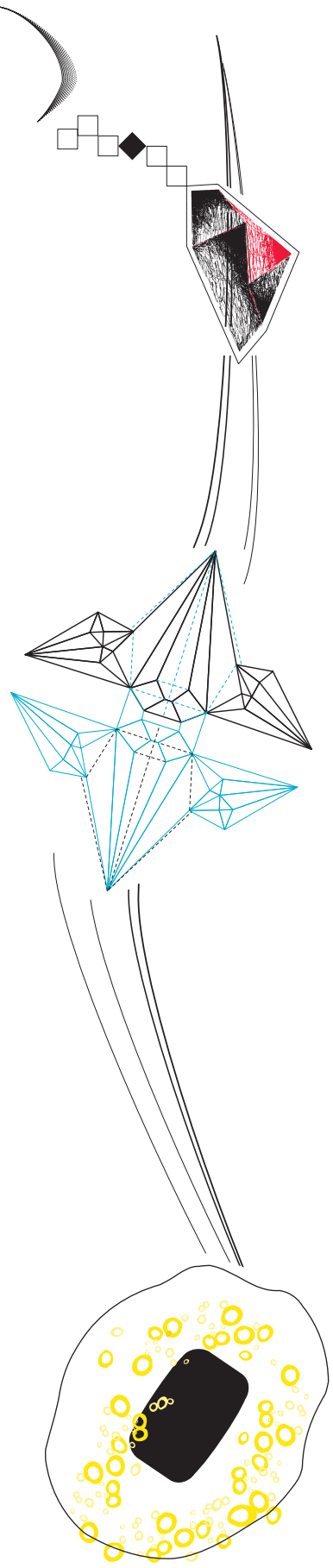

MSc thesis in Technical Medicine

Electrical brain responses during processing of nociceptive stimuli around the detection threshold:

an explorative study in pain-free subjects and failed back surgery syndrome patients

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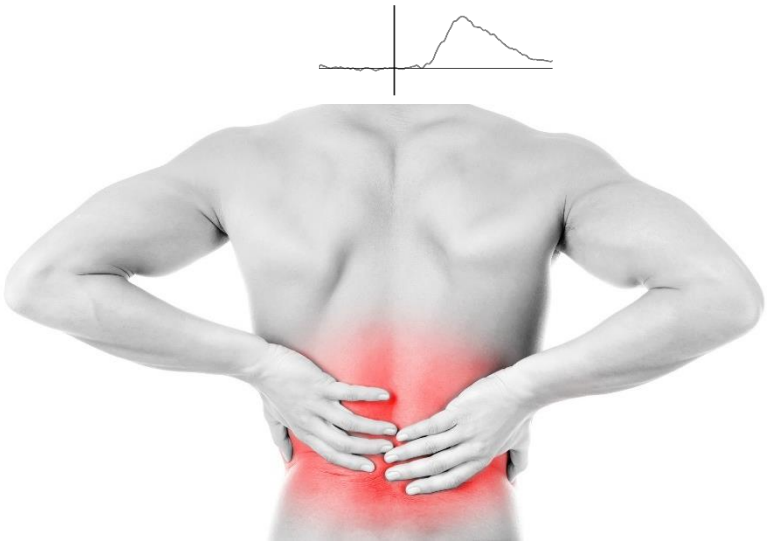

Electrical brain responses during processing of nociceptive stimuli around the detection threshold:

an explorative study in pain-free subjects and failed back surgery syndrome patients

A dissertation submitted to the University of Twente for the degree of

Master of Science in Technical Medicine

by

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UNIVERSITY OF TWENTE.

ZIEKENHUIS ST ANTONIUS

I would like to dedicate this thesis to my family.

Acknowledgements

When I started working on my thesis, my experience with the high-tech experiment for monitoring nociceptive processing was limited to participation in an earlier study about this technique by Boudewijn at the University of Twente. From that moment I was enthusiastic about the possibilities for giving new insights into the neurophysiologic mechanisms in human. Fortunately, I was asked to continue his work to the next step by applying this technique in medicine, knowing that I was to face significant challenges. Apart from being familiar with the technique, I had to prepare the process in detail, including writing a study protocol approved by the medical ethical research committee and leading the study project in St. Antonius Hospital. On the way, I have learned to develop academic skills, but also gained more experience in clinical skills by spending a lot of time in Operation Room. The many possibilities offered by the St. Antonius Hospital was huge. I grew in the role of the technical physician, which is all because I was driven by passionate people around me this year.

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Acronyms

ACC	anterior cingulate cortex
AIC	anterior insular cortex
Ag/AgCl	silver chloride
BMI	body mass index
BPI	brief pain inventory
BSS	biomedical signal and systems
CNS	central nervous system
CRPS	complex regional pain syndrome
CSI	central sensitivity inventory
D	stimulus detection
DN4	Douleur neuropathic 4,
EEG	electroencephalography
EP	evoked potential
eQST	electrical quantitative sensory testing
FBSS	failed back surgery syndrome
GCT	gate control theory
GLMM	generalized linear mixed model
IASP	international association for the study of pain
IES	intra-epidermal electrocutaneous stimulation
IPI	inter-pulse-interval
LMM	linear mixed model
MTT	multiple threshold tracking
MTT-EP	multiple threshold tracking – evoked potential
N1	first negative evoked potential component

N2	second negative evoked potential component
NDT	nociceptive detection threshold
NMDA	N-methyl-D-aspartate
NoP	number of pulses
NPQ	neurophysiology of pain questionnaire
NRS	numeric rating scale
P1	first positive evoked potential component
PAG	periaqueductal gray
PFS	pain-free subjects
pQST	pressure quantitative sensory testing
PW	pulse width
QST	quantitative sensory testing
S1	primary somatosensory cortex
S2	secondary somatosensory cortex
SD	standard deviation
SNR	signal-to-noise-ratio
SP1	single-pulse stimuli
SP2_10	double-pulse stimuli with 10 ms inter-pulse-interval
SP2_40	double-pulse stimuli with 40 ms inter-pulse-interval
StA	St. Antonius hospital
TENS	transcutaneous electrical nerve stimulation
TRL	number of trials/number of received stimuli
UT	University of Twente
VAS	visual analog score
WDR	wide dynamic range

Abstract

Multiple threshold tracking (MTT) has been shown to be effective in measuring the effect of stimulus parameters on stimulus detection. In addition, the evoked potential (EP) has been shown to reflect neurophysiological activity related to stimulus processing. Therefore, a combination of both techniques, known as the MTT-EP experiment, is a promising diagnostic method which may provide objective insight into the processing of nociceptive stimuli. Stimulus-related EPs were recently investigated using the MTT-EP experiment in pain-free subjects at the University of Twente, but its applicability has not been explored yet in a hospital environment and in chronic pain patients.

Firstly, therefore, we explored the replicability of the MTT-EP experiment in twenty pain-free subjects at St. Antonius Hospital. Secondly, we observed the neurophysiological responses during processing of nociceptive stimuli around the detection threshold in seven failed back surgery syndrome (FBSS) patients.

Results show that (initial) NDTs and EPs present profiles and phenomena (such as habituation and paired-pulse facilitation), which are in line with results from the University of Twente. Also, it is seen that the EP is rather modulated by stimulus detection, amplitudes and the number of received stimuli. Strikingly, we found higher NDTs in FBSS patients, in whom we assumed they suffered from a central sensitization syndrome (CSI-score = 49.0), comparing to results of pain-free subjects (CSI-score = 14.6). These NDTs in FBSS patients may implicate that additional facilitating effects occurred in the central nervous system. However, the influence of analgesics is uncertain. Additionally, an early phase component of the EP was found at CPz-A1A2 for detected stimuli, which might indicate that it can be a potential biomarker of brain processing in FBSS patients.

Since similar phenomena in NDTs and EPs were observed during nociceptive stimulation in pain-free subjects at St. Antonius Hospital, it can be concluded that results of MTT-EP experiment can be replicated. Secondly, since an altered behavior of NDTs and EPs seems to be observed in FBSS patients compared to pain-free subjects, it is recommended to continue this study in these chronic pain patients.

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1 Introduction

1.1 Problem statement

Chronic pain is a health issue with a dramatic impact on European society. Since the pathophysiology of malfunctioning nociceptive systems is still poorly understood, roughly one out of five adults suffer from moderate-to-severe non-cancer chronic pain in Europe[1]. Moreover, more than two million people in the Netherlands continue to suffer from chronic pain. Besides the fact that chronic pain has an impact on patient-perceived health status, such as significantly affected everyday activities, personal relationships and depressive symptoms, it also entails a significant economic burden on society. Annual costs are about 20 billion euros and. Notably, 40% of chronic pain patients do not receive adequate treatment for their pain[2]. This emphasizes the importance of improving treatments for chronic pain syndromes. Current therapies are mostly based on symptom spreading and are made to provide pain relief. However, this approach is rarely successful[3]. More important, there is a lack of reliable methods monitoring nociceptive processing which can explain the nature of chronic pain disorders objectively. Conventional pain monitoring is based on subjective pain reports, analgesic intakes or questionnaires (e.g. DN4, NPQ or CSI). These methods are limited to clinical diagnostics because changes in neuroplasticity are not recognized. Early detection of maladaptive mechanisms in the nociceptive system should decrease the inadequate treatments. Consequently, identification of nociceptive system properties enables mechanism-based monitoring of chronic (low back) pain disorders.

For this reason, we need to investigate possibilities to improve prevention, diagnosis, and treatment of chronic pain. How and why chronic pain is caused and maintained is often unclear. Therefore, it is important to study the underlying mechanisms (central and peripheral), and how they are altered in chronic pain patients compared to healthy subjects. Clinical studies reveal that a changed sensitivity can be interpreted as an important contribution of central sensitization to chronic pain patients[4]. Exploring objective biomarkers for central sensitization will be extremely helpful. One major obstacle is the lack of an objective measure of central and peripheral sensitization. Recently, a method for measuring nociceptive detection thresholds (NDTs) has been developed. These are stimulus amplitude thresholds for a detectable sensation, which use intra-epidermal electrocutaneous stimulation (IES) of the skin. IES preferentially activates nociceptive nerve fibers in the superficial skin (pin-prick sensation at detection level) without initial activation of tactile nerve fibers (a non-painful sensation at detection level). Therefore, IES can be used to estimate pain sensitivity measuring the NDT. The NDTs can be determined using a multiple threshold tracking (MTT) algorithm, which was developed in earlier studies[5,6]. Tracking NDTs can facilitate the investigation of the underlying mechanisms of sensitization. The paradigm has been used to determine NDTs with single and multiple pulses[5], demonstrate the sensitivity to short-term changes in nociceptive processing[6], demonstrate a variety of the NDT related to stimulus parameters[7], and measure the effect of capsaicin-induced peripheral sensitization on the NDT[8]. Since MTT measures the subject's psychophysical response, it does not provide a completely objective measure of nociception.

A possible objective measure of nociception related activity in the central nervous system (CNS) is electroencephalography (EEG). Multiple-trial averages of this EEG signal, referred to as evoked potentials (EPs), have been shown to be sensitive to changes in stimulus parameters such as the number of pulses[9,10] or number of trials[11]. Firstly, MTT has been shown to be effective in measuring the effect of stimulus parameters on stimulus detection. Secondly, the EP has been shown to reflect neurophysiological activity related to stimulus processing. Therefore, a combination of both techniques, known as the MTT-EP experiment, might provide insight into the relationship between neurophysiological activity and nociceptive stimuli. Recently, this relationship has been investigated in a study from the Biomedical Signals and Systems (BSS) research group at the University of Twente. That study showed that components of the EP were closely related to the stimulus detection and stimulus amplitudes[12]. The next step is to investigate the applicability of the MTT-EP experiment in a hospital. We need to explore if these results are a replication of results from university lab and observe if the experiment is feasible to be performed by patients.

1.2 Research objective

Therefore, the aim of this explorative study is (1) to explore whether results of the MTT-EP experiment in pain-free subjects at St. Antonius hospital are a replication of results in pain-free subjects at the University of Twente and (2) to observe neurophysiological responses during processing of nociceptive stimuli around the detection threshold in chronic pain patients.

1.3 Thesis outline

Organization of this thesis is as follows. The background chapter introduces the clinical aspects of pain, the (patho)physiology and explanation of central sensitization. We focused on one specific type chronic pain patient, namely the FBSS patients who are scheduled for neuromodulation therapy. The underlying pathophysiology of these patients is convenient to discover for this study because we expect that these patients are suffering from central sensitization. Additionally, this population is often seen by a pain specialist at the outpatient pain clinic of St. Antonius Hospital. The MTT-EP experiment from the University of Twente will be translocated to the hospital to give insight into nociceptive processing in both pain-free subjects and FBSS patients. Therefore, background information about the MTT-EP experiment elaborates the technique as an observational method for nociceptive processing. Finally, the implication addresses the hypothesis and operational research questions. The subsequent chapter discusses the subjects, materials, and methods applied in this study including the procedure of the experiment, followed by a description of the (offline) data analysis. Findings of this study are described in the next chapter. Results of neurophysiological responses in pain-free subjects and FBSS patients are represented. The next chapter discusses whether results of the MTT-EP experiment in pain-free subjects at St. Antonius Hospital are a replication of results from the previous study. Also, neurophysiological effects observed in chronic pain patients are related to other research and a possible interpretation of mechanisms is given. This chapter ends with strengths and limitations. The following chapter answers the research questions and describes a conclusion of this study. This thesis concludes with strategic recommendations and future perspectives.

2 Background

2.1 Pain

Pain is defined as 'An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage', according to the International Association for the Study of Pain (IASP)[13]. It plays an important role in normal defense mechanisms, warning of potentially damaging environment actions and initiating behavioral strategies[14]. Pain is always subjective and is best regarded as an experience involving both a physiologic sensation and an emotional reaction to sensation[13,14].

Pain can be categorized into nociceptive, neuropathic and mixed pain. Nociceptive pain is pain arising by activation of specialized peripheral sensory receptors (nociceptors)[15]. Neuropathic pain is pain initiated by a direct consequence of a lesion or disease affecting the somatosensory system[15]. If both nociceptive and neuropathic pain occur in the same patient, it is known as mixed pain.

2.1.1 Nociceptive processing of pain

Nociception is a primary physiologic mechanism of pain, which consists of the process of transduction, transmission, central modulation and perception (Figure 1)[14]. Processing of pain signals is a complex process in the nociceptive system, which is roughly regulated by ascending and descending pathways. The route of signals from peripheral nociceptors through the spinal cord going up to the brain are referred to as the ascending pathway. Neurons of the descending pathway are connected from the brain stem through the spinal cord to the dorsal horn. While the ascending pathway is responsible for transmitting the pain signal up to the brain, the descending pathway is responsible for controlling and inhibiting the ascending pathway essentially.

