free individuals

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ST ANTONIUS

Characterization of chemotherapy and sarcoidosis induced peripheral neuropathy through combined nociceptive detection thresholds and brain responses

Explorative investigations with the novel NDT-EP measurement method in mamma carcinoma, sarcoidosis patients and healthy, pain-free individuals

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Voorwoord

Na een jaar hard werken in het St. Antonius Ziekenhuis mag ik dan eindelijk dit proefschrift presenteren. Het afgelopen jaar heb ik de mogelijkheid gekregen om mee te helpen aan het exploreren van een potentiële meetmethode voor het verkrijgen van nieuwe inzichten in het nociceptieve systeem. Dit was het sluitstuk van de opleiding Technische Geneeskunde (Universiteit Twente). Per september 2020 ben ik begonnen met deze afstudeerstage bij de afdeling Anesthesiologie, Intensive Care en Pijngeneeskunde. Ik heb ontzettend veel geleerd dit jaar. Ik heb twee pilotstudies opgezet waarin ik een protocol uit moest breiden, ethische goedkeuring moest verkrijgen, werving en metingen van deelnemers uitvoerde, analyserichtingen bepaalde om ten slotte de resultaten en conclusies te presenteren. Naast dat ik veel heb geleerd over chronische pijn heb ik ook veel geleerd over sarcoïdose en Chemotherapie-geïnduceerde perifere neuropathie patiënten en hoe zij hun klachten ervaren. Deze verantwoordelijkheden hebben mij afgelopen jaar geholpen om vaardigheden verder te ontwikkelen die passen bij een technische geneeskundige die ik wil ik zijn. Dit had niet gekund zonder de mensen die mij hierbij hebben gesteund.

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Abstract

Introduction. Small fiber neuropathies (SFN) are a diverse group of disorders affecting thinly myelinated Aδ-fibres and unmyelinated C-Fibres. The cause of SFN up to 50% of the cases remains unknown. An intriguing candidate for this purpose is a novel measurement approach ('NDT-EP'), which allows for the evaluation of tracked reactions and evoked potentials (EP) in response to intraepidermal electrical stimuli. The preliminary results of the MTT-EP technique in diabetic patients with SFN are encouraging. As a result, the feasibility and efficacy of this technique in CIPN and sarcoidosis patients were studied.

Methods. The participants were split into four groups. The first group was made up of two groups of persons who were both healthy and pain-free. The second case involved CIPN patients. The third group consisted of sarcoidosis patients with SFN symptoms. The EPs and stimulus detection probabilities were obtained by stimulating the dorsa of the hands. Data from prior research of healthy persons was utilized as a control. (Generalized) linear mixed regression was used to compare measurement outcomes between study groups.

Results. The study included 18 healthy patients matched to the CIPN group (average age: 56.3 ± 11.1 years, 18 females), 18 CIPN patients (average age: 58 ± 10.1, 18 females), 19 sarcoidosis patients (average age: 50.2 ± 12.5, 12 females), and sarcoidosis patients (average age: 49.6 ± 10.9, 12 females). There were no significant differences in detection probability between CIPN patients and healthy control data. Sarcoidosis patients had a significant decreased detection probability ($P < 0.05$) when compared to healthy control data. EPs produced equivalent results, with smaller amplitudes for CIPN ($P < 0.05$ for Double pulses with a 10ms inter-pulse interval) and sarcoidosis ($P < 0.05$ for Double pulses).

Conclusions. According to the study, NDT-EP assessments are generally accurate and so appear to be feasible in CIPN and sarcoidosis patients with a variety of neuropathic symptoms. They reveal that patient outcomes differ from healthy controls in terms of latency and amplitude anomalies, as well as modified nociceptive detection thresholds. On the other hand, there is currently limited evidence that altered detection probabilities reflect the same condition. Overall, our findings suggest that (parts of) this technique may be useful in the future search for quantitative small fiber diagnostic markers. More study is needed to investigate demographic characteristics, experiment with other measurement settings, and test the approach in other diseases defined by SFN and chronic pain syndromes.

Keywords: *chronic pain, small fiber neuropathy, detection threshold, evoked potential, nociception, linear mixed regression, chemotherapy-induced peripheral neuropathy, sarcoidosis, psychophysics*

Contents

List of abbreviations

CNS	central nervous system
CS	central sensitization
CSI	central sensitization inventory
CSS	central sensitization syndrome
CIPN	chemotherapy-induced peripheral neuropathy
GFP	global field power
DRG	dorsal root ganglia
DP10	double pulse with an inter-pulse interval of 10ms
DP40	double pulse with an inter-pulse interval of 40ms
EEG	electroencephalography
ER	endoplasmic reticulum
EP	evoked potentials
GLMM	generally linear mixed models
HRQL	health-related quality of life
HC	healthy controls
ICA	independent component analysis
IPI	inter-pulse interval
ILD	interstitial lung diseases
IES	intra-epidermal electrical stimulation
LMM	linear mixed-effects models
MTT	multiple threshold tracking
NCS	nerve conduction studies
NDT	nociceptive detection threshold
NRS	numerical rating scale
QSART	quantitative sudomotor axon reflex testing
SFN	small fiber neuropathies
SFNSL	small Fiber neuropathy screening List
SP	single pulse
DN4	the douleur neuropathique
LMR	linear mixed regression

1. Introduction

Small fiber neuropathies (SFN) are a diverse group of disorders affecting thinly myelinated Aδ-fibres and unmyelinated C-fibres. SFN appears in a variety of different diseases and often results in symptoms of burning pain, shooting pain, allodynia, and hyperesthesia[1]. Due to lack of understanding among physicians, the diagnosis of SFN is probably highly underreported. In the Netherlands, a minimum estimated incidence of 12 per 100 000 population and a prevalence of 53 per 100 000 population have been reported[2]. Despite extensive diagnostic evaluation, the cause of SFN up to 50% of the cases remains unknown[3]. Assessment of SFN is challenging, and it remains challenging to diagnose and quantify across disorders. Many conventional tools to diagnose and quantify peripheral neuropathy do not provide insight into small fiber function[4,5].

Chemotherapy-induced peripheral neuropathy (CIPN) is one of the most common side effects of chemotherapy[6]. In CIPN, small nerve fibres transporting temperature and pain information (unmyelinated C-fibres and thinly myelinated Aδ-fibres) undergo early, selective degeneration (SFN)[7,8]. As such, peripheral nerve degeneration or SFN is generally accepted as underpinning the development of CIPN[9,10,11,12]. Given the challenges of measuring small fiber nerve damage, the prevalence and pathophysiology of SFN amongst cancer survivors remain poorly understood. As a result, the prevalence of autonomic dysfunction and neuropathic pain for patients treated with neurotoxic chemotherapy remains poorly defined. Quantification of CIPN remains a challenge, with many of the current assessment tools often showing a lack of concurrence with patient report, lacking sensitivity and the subjective evaluation by patients or physicians[13].

Another disease underlying SFN includes sarcoidosis[14,15]. SFN is prevalent in a large proportion of patients with sarcoidosis, but the exact number remains unknown. Some studies suggest it might be as high as approximately 75%[16–18] and may be the underlying cause of the poor health-related quality of life (HRQL)[14,15,19]. Sarcoidosis is a multisystem inflammatory disorder of unknown aetiology which results in granuloma formation into various organs. Fatigue and pain are frequent complaints in sarcoidosis but seem to be an underestimated problem in clinical practice[17]. Neither the type nor intensity of pain in SFN have been studied systematically in this condition. Although some groups have investigated possible causes of pain, the exact (nociceptive) system mechanism remains unclear[15]. However, it might have involvement of both central and peripheral dysfunctions[20,21]. Nevertheless, few objective measures are available for the assessment of small nerve fibers. The most commonly

available diagnostic tools for SFN are nerve conduction studies (NCS) to exclude large fiber neuropathy, skin biopsy, and quantitative sudomotor axon reflex testing (QSART)[18].

Recently, a method was developed for tracking the nociceptive detection threshold (NDT). This measure of pain sensitivity can be acquired using intra-epidermal electrical stimulation (IES), which predominantly activates small-diameter nociceptive Aδ-fibers[22]. One of the tracking methods that employs IES is Multiple Threshold Tracking (MTT)[23,24]. MTT estimates NDTs by iterative administration of electrical stimuli with different properties. This approach has several purposes considering the influence of the stimulus parameters on NDTs and the registration of transient nociceptive behavior. However, a major limitation of this approach is that it is unknown whether characteristics of the detection probability are due to physiological or psychological factors.

An objective measure for more specific insight into nociceptive processing and related activity in the central nervous system is electroencephalography (EEG). It was recently suggested that evoked potentials (EPs) could be used to observe altered central processing[25]. Furthermore, it is indicated that the effects of stimulus properties on the EPs can be quantified using linear mixed regression (LMR)[26]. A recent study showed altered behavior of NDTs and EPs in patients with failed back surgery and chronic pain[27]. As such, a combination of EP measurements with EEG and NDT measurements using an IES-MTT approach might create a hybrid (NDT-EP) method for observing nociceptive processing.

Preliminary results of the MTT-EP method in diabetic patients with SFN are promising and might contribute to detect possible changes between SFN and healthy controls in the future. This suggests that the MTT-EP method is applicable for the evaluation of SFN in CIPN and sarcoidosis. Exploration of (underlying) mechanisms is important, and CIPN and sarcoidosis patients may benefit from timely recognition of SFN. A better understanding of the underlying mechanisms could be of clinical relevance[19]. The present report aims to explore the feasibility of the NDT-EP measurement method and its outcomes in CIPN and sarcoidosis patients. Peripheral dysfunctions are hypothesized to be observed, and possible central nociceptive alterations might be monitored using EEG.

2. Background

2.1 Pain

Burning pain, shooting pain, allodynia, and hyperesthesia are common symptoms of small-fiber neuropathy, occurring in various disorders[1]. When noxious stimuli are present, the primary pain mechanism performs three events: transduction, transmission, and modulation. For example, along the nociceptive pathway, transduction occurs in the following order. First, stimulus events are converted to chemical tissue events. Second, chemical tissue and synaptic cleft events are then converted to electrical events in neurons; and third, electrical events in neurons are transduced as chemical events at synapses. The transmission mechanism would come after the completion of transduction and occurs when electrical events are sent along neural pathways, and neurotransmitters in the synaptic cleft transport information from one cell's postsynaptic terminal to another's pre-synaptic terminal. In the meantime, modulation happens by up-or down-regulation at all nociceptive routes via the primary afferent neuron, dorsal horn, and higher brain center. Finally, all of these events culminate in establishing and completing the pain pathway, allowing us to experience the painful feeling elicited by the stimulus[28].

2.1.1 Nociceptive system

A nociceptor is a type of nerve ending that detects potentially harmful stimuli to the body. These stimuli frequently elicit pain, and external nociceptive stimulation can activate a nociceptor. The two main types of afferent nerve fibres that transmit nociceptive information are fast Aδ-fibres and slow C-fibres. Electrical currents up to 1.5mA can be used to stimulate A-fibres. The dorsal horn of the spinal cord is the synaptic terminal of peripheral afferent nerve fibres[29]. Dorsal root ganglia are in charge of ascending sensory transmission to the CNS, which results in pain modulation and localization. Synapses are formed in this area by primary and secondary sensory neurons[30]. The spinothalamic tract allows secondary neurons in the spinal cord to cross the midline and send impulses to the thalamus[29]. The primary and secondary somatosensory cortex processes all incoming nociceptive signals. Furthermore, the cortex insularis and cortex cingularis anterior are involved in negative emotions that can arise during pain experience. Moreover, the prefrontal cortex is linked to long-term pain perception[30].

After nociceptive information has reached the higher centres, descending pathways play a role in pain modulation[28]. The dorsal horn is one of the locations where the descending pathway can modulate the incoming signal[31]. A signal can be modified during its transmission through the body by using pain modulation[32]. As a result, it is not solely determined by the amount of excitatory and inhibitory input[28].

This mechanism may explain why people do not always respond equally to pain stimuli[32]. Since it is not exactly clear how the signal is adjusted, the nociceptive system can be approached as a a black-box input-output system with the nociceptive stimulus as (controlled) input and the pain perception (measuring) as output.

2.1.2 Quantification of pain

SFN accompanies generalized sensory polyneuropathy and cannot be detected with conventional nerve conduction studies. Nevertheless, few objective measures are available for the assessment of small nerve fibers. The most commonly available diagnostic tools for SFN are nerve conduction studies (NCS) to exclude large fiber neuropathy, skin biopsy, and quantitative sudomotor axon reflex testing (QSART)[18]. The lack of ability to determine modulatory and modificatory effects of CIPM and sarcoidosis on the nervous system may be a factor for the development of chronic pain. It is important to study these underlying mechanisms (central and peripheral) and how they are altered in chronic pain patients compared to healthy subjects. One major obstacle is the lack of an objective measure of central and peripheral sensitivity.

Conventional methods for pain monitoring are mainly based on subjective pain reports (Numeric Rating scale (NRS)), palliative intakes or surveys (DN4, NPQ or Central Sensitization Inventory; CSI). These methods do not identify neuroplasticity and can, therefore, not be directly related to a clinical diagnosis. The early detection of malfunction mechanisms in the nociceptive system could reduce these inadequate treatments. Consequently, it is essential to perceive properties from central nociceptive processing by assessing neurophysiological responses to cutaneous nociceptive stimuli. The deficiencies listed above raise the clinical question if there are more objective and competent methods for (timely) recognition of SFN and characterization of chronic painful features.

Patients are asked to circle the number that best reflects their pain level on a Numerical Rating Scale (NRS), ranging from 0 to 10, 0 to 20, or 0 to 100[33]. The lower limit is frequently associated with "no pain at all," while the upper limit is associated with "the greatest conceivable pain ever." In numerous research, numerical rating scales have shown a significant association with other pain evaluation measures[34,35]. Furthermore, the feasibility of use and good compliance have been demonstrated[36,37].