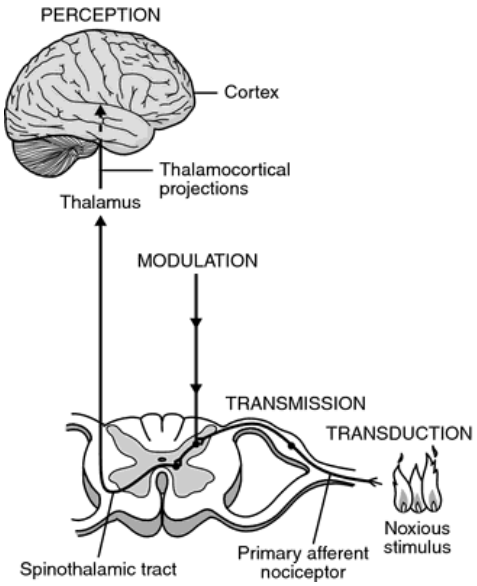

Figure 1. In nociceptive aspect of pain perception, damaging (noxious) stimuli are detected by nociceptors. Free nerve endings of the nociceptors can transduce noxious stimuli into electrical signals, which are transmitted to the dorsal horn of the spinal cord[16]. From here, second-order neurons are activated by released neurotransmitters. The pain signal crosses the spinal cord to the contralateral side and travels up the spinothalamic tract to the thalamus. Then, the third-order neuron is activated, which relays the signal to the somatosensory cortex, to percept pain. Descending pathways modulates the excitatory activity of the ascending components.

Peripheral processing of nociceptors

The first process of nociception is transduction by which noxious thermal, mechanical or chemical stimuli are selectively converted to electrical signals in the nociceptors. The specialized free nerve endings reside mostly in the epidermis and function to protect tissue to injury. Nociceptor stimulus thresholds have a relatively high threshold of activation. Pain receptors can be activated by several neurotransmitters (acetylcholine, serotonin, histamine, bradykinin, substance P, cholecystokinin, adenosine, glutamate, bombesin, neuropeptide-Y, prostaglandin E and endogenous opioids) and other influences (acidity and temperature). Activation of afferent nociceptors results in generating action potentials transmitting to their synapses in the dorsal horn. This second process is also called a transmission of information. Nociceptors are responsible for transmission from the periphery to the spinal cord. These first-order neurons are classified into unmyelinated C-fibers and finely myelinated Að-fibers, with small diameter axons (2 – 5 µm and < 5 µm, respectively)[17,18]. C-fibers have a conduction velocity of 0.5 to 2 m/s, which characterize as a slow, diffuse, dull and aching pain sensation. Að- fibers have a conduction velocity of 5 to 15 m/s and show a rapid, pricking and well-localized pain sensation[18].

Dorsal horn neurons

Cell bodies of both afferent nociceptive fibers are included in the dorsal root ganglia, which contain connections to synapses with dorsal horn neurons in the grey matter in the dorsal horn. The dorsal horn is a complex relay station, which can be seen as the first decision point. Once the nociceptive signal arrives the dorsal horn of the spinal cord, transduction of the first-order neuron to the second-order neuron takes place by neurochemistry. Several neurotransmitters are involved in this process, such as cholecystokinin, substance P, glutamate and γ-Hydroxybutyric acid (GHB). The N-methyl-D-aspartate (NMDA) receptor plays an important role in transduction as well[18].

The dorsal horn is divided into several laminae with multiple interconnections (Figure 2). C-fibers terminate in lamina I and II (substantia gelatinosa of Rolando), whereas Að-fibers terminate in lamina I and V and non-nociceptive Aβ-fibers terminate in lamina III to V[17–19]. Dorsal horn neurons are either classified as nociceptive-specific neurons, wide dynamic range (WDR) neurons, low threshold mechanic (LTM) neurons or interneurons. Nociceptive-specific neurons synapse with Að- and C-fibers in lamina I, WDR neurons synapse with Að- and Aβ-fibers in lamina V. LTM neurons synapse with (tactile) Aβ-fibers in laminae IV. Interneurons are situated in and connected with all laminae and receive input from afferent fibers, as well as from descending pathways. Interneurons can be subdivided into exciting and inhibiting interneurons, which influence e.g. other interneurons (processing) or ascending neurons (sensation)[20]. Descending fibers terminate in (several laminae of) the dorsal horn, which modulate pain signals by inhibition[21].

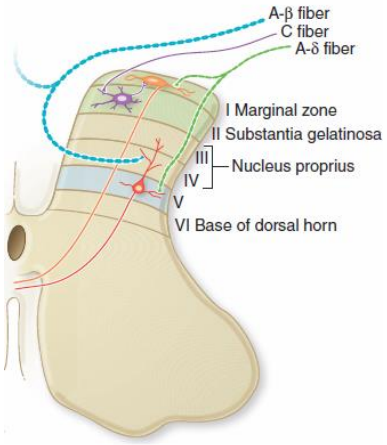

Figure 2. Nociceptive fibers terminate in the Laminae of Rexed. Adapted from The McGraw-Hill, 'Adams and Victor's Principles of Neurology', 11th edition (2015).

Central modulation

Subsequently, the nociceptive process is continued by central modulation. The dorsal horn is an important area in the CNS, which can be determined as a gate control system (GCT)[22]. This GCT, described by Melzack and Wall in 1965, supposes that a network of nociceptive neurons in the dorsal horn can modulate sensory input and therefore influences the transmission of pain signals to the brain. They describe in a simplistic view that activation of inhibitory interneurons in the substantia gelatinosa can be caused by stimulation of non-painful large afferents (Aβ-fibers) that would suppress transmission in small afferents (C-fiber)[18]. This mechanism could describe why rubbing the painful area decreases pain.

Ascending spinal tracts

The spinothalamic tract is the main second-order neuron which is responsible for carrying the nociceptive signals for pain and temperature to higher centers of the brain (Figure 1). This tract is located in the anterolateral white matter of the spinal cord[18]. The spinothalamic tract consists of a lateral (neospinothalamic) and medial (paleospinothalamic) tract for fast and slow pain, respectively.

Supraspinal centers

Supraspinal centers are reached using parallel distributed systems. One of the centers is the reticular formation consisting of complex core groups at the brainstem. This area is important for pain experience, as nociceptive input has a deep effect on reticular activity. It plays a role in consciousness and arousal, autonomic functions and pain suppression via the periaqueductal gray (PAG) matter of the midbrain. Besides pain suppression via descending modulatory tracts, PAG delivers ascending projections to the hypothalamus and thalamus. The thalamus is the key area for nociceptive processing, which serves as relay point. The tracts terminate in their respective lateral and medial located thalamic nuclei and from here the neurons project to regions of the cerebral cortex[14,18,23]. The amygdala is an important part of the limbic system. Also, the hippocampus, septal nuclei, preoptic region, hypothalamus, and some thalamic parts belong to limbic structures. The limbic system supports functions including emotion and motivation, which determine purposeful behavior[14].

Cerebral cortex

The primary somatosensory cortex (S1) has a prominent and highly modulated role for perception of pain[24]. Also, the secondary somatosensory cortex (S2), insula, orbitofrontal cortex, dorsal-lateral prefrontal cortex, extended amygdala, and cingulate cortex activate by painful stimuli[14,18,23]. The S1 is build up by somatotopic organization following Penfield's homunculus pattern[23]. Note, that activation of the cortical area is related to the origin of the peripheral nociceptive stimulus location.

Descending modulatory pathways

Descending modulatory pathways are important for pain modulation. This modulatory system originates in the somatic sensory cortex, hypothalamus, amygdala, midbrain PAG, raphe nuclei, and other nuclei of the rostral ventral medulla[25]. The PAG is the primary control center for this inhibiting system. From here, tracts are connected to the dorsal horn via parabrachial nucleus,

medullary reticular formation, locus coeruleus, and raphe nuclei. Serotonin and noradrenaline are key neurotransmitters involved in descending inhibition. Descending tracts can be activated by endogenous opioid peptides, such as enkephalins and β-endorphins. Activation of these tracts results in a decreased release of substance P, and therefore inhibition of pain signal transmission.

2.2 Pathophysiology of pain

2.2.1 Chronic pain

Chronic pain is described as that pain that persists beyond the normal healing[13,26]. It is characterized by an enhanced perception of pain to a nociceptive stimulus (hyperalgesia) and the novel perception of a normally innocuous stimulus as being painful (allodynia)[16]. Chronic pain is a pathophysiological function of the peripheral and/or central sensory pathways, which results in an altered sensitization. The exact pathophysiology underlying chronic pain problems are largely unknown. The prevalence of chronic pain depends on when, where and how it is measured. Three months are often taken as the point beyond the normal healing. The back is the commonest location of chronic pain. A large survey of chronic pain in Europe shows that nineteen percent of the responders suffer from pain for more than six months[2]. The impact of chronic pain is often determined by extent and duration. It is correlated with poor (psycho)physical and social aspects of health. General factors associated with chronic pain are female gender, increasing age, acute uncontrolled pain and deprivation of household income, education and cultural- and geographical properties[27].

2.2.2 Central sensitization

Since the early 1980s, it was discovered that central sensitization plays a role in chronic pain. After decades of research, Latremoliere and Woolf defined central sensitization as '*an enhancement in the function of neurons and circuits in nociceptive pathways caused by increases in membrane excitability and synaptic efficacy as well as to reduced inhibition and is a manifestation of the remarkable plasticity of the somatosensory nervous system in response to activity, inflammation, and neural injury*'[28]. In other words, central sensitization is use-dependent plasticity of neural signaling within the CNS that is associated with development and maintenance of chronic pain[4,29] (Figure 3). It is suggested that the central sensitization is altered in chronic pain patients, such as FBSS patients[4].

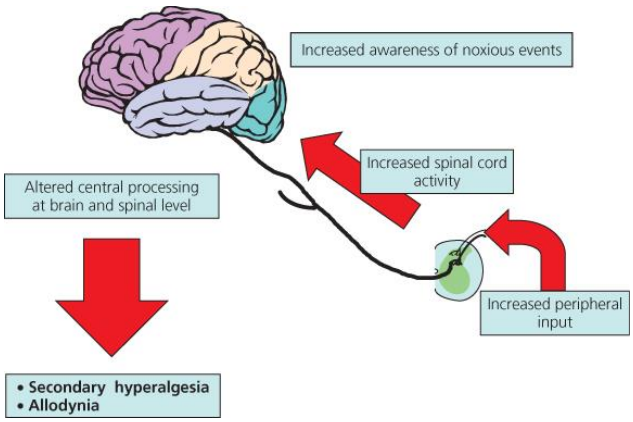

Figure 3. A schematic overview of the term central sensitization.

2.2.3 Failed back surgery syndrome

Chronic low back pain is a socioeconomic burden with a prevalence in general adult population of 37% and a lifetime prevalence of between 60% and 80%[30,31]. Increasing rates of spine surgeries have increased the number of patients suffering from FBSS. FBSS is a diagnosis that describes persistent or recurrent low back pain following spine surgery[31,32] and has a prevalence of 10-40%[33]. Patients suffering from FBSS are supposed to have an altered central sensitization forced by constant stimulation of nociceptive circuits[3,4,33–36]. Recent literature shows that the sympathetic nervous system is overstimulated in FBSS patients[37]. This may be a contributing factor in maintenance of pain. Although the complex pathophysiology is poorly understood, it involves both nociceptive and neuropathic factors[38]. One of the mechanisms involved is the abnormal ectopic activity in neurons, caused by disturbed expression and distribution of ion channels[3,39]. Another change is the loss of inhibitory mechanisms, resulting in increased activity of second- and third-order neurons[3].

Many of these patients are seen by pain specialists every year at the outpatient pain clinic of St. Antonius Hospital. Symptoms of (low) back pain radiating to the leg(s) are common for FBSS patients. They are often treated by strong opioids, such as morphine and pregabalin, because other interventions and pharmacologic strategies have failed. Since FBSS is a complex condition, it is difficult to treat. These patients are often suffering multiple years from pain. Some of them are eligible for neuromodulation, which is seen as a last-resort treatment. However, this does not work always effective for everyone. In practice, there are about 60 FBSS patients out of 120 chronic pain patients scheduled for neuromodulation each year, of which not all of them are new because these interventions include also battery replacements or retreatments due to broken leads or infections. Nevertheless, the waiting list is between three and six months. Previous experience at St. Antonius Hospital shows that this category is very helpful to contribute to clinical research. These FBSS patients have tried all the possible treatments and understand how important it is to investigate the underlying pain mechanisms.

2.3 Observation of nociceptive processing

To understand the pain mechanisms, quantification of pain perception is needed. Unfortunately, there is no ‘golden standard’ available for determination of pain. For clinical application, pain perception is currently often observed by visual analog scores (VAS) and numeric rating scales (NRS). However, it is hard to quantify exactly pain perception, because of the subjective nature. Questionnaires are often used for clinical application. For example, the Brief Pain Inventory (BPI) is a pain questionnaire used to evaluate the severity of pain and the impact on daily life.

A more focused questionnaire is the central sensitization inventory (CSI), which is a screening document to identify patients with central sensitization syndromes[40]. The severity of central sensitization was determined by a scoring system resulted from 25 closed questions related to central sensitization. The subject has to answer ‘Never’, ‘rarely’, ‘sometimes’, ‘often’ or ‘always’, which is scored by a number ‘0’, ‘1’, ‘2’, ‘3’ and ‘4’, respectively. The total sum quantifies the level of sensitization, in which a cutoff score of 40 is used for a central sensitization syndrome. Severity ranges are defined as follows: Subclinical = 0-29; Mild = 30-39; Moderate = 40-49; Severe = 50-59; and Extreme = 60-100.

2.3.1 Psychophysical methods

While questionnaires are useful, psychophysical methods provide more objective information about nociceptive processing of pain. Quantitative sensory testing (QST) is an upcoming method for quantifying changes in somatosensory neural function. This psychophysical method can be subdivided into pressure QST (pQST), electrical QST (eQST) and nociceptive detection threshold (NDT) measurements. Each method is based on stimulation of specific peripheral nerve fibers. However, NDT measurements are mainly usual for quantifying nociceptive pain processing in clinical research. NDT experiments are used in combination with IES of the skin, in case of nociceptive nerve fiber stimulation.

2.3.2 Stimulation of nociceptive pathways

Intra-epidermal electrical stimulus electrode

Nociceptive fibers can be activated by IES applied using an IES-5 electrode, which contains an array of 5 micro-needles (Figure 4)[41]. These electrodes protrude only 0.2 mm through the stratum corneum of the skin. The electrodes do not penetrate the epidermis and are therefore considered non-invasive. Such a superficial intrusion in the epidermis permits specific activation of superficial (Aδ) nociceptive skin fibers, which has been shown by Inui et al.[42,43] and confirmed independently by Mouraux et al.[44]. Making use of this specificity, Steenbergen et al. have been using BiModEl electrodes (similar to the IES-5 and also produced at the University of Twente), to study the somatosensory topography of Aδ fibers in human subjects[45–47]. Similarly, Doll et al. have been using the IES-5 electrodes to characterize peripheral and central changes of the nociceptive system with respect to stimulus parameters[6,7]. Also, van den Berg et al. have been using the IES-5 electrodes for analyzing stimulus-related evoked potentials around the nociceptive detection threshold. The IES-5 electrode is a medical accessory of the stimulator, which supplies stimuli to the electrodes.