The Central Sensitization Inventory (CSI) is a questionnaire that assesses the physical and mental symptoms that are typical in central sensitization syndrome (CSS). Part A assesses 25 symptoms and provides five responses (0 to 4). An overall score is a number between 0 and 100. Part B asks if the

patient has ever been diagnosed with any of the ten CSS diagnoses[38]. The CSI is designed to alert physicians to the likelihood of central sensitization as a starting point for more practical therapeutic solutions[39].

The Douleur Neuropathique (DN4) questionnaire includes four questions and ten items with Yes or No answers. (A contribution of) neuropathic pain is probable if four or more positive answers are given. The majority of structured questionnaires (in which a physical examination is occasionally included as part of the screening instrument) are inadequately researched and validated, particularly in the Dutch scenario. The DN4 is the only well-researched questionnaire with sufficient sensitivity (74%) and specificity (79%) for detecting the presence or absence of neuropathic pain in a Dutch translation. However, the DN4 has been thoroughly tested for reproducibility in patients with neuropathic pain in the original language (French) and confirmed to be accurate (Bouhassira, 2005). The DN4 is made up of a series of standardized questions and some aspects of the physical examination[40].

The Small Fiber Neuropathy Screening List (SFNSL), a short and easy-to-administer questionnaire, was developed in a sarcoidosis population to assess the presence of SFN in clinical practice. The SFNSL is a self-administered 21-item questionnaire that is used to assess for SFN symptoms. On a five-point scale (0 never/not to 4 always/severe), the SFNSL scores can vary from 0 to 84. A score of 11 or less indicates that there are few or no SFN-related symptoms, 11 to 48 indicates that SFN is plausible or likely, and 48 or more indicates that SFN is present[41].

2.2 Small fiber neuropathy

2.2.1 Small fibers

The skin is a highly specialized organ for receiving sensory input and maintaining homeostasis in the human body. These tasks are handled by cutaneous small nerve fibers, which have a complicated anatomical architecture and are classified as cutaneous Aβ, Aδ, and C-fibers depending on their diameter, myelinization, and action potential conduction velocity[42]. Although some nociceptors are thinly myelinated (Aδ-fibers), the bulk of sensory neurons in the peripheral nervous system are unmyelinated (C fibers)[43].

According to the modality and location of the stimulus, pain has different qualities and temporal features: first pain is described as lancinating, stabbing, or pricking; second pain is more pervasive and includes burning, throbbing, cramping, and aching, and recruits sustained affective components with

descriptors such as "sickening." [44]. A-fibers mainly receive and convey information regarding acute pain (sharp, immediate, and relatively short-lasting). The second, burning pain, is caused by C-fiber nociceptors[45].

2.2.2 Symptoms

Sensory and autonomic symptoms are the two types of SFN symptoms, with sensory symptoms classified as either positive or negative[46]. Patients frequently experience positive sensory symptoms such as tingling, burning, prickling, shooting pain, or aching. The pain is usually worse at night and can make it difficult to sleep. Pain is not always a symptom of small-fiber neuropathy. Some of the negative symptoms that patients may experience include numbness, tightness, and coldness.

As part of their autonomic symptoms, patients may experience increased or decreased sweating. Facial flushing, skin discoloration, dry eyes and mouth, and changes in skin temperature affect less than half of the population[47]. In clinical findings, thermal and pain sensitivity is frequently reduced in association with normal strength, proprioception, and tendon reflexes. Although patients are "allowed" to have some vibratory loss at the great toes, consistent with mild large-fiber involvement, vibration is usually normal. In many patients, atypical clinical findings are rare or nonexistent[48].

2.2.3 Pathophysiology

A variety of pathophysiological mechanisms are thought to be responsible for peripheral neuropathy. However, the pathogenesis of these disorders remains unknown, and much remains to be discovered about the pathophysiology of isolated SFN. Some pathophysiological mechanisms in mixed polyneuropathies have been identified, and these mechanisms may also play a role in SFN[4].

SFN can be caused by various factors such as metabolic, immunologic, toxic, viral, paraneoplastic, or genetic factors[49]. The most common underlying illness caused by SFN is diabetes mellitus[50]. The density of intraepidermal axons is drastically reduced in skin biopsies from DM patients, and axon twisting and swelling have also been reported. The precise nature of this process is unknown. Two mechanisms currently known to cause harm in SFN are disrupted nociceptive perceptions and axonal injury. These mechanisms can occur independently or concurrently[51] and are discussed in more detail in the paragraphs below.

2.2.4 Altered nociceptive sensations

Some SFN patients have been found to have mutations in the voltage-gated sodium channels Nav1.7 and Nav1.8, encoded by the SCN9A and SCN10A genes[52]. In the nociceptive pathway, these channels are in charge of conducting and generating action potentials. Gain-of-function sodium channel Nav1.7 variants have been found in up to 30% of cases of idiopathic painful SFN[53]. It is hypothesized that a Nav1.7 channel mutation results in a reduced transmembrane sodium gradient. This decreased gradient may result in a sodium-calcium pump exchange and the emergence of a toxic increase in intra-axonal calcium[52]. As a result, the dorsal root ganglia (DRG) detection threshold is lower, resulting in more frequent and occasionally spontaneous signals[52,53]. Consequently, the DRG becomes more excitable, increasing pain sensitivity, which can lead to central sensitization (CS)[54].

Furthermore, Faber et al.[55] demonstrated that sodium channels have altered transmission properties in patients who do not have a Nav1.7 mutation but have a Nav1.8 mutation. Nine Nav1.8 mutations were discovered in a group of 104 patients with painful small-fiber neuropathy. The pathogenicity criteria were met by three mutations, two of which improve the channel's response to depolarization and cause hyperexcitability in DRG neurons. Mutations in Nav1.7 and Nav1.8 cause sodium channel dysfunction in nerve fibers, resulting in earlier nociceptive conduction activation. The Nav1.8 and 1.7 mutations might explain SFN pain and allow for targeted treatment interventions[53].

2.2.5 Axonal damage

The axons are no longer able to dissipate all of the sodium properly due to the reversed action of the sodium-calcium pump. Increased sodium influx is a well-known phenomenon that places an energetic burden on neurons, particularly those with small diameters. This increase may cause axonal damage[55], as a reversal of the pump results in toxic intracellular calcium concentrations. This calcium is absorbed by the endoplasmic reticulum (ER) and mitochondria. As a result, adenosine triphosphate (ATP) production is reduced, and a process in which ATP decays faster than it is produced occurs. Finally, this process can result in axon swelling or degeneration[51].

2.3. Chemotherapy-induced peripheral neuropathy

Similar to SFN, Aδ- and C-fibers are affected by CIPN[8,56], which is a common side effect of neurotoxic chemotherapy[57–60]. Due to previously mentioned difficulties in measuring damage to Aδ- and C-fibers, the prevalence and pathophysiology of SFN amongst chemotherapy-treated patients continue to be poorly comprehended[56]. According to a recent meta-analysis amongst 4179 cancer survivors, CIPN

occurs in approximately 30% of all patients six months after completing chemotherapy[57]. Chemotherapy treatment with Paclitaxel has one of the highest reported incidences of CIPN (76-87%)[61,62]. The pathomechanisms of CIPN due to Paclitaxel are multifactorial and result in neuroinflammation and altered excitability of peripheral neurons[56]. CIPN symptoms such as paresthesia, painful sensations and, in severe cases, complete patient immobilization, interfere with daily living activities and reduce quality of life[56,60]. Symptoms of CIPN usually progress faster than other peripheral neuropathies, such as painful diabetic polyneuropathy (PDPN)[63].

Early detection of CIPN is vital to enable adjustment of therapy to prevent worsening of CIPN[58,60]. Moreover, accurate detection is essential since early cessation or dose reduction may negatively influence treatment effect and survival[57–59]. Current CIPN assessment tools include the Common Toxicity Criteria scales and several validated patient-reported outcome measures[58]. However, these tools mainly rely on subjective assessment and do not provide insight into small fiber function[64]. In conclusion, there is an urgent need to diagnose CIPN early, accurately, objectively, and according to a standardized approach[57–60].

Depending on the specific drug, the combination of medications, the dosage, the methods of pain assessment, and the patient situation. The prevalence of CIPN varies depending on the agent, with reported rates ranging from 19% to more than 85%. Platinum-based drugs (70–100%), taxanes (11–87%), thalidomide and its analogues (20–60%), and ixabepilone (60–65%) have the highest rates[65]. Toxicity can occur as a result of a single high dose or over a long period of time. Taxanes include the drug paclitaxel used to treat breast, ovarian and lung cancer, among others. Paclitaxel is a very effective drug against tumor progression. However, in 60-70% of patients treated with Paclitaxel, it causes peripheral neuropathy[66]. Acute, transient thermal sensations to permanent changes in peripheral nerves, chronic pain, and irreversible nerve damage are all possible symptoms. According to recent research, the prevalence of CIPN is approximately 68.1% in the first month following chemotherapy, 60.0% at three months, and 30.0% at and after six months[57].

2.3.1 Pathophysiology

Any part of the peripheral nervous system, including the autonomic and DRG, as well as the axon and any type of peripheral nerve fiber, can be affected by CIPN. Sensory myelinated (Aβ) fibers with large diameters are the most commonly affected, but motor, small myelinated (Aδ), unmyelinated (C), or autonomic fibers can also be affected[67].

Over the last two decades, a compelling body of evidence has emerged from preclinical research on CIPN, pointing to four major trends: neurotoxic anticancer drugs affect the peripheral sensory nerve by (1) directly targeting mitochondria and causing oxidative stress, (2) functionally impairing ion channels, (3) triggering immunological mechanisms through activation of satellite glial cells, and (4) disruption of microtubules[68,69]. Axonal degradation, which is a persistent pathogenic process in most drug-induced neuropathies, is the most common histomorphologic alteration seen during CIPN. More specifically, paclitaxel use has been linked to significant axonal mitochondrial abnormalities. Although experimental studies have contributed to a better understanding of the pathogenesis of some drug-induced peripheral neuropathies, the underlying mechanisms remain unknown[68].

2.4 Sarcoidosis induced peripheral neuropathy

Sensory problems in the trunk, face or more proximal sections of the limbs are common symptoms of sarcoidosis-induced peripheral neuropathy. Unilateral flank numbness, chest tingling, thigh pain, and continuous facial paresthesias are common symptoms of sarcoidosis-induced peripheral neuropathy. During periods of inactivity, such as extended sitting, standing, or lying down, some patients' symptoms can worsen. Fatigue is a crucial problem that has a negative influence on individuals with sarcoidosis who have neuropathy and those who do not have neuropathy. Chronic pain, adverse drug effects, involvement of other organ systems, and psychosocial factors are all likely to contribute to fatigue[70].

2.4.1 Pathophysiology

Granulomatous deposition in and around the nerves, microvasculitis, and/or necrotizing vasculitic alterations are signs of sarcoidosis. The specific cause of sarcoidosis-induced peripheral neuropathy axon loss is unknown. In one study, patients with small-fiber neuropathy symptoms had considerably lower IENFs than those with asymptomatic sarcoids. This is consistent with lower IENF density findings in skin biopsy samples from patients with various disorders[70].

2.5 NDT-EP method

Lately, a method has been established for measuring nociceptive detection thresholds (NDTs), which are stimulus amplitude thresholds for a detectable sensation, applying intra-epidermal electro cutaneous stimulation (IES) on the skin. The NDT for IES stimuli can be assessed using a multi-threshold tracking algorithm (MTT) developed in previous studies[23,24]. The investigation of the underlying

mechanisms of sensitization can be simplified by tracking the NDTs. The concept has been used to measure NDTs of IES stimuli with single and multiple pulses[23], demonstrate the sensitivity to short-term changes in nociceptive processing[24], show changes of the NDT related to stimulus parameters[71], and measure the effect of capsaicin-induced peripheral sensitization on the NDT[72]. Since MTT measures the subject's psychophysical response, it does not provide an objective measure of nociception.

2.5.1 Intra-epidermal electrical stimulation

Intra-epidermal electrical stimulation (IES) has been offered as an alternative to thermal stimulation for selectively activating nociceptors. An IES-5 electrode with an array of 5 microneedles is used to deliver intra-epidermal electrocutaneous stimulation[73]. These electrodes merely protrude 0.2 mm into the skin's stratum corneum. The electrodes are non-invasive since they do not pierce the epidermis. Inui et al.[74,75] demonstrated that such a superficial intrusion in the epidermis allows for selective activation of superficial (A) nociceptive skin fibers, corroborated independently by Mouraux et al.[76].

In a preliminary experiment, Mouraux et al.[76] used capsicin to elicit selective denervation of capsaicin-sensitive nociceptors to see if this population of afferent fibers mediates IES reactions. According to the researchers, capsicine decreases behavioral and electrophysiological responses to electric currents spatially confined to the epidermal layers. Their findings suggest that IES can selectively activate Ad-nociceptors as long as low levels of stimulation are used.

Steenbergen et al. studied the somatosensory topography of A-fibers in human subjects with BiModEl electrodes (identical to the IES-5 and also made at the University of Twente)[77–79]. Similarly, Doll et al. used the IES-5 electrodes to describe peripheral and central nociceptive system changes in response to stimulus parameters[24,71].

2.5.2 Implementation NDT-EP method

The BSS group developed the AmbuStim 1-channel stimulator at the University of Twente. It is a cathodic square-wave electrical current pulse with a pulse width of 0.21 ms and a variable inter-pulse interval (IPI). The stimulus amplitude is set at an initial maximum current of 1.5 mA. Two different IPIs will be assessed: 10 and 40 ms. Since earlier studies applying IES show a sensory threshold between 0 and 1.0 mA, the maximum current in the software is set to 1.5 mA. This limit can be further constrained by setting a limit in the stimulation software.

The AmbuStim 1-channel stimulator was developed and tested by the BSS group at the University of Twente. A desktop computer running a custom computer program written in LabVIEW 2013, SP1 controls all stimulation procedures and registers the applied stimulus amplitudes (in mA) and their trigger codes, the responses to stimuli, and the stimulus times in milliseconds. All communication between software and stimulator is logged.