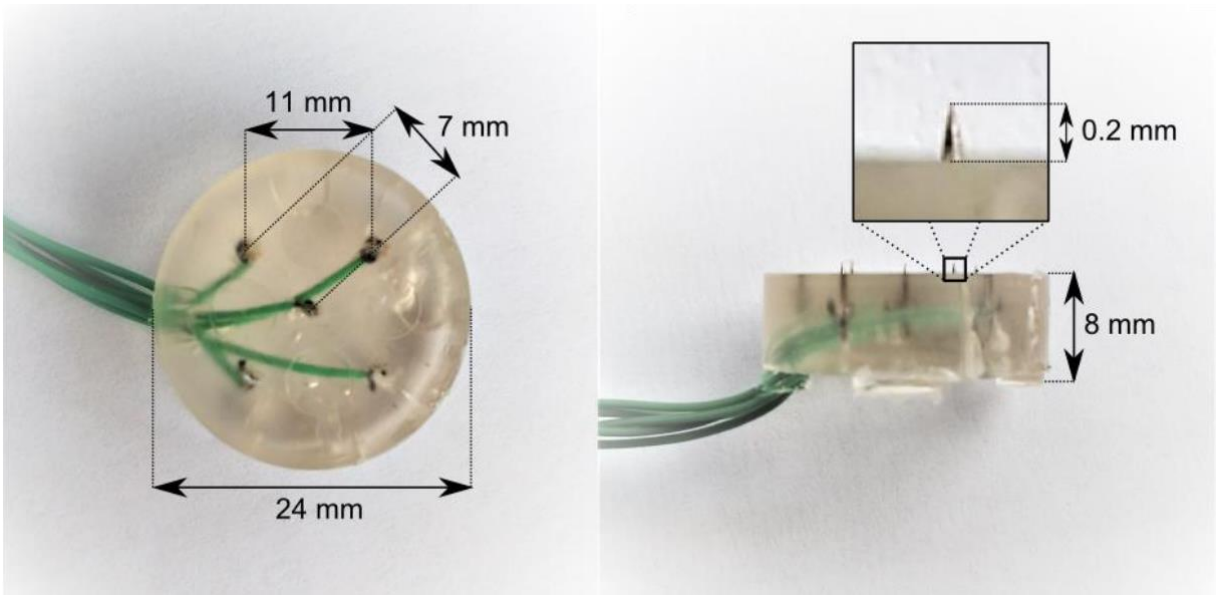

Figure 4 IES preferentially activates nociceptive nerve fibers in the superficial skin (pin-prick sensation at detection level) without initial activation of tactile nerve fibers (non-painful sensation at detection level). Therefore, IES can be used to estimate pain sensitivity measuring the NDT.

Stimulator

The stimulator is an AmbuStim 1-channel stimulator, developed and thoroughly 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. In addition to registering stimulus and threshold data, all communication between software and stimulator is logged.

2.3.3 Psychometric curve

The psychometric curve (Figure 5,) was estimated by the stimulus-response pairs from the experiment, which was based on the psychometric function. The psychometric function is a constructive model applied for psychophysical data. It explains the relationship between electrical stimuli and the responses of the subject expressed in detection probability. This model provides information about detection thresholds related to different stimulus settings.

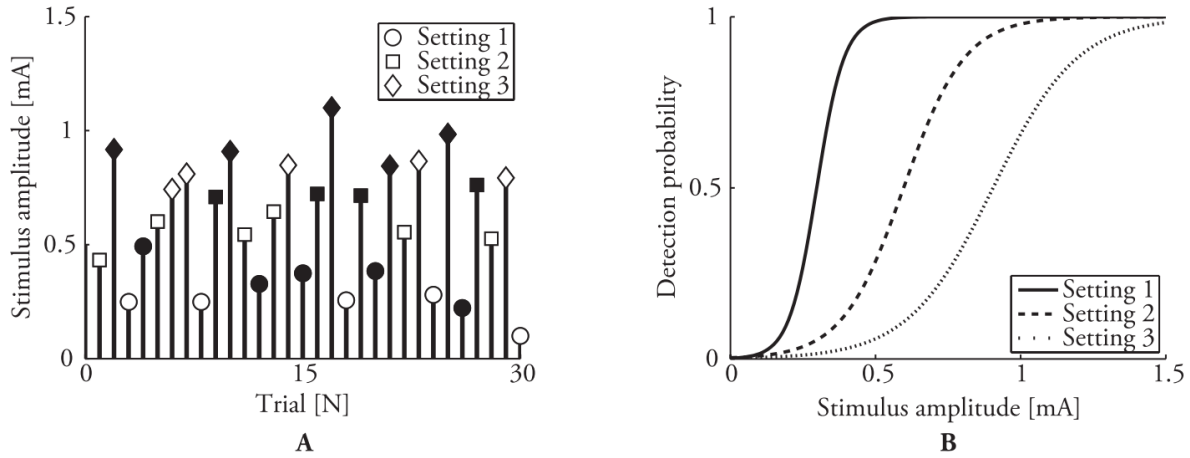

Figure 5. A). Detected (closed marker) and undetected (open marker) stimuli can be shown for each stimulus setting, when the stimulus was perceived or unperceived, respectively. B). The psychometric curve was estimated by the stimulus-response pairs from the experiment. Adapted from Doll et al. (2016).

2.3.4 Multiple threshold tracking

Stimuli can be selected using the multiple threshold tracking (MTT) procedure, which is developed by Doll et al. in the BSS group at the University of Twente[5,6]. Doll et al. introduced the possibility to include different combinations of stimulus properties (setting 1, 2 and 3). The settings consist of different temporal properties (i.e. pulse width (PW), number of pulses (NoP) and IPI), which lead to different threshold tracks (Figure 5). In fact, this method was based on a new adaptive stimulus selection procedure, described as a random staircase procedure. The stimulus selection in this MTT procedure works as follows: the set stimuli (setting 1, 2 and 3) are randomly selected from a predefined set of stimulus amplitudes and applied in random intermingled order to the subject. If the stimulus was unperceived, the amplitude of the stimulus setting increased by a fixed step, whereas it decreased after the stimulus was perceived[48]. Doll et al. has researched that the MTT procedure has benefits over other methods. Therefore, it was recommended to track the nonstationary nociceptive thresholds using the random staircase procedure in combination with logistic regression[5]. The MTT procedure is intended to decrease observer and subject bias by varying the stimulus type randomly.

2.3.5 Nociceptive detection threshold

NDT values (Figure 6) can be determined using the MTT paradigm by detecting the subject's response (detected or not detected) to multiple stimuli with different amplitudes and subsequently derived using the psychometric function. The stimulus amplitude with a detection probability of 0.5 is generally used as NDT value in clinical research. Values of the NDT estimate the degree of pain perception and is a psychophysical parameter reflecting nociceptive processing.

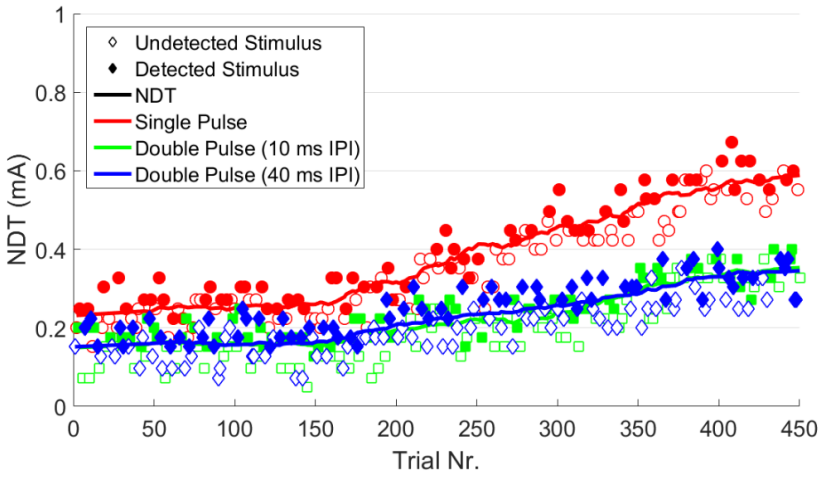

Figure 6. Nociceptive detection thresholds from 450 (150 of each setting) electrical stimuli applied to a pain-free subject at the University of Twente.

2.4 Electro-encephalography

2.4.1 Physiology

The physiology of the EEG is based on voltage differences across cell membranes. Neurons and myocytes are specialized to generate rapidly voltage differences by opening and closing ion channels. Neurons have a resting membrane potential between approximately -50 and -70 mV. This is caused by concentration gradients of sodium, potassium, and calcium across cell membranes, and semipermeable membrane properties. Communication between neurons is mainly driven by chemical synapses, in which neurotransmitters interact with ionotropic receptors. Neurons are excitable cells, in which the membrane potential can be modified by activity of voltage-dependent ion channels or interactions of neurotransmitters. In case of sufficient voltage change, an action potential can be generated. Electrical rhythms occur in multiple spatial scales by neuronal interactions. The EEG reflects mainly activity of cortical pyramidal cells, because of countless partly synchronous excitatory and inhibitory postsynaptic currents that give rise to voltage differences between 10 and 100 µV on the skull[49].

Electrical activity of the EEG signal can be determined by an electrical dipole consisting of three characteristics: (1) the dipole is localized at a specific location in the brain, (2) has a specific size and (3) has a specific direction, which is given in a three-dimensional space[50]. A dipole can be described by a potential field, which overviews the temporary field strengths. These potential fields can be determined by analyzing the derivations of the EEG. Derivation of the EEG signal is characterized by a difference in measurement of bio-electricity. Voltage differences between channels describe a potential field.

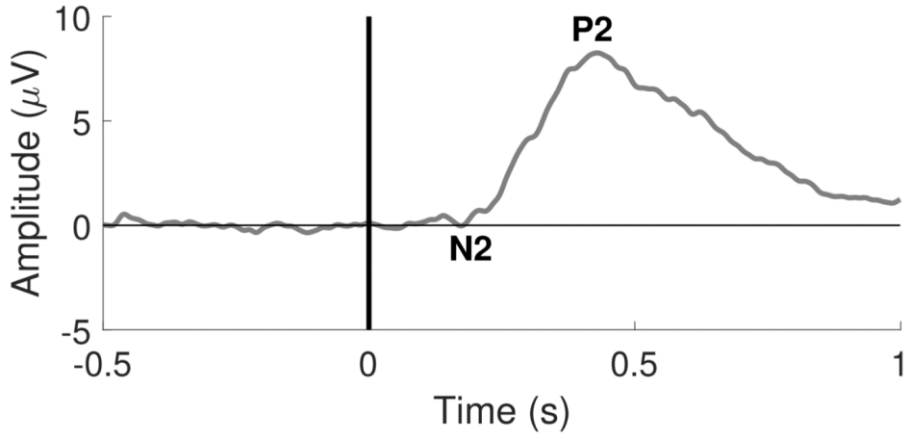

Figure 7. Grand average evoked potential from 12 pain-free subjects at the University of Twente. The evoked potential was derived at CPz-A1A2 (with a band-pass filter of 0.1 Hz to 40 Hz) in response to a nociceptive stimulus. Adapted from B. van den Berg (2018)[12].

2.4.2 Evoked potentials

An evoked potential (EP, Figure 7) is defined as a time-locked neurophysiological signal in response to a stimulus of peripheral nerve fibers. EPs are objectively physiological markers derived from EEG signals, which can be used for clinical purposes (sensor and motor systems). EPs can be characterized by waveform amplitude (μV) and latency (ms)[51]. The EP is a psychophysical parameter reflecting nociceptive processing. Each peak (positive or negative) is given a letter (P or N) and a number (1, 2, 3, etc.) in its name. The number describes the order of the peak. For example, P1 is the first positive wave and N2 is the second negative wave. Another way to describe peaks in the EP is by its latency. For example, P100 is a positive wave at a latency of about 100 milliseconds and N100 is a negative wave. Peak to peak and interpeak changes can reflect clinically relevant characteristics.

Lee et al. explained characterizations of EPs during nociceptive stimulation which pain emerges. Findings show that three components are clearly identified: N1, N2, and P2 waves. N1 is suggested to illustrate the early stage of sensory processing. In this stage, latency and amplitude of the perceived and unperceived laser stimuli are similar. Therefore, it is thought that stimulus perception do not occur this phase and probably reflects deeper brain area[52]. N2 and P2 waves reflect later stages of sensory processing. These waveforms perceived the cortex and explained the conscious perceptual outcome of the nociceptive stimulus. N2 and P2 waves originate from multiple cortical areas, including sources in the bilateral operculo-insular regions and anterior cingulate cortex (ACC) are activated in latencies between N2 and P2, displayed at vertex region[52,53]. Some regions, such as the anterior insular cortex (AIC), play a role in stimulus awareness and attention[52,54]. This can be observed at P400[55]. The important waveforms of the EP can be localized by selecting accurate derivations. For example, early components of the EP can be found in the lateral component derived from e.g. Tc-Fpz (T7 or T8 on the contralateral side). Central activation in the area around the vertex can be derived from CPz minus the reference channels A1A2. Since the EPs describe the behavior of underlying involved brain areas, also clinically relevant abnormalities can be characterized.

2.5 MTT-EP experiment

EPs can be registered using an EEG recording system. Earlier studies from the University of Twente used the MTT-EP experiment to measure nociceptive processing during electrical stimulation[12,56]. The MTT-EP set-up was created by Schooneman et al. and adapted by B. van den Berg[12,56], which combines nociceptive stimulation with EEG registration. The experiment uses the procedure of the MTT set-up developed by Doll et al. to perform MTT[5].

2.5.1 Experiment set-up

The subject sits in a chair and manually controls the NociTrack stimulator (Figure 8). The stimulator is connected by cables with the cathodic IES-5 stimulus electrode and anodic transcutaneous electrical nerve stimulation (TENS) electrode on the subject's hand. Using Bluetooth, the stimulator receives information from a dedicated laptop running software for stimulus pattern generation and triggering stimuli. Thereafter, the stimulator sends the recording response data back to the computer. The NociTrack is connected by a cable with the EEG amplifier to send trigger codes. At the same time, the scalp EEG records continuously cortical activity using an ANT Neuro Waveguard 64 EEG cap containing 64 Ag/AgCl electrodes. This 64 channel EEG is connected with an ANT Neuro 72-channel Refa EEG amplifier. Subsequently, this amplifier is connected by optical fibers to communicate with a dedicated laptop containing software for EEG recording.

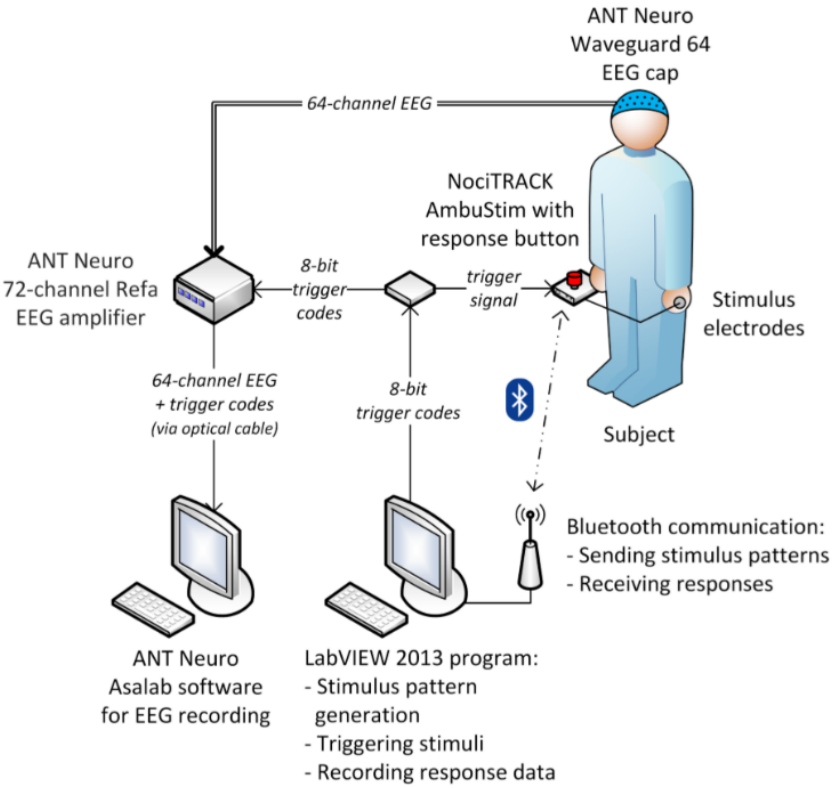

Figure 8. MTT-EP setup. Adapted from Schooneman[56].

2.5.2 Data analysis

Nociceptive detection probabilities and thresholds estimated the average NDTs and probability curve by including all data in a generalized linear mixed model (GLMM). This statistical model analyzed successfully average thresholds for this longitudinal data set, which was researched by B. van den Berg in previous research.

Due to bad signal-to-noise-ratio (SNR) of the EP, averaging does not provide noise reduction. Van den Berg studied that linear mixed models (LMM) can improve the analysis of EPs during MTT. LMM is useful because the longitudinal data from MTT-EP experiments consists of repeated-measurements which is clustered within subjects.

LMM can be used to estimate relationships within data. This is useful in a design with multiple measures per subject. Linear and random structures are formulated in an LMM, which ensures that all data can be used in a single regression. Fixed effects are parameters that do not vary. Random effects, also known as stochastic part of the model, are parameters which cannot be controlled experimentally. Using a complex mathematical computation based on linear regression, it is possible to estimate the outcome variable and fixed effects.

EP is the outcome variable of the LMM in the MTT-EP experiment. Stimulus detection (D), amplitudes of the three stimulus types (SP1, SP2_10, and SP2_40), and the total amount of received stimuli (TRL) are defined as fixed effects. The experiment of the subject is set as a random effect. The full mathematical background behind LMMs is out of the scope of this thesis, so please refer to literature of Jiang[57]. A summarized mathematic version for this MTT-EP experiment is elaborated by B. van den Berg[12].

2.5.3 Results of pain-free subjects at the University of Twente

Nociceptive detection thresholds

Results of NDTs from 25 pain-free subjects demonstrated that average initial NDTs for single-pulse stimuli were 0.2 mA and increased to approximately 0.5 mA during the last trial (Figure 11, left upper panel). At the same time, initial NDTs for double-pulse stimuli were around 0.1 mA and increased gradually to approximately 0.2 mA. Note that NDTs for single-pulse stimuli are higher than for double-pulse stimuli and that the difference in IPI did not affect the NDTs. It was observed that habituation and paired-pulse facilitation played a role during the MTT-EP experiment.

Evoked potentials

Next, it was identified that three components of the EP were important, namely at 165 ms (N1), 205 ms (N2) and 420 ms (P2) after the stimulus (Figure 12, Butterfly plot). However, the N1 peak was difficult to identify. EPs were derived by analysis from two derivations: (1) CPz-A1A2 and (2) Tc-FPz (contralateral channel, T7-FPz or T8-FPz), because it was investigated that the variance of the EP was the largest at these derivations. Results from the LMM showed that the EP is rather modulated by stimulus detection, amplitudes and the number of received stimuli (Figure 13, left panels)[12].

2.6 Implications

Research on underlying pathophysiology of chronic pain diseases is needed because 19% of the European people are still suffering from chronic pain[1]. The number of ineffective treatments is enormous, which results in high costs and social-economic burden. Great steps should be taken in the field of diagnostics and treatments for chronic pain. Although the underlying pathophysiology is still not completely understood, literature describes that central sensitization is seen in FBSS patients. At St. Antonius Hospital, approximately 60 patients diagnosed with FBSS are placed on the waiting list for neuromodulation each year. These chronic pain patients have passed all the possible therapies and are still suffering from pain, so it is assumed that these patients are certainly suffering from a central sensitization syndrome.

Since earlier studies from the University of Twente showed that the MTT-EP experiment might be promising for objective observation of nociceptive processing in pain-free subjects. Therefore, the first step is to dislocate the experiment from the university lab to the hospital environment. Then, it is recommended to explore whether results of the experiment at St. Antonius Hospital are a replication of results from the previous study and observe if it is feasible to perform by FBSS patients. At the same time, results of pain-free subjects the MTT-EP experiment at St. Antonius can be helpful to use as healthy control group for results of chronic pain patients in the future.

The MTT-EP experiment is hypothesized to be applicable when phenomena of the NDTs and EPs measured in the hospital are in line with results from the previous study at the University of Twente. Furthermore, it is hypothesized that neurophysiological responses might be different in chronic pain patients compared to pain-free subjects due to an altered central sensitization.

2.6.1 Primary research objectives

The primary objective is to explore whether results of the MTT-EP experiment in pain-free subjects at St. Antonius hospital are a replication of results in pain-free subjects at the University of Twente. Also, the objective is to describe how NDTs and EPs for electrocutaneous stimuli using an MTT paradigm behave in both pain-free subjects and FBSS patients.

Primary research questions:

a. **Are results of the MTT-EP experiment in pain-free subjects replicable in a hospital environment?**
   - **How present the average NDT and EP profiles?**
   - **How behave neurophysiological effects such as habituation and paired-pulse facilitation?**
   - **How are detected nociceptive stimuli related to the EP?**

b. **How behave NDTs and EPs using the MTT-EP experiment in FBSS patients at St. Antonius Hospital?**

2.6.2 Secondary research objectives

Secondary objectives are (1) to see if differences in behavior of NDTs and EPs can be found between FBSS patients and pain-free subjects, and (2) to analyze how the NDT and EP are related to central sensitization in FBSS patients.

Secondary research questions:

a. **Is an altered behavior of NDTs and EPs found in FBSS patients in comparison to pain-free subjects at St. Antonius Hospital?**
b. **How are NDTs and EPs in FBSS patients related to central sensitization?**

3 Methods

3.1 Subjects

Twenty pain-free subjects (PFS@StA) and seven FBSS patients (FBSS@StA) were enrolled in the study between September 2018 and November 2018. The subjects were included according to the inclusion- and exclusion criteria (Table 1). Verbal and written informed consent was obtained prior to inclusion. None of the pain-free subjects took analgesic medication. FBSS patients were allowed to continue the medication intake, if necessary. Not completing the MTT-EP experiment or analyzing EEG electrode 'M1', 'M2', 'CPz', 'FPz', 'T7', or 'T8' impedance higher than 5 kOhm was the exclusion criterion. The study was approved by the Medical research Ethics Committees United (MEC-U, file number: NL66136.100.18).

Table 1. Inclusion/exclusion criteria for pain-free subjects (PFS) and failed back surgery syndrome (FBSS) patients at St. Antonius Hospital (@StA).

	PFS@StA	**FBSS@StA**
Inclusion	• No pathological pain in history • Age: 18+ years	• Chronic low back pain • Age: 18+ years • Diagnosed with FBSS • Placed on waiting list for neuromodulation
Exclusion	• Diabetes Mellitus • Implanted stimulating device • Pregnancy • Alcohol 24 hours before experiment	• Diabetes Mellitus • Implanted stimulating device • Pregnancy • Alcohol 24 hours before experiment

3.2 Design

The study was a mono-center, explorative cross-sectional study, which was carried out in the Pain Clinics department at St. Antonius Hospital Nieuwegein, The Netherlands. This study monitored electrical brain responses during processing of nociceptive stimuli around the detection threshold. Each subject underwent one session of the MTT-EP experiment, consisting of two measurements (Figure 9).

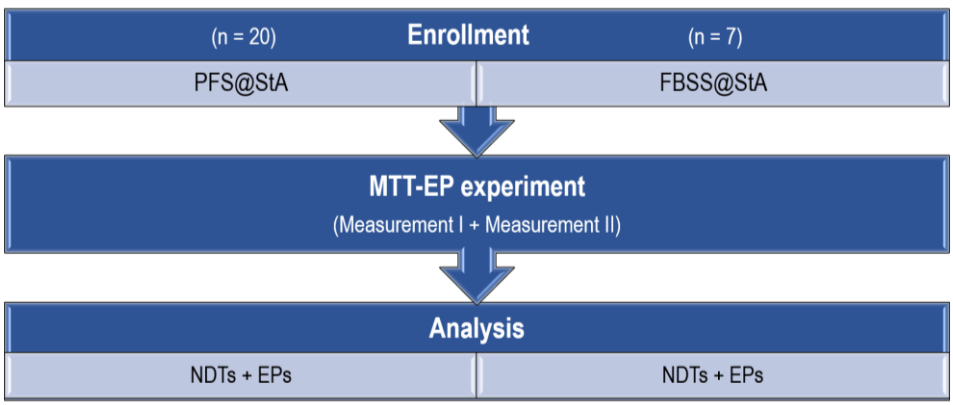

Figure 9. Study design. Twenty pain-free subjects (PFS@StA) and seven FBSS patients (FBSS@StA) were enrolled. Nociceptive detection thresholds (NDTs) and evoked potentials (EPs) were analyzed in both groups.

3.3 Materials and methods of measurement

3.3.1 Stimuli

The AmbuStim 1-channel stimulator was used for stimulation, which was connected to a cathodic electrode. This sterilized IES-5 electrode contained an array of five 0.2 mm needles. The electrode was placed gently on the dorsal hand and fixed with tape. Nociceptive (Að) fibers in the epidermis were specifically activated by IES[6,7,42–47]. A rectangular 9 x 5 cm TENS electrode served as an anode and was placed proximal to the IES-5 electrode at the wrist. A personal computer was wirelessly connected to the stimulator using Bluetooth. Moreover, the computer ran a custom computer program written in LabVIEW 2013. All stimulation procedures, stimulus amplitudes (and their trigger codes), responses to the stimuli and stimulus times were controlled and registered by the program code.

Table 2. Three stimulus types were executed: (1) the stimulus consisted of one cathodic square-wave electrical current pulse with a pulse width of 0.21 ms (setting 1); (2) the stimulus consisted of two cathodic square-wave electrical current pulses with a pulse width of 0.21 ms and an IPI of 10 ms (setting 2); (3) the stimulus consisted of two cathodic square-wave electrical current pulses with a pulse width of 0.21 ms and an IPI of 40 ms (setting 3). IPI of 10 ms and 40 ms were chosen because of the electrophysiology of synapses in the nociceptive system. The stimulus amplitude was limited to a maximum current of 2.0 mA in the stimulation software, because the detection threshold was expected between 0.0 and 1.0 mA.

Stimulus types	NoP	IPI (ms)	Pulse width (ms)
Setting 1	1	-	0.21
Setting 2	2	10	0.21
Setting 3	2	40	0.21

3.3.2 Multiple threshold tracking paradigm

During both measurements, a total of 450 stimuli consisting of 150 stimuli for each stimulus type (setting 1, setting 2 and setting 3) were applied to the subject. The MTT paradigm tracked NDTs for these three types of stimuli (Table 2)[5,7]. Thresholds for each combination of NOP and IPI was tracked simultaneously by measuring the subject's response (detected or not detected) to a randomized set of stimulus amplitudes. All types of stimuli were selected the same number of times, but in a random order. Observer and subject bias were decreased by varying the stimulus type randomly.

3.3.3 EEG recording

Simultaneously to the stimulation, electrical brain activity was recorded continuously with a sampling frequency of 1 kHz. This was performed using an ANT Neuro Waveguard EEG cap containing 64 Ag/AgCl electrodes in combination with a TMSi 72-channel Refa EEG amplifier. The EEG and the trigger codes were recorded on a dedicated computer running TMSi Polybench (Polybench Designer 1.30.0) software. The EEG cap was adjusted to the size of the head, before it was applied on the head. The C_z electrode was set in the middle between the nasion and inion and between both mastoids. A ground electrode was placed on the forehead and earlobe electrodes were also applied (A_1A_2) for CP_z-A_1A_2 analysis. All cap electrodes were filled using a needle and syringe with gel to meet the conductivity. The scalp electrode impedance was screened to be below 5 kOhm.

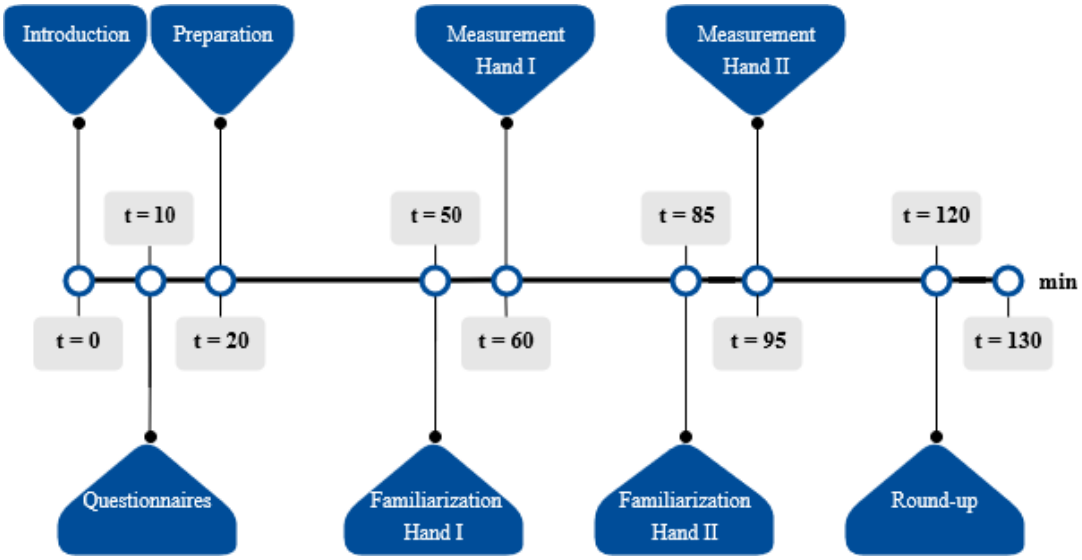

Figure 10. Timeline of the procedure. The CSI was part of the questionnaires. Preparation of the EEG cap was dependent of the subjects hair, and therefore preparation time varied to meet the required electrode impedances (<5 kOhm). The MTT-EP experiment consisted of two measurements (in which the order was determined by randomization). The whole session took about 130 minutes.