2.6 Nociceptive detection threshold

Stimuli have unique detection thresholds in psychophysics, which indicate the lowest physical intensities at which they elicit a psychological reaction. A psychophysical (or psychometric) curve is the visualization of the link between physical intensity and psychological reaction when stimulus intensity and corresponding responses are plotted against each other. This relationship can be interpreted in various ways, such as the percentage of accurate detections or the response amplitude compared to the predicted maximum. However, each psychological reaction variable requires an arbitrary cut-off value at which the detection threshold can be specified to calculate the detection threshold[80].

The psychophysical approach equips researchers with the skills they need to comprehend the mental correlates of varying-intensity environmental stimuli. However, this method fails to account for a critical property of human stimulus detection: non-stationarity. When it comes to body signals, such as those involved in the neurophysiological processing of received stimuli, strong non-stoically persistent signals are standard. This finding prompted researchers to extend single-repetition threshold determinations to studies in which the thresholds are tracked, ideally considering the influence of various experimental parameters[81].

2.6.1 Multiple threshold tracking

Doll et al. (2014)[82] explored which stimulus selection technique and threshold estimation approach resulted in the highest precision and most negligible bias of monitored thresholds. They discovered that using a 'random staircase' (or adaptive probing) technique to choose stimulus intensities and logistic regression to estimate detection thresholds produced the most reliable findings. In this study, a logistic psychophysical relationship between stimulus intensity and detection probability was introduced.

The MTT approach is used to choose stimuli[23,24]. According to MTT methods, the threshold for each combination of NOP and IPI is determined by evaluating the subject's response (detected or not

detected) to a randomized set of stimulus amplitudes. Each time, the same number of stimuli are chosen, but in a different order. Observer and subject bias are reduced when stimulus types are changed at random.

2.7 Evoked potentials

An objective measure of nociception-related activity in the central nervous system is the electroencephalographic (EEG) signal. Multiple-trial averages of this signal, referred to as evoked potentials (EPs), have been shown sensitive to changes in stimulus parameters such as the number of pulses[83,84] or number of trials[85]. Since MTT has been shown to be helpful in measuring the effect of stimulus parameters on stimulus detection, while the EP has been shown to reflect neurophysiological activity related to stimulus processing, a combination of both techniques might provide insight into the relation between neurophysiological activity and nociceptive stimuli.

2.7.1 Interpretation evoked potentials

Van den Berg (2020)[86] combined the threshold estimation experiment with simultaneous recordings of electroencephalographic (EEG) activity around stimulus administration. The evoked potential, or EP, is a measure of cortical activity in response to sensory input. It can be observed for stimuli of many origins. Peak amplitudes and related latencies can be used to classify EPs. The viability of a combined psychophysical and neurophysiological analysis of nociceptive properties was proven by combining Generally Linear Mixed Models (GLMM) of detection probability with linear mixed-effects models (LMM) of EP amplitude. This indicated that specific stimulus features influenced the chance of detecting a stimulus (contrary to previous research by the group) as well as the amplitudes of EPs.

2.8 Clinical and technical-medical question

Evaluation could provide valuable insight into the feasibility of measuring NDTs and EPs within a simulated region of small fiber dysfunction and their behavior. Therefore, the technical-medical question in this graduation research is as follows:

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

2.8.1 Objectives

The central aim of previous explorative studies was to combine measurements of the NDT with EEG recording and analysis to observe neurophysiological activity during nociceptive processing. During the delivery of multiple types of stimuli, EEG signals were recorded in pain patients and healthy subjects, which were analyzed concerning the stimulus parameters. In this study, pain patients are CIPN and sarcoidosis patients. Therefore, the central aim of this graduation research is as follows:

Central aim:

Explore the feasibility of the NDT-EP measurement method and its outcomes in CIPN and sarcoidosis patients.

Furthermore, primary and secondary objectives have been formulated:

Primary study objectives:

Explore the feasibility and describe the outcomes of NDT-EP measurement in CIPN and sarcoidosis patients.

Secondary study objective:

Compare the outcomes from CIPN and sarcoidosis patients with healthy controls

3. Methods

Only the most essential information is highlighted in this section. For the full report, please refer to the research protocol from Berfelo et al. (NL66136.100.18).

3.1 Study design

In this explorative retrospective study, obtained psychophysical and neurophysiological recordings from the NDT-EP method were used to investigate the feasibility of the NDT-EP measurement method and its outcomes in CIPN and sarcoidosis patients. These recordings were obtained in the period between January and April 2021 at the Department of Anesthesiology and Pain Clinics, St. Antonius Hospital, Nieuwegein, Netherlands. The recordings were collected during a two-hour visit.

3.2 Study population

CIPN

Between February and May 2021, 18 CIPN patients were recruited from St. Antonius Hospital in conjunction with the Department of Oncology. Individuals between the ages of 18 and 75 who had previously undergone Paclitaxel treatment for breast cancer and had CIPN symptoms of paresthesia, numbness, or dysesthesia and had written informed consent were eligible to participate. Nurse practitioners who were involved in their care identified these patients.

Following the inclusion of the CIPN patients, the population was matched to a group of 18 healthy controls (HCs) from an existing dataset based on sex, age, and handedness. Except for the inclusion criteria of Paclitaxel treatment and CIPN symptoms, the inclusion and exclusion criteria were comparable to those of the CIPN group.

Sarcoidosis

In collaboration with the Department of Interstitial Lung Diseases (ILD) at St. Antonius Hospital, 19 sarcoidosis patients were recruited between February and May 2021. Participants must be between the ages of 18 and 75, have signed informed consent, have an SFNSL score of at least 30, and be judged by a competent neurologist to have a possibility/likelihood of SFN. Following the inclusion of the sarcoidosis patients, the population was matched to a group of 19 healthy controls (HCs) from an existing dataset based on sex, age, and handedness.

General

The exclusion criteria for healthy controls were any history of pathological pain and diabetes. Other general exclusion criteria were participants' refusal during the study, communication problems, an implanted stimulation device, pregnancy, consumption of alcohol or drugs within 24 hours before the experiment, non-intact, inflamed, or otherwise affected skin on (at least) one of the hand dorsa. Participants were recruited via advertisements and flyering in the St. Antonius Hospital. General recruitment was done in collaboration with the Department of Interstitial Lung Diseases (ILD) and Oncology Department in the St. Antonius hospital in Nieuwegein. Eligible volunteers were invited for a visit in the St. Antonius Hospital, where they were asked to sign written informed consent.

3.3 Materials and procedures

Experimental Procedure

First, the subject filled in a set of questionnaires related to primary and secondary study objectives. These questionnaires included a standard Numeric Rating Scale (NRS), Central sensitization inventory (CSI), and DN4[87]. Moreover, neurological evaluation and preparation on both hands were performed before the NDT-EP measurement started. Both hands were measured separately for 30 minutes per side. During these measurements, nociceptive stimulus-response pairs were measured for a variety of nociceptive stimuli. This stimulation was performed conform the IES procedure.

Intra-Epidermal Electrical Stimulation (IES) & threshold tracking

A method of IES established at the University of Twente was used for selective stimulation of nociceptive Aδ-fibers. The stimulus amplitude is set at a maximum current of 1.5 mA. Two different inter-pulse intervals (IPIs) were assessed:10 and 40 ms. A total of 450 stimuli per hand were applied, consisting of 150 stimuli for three different settings: (1) single pulse, (2) two pulses with an IPI of 10 ms, and (3) Two pulses with an IPI of 40 ms. The selection of these stimuli is performed with the MTT procedure. The stimulus was labelled as detected if the participant confirmed the stimulus by releasing the button. The stimulus was labelled as undetected if the button was still pressed. The subject's response to a randomized set of stimulus amplitudes resulted in three simultaneously tracked NDTs. All types of stimuli were selected the same number of times but in random order. Moreover, the inter-stimulus interval was randomized with a 1-second uniform distribution for reducing bias.

Electroencephalographic Recordings

The EEG electrodes were positioned using an EEG cap with 64 Ag/ AgCl electrodes. The ground electrode was placed on the forehead, and the recordings were made using TMSi Polybench software (TMSi B.V., Oldenzaal, The Netherlands) at a sampling rate of 1000 Hz. The recordings were performed simultaneously with the stimulations.

Sample size calculation

Sample size calculations were not applicable due to the explorative design of the study.

3.4 Data preparation

Nociceptive Detection Threshold

The detection thresholds for each stimulus setting were estimated by using individual NDT values. This estimation was conducted using a moving window (30 trials) and psychophysical functions. Considered values that were twice the detection threshold were removed from the analysis. The detection rate is usually around 50% because the detection threshold is defined as the stimulus amplitude resulting in a detection probability of 0.5.

Evoked Potentials

EEG data were analyzed offline using MATLAB and FieldTrip (*Matlab R2015b, Mathworks, Inc, Massachusetts, US*). The EEG data were band-pass filtered between 0.1 and 40 Hz and baseline-corrected using the mean amplitude within the period until the stimulus. Other artefacts, such as signal drift and eye blinks, were removed manually. The time window (epoch) of interest was -0.50 – 1 sec. Next, epochs were created using an independent component analysis (ICA) in EEGLAB [88].

Following data cleaning with ICA per group, a butterfly plot was created for all EEG data. For each subject group, a corresponding global field power (GFP) [89] was determined and used to calculate the latencies. Thus, a maximum GFP can be identified. Such latencies tended to differ between the subject groups. This averaged latency of the entire time series was used to prepare the data for a statistical model fit. For every measurement, the EP values for this latency were averaged per subject for a central derivation (CPz-A1A2) and contralateral derivation (T7-F4). One of the EEG caps commonly used to assess CIPN and sarcoidosis patients appeared to have poor electrode CP6 quality. As a result, electrode CP6 was removed from the cleaned epochs of both morbid obese study groups. Because healthy controls were compared to CIPN and sarcoidosis, CP6 was also removed from these EEG data.

3.5 Statistical analysis

Statistical analysis will be performed at the University of Twente and the St. Antonius Hospital, using appropriate statistical software (SPSS, R, MATLAB and/or FieldTrip).

Nociceptive Detection Threshold

Comparing coefficients of the GLMM computed using the NDTs. Mean, median, minimum, maximum, and standard deviation (SD) were compared.

NDTs were re-estimated using GLMM, allowing statistical analysis of the relationship between different parameters and the subjects' response as a dependent variable. Such parameters included the stimulus setting, study group, and trial number. Equation 1 (Eq. 1) contains the GLMM that describes the subjects' responses. Random effects between subjects were included in all fixed effects. Next, all the effects' coefficients were computed, and linear prediction of these coefficients resulted in detection thresholds per trial for each stimulus setting. Thresholds that were twice the value of the previous trial were removed from the threshold analysis. Data from all subjects was used to analyze nociceptive detection probabilities and thresholds. Finally, NDTs for all study groups were displayed to allow for visual exploration of the GLMM results.

GLMM effects with average detection thresholds and slopes were statistically examined using R-Studio 1.4.1 (R Foundation for Statistical Computing, Vienna, Austria) and the lme4 library for multilevel analysis. A logit link function was created to estimate detection probability (see Equation 1). Two different GLMMs based on Equation 1 were created to compare the study groups: HC with CIPN and HC with SAR.

The type III Wald Chi-square test was used to determine the significance of the GLMM main and interaction effects. Post-hoc analysis was used to calculate the average NDTs and psychometric slope for each group. Average NDTs were calculated using the delta method. Using general linear hypotheses, the average psychometric slopes for each group were calculated. Z-statistical testing was used to compare average NDTs and slopes between study groups. The significance threshold for all statistical test results was set at 0.05.

Per subject, the mean NDT value was estimated using a Generally Linear Mixed Model (GLMM).

*NDT ~ 1 + Amplitude*D + Amplitude 2*D + Amplitude 3*D + Trial nr*D + Measurement nr + (1 + Amplitude*D + Amplitude 2*D + Amplitude 3*D + Trial nr*D + Measurement nr | SubjectNR)* Eq.1

In Eq.1, Amplitude represents the stimulus amplitude, whereas Amplitude 2 and Amplitude 3 respectively represent the stimulus amplitude for settings 2 and 3. Trial nr. represents the total number of stimuli applied. Measurement nr denotes either the first or second measurement, and D denotes whether the patient is healthy, has CIPN, or has Sarcoidosis. Finally, for each fixed effect, SubjectNR describes intersubject random effects.

Evoked Potentials

Comparing coefficients of the LMM computed using the measured EEG signals, describing the influence of stimulus parameters on the EP. The following parameters were compared: model coefficients, significance of model coefficients from Wald Chi-square test, and coefficient interactions.

A LMM was used to assess the effect of stimulus-response, study group, stimulus properties, and trial number (habituation) on maximum EP amplitude. This LMM was carried out using MATLAB. As shown in Equation 2 (Eq.2.), random effects between subjects were included for all fixed effects. To analyze EPs, CPz was referenced with A1A2 and T7-F4 derivations. Two LMM analyses were carried out. First, to determine potential differences between a healthy control group and the CIPN group. Second, to see if there are any differences between a healthy control group and those with sarcoidosis. In MATLAB, the t-test was used to statistically test the resulting grand average EPs at the pre-specified latencies. The threshold for significance was set $\alpha=0.05$.

Per subject, the EP was estimated using a Generally Linear Mixed Model (GLMM).

*EP ~ 1 + Amplitude*D + Amplitude 2*D + Amplitude 3*D + Trial nr*D + Measurement nr + (1 + Amplitude*D + Amplitude 2*D + Amplitude 3*D + Trial nr*D + Measurement nr | SubjectNR)* Eq.2

In Eq.1, Amplitude represents the stimulus amplitude, whereas Amplitude 2 and Amplitude 3 respectively represent the stimulus amplitude for settings 2 and 3. Trial nr. represents the total number of stimuli applied. Measurement nr denotes either the first or second measurement, and D denotes whether the patient is healthy, has CIPN, or has Sarcoidosis. Finally, for each fixed effect, SubjectNR describes intersubject random effects.

4. Results

As mentioned earlier, the feasibility of the NDT-EP measurement method and its outcomes in CIPN and sarcoidosis patients was investigated. Due to the dichotomy in main SFN groups, the results will be separated in CIPN and sarcoidosis. Following the exclusion of subjects, 74 were ultimately included in the analysis. In section 'Subject Characteristics', we compare different characteristics overall and within the groups. Furthermore, the results are divided into three parts: subject characteristics, the influence on response variable Nociceptive Detection Thresholds, and the influence on response variable Evoked Potentials.