3.3.4 Procedure

First, the subject was informed about the purpose of the study (Figure 10). After completion of the informed consent, the subject was asked to fill in a set of questionnaires, including the CSI to quantify central sensitization. One session was divided into two measurements: measuring NDTs and EPs during IES of the dominant hand followed by IES of the nondominant hand, in which the order was determined by randomization. Mobile phones were not allowed in the room of the MTT-EP experiment to prevent artifacts in the EEG signal. The subject was asked to sit in the chair during the measurements. Before starting the first measurement, the software of the EEG system was prepared, and the EEG electrodes were placed. All scalp-electrode impedances were verified and recorded to be less than 5 kOhm. Next, the stimulator system was prepared, and the stimulation electrodes were attached on the dorsal hand while the stimulator was held in the other hand. Then, the subject was familiarized with test stimuli and detection tasks before the start of each measurement.

Familiarization

The subject received four series of test stimuli using a short detection threshold estimation method consisting of one sequence of ten ascending stimuli for each stimulus type[41]. During the first series, the subject pressed and held the button on the stimulator triggering a series of stimuli of setting 1 with rising amplitude applied at a rate of one stimulus per second. During the first series, the subject was instructed to release the button (terminating the series of stimuli) when the stimulus-related sensation was clearly felt and recognized multiple times. During the second series, the subject was instructed to release the button as soon as any sensation was felt, which was ascribed to the application of a stimulus from setting 1. This procedure was repeated also a third and fourth time for setting 2 and setting 3, respectively. Besides familiarizing the subject with the measurement, an initial estimate of the detection threshold was obtained using the stimulus amplitude of the last familiarization series for each stimulus setting. The MTT paradigm used this value to initialize tracking.

Nociceptive detection tasks during both measurements

The subject was instructed to initiate a perceived stimulus by pressing the response button and releasing it as soon as any sensation was felt, which was ascribed to the application of a stimulus. The subject was asked to repeat the task after about half a second. If the button was released within 1000 ms after the stimulus, the stimulus was labeled as 'detected'. The stimulus was labeled as 'undetected' if the button was still pressed. Simultaneously, the EEG signals were recorded when the button was pressed. Therefore, the subjects were asked to focus their eyes on one point and avoid muscular face movements (e.g. talking and swallowing).

3.4 Data analysis

3.4.1 Nociceptive detection threshold

Individual NDT values were determined using the psychometric function, which estimated the subjects' detection threshold for each stimulus setting. Trials consisting twice the previous detection threshold were removed from threshold analysis. Nociceptive detection probabilities and thresholds were analyzed for every stimulus type by including the information from all subjects in a generalized linear mixed model (GLMM). The estimated average threshold was determined by a linear predictor consisted of fixed effects and random effects. The intercept, stimulus amplitude (for each stimulus type) and total received stimuli were defined as fixed effects. Between-measurement random effects were applied for every fixed effect. A moving window of 30 stimulus-response pairs was used. The GLMM approached the subject's response using a logit link function which was regulated by the stimulus amplitude and habituation. The predicted model response was approached by Equation 1:

$$Logit(D) \sim 1 + SP1 + SP2\_10 + SP2\_40 + TRL + (1 + SP1 + SP2\_10 + SP2\_40 + TRL \mid Measurement) \quad (1)$$

The subject's response was described by the stimulus detection (D). The stimulus was specified by the amplitude of a single-pulse stimulus (SP1), and a double-pulse stimulus with an IPI of 10 ms (SP2_10) and an IPI of 40 ms (SP2_40). Habituation was included with respect to the total amount of received stimuli (TRL). To determine the significance, the coefficient of the GLMM were statistically tested against the null-hypothesis using a Wald t-test. The predicted psychophysical curve based on probability summation of two pulses was calculated by the probability of the detection threshold (from available data) using logistic regression.

Pre-processing of EEG data

The offline EEG data were re-referenced to the A1A2-electrodes. Second, EOG components from the raw EEG-signals were filtered by the application of an independent component analysis. Then, the EEG was preprocessed on the clean MTT trials using FieldTrip, which is a MATLAB toolbox for signal processing[58]. Trials for the EP analysis were segmented using a time window range from 0.5 s before the stimulus to 1.0 s post-stimulus. The data were offline band-pass filtered from 0.01 – 40 Hz and baseline-corrected in the period from -0.5 s to 0.0 s. Eye blinks, muscular activity and artifacts in the EEG data were cleaned using an independent component analysis algorithm in MATLAB (version 2015b; The MathWorks Inc, Natick, Massachusetts, US). In addition, trials consisting of outliers and EEG channels consisting many artifacts and impedances higher than 5 kOhm were excluded manually from analysis as well.

3.4.2 Evoked potential

The central and lateral component of the EPs were respectively derived from two derivations: (1) CPz-A1A2 and (2) Tc-FPz (contralateral channel, T7-FPz or T8-FPz). Grand average EPs over all stimuli were displayed for both derivations. Whether grand average EPs from detected stimuli differed from undetected stimuli were assessed by nonparametric statistical testing[58,59].

An overview of grand average EPs from all channels was illustrated using a butterfly plot. Timestamps for grand average scalp topographies were based on the N2 and P2 peaks in both pain-free subjects and FBSS patients. In this study, the N2 peak was defined as the maximal negative peak approximately 200 ms post-stimulus at Tc-FPz. The P2 peak was defined as the first maximal positive peak approximately 400 ms post-stimulus at CPz-A1A2.

The variation of EPs was analyzed using a linear mixed model (LMM). The EP was approached by Equation 2:

$$EP \sim 1 + D + SP1 + SP2\_10 + SP2\_40 + TRL + (1 + D + SP1 + SP2\_10 + SP2\_40 + TRL \mid Measurement) \quad (2)$$

Stimulus detection (D), which reflected the subject's response (detected or not detected), was used as a fixed effect in this statistical model. Besides this parameter, three stimulus amplitudes (SP1, SP2_10, and SP2_40) and total amount received stimuli (TRL) were selected also as fixed effects. Again, between-measurement random effects were included for these fixed effects.

Grand average EPs described by these fixed LMM coefficients were displayed at CPz-A1A2 and Tc-FPz. To determine the significant components of the grand average EPs, the coefficient of the LMM were statistically tested against the null-hypothesis using a t-test[59].

3.5 Replicability in pain-free subjects

The replicability of the MTT-EP experiment at St. Antonius Hospital was explored by multiple parameters; average NDTs, psychometric curves, grand average EPs and their scalp topographies, and fixed coefficients of the LMM which influenced the detected grand average EPs, were observed in both pain-free subjects' groups. All the data of pain-free subjects at the University of Twente (PFS@UT) was imported from the previous study[12].

3.6 Behavior of neurophysiological effects in FBSS patients

Behavior of NDTs and EPs were described by the same parameters as mentioned for the replicability to explore neurophysiological effects in FBSS patients. The CSI-score (>40) was used to analyze if the FBSS patients were suffering from a central sensitization syndrome. Differences in subject characteristics, such as age, BMI, NRS-score (before and during the experiment) and CSI-score were tested between pain-free subjects and FBSS patients at St. Antonius Hospital using an independent sampled t-test. Significance level $p < 0.05$. Subsequently, the same parameters as described for the replicability were visually compared between both groups.

4 Results

Table 3. Characteristics of pain-free subjects (PFS) at the University of Twente (UT) and St. Antonius Hospital (StA) and characteristics of failed back surgery syndrome (FBSS) patients at St. Antonius Hospital. Note that the number of measurements is twice times the number of subjects included for analysis at St. Antonius Hospital, because they were measured at both hands. Differences in age, BMI, NRS and CSI between PFS@StA and FBSS@StA were tested using an independent sampled t-test. Significance level $p < 0.05$.

	PFS@UT	PFS@StA	FBSS@StA	*p*-value
Number of subjects	25	17	7	-
Number of measurements	25	34	14	-
Sex (M/F)	16/9	3/14	3/4	-
Age (mean ± SD)	23.0 ± 3.6	35.9 ± 11.9	54.3 ± 11.3	0.003*
Handedness (R/L)	24/1	15/2	5/2	-
BMI	-	22.2 ± 2.8	24.7 ± 3.4	0.070
NRS last week (mean ± SD)	-	1.4 ± 0.7	7.4 ± 1.6	0.001*
NRS during MTT-EP experiment	-	1.0 ± 0.0	6.7 ± 2.1	0.001*
CSI score (mean ± SD)	-	14.6 ± 8.8	49.0 ± 15.5	0.001*
Medication intake (Yes/No)	-	1/16	6/1	-
Duration of FBSS in months (mean ± SD)	-	-	35.0 ± 21.2	-
Duration of pain in years (mean ± SD)	-	-	18.6 ± 17.5	-

4.1 Subject Characteristics

4.1.1 Pain-free subjects

In total 17 pain-free subjects at St. Antonius Hospital (PFS@StA) were analyzed. Three other participants were excluded due to one of the following reasons: M1/M2 electrodes showed impedances higher than 5 kOhm or the subject could not complete the MTT-EP experiment because of uncertain reasons. The group characteristics are summarized in Table 3. Three men and fourteen women are shown as pain-free subjects. The mean and standard deviation (SD) of these subjects' age is 35.9 ± 11.9 years, ranging from 18 to 63 years. The CSI scores (14.6 ± 8.8) demonstrate that none of the subjects suffered from a central sensitization syndrome (CSI-score >40). The body mass index is 22.2 ± 2.8 kg/m^2. Fifteen subjects (88%) reported being right handed. Four subjects mentioned suffering from pain in the week before the MTT-EP experiment (NRS: 1.4 ± 0.7) and one of them took pain analgesic medication (paracetamol) on the day of the MTT-EP experiment. None of the subjects reported pain on the questionnaires before the MTT-EP experiment (NRS: 1.0 ± 0.0).

4.1.2 FBSS patients

Next to the pain-free subjects, seven FBSS patients at St. Antonius Hospital (FBSS@StA), who all suffered from a central sensitization syndrome (mean: 49.0 ± 15.5) were included for analysis (Table 3). The subjects reported a mean NRS of 7.4 ± 1.6 as average pain in the week before the MTT-EP experiment and 6.7 ± 2.1 on the experimental day itself. Six of these patients took analgesic medication because the pain was unbearable. All of them were treated by a variety of interventions and finally by laminectomy. Still, the pain was not resolved, so

they were placed on the waiting list for a neurostimulator, but they did not have an implanted stimulation device on the day of the MTT-EP experiment. The patients suffered 18.6 years from pain on average, ranging from 3 to 54 years. In addition, these chronic pain patients were diagnosed with FBSS for 35.0 ± 21.2 months. Among the patients, the mean and standard deviation age is 54.3 ± 11.3 years, varying from 39 to 76 years. Five patients noted being right handed. Table 1Table 3 presents that age (p = 0.003), NRS-score (before (p = 0.001) and during the MTT-EP experiment (p = 0.001) and CSI-score (p = 0.001) are significantly higher in the FBSS patients compared to the pain-free subjects at St. Antonius Hospital. BMI does not show significant (p = 0.070) differences between both groups.

4.2 Replicability in pain-free subjects

4.2.1 Nociceptive detection thresholds

The most striking observation to emerge from the estimated average NDT data is the effect of habituation, which is similar to previous results from the University of Twente (Figure 11, upper panel). Next to this, it is crucial to note that single-pulse stimuli show higher NDTs than double-pulse stimuli. The estimated average initial thresholds for single-pulse stimuli are approximately 0.2 mA and 0.15 mA for double-pulses. These are in line with previous results.

Psychometric curves of pain-free subjects

The most striking result to emerge from the psychometric curve is the effect of paired-pulse facilitation, which occurred in both pain-free subject groups (Figure 11, lower panel). Last trial curves show higher stimulus amplitudes than first trials for all stimuli. The predicted double-pulse curve estimates the threshold purely based on probability summation. Since single-pulse curves show higher stimulus amplitudes than the predicted double-pulse curves, it is seen that there is a facilitating effect involved due to the paired-pulse. Furthermore, the steepness of the curves suggests that double-pulse stimuli are more reliably detected than single-pulse stimuli.

4.1.3 Evoked potentials

First, a significant difference between detected and undetected stimuli is shown in the grand average EP of both pain-free subjects. Second, no substantial differences between both pain-free subjects seem to be found for grand average EP at CPz-A1A2 and Tc-FPz derivations with respect to the detected stimuli (Figure 12). The maximum peak of the grand average EP from detected stimuli at CPz-A1A2 and Tc-CPz is at 198 ms and 444 ms after the stimulus, respectively (Figure 12). These concur well with previous results from the University of Twente (205 ms and 420 ms). Furthermore, grand average scalp topographies are roughly similar with respect to detected stimuli (Figure 12). In both topographies, it is illustrated that there is a negative contralateral focus in the area around T7 or T8 at the N2 peak and a central component in the area of CPz for the P2 peak. In addition, the grand average EP is significantly influenced by all fixed coefficients of the LMM at CPz-A1A2 for both pain-free subject groups (Figure 13Figure 13). It is shown that the EP is significantly related to stimulus detection (D) at Tc-FPz. However, this derivation shows that components of the EP are significantly related to double-pulse stimuli with an IPI of 10 ms, while this observation was not found in previous results from the University of Twente.