4.1 CIPN

4.1.1. Subjects

Measurements for CIPN patients were conducted between February and May 2021, whereas HC measurements were conducted between October 2018 and April 2021. Measurement data from a total of 36 subjects (18 CIPN and 18 HC) was included for analysis.

4.1.2. Subject characteristics

Table 4.1. compares the demographics of healthy controls (HC) and patients with chemotherapy-induced peripheral neuropathy (CIPN). A Paired Sample T-Test with a significance level of (=0.05) was used to test differences between subgroups. * denotes statistically significant differences.

Parameter	**HC (n=18)**	**CIPN (n=18)**	**p-value**
Age, years	56.3 (11.1)	58.0 (10.1)	0.602
Female, n (%)	18 (100%)	18 (100%)	1.000
BMI, kg/m^2	24.5 (2.6)	28.5 (4.2)	<0.001*
Right handedness, n (%)	16 (89%)	14 (78%)	0.163
Pain medication use, n (%)	0 (0%)	2 (11%)	0.163
Other medication use, n (%)	2 (11%)	13 (72%)	<0.001*
NRS score last week	1.4 (1.2)	3.7 (2.8)	0.005*
NRS score today	0.0 (0.0)	3.4 (3.0)	0.003*
CSI score	19.9 (8.4)	37.9 (13.5)	<0.001*

Numeric variables are presented as mean (standard deviation) unless stated otherwise.
Abbreviations: BMI – Body Mass Index; NRS – Numeric Rating Score; CSI – Central Sensitization Inventory.

The demographic characteristics of the HCs and CIPN groups are shown in Table 4.1. There are significant differences between groups in BMI, medication use other than pain medication, NRS score in the previous seven days, NRS score on the measurement day, and CSI score. Notably, there was no statistically significant difference between the two groups in terms of pain medication use. The

medications reported by HCs were anticoagulant medication (n=1) and cholesterol synthesis inhibitors (n=1). Table 2 delves deeper into the medication use of the CIPN group.

Table 4.2: Characteristics of Chemotherapy-Induced Peripheral Neuropathy Patients (CIPN).

Parameter	
DN4 score	4.6 (2.4)
Prior therapy for breast cancer	
Radiation	7 (39%)
Surgery	7 (39%)
Hormone therapy	1 (6%)
Number of Paclitaxel doses	11.2 (3.5)†
Duration of Paclitaxel doses, months	2.4 (1.5)
Dose reduction, n (%)	12 (67%)
Premature cessation, n (%)	9 (50%)
Cumulative Paclitaxel dose, mg/m^2	801.5 (263.7)†
Time since last Paclitaxel dose, months	15.8 (9.7)
Time since start CIPN, months	15.9 (9.6)
Neuropathy location, n (%)	
Feet	16 (89%)
Hands	8 (44%)
Other‡	5 (28%)
Neuropathy sensation, n (%)	
Paresthesia	10 (56%)
Numbness	7 (39%)
Dysesthesia	5 (28%)
Medication use, n (%)	
Hormone therapy	10 (56%)
Mucosal protective agents	3 (17%)
ACE inhibitors	2 (11%)
Antidepressants	2 (11%)
Anticoagulation	1 (6%)
Antiepileptics	1 (6%)
Cholesterol synthesis inhibitors	1 (6%)
Diuretics	1 (6%)
Laxatives	1 (6%)
Opioids	1 (6%)
Thyromimetics	1 (6%)

Numeric variables are presented as mean (standard deviation) unless stated otherwise.
Abbreviations: DN4 – Douleur Neuropathique in 4 questions.
† Two patients received more doses than regular (21 and 17) due to participation in other scientific research.
‡ Other locations were the back of the neck, fifth digits, left calf, three middle toes, and general background pain.

Table 4.2. lists the disease-specific characteristics of the CIPN group. Compared to a typical cumulative Paclitaxel dose of 960 mg/m2 after 12 treatments, the mean cumulative Paclitaxel dose was 801.5 mg/m2. Furthermore, 50 percent of CIPN patients completed their Paclitaxel treatment prematurely, and 67 percent of CIPN patients required a dose reduction due to CIPN symptoms. The feet (89

percent) are the most common site for these symptoms, and paresthesia is the most common sensation (56 percent). Hormone therapy was the most commonly prescribed medicine in the CIPN group (56 percent). Notably, two of the 18 patients received higher doses than usual (21 and 17 vs. 12 generally) because they were also involved in other scientific studies.

4.1.3. Nociceptive Detection Thresholds

Table 4.3. Average percentages of detected stimuli divided by the total amount of stimulus type for healthy controls (HC) and CIPN.

Stimulus type	HC	CIPN
General	44.8	43.4
SP	39.9	39.5
DP10	47.7	45.7
DP40	47.0	45.1

Table 4.3. shows the characteristics of the NDT experiments for the HC and CIPN groups. The CIPN group has a lower percentage of detected stimuli in settings 2 and 3 than the HC group. As can be seen, setting 1 has a lower percentage of detected stimuli than settings 2 and 3.

Table 4.4. Coefficient estimates and fixed effects for three generalized linear mixed models (degrees of freedom = 1) for detecting the probability of three different types of stimuli. The model was fitted to (1) CIPN data, with healthy controls (HC) serving as a reference. The intercept and trial number had significant effects on the model. The measurement numbers of the participants were not significant. These coefficient estimates and their effects were not displayed.

Fixed model factor		**Coefficient estimate**	**Effect X^2**	**P-value**
	Interaction			
Model 1 (HC)				
CIPN				
	x SP	-2.12	1.32	0.250
	x DP10	-0.36	0.05	0.825
	x DP40	-0.41	0.09	0.770
	x Trial number	0.11	1.09	0.297

SP= single pulse, DP10= double pulse with an inter-pulse interval of 10ms., DP40= double pulse with an inter-pulse interval of 40ms. x indicates interactions between effects. The p-value that is statistically significant is <0.05. *= significant effect between interactions, based on Type III Wald Chi-square test to assess the significance of fixed effects

The regression parameter estimates of the GLMM's fixed effects are shown in Table 4.4. The detection probability is demonstrated to be affected by all stimulus amplitudes as well as the trial number. When these factors were combined with the diagnosis of CIPN (Table 4.4), however, they did not affect the detection likelihood. No substantial influence is also seen in the measurement number.

Table 4.5. Average nociceptive detection thresholds (NDTs, orange) and slopes of psychophysical curves (blue) describing intraepidermal stimuli to detection probability for three stimulus types were calculated for each group. The averages and differences for healthy controls (HC) and CIPN are shown. Between parentheses, upper and lower bounds are provided. A generalized linear mixed model was used to generate the results.

		HC	**CIPN**	**P-value HC-CIPN**
SP	*Avg. NDT (mA)*	0.47 (0.27 – 0.68)	0.59 (0.34 – 0.85)	0.433
	Avg. Slope (mA^{-1})	5.24 (2.24 – 8.24)	3.12 (1.01 – 5.18)	0.250
DP10	*Avg. NDT (mA)*	0.24 (0.15 – 0.34)	0.24 (0.15 – 0.33)	0.945
	Avg. Slope (mA^{-1})	10.14 (5.15 – 15.14)	7.66 (3.26 – 12.06)	0.459
DP40	*Avg. NDT (mA)*	0.26 (0.16 – 0.36)	0.26 (0.16 – 0.36)	0.945
	Avg. Slope (mA^{-1})	9.58 (4.98 – 14.18)	7.05 (2.87 – 11.23)	0.419

SP= single pulse, DP10= double pulse with an inter-pulse interval of 10ms., DP40= double pulse with an inter-pulse interval of 40ms. mA= milliampere.
*= significant difference (P<0.05) between two groups based on two-tailed independent samples Z-test

Table 4.5 shows the average group NDTs as well as the slopes of the psychophysical curves. The CIPN group's mean slopes appear to be lower than the HC group's. However, neither the mean slopes nor the mean NDTs showed any significant differences between the two groups. Surprisingly, both groups' mean NDTs for setting 2 and 3 are practically comparable.

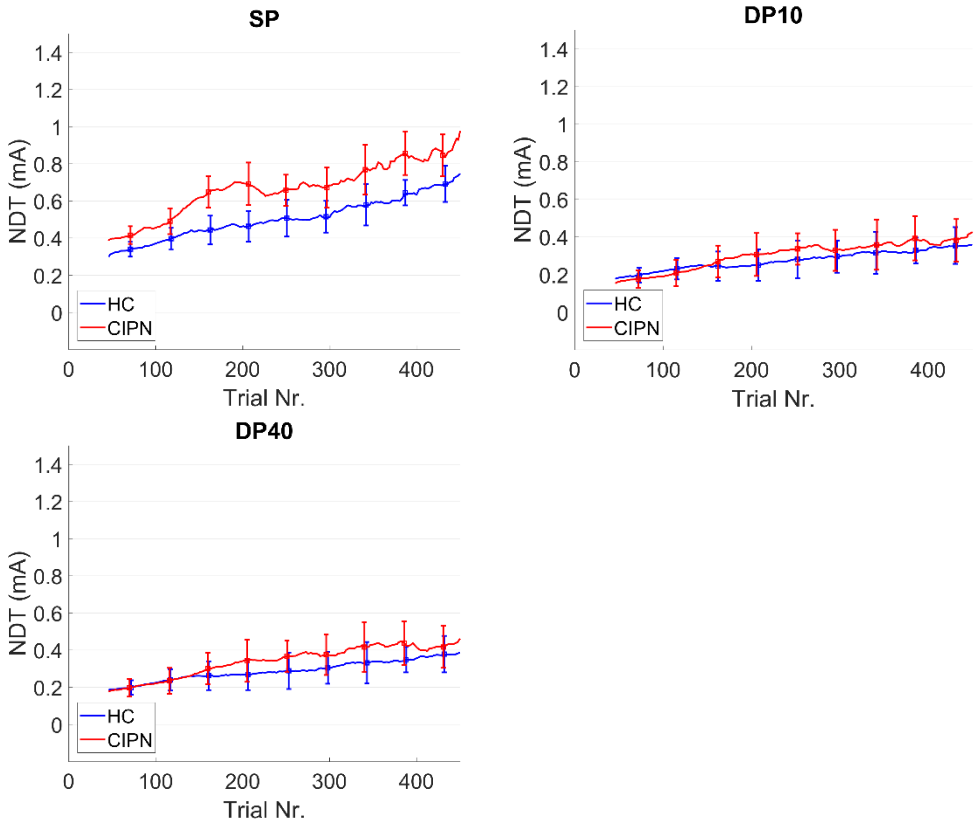

Figure 4.2. Estimated average nociceptive detection thresholds (NDTs) in milliampere (mA) tracked over trial number for (**A**) single-pulse stimuli, (**B**) double-pulse stimuli with an inter-pulse interval of 10ms, (**C**) double-pulse stimuli with an inter-pulse interval of 40ms. Per stimulus type, 150 stimuli were given. In each graph NDTs for the three study groups are visualized: healthy controls (blue) and CIPN (red). Note: NDTs used for statistical analysis differ from these NDTs used to visualize.

The NDTs for the entire study population were calculated using the GLMM described in Eq.1 (i.e., both HC and CIPN groups). The NDTs for each setting are depicted in Figure 4.2. The NDT of setting SP in the CIPN group decreases around trial number 200. The CIPN group has a higher initial threshold and a steeper angle at the start than the HC group. However, as the CIPN group decreases, the NDT angles of both groups begin to converge. As shown in 4.2, the angles and amplitudes of settings DP10 and DP40 appear to be comparable between groups, with the CIPN group having a higher endpoint.

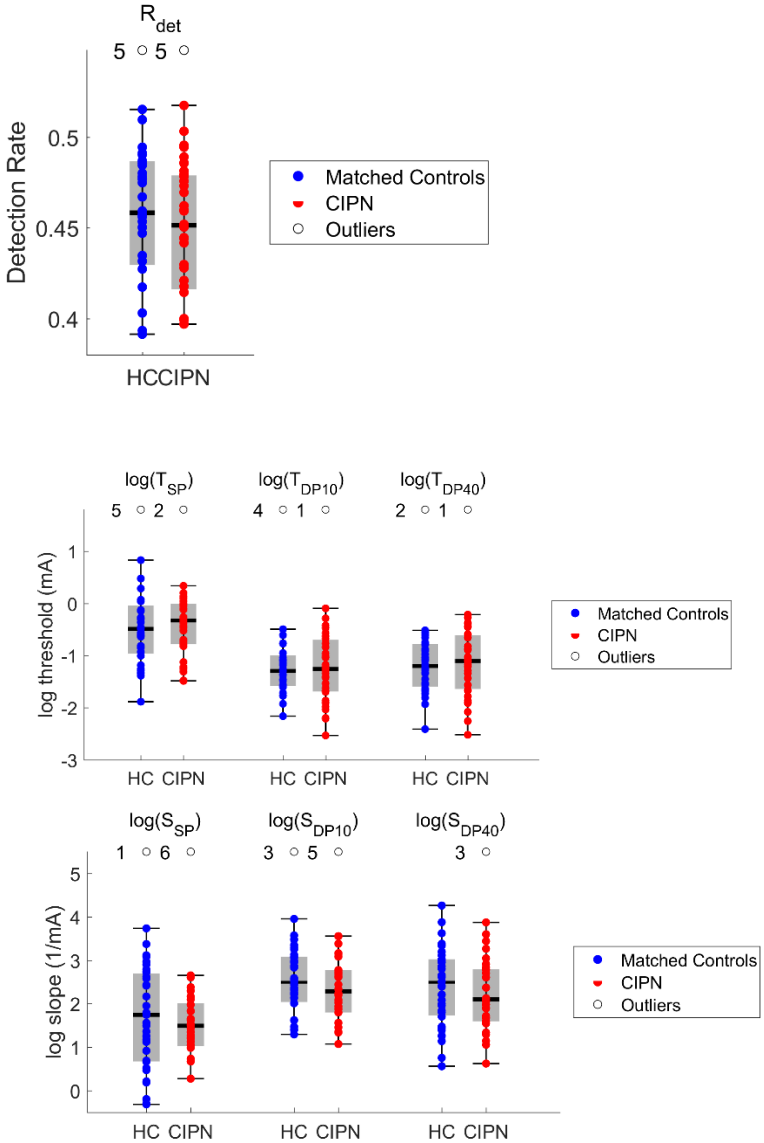

Figure 4.3. Individual psychophysical detection rate, derived detection thresholds and slopes of the psychophysical curve from 18 subjects of each group (i.e., HC and CIPN). The detection rate, threshold, and slopes were averaged over the entire measurement. These values were shown for both measurements. No significant altered psychophysical values were seen. This was found for all stimulus types.