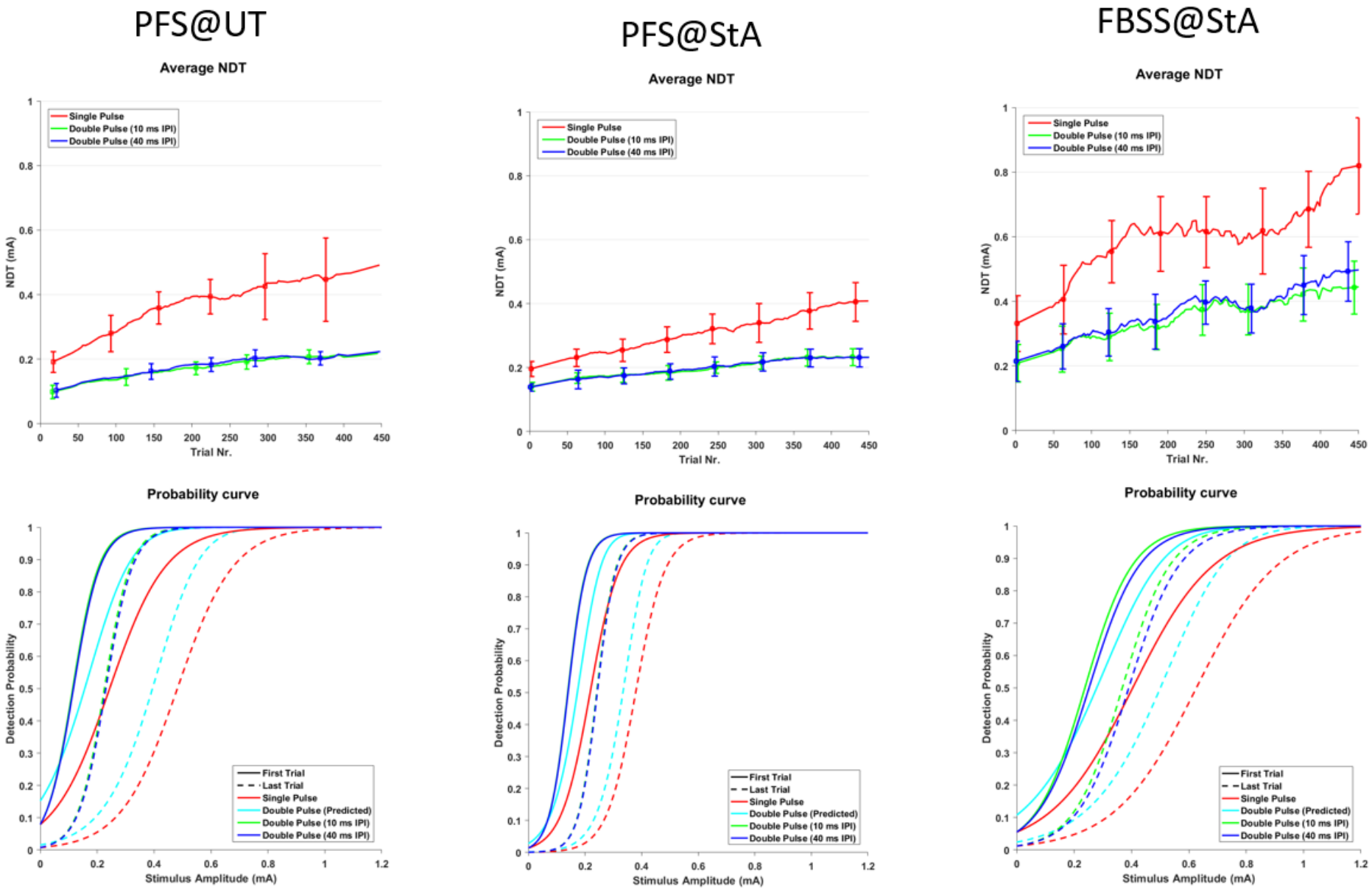

Figure 11. Estimated average NDTs (upper panels) and estimated psychophysical curves (lower panels) from 25 pain-free subjects at University of Twente (PFS@UT), 17 pain-free subjects at St. Antonius Hospital (PFS@StA) and 7 failed back surgery syndromes patients at St. Antonius Hospital (FBSS@StA). Note that measurements were performed twice (on each hand once) at St. Antonius Hospital, which ensures that PFS@StA and FBSS@StA include 34 and 14 measurements, respectively. Strikingly, the effect of habituation is seen in each group by the rising thresholds (upper panels). In addition, paired-pulse facilitation is seen in each group (lower panels) by the double pulse stimuli (green and blue lines), which show lower stimulus amplitudes than the predicted curve based on probability summation (light blue lines). The first trial is illustrated by a solid line and last trial by a dotted line.

4.2 Behavior of neurophysiological effects in FBSS patients

4.2.2 Nociceptive detection thresholds

Average NDTs estimated by GLMM analysis are higher in FBSS patients than in pain-free subjects (Figure 11, upper panel). The average initial threshold for single-pulse stimuli is more than 0.3 mA, and for double-pulse stimuli, it is more than 0.2 mA. Although the NDTs increase generally over time in both groups, detection thresholds rise faster in FBSS patients. Also, detection thresholds in FBSS patients are less constant than in pain-free subjects. For all participants, stimulation by single-pulses shows higher NDTs than by double-pulses. Thresholds for single-pulse stimuli rise faster over time as well. Varying of IPIs present almost identical detection thresholds.

Psychometric curves of FBSS patients

Altered behavior of psychometric curves can be observed in chronic pain patients (Figure 11, lower panel). In general, it is seen that the stimulus amplitude shifts from the left to the right in a period between the first and last trial, which implies that the stimulus intensity increases during the measurement. Also, these curves demonstrate that higher stimulus amplitudes were required for stimulus detection in chronic pain patients compared to pain-free subjects. Additionally, a lower steepness of the curves is shown in these patients.

4.2.3 Evoked potentials

Results show that EPs from detected stimuli are significantly different from undetected stimuli at both derivations in FBSS patients. An early phase component (i.e. positive peak at 150-200 ms) of detected stimuli is seen at CPz-A1A2, which is not noted in pain-free subjects (Figure 12). Also, it is demonstrated that the amplitude of the N199 peak shows a higher amplitude in the grand average EP at Tc-FPz than the amplitude of the N198 peak of pain-free subjects (Figure 12). Grand average scalp topographies illustrate that a contralateral peak occurs 199 ms after stimulus around T7 or T8. This shifts subsequently toward the central side around CPz at 437 ms after the stimulus was given. Grand average scalp topographies illustrate that the stimulus distributes from a contralateral component in the area at N199 towards the central part of the scalp at P437. With respect to the fixed coefficients, it is shown that the grand average EP is only significantly influenced by stimulus detection at CPz-A1A2 (Figure 13). All other parameters do not show a significant relationship with the EP. This implicates that stimulus amplitudes of these settings and a total number of received stimuli do not affect significantly the EP in FBSS patients.

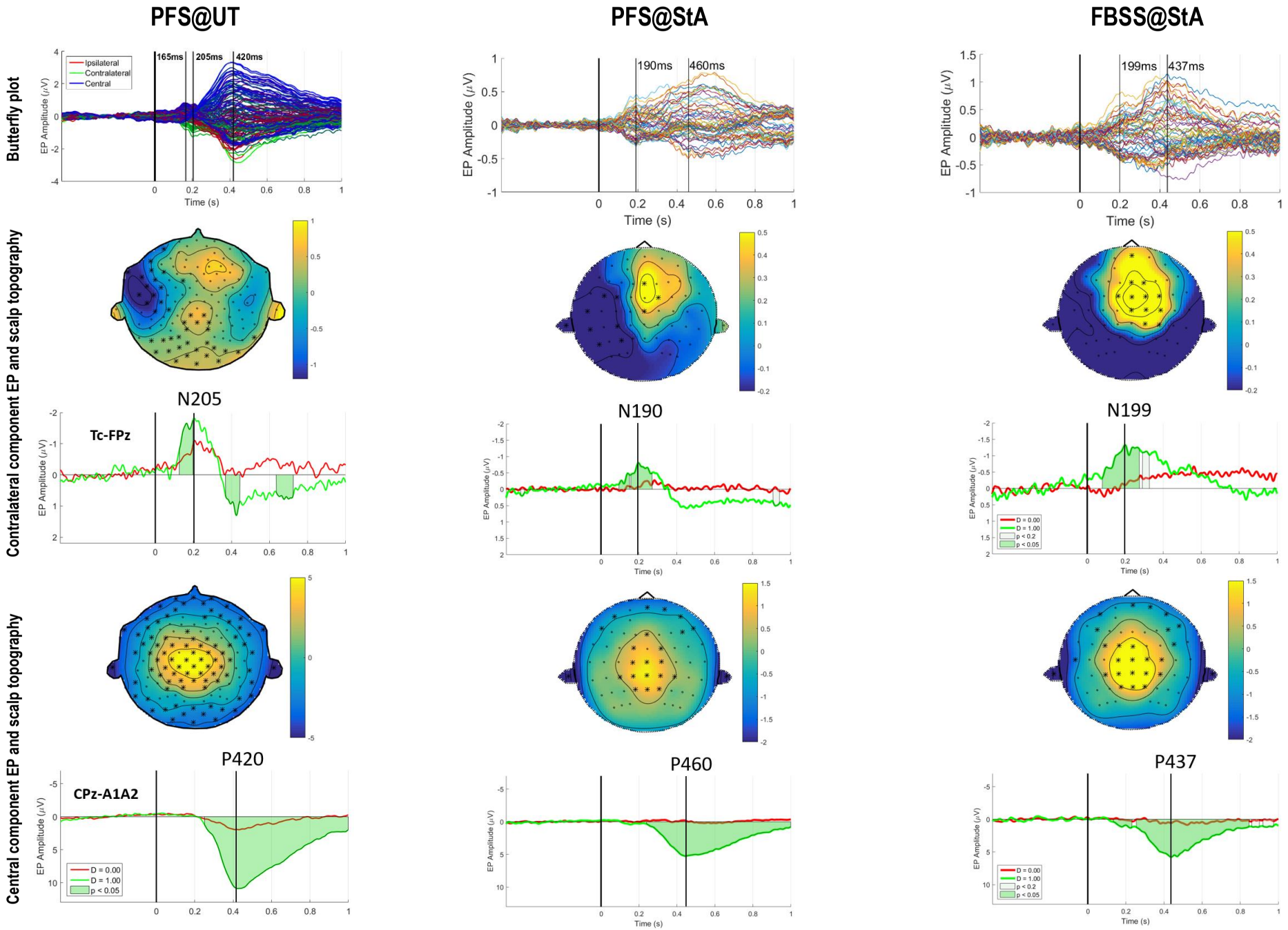

Figure 12. Butterfly plots (upper panels), including grand average scalp topographies and grand average EPs of the contralateral component (middle panels) and grand average scalp topographies and grand average EPs of the central component (lower panels) from pain-free subjects at University of Twente (PFS@UT), pain-free subjects at St. Antonius Hospital (PFS@StA) and failed back surgery syndrome patients at St. Antonius Hospital (FBSS@StA). Note that the contralateral component is derived from Tc-FPz (T7 or T8 channel on the contralateral side of stimulation) and the central component is derived from CPz-A1A2. The N2 peak is seen at the contralateral component, whereas the P2 peak is seen at the central component. The scalp topographies illustrate the dipole described by a potential field around the peaks with a time window of 20 ms. When the EP from detected stimuli (green line) are significantly ($p < 0.05$) different from undetected stimuli (red line), the channel is marked by a star (*) in the topography and the component is colored in green on the grand average graph.

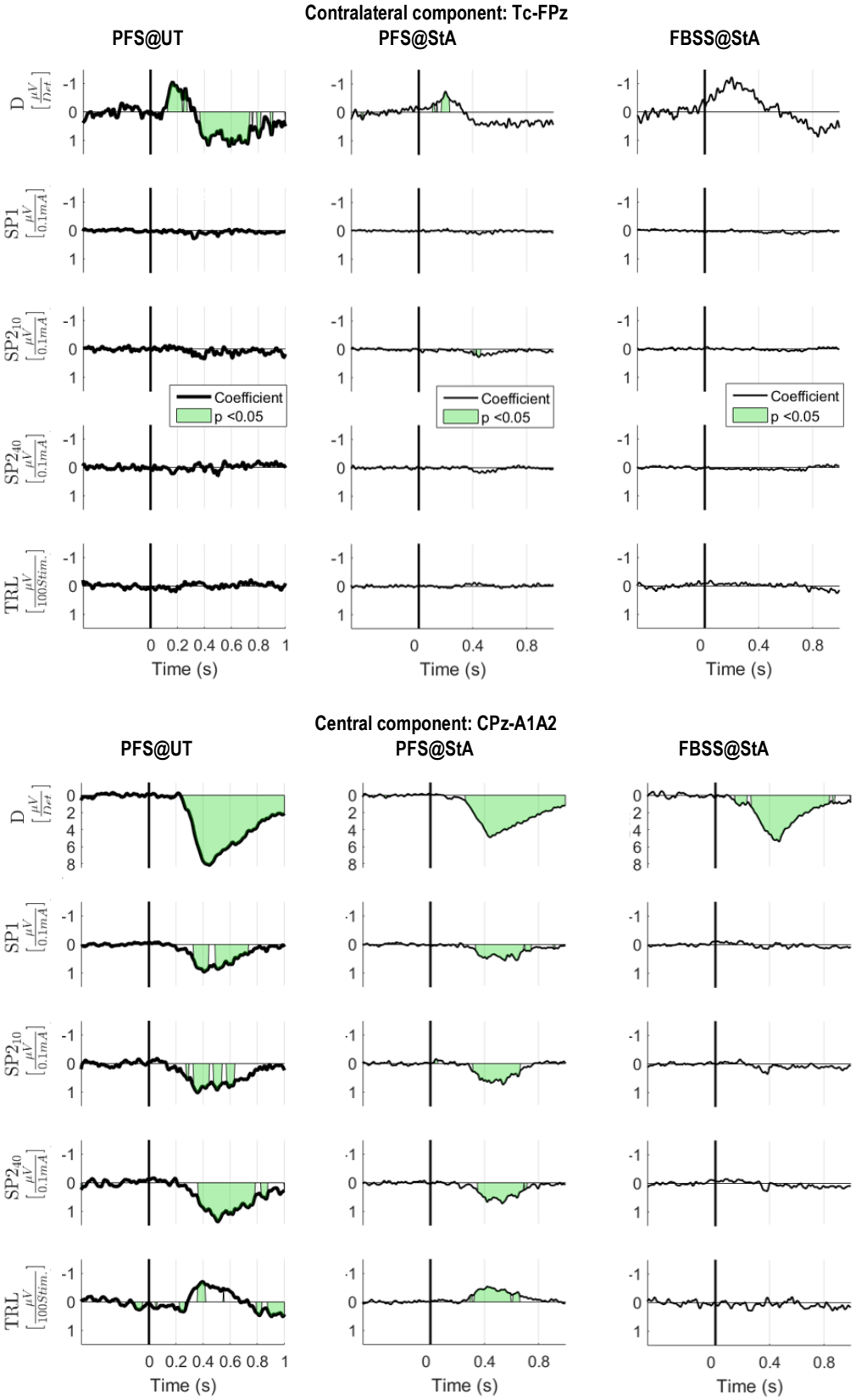

Figure 13. Fixed coefficients of the linear mixed model related to the grand average EPs for detected stimuli. Stimulus detection (D), the amplitude of single pulse stimuli (SP1), the amplitude of double-pulse stimuli with 10 ms IPI (SP2_10) and 40 ms IPI (SP2_40), and total received number of stimuli (TRL) were set as fixed coefficients. The EPs were derived from the contralateral component (Tc-FPz) upper panel) and central component (CPz-A1A2, lower panel) in pain-free subjects at University of Twente (PFS@UT, left), and both pain-free subjects (PFS@StA, middle) and FBSS patients (FBSS@StA, right) at St. Antonius Hospital.. Components of the EP for detected stimuli which were significantly different from the EP for undetected stimuli are colored in green. This implicates that the EP is modulated by the coefficient.