Figure 4.3 shows the individual detection rate, thresholds, and slopes of the psychophysical curve. Individual values did not differ significantly (p>0.001) between groups. The detection rate appeared to be lower in CIPN than in HC. NDTs were not found to be significantly larger in CIPN than in HC. In addition, the slope's steepness did not differ significantly between groups.

4.1.4. Evoked Potentials

Table 4.6. T-statistics for fixed effects of a linear mixed-effects model of the amplitude of evoked potentials (EPs) for CIPN referenced to healthy controls (HC) in model 1. EPs followed from intraepidermal stimulations with three types of stimuli. EP amplitude was evaluated at the central component with a latency of 400 ms. (Non-)significant p-values in orange.

Fixed effect	**Interaction**	**Effect size**	**t-value**	**P-value**
Model 1 (HC)				
CIPN		0.81	3.28	0.03*
	Response	-0.03	-0.04	0.97
	Trial number	0.002	1.12	0.27
	Amplitude SP	0.52	0.61	0.55
	Amplitude DP10	-1.59	-2.64	0.01*
	Amplitude DP40	-0.31	-0.56	0.58

SP= single pulse, DP10= double pulse with an inter-pulse interval of 10ms., DP40= double pulse with an inter-pulse interval of 40ms. The p-value that is statistically significant is <0.05.
*= significant effect between interactions, based on t-statistics for fixed effects of linear mixed models.

The LMM with EEG data from the CIPN group and healthy controls as a reference group is summarized in Table 4.6. The most important findings of this model were significant negative t-statistics for CIPN group interaction with DP10 amplitude. This suggests that the CIPN group has smaller EP amplitudes than healthy controls when stimulated with double pulses with an IPI of 10ms. Compared to healthy controls, EPs in CIPN did not appear to be affected by response or trial number. The resulting grand average EPs from the central derivation are shown in Figure 4.4.

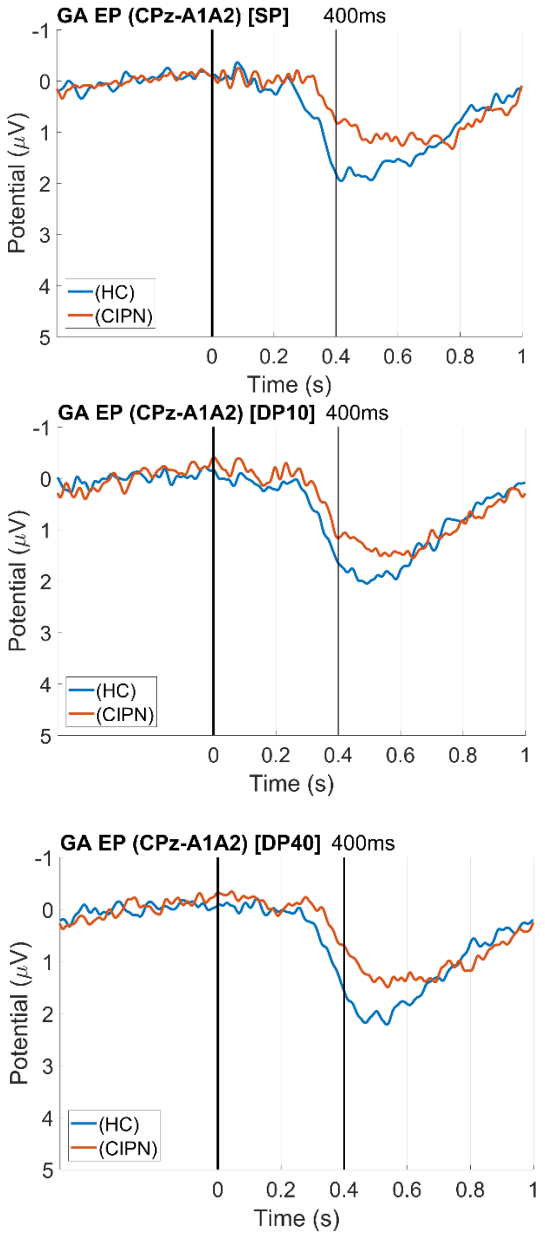

Figure 4.4. Grand average (GA) evoked potentials (EPs) in EEG derivation CPz-A1A2 as a result of intraepidermal stimulation by three types of stimuli: **A**. Single-pulse (SP), **B**. Double pulse with an inter-pulse interval of 10ms (DP10), **C**. Double pulse with an inter-pulse interval of 40ms (DP40). In all panels, EPs for healthy controls (HC) and CIPN are visualized. EP amplitude of the CIPN group was compared with HCs at P400. T-statistics ($P<0.05$) modulated significantly lower EP amplitudes for the CIPN group compared to HCs for double pulse stimuli with an inter-pulse interval of 10ms.

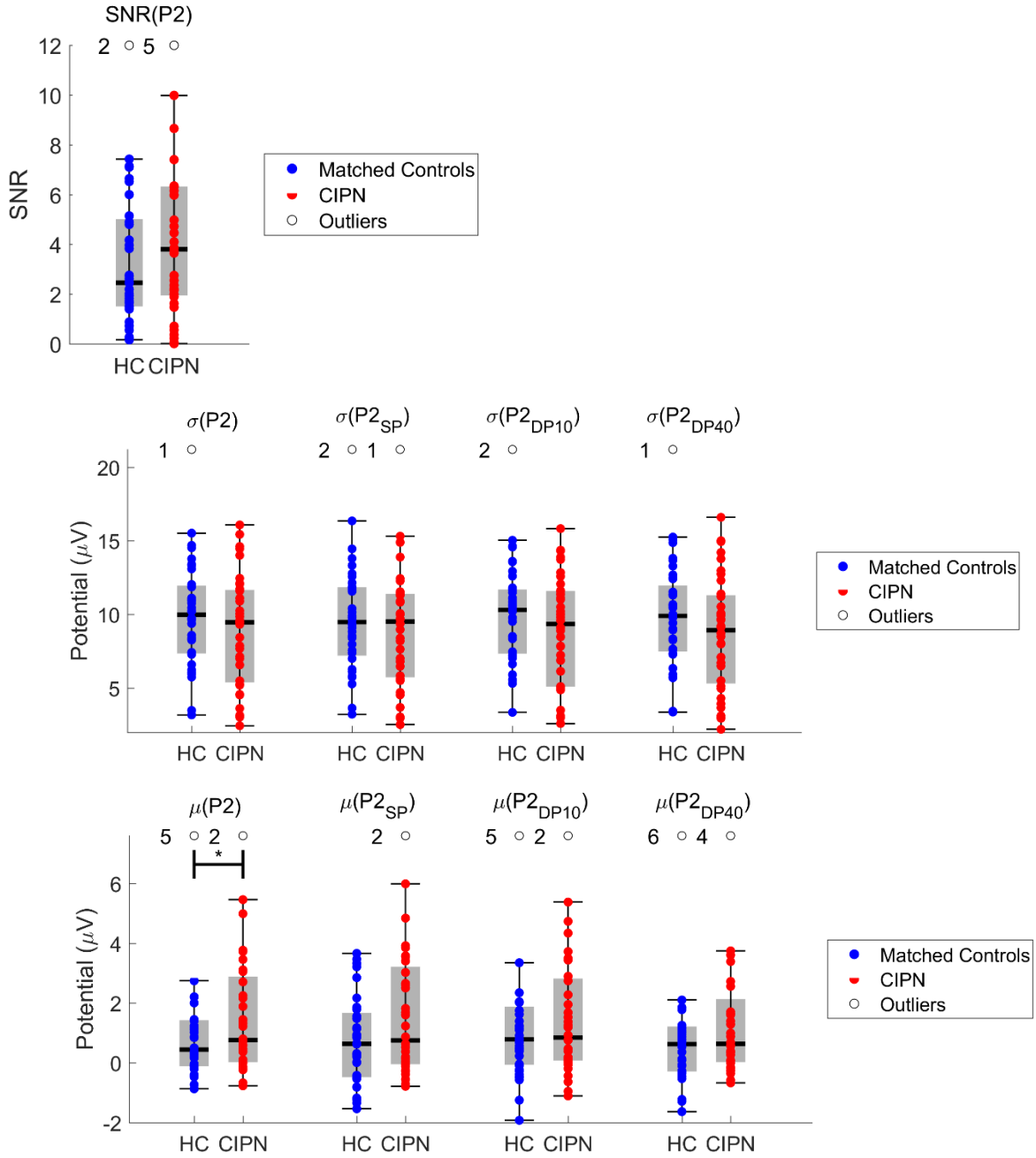

Figure 4.5. Individual Eps and the grand average EP (bold) from 36 measurements (i.e., two measurements per subject) with a P2 latency determined at 400 ms post-stimulus. The mean (μ) and the average standard deviation (σ) of the P2 amplitude did non differ significantly between groups.

The P2 peak was discovered 400 ms after the stimulus (Figure 4.5). The SNR of the P2 appears to be reduced in HC, but it was still adequate for EEG analysis. The variation and mean of the EP P2 did not differ significantly between groups.

4.2. Sarcoidosis

4.1.1. Subjects

Measurements for sarcoidosis patients were conducted between February and May 2021, whereas HC measurements were conducted between October 2018 and April 2021. Measurement data from 38 subjects (19 sarcoidoses and 19 HC) was included for analysis.

4.1.2. Subject characteristics

Table 4.7. Demographic-related subject characteristics for healthy controls (HC) and SAR subjects. Disease-related characteristics for the SAR subject group were not statistically tested. P-values (significant and non-significant) are showed in orange.

Parameter	HC	SAR	p-value
n	19	19	
Sex = M (%)	7 (36.8)	7 (36.8)	1.000
Age (mean (SD))	50.16 (12.51)	49.58 (10.88)	0.880
BMI (mean (SD))	24.06 (2.21)	26.36 (5.21)	0.085
Handedness = R (%)	17 (89.5)	18 (94.7)	1.000
Pain Years (mean (SD))	NaN (NA)	8.32 (8.27)	NA
NRS Last week (mean (SD))	1.37 (0.76)	5.95 (1.78)	<0.001*
NRS now (mean (SD))	1.00 (0.00)	4.53 (2.17)	<0.001*
CSI - score (mean (SD))	17.84 (7.68)	57.26 (11.32)	<0.001*
DN4 - score (mean (SD))	NaN (NA)	6.74 (2.33)	NA
SFNSL (mean (SD))	NaN (NA)	49.79 (13.75)	NA

BMI= body mass index, NRS= numeric rating scale, CSI= central sensitization inventory, N.A.= not applicable. *= significant effect between HC and SAR group.

The demographic characteristics of the HCs and sarcoidosis groups are shown in Table 4.7. There are significant differences between groups in NRS score in the previous seven days, NRS score on the measurement day, and CSI score. Notably, there was no statistically significant difference between the two groups in age and BMI.

4.1.3. Nociceptive Detection Thresholds

Table 4.8. Average percentages of detected stimuli divided by the total amount of stimulus type for the three subject groups: healthy controls (HC) and SAR.

Stimulus type	HC	SAR
General	45.4%	40.3%
SP	40.9%	35.4%
DP10	48.0%	43.5%
DP40	47.4%	42.1%

The characteristics of the NDT experiments for the HC and sarcoidosis groups are shown in Table 4.8. In settings 2 and 3, the sarcoidosis group has a lower percentage of detected stimuli than the HC group. Setting 1, as can be seen, has a lower percentage of detected stimuli than settings 2 and 3.

Table 4.9. Coefficient estimates and fixed effects for three generalized linear mixed models (degrees of freedom = 1) for detection probability of three stimulus types. The first two models (orange) were fitted to data from (1) SAR, with healthy controls (HC) as reference. For the two models, intercept and trial number were significant effects. Participants' measurement numbers were not significant in the two models. These coefficient estimates and effects were not shown.

Fixed model factor	**Interaction**	**Coefficient estimate**	**Effect X^2**	**P-value**
Model 1 (HC)				
SAR				
	x SP	-2,91	2,12	0,146
	x DP10	-3,18	5,81	0,016*
	x DP40	-2,92	7,14	0,008*
	x Trial number	0,05	0,40	0,529

SP= single pulse, DP10= double pulse with an inter-pulse interval of 10ms., DP40= double pulse with an inter-pulse interval of 40ms. x indicates interactions between effects. The p-value that is statistically significant is <0.05. *= significant effect between interactions, based on Type III Wald Chi-square test to assess the significance of fixed effects

The regression parameter estimates of the GLMM's fixed effects are shown in Table 4.9. When these factors were combined with the diagnosis of sarcoidosis (Table 4.9), they did affect the detection likelihood significantly for setting DP10 and DP40.

Table 4.10. Group average nociceptive detection thresholds (NDTs, orange) and slopes of psychophysical curves (blue) describing intra epidermal stimuli to detection probability for three stimulus types. Average values and differences are displayed for healthy controls (HC) and SAR. Upper and lower values are provided between parentheses. Results were obtained from a generalized linear mixed model.

		HC	**SAR**	**P-value HC-SAR**
SP	*Avg. NDT (mA)*	0.43 (0.34 – 0.52)	0.69 (0.24 – 1.15)	0.25
	Avg. Slope (mA^{-1})	6.32 (3.57 - 9.07)	3.41 (0.62 - 6.21)	0.015

DP10	*Avg. NDT (mA)*	0.22 (0.18 – 0.26)	0.37 (0.18 - 0.56)	0.1
	Avg. Slope (mA^{-1})	12.48 (8.28 - 16.68)	6.39 (2.32 - 10.46)	0.04*
DP40	*Avg. NDT (mA)*	0.23 (0.20 - 0.27)	0.40 (0.19 - 0.62)	0.1
	Avg. Slope (mA^{-1})	11.71 (7.76 – 15.66)	5.88 (1.90 – 9.86)	0.04*

SP= single pulse, DP10= double pulse with an inter-pulse interval of 10ms., DP40= double pulse with an inter-pulse interval of 40ms. mA= milliampere.
*= significant difference (P<0.05) between two groups based on two-tailed independent samples Z-test

Table 4.10. displays the average NDTs for each group as well as the slopes of the psychophysical curves. For setting DP10 and DP40, the sarcoidosis group's mean slopes are significantly lower than the HC group's. However, there were no significant differences between the two groups regarding the slope in SP and the mean NDTs for all settings.

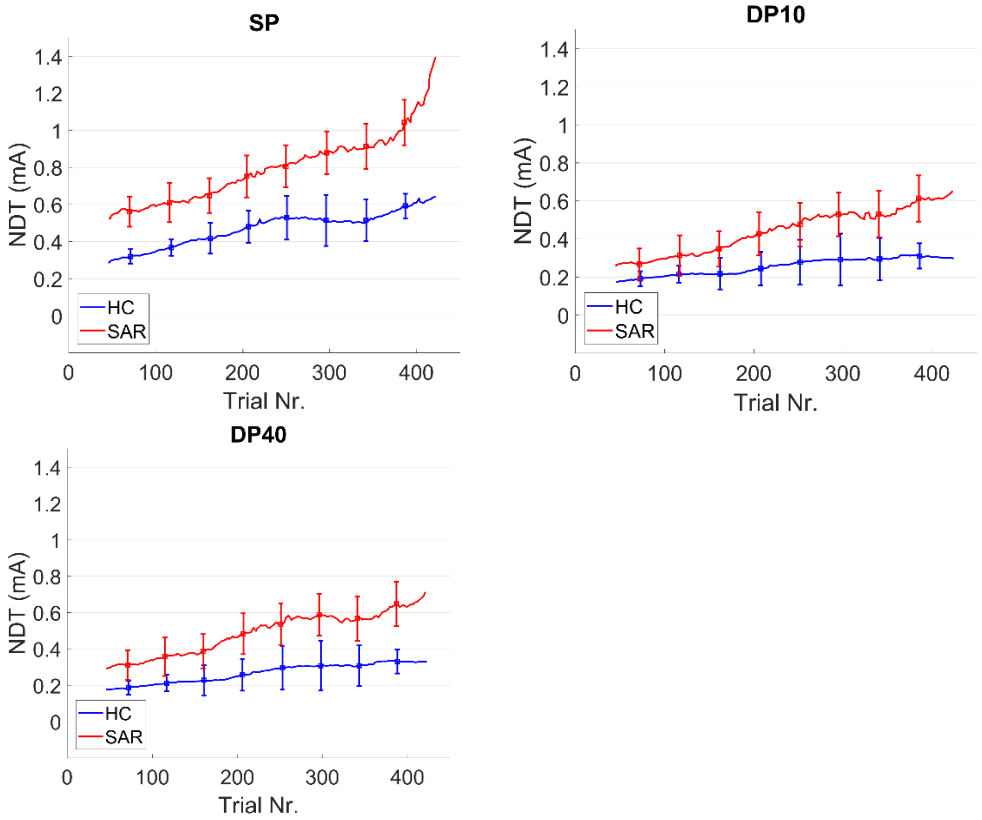

Figure 4.6. Estimated average nociceptive detection thresholds (NDTs) in milliampere (mA) tracked over trial number for (**A**) single-pulse stimuli, (**B**) double-pulse stimuli with an inter-pulse interval of 10ms, (**C**) double-pulse stimuli with an inter-pulse interval of 40ms. Per stimulus type, 150 stimuli were given. In each graph NDTs for the three study groups are visualized: healthy controls (blue) and SAR. Note: NDTs used for statistical analysis differ from these NDTs used to visualize.

The NDTs for the entire study population were calculated using the GLMM described in Eq.1 (i.e., both HC and SAR groups). The NDTs for each setting are depicted in Figure 4.6. Around trial number 380, the NDT of setting SP increases in the SAR group. As shown in Figure 4.6., the angles and amplitudes of settings DP10 and DP40 appear to be comparable between groups, with the SAR group having a higher endpoint. In all settings, it is clear that the two groups differ in terms of nociceptive detection thresholds.

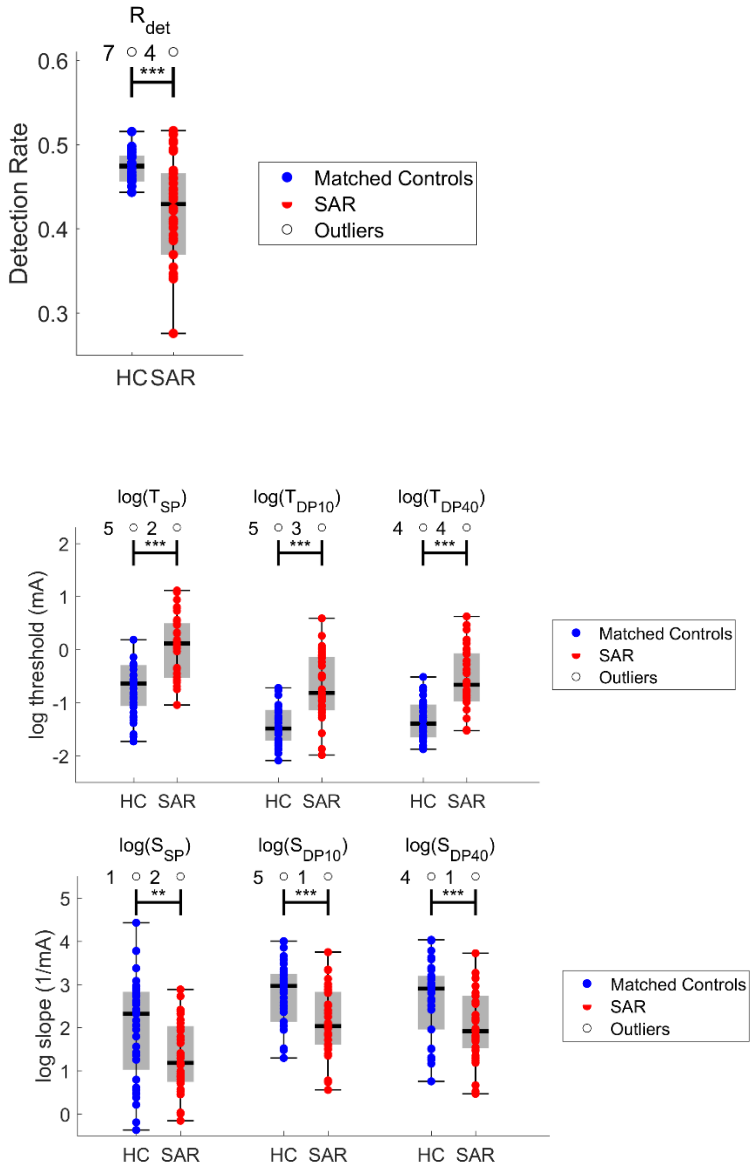

Figure 4.7. Individual psychophysical detection rate and derived detection thresholds and slopes of the psychophysical curve from 19 subjects of each group (i.e., HC and SAR). The detection rate, threshold, and slopes were averaged over the entire measurement. These values were shown for both measurements. Significant altered psychophysical values were seen in patients with sarcoidosis compared to healthy controls (** p <.01, *** p < .001). This was found for all stimulus types.

Figure 4.7. depicts the individual detection rate, thresholds, and slopes of the psychophysical curve.

Individual values varied significantly (p<0.001) across groups. When sarcoidosis was compared to HC, the detection rate was significantly (p<0.001) lower. Sarcoidosis was found to have significantly higher NDTs than HC. In addition, the steepness of the slope was significantly reduced across the groups.

4.1.4. Evoked Potentials

Table 4.11. T-statistics for fixed effects of a linear mixed-effects model of the amplitude of evoked potentials (EPs) for SAR referenced healthy controls (HC) in model 1. EPs followed from intra epidermal stimulations with three types of stimuli. EP amplitude was evaluated at the central component with a latency of 400 ms. (Non-)significant p-values in orange.

Fixed effect	**Interaction**	**Effect size**	**t-value**	**P-value**
Model 1 (HC)				
SAR		-0.28	-0.74	0.47
	Response	-0.28	-0.37	0.72
	Trial number	0.003	2.17	0.03*
	Amplitude SP	-0.29	-0.61	0.54
	Amplitude DP10	-3.57	-5.45	5.34E-08*
	Amplitude DP40	-1.35	-2.19	0.03*

SP= single pulse, DP10= double pulse with an inter-pulse interval of 10ms., DP40= double pulse with an inter-pulse interval of 40ms. The p-value that is statistically significant is <0.05.
*= significant effect between interactions, based on t-statistics for fixed effects of linear mixed models.

The LMM with EEG data from the sarcoidosis group and healthy controls as a reference group is summarized in Table 4.11. The most important findings of this model were significant negative t-statistics for sarcoidosis group interaction with DP10 and DP40 amplitude. This suggests that the sarcoidosis group has smaller EP amplitudes than healthy controls when stimulated with double pulses with IPIs of 10ms and 40ms. When compared to healthy controls, EPs in sarcoidosis did not appear to be affected by the response. The trial number interaction, on the other hand, affects EPs. The grand average EPs derived from the central derivation are shown in Figure 4.8. The sarcoidosis group has shifted earlier latency with all settings.

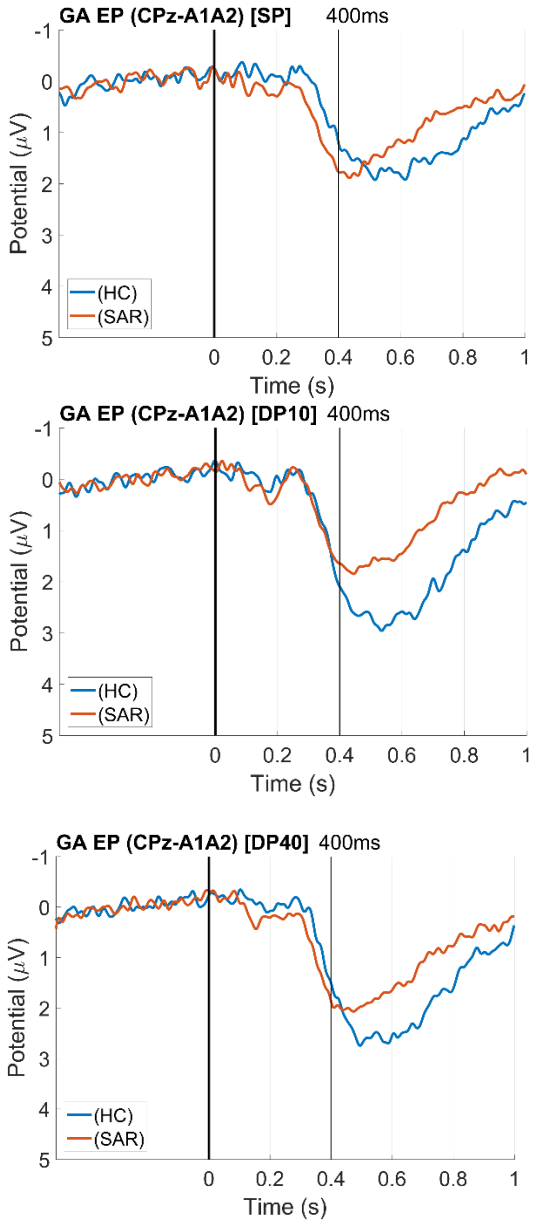

Figure 4.8. Grand average (GA) evoked potentials (EPs) in EEG derivation CPz-A1A2 as a result of intraepidermal stimulation by three types of stimuli: **A**. Single-pulse (SP), **B**. Double pulse with an inter-pulse interval of 10ms (DP10), **C**. Double pulse with an inter-pulse interval of 40ms (DP40). In all panels, EPs for healthy controls (HC) and SAR are visualized. EP amplitude of SAR group was compared with HCs at P400. T-statistics ($P<0.05$) modulated significantly lower EP amplitudes for SAR group compared to HCs for double pulse stimuli with an inter-pulse interval of 10ms and 40 ms.