5 Discussion

In this study, we explored the replicability of the MTT-EP experiment in pain-free subjects at St. Antonius Hospital. Secondly, we observed the neurophysiological responses during processing of nociceptive stimuli around the detection threshold in chronic pain patients. The study yielded two main findings. (1) Results of the MTT-EP experiment in pain-free subjects at St. Antonius Hospital are a replication of results from the previous study at the University of Twente based on observations of NDTs and EPs during nociceptive stimulation. (2) Behavior of neurophysiological effects seems to be altered in FBSS patients compared to pain-free subjects.

5.1 NDTs and EPs

The estimated average (initial) NDT thresholds in pain-free subjects were quite similar to previous results for all stimulus settings. Results of NDTs from 17 pain-free subjects demonstrated that average NDTs for single-pulse stimuli started around 0.2 mA and increased to 0.4 mA during the last trial (Figure 11, upper panel). At the same time, initial NDTs for double-pulse stimuli were around 0.15 mA and increased gradually to approximately 0.2 mA. Strikingly, in all cases the NDTs were increasing over the number of trials and, moreover, NDTs for single-pulse stimuli were higher than for double-pulse stimuli. The difference in IPI did not affect the NDTs. These results suggest that the MTT-EP experiment can replicate similar NDTs in pain-free subjects at St. Antonius Hospital using a changed observer, (pain-free) population and environment.

Next, the estimated grand average EPs in pain-free subjects concur well with previous results from the University of Twente. The most important observation was the similarity in behavior of detected stimuli compared to undetected stimuli. The N2 and P2 waveforms were well recognizable for detected stimuli and their latencies were approximately the same. Similar latencies implicate a similar conduction time for ascending signals reaching the cortex. However, in our butterfly plot can be seen that the strategy for choosing scalp topographies can be improved in pain-free subjects at St. Antonius Hospital by choosing another derivation because our selected latencies (190 and 460 ms) are not exactly in the maximal peak of the butterfly plot (Figure 12). Namely, our latencies are defined as maximal negative peak (N2) at Tc-FPz and maximal positive peak (P2) at CPz-A1A2. We have chosen to illustrate the grand average scalp topography using the same derivations as before (hence Tc-FPz for contralateral component and CPz-A1A2 for central component), with a time window of 20 ms around the N2 and P2 peaks. The reason for similar derivations was to compare respectively the N2 and P2 peaks optimally with results from pain-free subjects at the University of Twente. However, the previous strategy to select the N2 and P2 peaks was based on results of the maximal amplitude in the butterfly plot (which consists of grand average EPs from all channels). Since it is shown that the same peaks (N2 and P2) can be illustrated using the same contralateral (Tc-FPz) and central (CPz-A1A2) derivations, it is suggested that grand average EP profiles can be replicated at St. Antonius Hospital (Figure 12). Additionally, using the coefficients of the LMM, it was shown that similar fixed effects were significant ($p < 0.05$) related to the EP (Figure 13).

Namely, the contralateral component was mainly modulated by stimulus detection and the central component was influenced by all fixed effects (stimulus detection, amplitudes of the single and double-pulse stimuli and the number of received stimuli) in both pain-free subject groups.

5.2 Behavior of neurophysiological effects

5.2.1 Habituation

The effect of habituation was found in virtually all participants, which was based on the rising NDTs during the measurements (Figure 11, upper panel). Interestingly, the coefficient for a total number of received stimuli (TRL) showed a negative effect on the grand average EP in pain-free subjects (Figure 13, lower panel), indicating that EP amplitude decreases for repeated stimuli due to habituation. This effect was also observed in the previous study at the University of Twente and earlier studies[11,12,52]. However, it is uncertain why thresholds in FBSS patients increased faster over time than in pain-free subjects (Figure 11, upper panel). Controversially, based on results of the LMM, it was seen that habituation does not significantly affect the EP in FBSS patients (Figure 13, lower panel). In addition, it is not likely that habituation occurred more extremely in FBSS patients compared to results from Vossen et al., because they concluded that habituation was less in FBSS[11]. Therefore, the strengthened habituating effect might possibly be a result of other reasons (i.e. medication intake, as discussed below). Since other literature studied different populations, using different stimulation paradigms and other analysis methods, it is in general difficult to compare the effect of habituation.

5.2.2 Paired-pulse facilitation

Paired-pulse facilitation was observed in pain-free subjects, which was in line with results from the University of Twente. In addition, paired-pulse facilitation was discovered in FBSS patients (Figure 11, lower panels). The presence of such a facilitating effect is in our knowledge a novel finding in FBSS patients, however, long-term potentiation is known in chronic pain patients[60]. Paired-pulse facilitation can be explored by the fact that estimated thresholds for double-pulse stimuli were clearly lower than predicted thresholds based on probability summation (Figure 11, lower panels). Therefore, this study showed that pure probability summation does not fully explain the decrease in threshold when the number of pulses is increased. Thus, it was suggested that the detection probability of the second pulse is facilitated by the first pulse[6] because R.J. Doll et al. also observed earlier this effect by processing of temporal stimulus properties on nociceptive detection probability in pain-free subjects[6].

Temporal summation of the postsynaptic potentials is one of the possible mechanism responsible for facilitation of the second pulse in the CNS. In addition, the short-term synaptic plasticity[61], such as paired-pulse facilitation or augmentation, were usually involved by a residual elevation in presynaptic Ca^{2+} concentration. This increases the probability of neurotransmitter release from the presynaptic membrane. Therefore, this effect might explain the improved postsynaptic response during depolarization of a single action potential, when a first pulse is followed by a second pulse. Luo et al. researched that this effect of facilitation occurred for IPIs ranging from 10 to 100 ms[62]. Results from our study confirmed that double-

pulse stimuli, consisting of IPIs with 10 and 40 ms, showed lower detection thresholds. Since the thresholds for both IPIs are similar, it is suggested that the facilitating mechanism was not influenced by the difference of these IPIs in pain-free subjects.

5.3 Detected stimuli related to evoked potential

Since the N2 and P2 peak amplitudes for detected stimuli are significantly ($p < 0.05$) different than undetected stimuli, it is indicated that there is a relationship between nociceptive input and cortical consciousness in both pain-free subjects at St. Antonius Hospital (Figure 12, grand average EPs). This notion also occurred in pain-free subjects at the University of Twente, which was in agreement with literature from Lee et al.[52].

Results of the LMM showed (at the central component) that the EP was significant ($p < 0.05$) influenced by all fixed parameters in pain-free subjects at St. Antonius Hospital (Figure 13, middle lower panel). The role of attention is thought to be reflected in the coefficient of stimulus detection because the subject was instructed to focus on the stimulus. When the subject should shift his focus to something else, it would probably be more difficult to detect stimuli around the detection threshold. Furthermore, the amplitude of all stimulus settings showed a positive relationship with the EP, suggesting that the amplitude of the EP increased for higher stimulus amplitudes (which might explain the early phase component in FBSS patients, as discussed below).

Next, N2 and P2 peak amplitudes for detected stimuli were also significantly ($p < 0.05$) different from undetected stimuli in FBSS patients (Figure 13, right lower panel). This novel finding means that detected stimuli from nociception can be characterized by cortical activity using the MTT-EP experiment in FBSS patients. Note that these patients are not treated by neuromodulation, which might influence the behavior of the EP. Results from the LMM in FBSS patients showed that the EP is only modulated by stimulus detection (Figure 13, right lower panel), however, it is uncertain why stimulus amplitudes and the number of trials are not significantly related to the EP. It has to be mentioned that the size of the group is quite low (N = 7, thus 14 measurements), which results in a less accurate estimated signal, and the pre-stimulus signal is a bit noisier than in pain-free subjects at St. Antonius Hospital.

5.4 Altered behavior of neurophysiological effects related to central sensitization

5.3.1 Central sensitization

We assumed that our FBSS patients suffered from a central sensitization syndrome (CSI-score > 40) since results from the CSI showed an average score of 49.0 and SD of 15.5. Strikingly, these results differed significantly from the pain-free subjects (CSI-sore: 14.6 ± 8.8). In literature, FBSS patients are supposed to have an altered central sensitization forced by constant stimulation of nociceptive circuits[3,4,33–36]. Since there is no golden standard for measuring nociceptive changes in the CNS, it is difficult to determine whether our patients show certainly an altered central sensitization. We have used CSI because it has described as a clinically useful outcome measure[71], but remember that this method is still objective. Note that differences of

NDTs and EPs between FBSS patients and pain-free subjects are difficult to indicate as the result of central sensitization.

5.3.2 Higher average NDTs

Strikingly, chronic pain patients demonstrated higher average initial detection thresholds than pain-free subjects (Figure 11, upper panel). Results about detection thresholds in central sensitized pain patients vary in literature. For example, decreased sensory and pain thresholds were found in low back pain patients using pQST[4,75]. On the other hand, elevated detection thresholds in chronic pain patients were found as well in literature, which supports a central mechanism[76]. Although there is not much research performed using IES around the detection thresholds in pain patients, it was shown that NDTs increase by capsaicin-induced nerve defunctionalization[8]. Still, it has to be mentioned that most of the FBSS patients (6/7) has used analgesics, such as paracetamol and opioids (i.e. oxycodone and pregabalin), which act in the central and peripheral nervous system. Therefore, it affects sensation and hence it might elevate NDTs. However, to which extend the behavior of NDTs were affected by medication is unknown.

5.3.3 Early phase component of the evoked potential

The presence of the early phase component (i.e. positive peak at 150-200 ms) for detected stimuli in FBSS patients is a novel finding, which was not found in pain-free subjects (Figure 12, grand average EP of the central component). The early phase component was seen at CPz-A1A2, which might indicate that it can be possibly a potential biomarker of brain processing in FBSS patients. This is in line with other literature, which also discovered that chronic pain was significantly related to changes of early and late latency EP amplitudes[63,64]. Early phase components (N1 waveforms) reflects the behavior of ascending nociceptive input, whereas late phase components (N2 and P2 waveforms) are more associated with pain emerges from nociception[52]. Nevertheless, note that these FBSS patients were stimulated with higher stimulus amplitudes because higher NDTs has been discovered. Moreover, it is known that EP amplitudes are linear related to the stimulus amplitude[11]. Therefore, this early phase component can be probably explained by the larger amplitudes of the N2 peak in the contralateral grand average EP for detected stimuli in FBSS patients (Figure 12, grand average EP of the contralateral component).

5.3.4 Changed amplitude of N2 peak

We identified interestingly different profiles in N2 peak amplitudes for detected stimuli between pain-free subjects and FBSS patients. Amplitudes of the N2 peak in FBSS patients are higher than in pain-free subjects at St. Antonius hospital but lower than in pain-free subjects at the University of Twente (Figure 12, grand average EP of the contralateral component). Remarkably, amplitudes of cortical activity in results of the butterfly plot from the University of Twente show also higher variations during the pre-stimulus phase compared to results from St. Antonius Hospital for both pain-free subjects and FBSS patients (Figure 12, butterfly plot). The reason for this observation is unknown. Probably, this might be declared by the different technical specifications of the EEG amplifiers, because in this study we used the TMSi 72-

channel Refa EEG amplifier instead of the TMSi 128-channel Refa EEG amplifier at the University of Twente. Keeping this in mind, it is difficult to discuss the amplitude of the N2 peak as well. Therefore, these technical specifications have to be researched in the future.

Nevertheless, the peak of the N2 wave might be a physiological correlate of (secondary) hyperalgesia[65], and hence it can be a potential biomarker for central sensitization[65,66]. Liang et al. and Ianetti et al. found that N2 amplitudes of subjects who are centrally sensitized were significantly larger than N2 amplitudes of subjects without central sensitization[65,66]. But, still then, there is evidence that modulation of N and P amplitudes were different by cognitive tasks[55,65,67] and have different neural generators[53]. Contribution of a bilateral source in operculoinsular areas and primary somatosensory cortex (contralateral to the stimulation side) were found in several studies[68–70]. EPs seem to be modulated by attention, which makes the N2P2 amplitude larger[55]. This observation can be confirmed by results of the LMM from our study because stimulus detection was significantly related to the grand average EP at central components in both pain-free subjects and FBSS patients (Figure 12). The fact that attention occurred can be explained since all subjects were instructed to focus on the stimulus during the measurements. Also, other fixed effects of the LMM are significantly associated with the EP at CPz-A1A2, indicating that stimulus properties affected the EP for detected stimuli.

5.4 Interpretation in terms of a facilitated conditioned pain modulation

In our study, we speculated that the instability of NDTs in chronic pain patients might be explained by the role of descending pathways, which are responsible for inhibiting peripheral nerve input. We hypothesized that the nociceptive control system was altered in pain patients. Probably, the psychophysical NDTs were modulated by conditioning stimuli: 'Conditioning Pain Modulation' (CPM), which is defined as a psychophysical paradigm in which a conditioning stimulus is used to affect a test stimulus[77,78].

In fact, we changed the condition by the number of measurements including multiple repeated trials. Therefore, the stimuli of the first measurement (which illustrate the sensation thresholds with a CPM in rest) can be determined as 'test-stimuli' and stimuli of the second measurement as 'conditioning stimuli'[77,78]. The phenomenon through which the last trial stimulus (of the first measurement) affects the first trial stimulus (of the second measurement) might, in this case, be modulated by the term CPM. It is already explained that NDTs increased over the number of perceived stimuli, because of habituation. After the first measurement, we changed the stimulation electrodes to the other hand and continued the familiarization procedure, which took approximately ten minutes in time. In pain-free subjects was seen that initial NDTs of the second measurement were similar to results from the first measurement, which is speculated that the regulatory system responsible for pain modulation 'was reset' due to the fact that the nociceptive input was stopped for a while (Figure 15, Appendix). However, in FBSS patients who suffer from chronic pain, initial thresholds of the second measurement seem to be higher than in the first measurement (Figure 16, Appendix). This observation is probably caused by the underlying pathophysiology, which might be explained by a facilitated CPM[79,80]. Since the CPM system seems to be 'overactivated' due to the MTT paradigm, it might be playing a role during the whole measurement. Probably this effect contributes to the phenomenon of unstable

NDTs for all settings in FBSS patients, mainly in the second measurement. Maybe, the facilitated CPM also explains why NDTs in FBSS patients are soaring faster (due to multiple received stimuli) than in pain-free subjects, and why single-pulse and double-pulse stimuli (due to a changed condition) show different behavior of NDTs in FBSS patients compared to pain-free subjects.