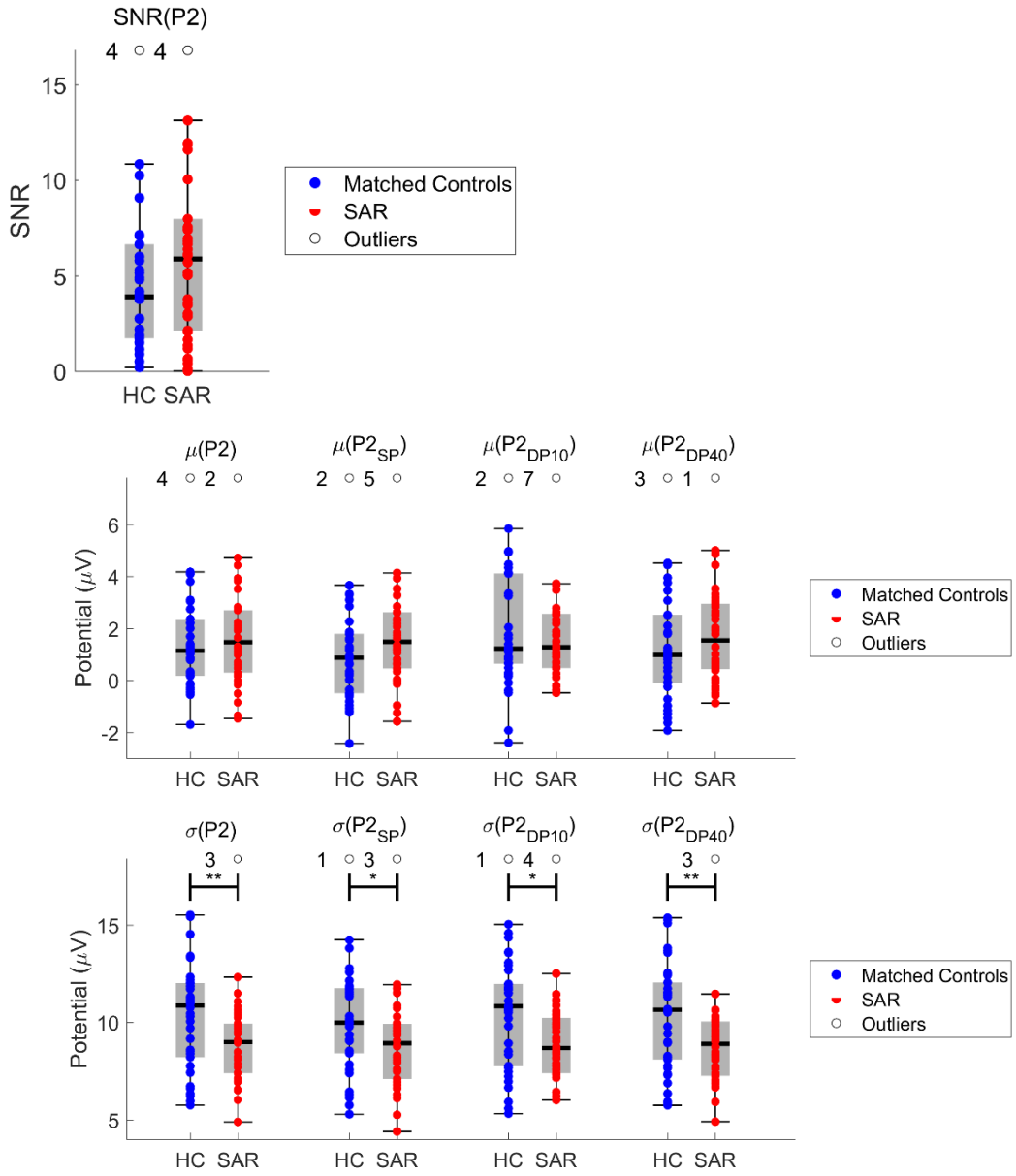

Figure 4.9. Individual EPs and the grand average EP (bold) from 38 measurements (i.e., two measurements per subject) with a P2 latency determined at 450 ms post-stimulus. The mean (μ) and the average standard deviation (σ) of the P2 amplitude did non differ significantly between groups.

The P2 peak was discovered at 400 ms post-stimulus (Figure 4.9). In HC, the SNR of the P2 appears to be reduced, but it was still adequate for EEG analysis. Even though the variation of the EP was significantly reduced in sarcoidosis, the mean P2 did not differ significantly between groups.

5. Discussion

This thesis investigated which outcomes the NDT-EP measurement method produces to stimulate dysfunctional small fibers in a clinical setting. In this regard, a two-part exploratory, prospective investigation was carried out. The first section indicated that CIPN patients do not appear to influence detection probabilities statistically, although they do lower maximum EP amplitudes (EEG derivation CPz-A1A2). The second portion demonstrated that sarcoidosis patients with SFN had different detection probabilities and EP amplitudes (CPz-A1A2) than healthy controls. Furthermore, sarcoidosis patients and healthy subjects had differing stimulus detection probabilities. It does, however, reduce maximum EP amplitudes (EEG derivation CPz-A1A2). These findings point to (1) the method's general applicability and feasibility in sarcoidosis patients and (2) the method's feasibility in this CIPN study group.

Our results are in general agreement with previous work (mentioned below) detection thresholds and evoked potentials as determinants. Since intra-epidermal electrical stimulation (IES) is a relatively new technique, we focus on nociceptive sensitivity to (electrical) stimuli. Due to the dichotomy in the NDT-EP outcomes, the relationship of these determinants to existing research will, therefore, be separated in NDT and EP.

5.1 Part 1: CIPN

In most studies, patients with CIPN have a higher mean age[90–95], comparable BMI[90,92,95,96], and comparable NRS[95] when the disease characteristics of our CIPN population are compared. The mean cumulative Paclitaxel dose in Oyama et al.[94] study is significantly higher than in this study: 1500 mg/m2 versus 801.5 mg/m2, respectively. Other disease characteristics in this study resemble those in Lycan et al. [96]. The mean cumulative Paclitaxel dose is nearly equal in both studies, with 801.5 mg/m2 in this study and 817.3 mg/m2 in Lycan et al. [96]. Furthermore, the number of patients who underwent breast cancer surgery before chemotherapy is comparable in this study and Lycan et al. [96], with 7 (39%) and 10 (50%) patients, respectively. They discovered that the proportion of patients who received radiation and hormone therapy before chemotherapy was higher: 95% and 40%, respectively, compared to 39% and 6%. Furthermore, the mean number of months since the last dose in this CIPN population is significantly higher than that in Lycan et al., at 15.8 months in this study versus 3.8 months in Lycan et al. Both Oyama et al.[97] difference in cumulative Paclitaxel dose and Lycan et al. [96] difference in time since the last Paclitaxel dose could explain why they found significant differences, which were not found in this study.

5.1.1. Interpretation of the NDT results

Looking at the NDTs in Figure 4.2, it is clear that the NDTs for settings 2 and 3 for the HC and CIPN groups are comparable, despite the CIPN group having a higher endpoint. The NDTs in Setting 1 reveal the most differences between the two groups. The CIPN group has a higher initial threshold, a steeper initial slope, and a decrease near trial 200. This curve shape was observed in multiple subjects in the CIPN group, and it was consistent with what was observed during measurements. As a result, this curve represents the CIPN population most likely. The curve shape could be caused by changes in concentration, external disturbances, or pausing the measurement. These possible explanations, however, were not mentioned in the measurement notes.

The detection probability is significant influenced by the number of trials and the amplitudes of all settings. These findings corroborate previous research[86,98,99]. No parameters, however, were found to have a significant impact on detection probability when used in conjunction with CIPN diagnosis or on CIPN diagnosis itself. One explanation could be the number of Aδ-fibers affected by CIPN. According to Gutiérrez-Gutiérrez et al.[100], the longest nerves are the most vulnerable to chemotherapy toxicity. As a result, CIPN symptoms typically begin in the fingers and toes, with Aδ-fiber damage and, consequently, CIPN symptoms progressing proximally[8,100]. While CIPN affects Aδ-fibers in the fingers and toes, fibers in the hand dorsum may be mostly unaffected, and CIPN patients without symptoms in the dorsum of the hand may perform similarly to HCs. However, no analysis was done to back up this claim.

For example, Oyama et al.[97] and Saito et al.[101] have developed methods to gain insight into nociceptive processing in CIPN patients. Oyama et al.[97] used IES to investigate a method for determining the pain threshold of CIPN patients at the dorsum of the hand. The pain threshold was defined as “a sensation felt three times at the same current intensity.” They discovered that there is a significant difference in pain threshold between all grades of CIPN[97]. Saito et al.[101] investigated the ulnar side of the forearm to determine the somatosensory threshold of electrical current perception. The minimum electrical current perceived by a subject is defined as their threshold. It was proposed that CIPN103 could be measured on the ulnar side of the forearm of the dominant hand. The studies by Oyama et al.[97] and Saito et al.[101] had shorter measurement periods and no comparison to HCs. Their measurements are similar to the familiarization phase of the NDT-EP method, in which an initial detection threshold is established. This initial detection threshold appears to correspond to the pain threshold reported by Oyama et al.[97] and the somatosensory threshold reported by Saito et al.[101]. While these initial

thresholds may provide enough insight into the severity of CIPN, the objectivity provided by EEG recordings has been shown to be beneficial in examining nociceptive processing abilities in other patient populations[98,99,102–104].

5.1.2. Interpretation of the EP results

CIPN patients resulted in significantly lower EP amplitudes after applying double pulse stimuli with an IPI of 10ms (Table 4.6). For the other types of stimuli no differences were found. The contralateral derivation revealed no differences. These results are in line with observed smaller EP amplitudes in CIPN patients compared to healthy individuals. Isak et al.[105], where thirty patients with possible CIPN after oxaliplatin or docetaxel treatment were compared to 27 healthy subjects. QST was used on all subjects to assess SF function and laser evoked potentials (LEP). Furthermore, SF-damage was evaluated using cutaneous silent periods elicited by electrical (El-CSP) and laser (Ls-CSP) stimuli. In terms of LEP, N2P2 amplitudes were significantly lower in patients than controls in both the upper (P = 0.007) and lower (P = 0.002) extremities, and the N1 amplitude in patients' upper extremities was significantly lower than in controls. Their results confirm that EPs were (significantly) lower in the upper extremities. However, in this research, LEP was used in the upper extremities instead of IES. Therefore, it is challenging to evaluate our results due to the specific stimulation type. Finally, only the EP interaction with DP10 (CIPN and HC) was significantly lower in our findings.

Instead of measuring on the hand dorsa, Narayanaswamy[106] measured heat evoked potentials on three sites: leg, arm, and face. The results from this study are partly in contradiction with our study. First, from the legs, CHEPS (Aδ) N2-P2 amplitudes were not significantly reduced compared with control values (p=0.4051). Second, CHEPS (Aδ) N2-P2 amplitudes from the arm were significantly reduced from the arm (p= 0.0130) compared with control values. Third, measured from the face, CHEPS (Aδ) N2-P2 amplitudes were not significantly reduced from the face (p=0.0736) compared with control values. However, contact heat evoked potential measurement may not be feasible given time pressures and constraints such as the wearing of wigs post-chemotherapy.

The question remains if there are possible mechanistic explanations for the cause of CIPN including changes to peripheral nerves, which potentially creates aberrant electrical signals to be communicated from the damaged peripheral cells to the brain. Other studies have suggested that central sensitization in CIPN could be caused by a number of mechanisms. Shim et al.[107] hypothesize that cisplatin-induced

mechanical hypersensitivity is caused by peripheral oxidative stress sensitizing mechanical nociceptors. In contrast, paclitaxel-induced mechanical hypersensitivity is caused by central (spinal) oxidative stress maintaining central sensitization that abnormally produces pain in response to Aβ fiber inputs.

Second, according to Boyette-Davis et al.[108], astrocyte activation, rather than microglia activation, contributes to CIPN symptoms in the spinal cord. Activated astrocytes' increased cytokine release contributes to neuropathic pain-like behaviors in mice. In rodents, astrocyte activation with minocycline or gap junction coupling with carbenoxolone can reduce chemotherapy-induced mechanical hyperalgesia.

Third, Nudelman et al.[109] discovered a connection between CIPN symptoms and cerebral perfusion and grey matter density. Frontal grey matter density decreased while cerebral perfusion increased[109]. Smit et al.[110] hypothesized that EEG measurements could be influenced in areas with lower grey matter density. As a result, it may be helpful in the future to monitor frontal EPs in CIPN patients.

5.1.3. Strengths and limitations

Most notably, this is the first study to our knowledge to explore the feasibility of the NDT-EP measurement method and its outcomes in CIPN.

The study's design may have resulted in several limitations. To begin, CIPN patients of all levels of neuropathic pain were invited to participate. As patients in severe pain may have been less inclined to participate, this could have resulted in a bias toward patients suffering from less severe forms. Furthermore, subject heterogeneity may have precluded additional significant findings. Second, because the study was exploratory, an SFN diagnosis was not included in the inclusion criteria. As a result, it was unclear which patients and which bodily extremities were likely to have functional small fiber abnormalities. Hence, considerable uncertainty remains regarding the extent to which group effects reflected dysfunctional small fibers or other factors, particularly for detection probabilities.

Third, outcome measures are related to evoked allodynia/ hyperalgesia and neurophysiological alterations in nerve function (electrophysiological measurements and/or damage to the peripheral nervous system), whereas many patients also report other symptoms such as numbness, tingling and ongoing pain.

Fourth, in order to avoid patient intolerance, we did not apply other tests (i.e., heart rate analysis, sudomotor function tests, or axon flare reflex tests, etc.) to diagnose SF damage. Lastly, a longitudinal rather than cross-sectional study design could have helped us observe the long-term changes of tests assessing SF-damage in CIPN patients.

5.1.4. Recommendations for further research

One suggestion for future research is to make a more apparent distinction between CIPN patients with different characteristics. The inclusion of a diverse group of CIPN patients in this study may have hampered the ability to detect a significant difference between the CIPN and HC groups. If many CIPN groups, each distinguished by a distinct feature, were compared to HCs, a significant difference between them might be discovered. The severity of the CIPN, the outcome of the DN4 questionnaire, and the duration of symptoms are all factors that could be used to make this distinction.

Another area of future research in CIPN patients is the site of stimulation. Symptoms of neuropathy were more common in the feet than in the hands. As a result, it may be worthwhile to investigate whether measuring symptoms in locations other than the dorsum of the hands is feasible and provides more reliable data. However, whether this can be done in conjunction with EEG recordings is unknown.

In a side analysis of the CIPN data with the influence of age, three findings were made that can be included in future inclusion or analysis. 1. It appears that age is highly significant. 2. Adding age does not make diagnosis or dose directly significant. 3. Strict exclusion of outliers makes diagnosis significant for the single-pulse threshold and the double pulse (10ms IPI) slope. Finally, the characteristic age should be carefully considered for future inclusion. Furthermore, there may be a correction in the (statistical) analysis. We choose to match healthy controls in this study, which reduced the difference in characteristics.

Measurement consistency may have been influenced by multiple examiners. Inter-rater reliability was not investigated in this study; however, in future studies, assessing the similarity with which caretakers

are included will ensure that clinical examinations are conducted consistently enough to alleviate concerns about individual rater variability introducing variability during study implementation.

It is critical to keep the clinical need in mind for future research and inclusion in mind. Early detection and assessment of first symptoms is critical for effectively preventing severe CIPN. For patients with CIPN, therapeutic options are still limited, and pharmacological treatment focuses primarily on reducing or relieving neuropathic pain. CIPN is typically treated acutely by reducing or discontinuing the causative chemotherapy, potentially jeopardizing treatment outcome. There is currently no causatively proven therapy for the prevention of CIPN.

As a result, in future studies, it is critical to examine a homogeneous group before, during, and after chemotherapy. Despite the fact that no statistically significant differences in detection probability were discovered, the method is considered feasible in this patient group. We would not, however, recommend this as a first step. We would advise looking at the technique (i.e. measurement site, duration of the experiment, etc.) with an improved inclusion first, and then determining whether these end parameters are actually suitable to extend to other studies.

Research on the pathophysiology of CIPN has focused on peripheral nerves because CIPN symptoms are felt in the hands and feet. However, better understanding the role of the brain in CIPN may accelerate understanding, diagnosing, and treating CIPN. A better balance between maintaining this EP parameter and the duration of the measurements should be found in future research. As previously stated, it is critical to detect (traces of) CIPN early. The question is whether there are already traces of altered central sensitization at that stage. For ease of measurement, we recommend leaving out the EEG measurements at first.

Finally, P300 measurement is commonly used in scientific research to investigate event-related potentials, particularly in decision-making. Because P300 changes are frequently associated with cognitive impairment, the waveform can be used to evaluate the efficacy of various treatments on cognitive function. For these reasons, some have proposed that it be used as a clinical marker. In clinical research, the P300 has a wide range of applications[111]. We would recommend investigating the P300 wave (Cz scalp location) besides the current wavevorms. Studies revealed chemotherapy treated patients having attenuated P300 waveforms compared to untreated controls[112].

5.2 Part 2: Sarcoidosis

5.2.1. Interpretation of the NDT results

Patients with sarcoidosis compared to healthy controls resulted in significantly higher detection probabilities after applying double-pulse stimuli (Table 4.9). Individual psychophysical detection rate and derived detection thresholds and slopes of the psychophysical curve demonstrated significant altered psychophysical values for all stimulus types (Figure 4.7). The results from our study are in line with a study performed by Hoitsma et al.[14]. They performed a study where they found that a substantial number of sarcoidosis patients report non-specific symptoms such as pain, for which no organic substrate has yet been found. They observed symptoms suggestive of small-fiber neuropathy in a group of sarcoids. The study aimed to verify this observation using various electrophysiological tests. Thermal threshold testing (TTT) revealed abnormalities in 51 of the 74 patients. Small fibers may be the cause of several hitherto unexplained symptoms in sarcoidosis.

Many published sarcoidosis with SFN studies used skin biopsy and quantitative sensory testing (QST)[70], which analyzes subjective thermal-threshold testing. Temperature threshold testing is included in quantitative sensory testing (QST). Thermal (cold and warm) and mechanical (tactile and vibration) detection thresholds are used to evaluate small-fiber function (including the central pathways). The cold detection threshold (CDT) examines the A-delta-fiber function, whereas the warm detection threshold (WDT) examines the C-fiber function (WDT). One of QST's major drawbacks is its psychophysical nature. As a result, malingering and other nonorganic factors could be involved[113].

Unlike the methods used in the literature above – we used another method for measuring and data analysis. Discrepancies in methodology, such as other types of stimulation and measurements limited to the lower extremities, should be considered. Nevertheless, it is interesting to consider psychosocial components and physiologic responses to noxious stimuli regarding sarcoidosis patients.

5.2.2. Interpretation of the EP results

Sarcoidosis patients compared to healthy controls resulted in significantly lower EP amplitudes after applying double-pulse stimuli with an IPI of 10ms and 40ms (Table 4.11). For the SP type of stimuli, no differences were found. Furthermore, a latency difference in the Cz-A1A2 potential (SAR vs. HC at SP) was discovered (Fig. 4.8).

An earlier latency of the maximal grand average EP peak (P2) was seen for sarcoidosis subjects. This 'shift' might provide insight into the selectivity of IES stimulation. It has been suggested that Aβ-fibres may be stimulated when high stimulus intensities are used. Figure 4.6 demonstrates the high NDTs after SP stimulation. Mechanical stimulation of non-nociceptive Aβ-fibres with stimulus intensities greater than twice the detection threshold is possible. These fibers have been shown to have earlier latencies. Because the P2 amplitude is still (around) 400ms, the early latency is assumed to be a grand average of stimulated Aβ- and Aδ-fibres. The stimulation of Aβ-fibres may be related to a study that found that reduced Aδ-fibre sensitivity[114].

Another study using the NDT-EP method in DM subjects with and without neuropathic pain also showed earlier latencies in pain-free DM subjects compared to DM patients with neuropathic pain and healthy controls. Others have found LEPS abnormalities in peripheral neuropathies; late LEPs may be absent, amplitude-attenuated, or latency-delayed. The severity of latency and amplitude changes, as well as the degree of hypoalgesia, were found to be related to the loss of small myelinated nerve fibers in sural nerve biopsy[115]. These researches, on the other hand, focused on LEPs.

The amplitude abnormalities discovered were consistent with the findings of Streletz et al.[116]. In 50 sarcoidosis patients, the visual evoked potential to pattern reversal was measured. Latency and amplitude abnormalities were discovered in 15 patients (30%), including all four patients with clinically evident brain disease and four of seventeen patients with overt ocular disease. In 29 patients, there was no clinical evidence of ocular or neurologic disease, and 7 (24 percent) had VEP abnormalities, implying subclinical sarcoid lesions in brain structures at the base of the brain. This study, however, only included sarcoidosis patients without proven SFN.

Gott et al. [117] investigated the impact of multimodality on potential sarcoidosis anomalies. In 25 patients with confirmed multisystem sarcoidosis, multimodality evoked potentials were obtained to determine central nervous system (CNS) involvement with regular CNS evaluation. In 12 patients, evoked potentials were abnormal: 5 had abnormal brainstem auditory evoked potentials (BAEP), 4 had abnormal median nerve somatosensory evoked potentials (SEP), and 6 had abnormal visual evoked potentials (VEP). Contrast-enhanced magnetic resonance imaging (MRI) of the brain revealed no structural abnormalities in two patients with abnormal evoked potentials. Because they can

detect subclinical neurosarcoidosis, multimodality evoked potentials are a valuable supplement to neuroradiology in diagnosing neurosarcoidosis. As a result, in our future research we should rule out neurosarcoidosis and keep in mind the multimodality evoked potentials. To understand the impact of SFN on the EPs, patients with multisystem sarcoidosis should be separated from sarcoidosis patients with SFN.

Finally, Heij et al.[113] addressed sarcoidosis and pain from Small-Fiber Neuropathy. To assess small nerve fibers objectively, laser-evoked potentials (LEPs) and contact heat-evoked potentials (CHEPs) were developed. Both laser and touch heat stimulation activate Thermo-nociceptive cutaneous nerves. Although the amplitude of Laser Evoked Potentials (LEPs) and Contact Heat Evoked Potentials (CHEPs) is influenced by attention and other cognitive processes, these tests provide valuable information about the functional state of nociceptive terminals. The CHEPs are heated to 53°C at a rate of 70°C/s using a thermofoil thermode stimulator. Patients with sensory neuropathy had lower amplitude CHEPs, which corresponded to the findings of other SFN tests[115] and with our study.

5.2.3. Strengths and limitations

Determination by a competent neurologist to have a possibility/likelihood of SFN and an SFNSL score of at least 30 were required for inclusion. As a result, it was clear which patients and body extremities were likely to have functional small fiber anomalies. As a result, there was less uncertainty about whether group effects were caused by defective small fibers or by other factors, mainly regarding detection probabilities.

The main limitation of the sarcoidosis measurements was the absence of sarcoidosis patients without SFN in addition to patients with SFN and healthy controls. This would allow researchers to investigate the possibility of (subclinically) altered small fiber function in people with the same underlying disease as chronic pain patients but without overt neuropathic (pain) symptoms.
Finally, sarcoidosis patients experiencing varying degrees of neuropathic pain were allowed to participate. Patients in excruciating pain may have been less willing to participate, which may have resulted in a bias favoring patients with less severe manifestations.

5.2.4. Recommendations for further research

At the time of inclusion, the duration of pain symptoms in this study group's participants was not used. The clinical need for observing peripheral function is not (entirely) about early detection in sarcoidosis patients. Making the diagnosis provides guidance to the sarcoidosis patient to interpret the daily experienced symptoms. In future studies, it is critical to examine homogeneous groups after different time points following exposure to sarcoidosis with small fiber neuropathy. Pain symptoms and length could be used to discriminate between groups of people with neuropathic sarcoidosis. This should ideally be done in conjunction with C-fiber stimulation to see if it improves the early detection of asymptomatic small-fiber impairment.

Furthermore, a diagnosis of SFN or significant clinical signs of SFN could be used as an inclusion criterion, as well as stimulation in the lower extremities. This modification focuses on the peripheral component of sarcoidosis patients' impaired nociception. Combining inclusion with diagnoses already in use in the clinic could be one way to do so (e.g., QST, autonomic testing, skin biopsy). Finally, future research should concentrate on sarcoidosis patients with acute rather than chronic neuropathic pain, as well as follow-up assessments. By removing the likely influence of (central) neuroplastic changes produced by chronic pain, it may be possible to clarify how recently generated pain symptoms are reflected in method outcomes. Follow-up measures could also reveal if the temporal evolution of these outcomes is linked to clinical worsening or improvement.

5.3. General Achievement and contribution

Most notably, this is the first study to our knowledge to investigate the feasibility of the NDT-EP measurement method and its outcomes in CIPN and sarcoidosis patients. Our results provide valuable information concerning the performance of the method and the phenomena of SFN. Remarkably, it could be inferred for the sarcoidosis group that the NDT-EP approach might complement other diagnostic tools for distinguishing SFN on a group level. This addition supports the idea of combining methods. Surprisingly, for the CIPN group, it was deduced that the NDT-EP approach is feasible (to perform) with the current study group. Furthermore, this insight SFN effect on detection probability and EP values offer an understanding of expanding and selecting future and existing participant groups. This distinctive technique and outcomes of this study may sometimes be considered contributing to the understanding of current phenomena of the peripheral and central nervous system (disorders).

5.4. General limitations/current and future research

Our results are encouraging and should be validated in a larger cohort. Nevertheless, by explorative character, the number of subjects was sufficient to perform a first investigation. It would be beneficial to validate in a larger cohort for a more accurate estimation of the NDT and EP values. At present, results from the (G)LMM are only indications for these specific patients and healthy controls.

Because of the round length measurements, the slightly older individuals had a more difficult time maintaining a high concentration level. As a result, some of the stimuli missed by elderly patients may have been due to insufficient attention. This could have reduced their detection possibilities beyond the influence of pathogenic causes, increasing the likelihood of accidentally discovering major group effects. Another disadvantage was that the IES method first targeted Aδ-fibers. This leaves the question of the role of c-fiber in CIPN and sarcoidosis concerning our measurement setup unanswered. As a result of this disparity, attempts to distinguish between research groups may have been hampered.

Worthwhile would be a more complex calculation of the latency for the EP amplitude. We would suggest a starting point by applying a standard procedure using butterfly plots to estimate the GFP for the entire group. This approach might change the current results and would, therefore, be more interesting for the overall group and corresponding latency.

In the future, care should be taken in selecting or expanding subject groups by variance in characteristics. It is advised that when specific subject characteristics depend on the concerning pathology of interest, a correction in de data analysis should be considered. Especially at present, where the results suggest a combination of the NDT and EP method has the potential for a possible link between the NDT-EP outcomes and subject characteristics such as age, dose, and pain length.

The detection probability decreases as the number of trials increases in Figs. 4.2 and 4.6, resulting in an increase in the detection threshold. Previous studies found this effect to be significant as well, but with a larger effect size[23,86]. When assessing nociceptive processing, it is important to look for altered habituation because it appears to play a role in several types of chronic pain syndromes. The

neurophysiological mechanisms underlying this effect, however, are unknown, and it could be due to a shift in task performance, attention, learning, or neuroplasticity.

As a result, we would want to propose recommendations based on (technical) changes to the NDT-EP method and protocol. This may aid future studies in (better) describing feasibility. Attentional aspects should be included in future studies to gain a better understanding of the NDT-EP method's results. Examine existing EEG data on modulation of the gamma or alpha bands during attentional shifts, for example, or use eye-tracking (an indirect cue concerning covert attention). Shortening the experimental process is advocated to lessen the attentional confounding component, which could help us get closer to clinical adoption. The first thing to consider is reducing the number of stimuli per setting. Furthermore, one sort of stimuli may be excluded from the experiments. It is worth noting that double pulses appear to be more effective at identifying a person's psychophysical state. More research could be done to establish which stimulus setting is less useful. The next stage in the investigation of the NDT-EP technique could be an improved definition for nociceptive stimulus detection. Apart from the detection rate, the maximal stimulus amplitude and the time it takes to obtain this value may be significant in defining the type of fiber stimulated.

Finally, in addition to expanding and altering existing study groups and inclusion and exclusion criteria, it may be worthwhile to investigate the feasibility of the NDT-EP strategy in small fiber neuropathy. Suggestions would be jögren's syndrome (autoimmune disorder), paraproteinemia, and paraneoplastic syndrome[1,118]. In hereditary amyloid neuropathy, small nerve fibers are also affected. Amyloid neuropathies are classified as multisystemic, cardiac-specific, or neuropathy-specific[119].

6. Conclusion

This thesis shows that NDT-EP measurements are generally accurate and thus appear to be feasible in CIPN and sarcoidosis patients with various neuropathic manifestations. They display that patient outcomes differ from healthy controls in terms of latency and amplitude abnormalities and altered nociceptive detection thresholds that are proportional to CIPN and Sarcoidosis. The altered stimulus detection probabilities in these patients, on the other hand, may reflect small fiber dysfunction less instantly. They are thought to describe other group differences, such as attentional levels, central dysregulation caused by persistent nociceptive input, or demographic characteristics. Overall, these findings suggest that (parts of) this method may be helpful in the future search for quantitative diagnostic markers of small fiber dysfunction. Following investigations that outline the influences of potential (disease) characteristics, alternative measurement strategies are considered, and preliminary findings and applicability in a similar (clinical) context need to be further investigated.

7. Bibliography

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