5.5 Strengths and limitations

The strength of this study is that the MTT-EP experiment provides new possibilities for getting insights into the processing of nociceptive stimuli in chronic pain patients. The MTT-EP experiment has never been performed in pain-free subjects in a hospital environment and in chronic pain patients. Measuring a clear pain-free subject population at St. Antonius Hospital has multiple advantages. First, the replicability of the MTT-EP experiment can be explored and hence results from the University of Twente can be validated. Second, the pain-free subjects can be used as a healthy control group for FBSS patients. FBSS patients, who are scheduled for neuromodulation therapy, are chosen because they are suffering from a central sensitization syndrome (CSI > 40). Results from this study are very valuable because a combination of NDTs and EPs provides unique neurophysiological information about the behavior of the CNS. The MTT-EP experiment can collect detailed information about these neurophysiological effects using an extensive MTT paradigm.

However, the low number of FBSS patients included is a limitation of this study. Based on the explorative character of this study, it is recommended to enlarge the group to a minimum of 15 participants. Then, the NDTs and EPs can be estimated more accurately. Therefore, results from the LMM in FBSS patients are just an indication and hence a comparison between FBSS patients and healthy controls cannot be made statistically yet.

A second weakness is that other influences, such as medication intake, might play a role in results from FBSS patients. Namely, six out of seven chronic pain patients took medication before the start of the MTT-EP experiment because the pain was unbearable. Most of the patients took oxycodone or pregabalin (Lyrica). Oxycodone is an opioid with an agonist activity on the endorphin receptors (Mu, Kappa, and Delta), which finally inhibits depolarization of afferent neurons in the nociceptive system and hence might result in higher NDTs. Pregabalin inhibits synaptic transmissions in calcium channels in the brain neurons, which ensures that hyperexcitable neurons in the brains are more relaxed.

Also, the difference of subject characteristics may affect the NDTs. Although BMI is not significantly different ($p = 0.070$), probably because of the small patient group, it is known that BMI can increase detection thresholds due to a lower density of nociceptors and altered hormonal regulation[72]. Moreover, age between pain-free subjects and FBSS patients are significantly different ($p = 0.001$), while literature shows a strong relationship between age and detection thresholds[73]. And finally, male/female-ratio is different, while it is investigated that females show lower detection thresholds than males[74].

In addition, there are some factors that limited the usability of the MTT-EP experiment. Firstly, the duration of the measurements was reported as too large (by five out of seven subjects), which limited probably the stability of attention. However, response times of the stimulus detection did not increase over time. Secondly, three patients responded that the design of the stimulator limited the usability because their hands were cramped during the measurements. Finally, the large number of EEG electrodes enlarged extremely the duration of the MTT-EP experiment, which limited the usability for the observer.

6 Conclusion

This study can conclude that the MTT-EP experiment seems to be replicable in St. Antonius Hospital because important phenomena that occurred in the NDT and EP during nociceptive stimulation of pain-free subjects are in line with results from the previous study at the University of Twente. For example, average NDTs and EPs presented similar profiles. Next, important phenomena, such as habituation and paired-pulse facilitation, are observed in both pain-free subject groups. Also, it is seen that the EP for detected stimuli is rather modulated by stimulus detection, amplitudes and the number of received stimuli.

In addition, an altered behavior of NDTs and EPs seems to be observed in FBSS patients compared to pain-free subjects, which suggests that the MTT-EP experiment might be applicable to give insights into these neurophysiological effects in central sensitized chronic pain syndromes. For instance, we found strikingly higher NDTs in FBSS patients, in which single-pulse stimuli behaved mainly unstable compared to results from pain-free subjects. These NDTs may implicate that additional facilitating effects occurred in the CNS of FBSS patients. Furthermore, it was demonstrated that detected stimuli from nociception can be characterized by cortical activity using the MTT-EP experiment in FBSS patients. An additional early phase component of the EP was found at CPz-A1A2 for detected stimuli, which might indicate that it can be possibly a potential biomarker of brain processing in FBSS patients. Although the CSI showed that these chronic pain patients suffered from a central sensitization syndrome (CSI-score = 49.0), it is unknown whether the behavior of neurophysiological effects is related to an altered central sensitization. It might be possible that analgesics affected the results yet. Since the results are still promising, it is recommended to continue this study in these chronic pain patients to compare them statistically with healthy controls.

7 Recommendations

7.1 Short-term recommendations

Our results about the MTT-EP experiment are encouraging since it seems to be replicable in a hospital and applicable in chronic pain patients. Therefore, it is highly recommended to continue this study under the same conditions using the following short-term recommendations. Firstly, it is recommended to enlarge the cohort of FBSS patients, because current results from these patients seem to be promising. Then, the average NDTs and grand average EPs should be estimated more accurately. The observer should continue to ask the patients specifically to avoid analgesic medication intake before the MTT-EP experiment, because it might affect the results. Of course, it is allowed when the pain is unbearable. Secondly, a new LMM should be coded in MATLAB including data from pain-free subjects at the University of Twente together with data from pain-free subjects and FBSS patients at St. Antonius Hospital. Then, this LMM has potential to provide the basic for statistical tests between all groups, which enables to answer the primary research question about replicability and secondary research question about the difference between FBSS patients and healthy controls. Data analysis of EPs can be expanded by analyzing effects in frequency domain, which would be beneficial to give more insight into behavior of EPs. Next, the relationship between average NDTs and central sensitization should be analyzed using results from the CSI. CSI-scores from both FBSS patients and healthy controls should be compared statistically using a t-test and can be shown by a scatter plot. Furthermore, the difference between the first and second measurement should be analyzed statistically, because the second measurement seems to be affected by the first measurement in FBSS patients. If so, it is recommended to use the first measurement (on dominant hand) for statistical analysis, because this was also performed in subjects at the University of Twente. Subsequently, the following research question arose: What is the effect of the measurement number in both pain-free subjects and chronic pain patients? Also, the results of response times can be included to analyze the influence of attention. For now, it does not seem to play a role. Lastly, characteristic factors, such as BMI, age and sex related to central sensitization should be analyzed within the pain patients.

7.2 Future perspectives

Future work concerning the MTT-EP experiment should focus on other chronic pain patients. The procedure of the MTT-EP experiment is already prepared to include the next population of chronic pain, namely complex regional pain syndrome (CRPS, please refer to Appendix A4 for additional information). It is recommended to include CRPS-I (type 1, developed without nerve lesions) patients following an injury of the arms, because in these patients both central and peripheral changes in pain processing are supposed. The reason for including only CRPS-I patients with pain in the hands or forearms is to be able to optimally compare them with healthy controls. Since we have already measured on both hands in pain-free subjects, our technique can be applied to compare psychophysical responses from delivered stimuli to the affected and unaffected hand within these patients. Analyzing neurophysiological responses during nociceptive processing in these patients would be very valuable, because it has never been done

anywhere in the world. More important, these results have potential to fill in gaps in our understanding about central sensitization. This research could eventually lead to the identification of novel biomarkers for diagnostics. It should be very worthwhile, therefore, to integrate this population in an LMM. Subsequently, it should be possible to compare behavior from CRPS-I patients with results from FBSS patients. Since we have performed already one MTT-EP experiment on a CRPS-I patient, it can be supposed that our technique seems to be applicable in this category. An amount of 19 other patients is recommended to recruit from the CRPS patient association and eventually from the outpatient pain clinic at St. Antonius Hospital. It is advised to complete this study before August 2020, because this was reported in the approval of the medical ethical research committee. Because of the technical nature of the MTT-EP experiment, it is recommended that the observer has a broad experience with computer programming in MATLAB when measurements are performed in patients. Unfortunately, the standard operational procedure of the MTT-EP experiments is still in its infancy and procedures for running the scripts of the software needs to be automated in future.

Next to this study, the MTT-EP experiment should create many new research opportunities for future clinical studies. Eventually, future investigations should shorten the duration of the measurement. Nowadays, three different stimulus settings are used, but it is explored that double-pulse stimuli with 10 and 40 ms IPI demonstrate almost similar NDTs. However, in results from the LMM in pain-free subjects at St. Antonius Hospital is seen that double-pulse stimuli with 10 ms IPI are significantly related to the grand average EP in both central and lateral components, whereas double-pulses with 40 ms IPI show only significant relationships with the EP on the central component. Therefore, it is recommended to exclude the setting for double-pulse stimuli with 40 ms IPI, because it would not provide additional information. This will lead to a reduction of approximately 20 to 30 minutes in total. In future, it is also advised to save time by excluding multiple EEG channels. Currently, 64 channels are prepared during the MTT-EP experiment, while only a few are analyzed. Therefore, it is recommended to find out which derivations (next to CPz-A1A2, FPz-T7 and FPz-T8) provide worthwhile information and which channels do not contribute. In addition, if a full automated algorithm of the LMM is written in MATLAB, our MTT-EP experiment means that results about behavior of central sensitization in other chronic pain patients can now be produced very quickly. It would be useful to investigate which population would be promising and remain to be identified next. From this starting point, new study protocols should be written for the medical ethical research committee. Eventually, additional pQST measurements next to the MTT-EP experiment can be helpful to validate the results from CSI in patients suffering from a central sensitization syndrome. Another possible direction, which would be very promising, is to continue these MTT-EP experiments in the same pain-free subjects and FBSS patients. First, it would be interesting to investigate the effects of a test-retest, because it yields unique results on the reliability of this MTT-EP experiment. Second, future work should be performed in the same FBSS patient, but then those who are just treated by neuromodulation. This chain of results would facilitate new insights into the underlying mechanisms of the CNS and reveal possibly a new approach to current treatment. Besides, the MTT-EP experiment might offer a better understanding of the effect of medication in chronic pain patients. All subjects can be categorized by different groups of analgesic medication and both NDTs and EPs should be

analyzed subsequently. However, it is seen that many more components, such as emotional state, degree of anxiety, attention and distraction, past experiences, memories, and other factors, influence the experience of pain[81]. Therefore, treatment of chronic pain can only be successful if the assessment focuses on the entire person, instead of just the pathophysiology[82]. It is known that emotional distress is often related to chronic pain experience. Thus, it would be interesting to modify emotional states of the subjects (by watching a video) during the MTT-EP experiment. Additionally, since our results show that EPs are highly affected by stimulus detection, it would be useful to research EPs when the subject is instructed to pay attention to another part of the body instead of the stimulating hand.

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Appendix

A1 Average NDTs and SD

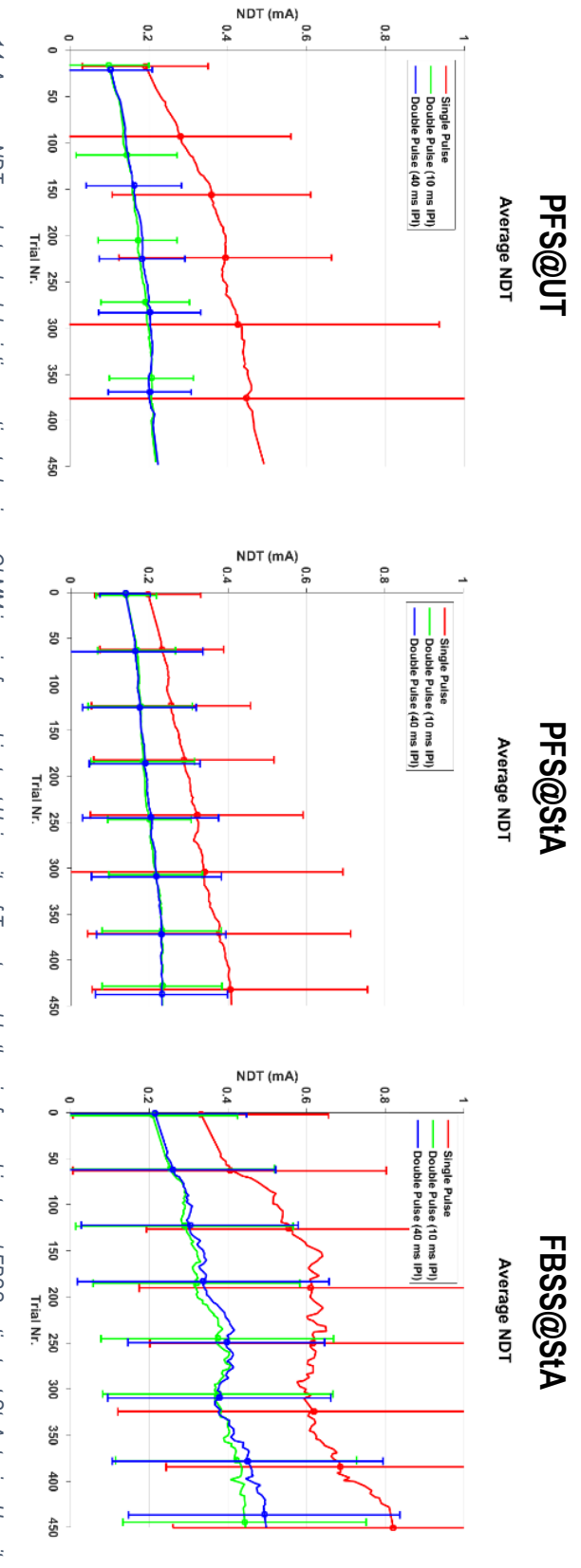

Figure 14. Average NDTs and standard deviations estimated using a GLMM in pain-free subjects at University of Twente, and both pain-free subjects and FBSS patients at St. Antonius Hospital.

A2 NDTs of pain-free subjects

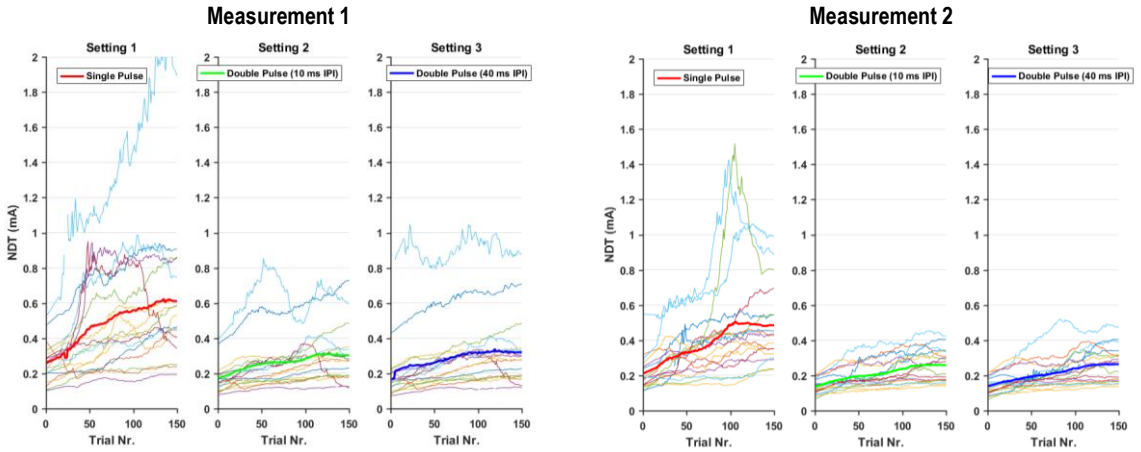

Figure 15. Individual NDTs without cleaning the data. Measurement 1 (left) and measurement 2 (right) from 17 pain-free subjects at St. Antonius Hospital.

A3 NDTs of FBSS

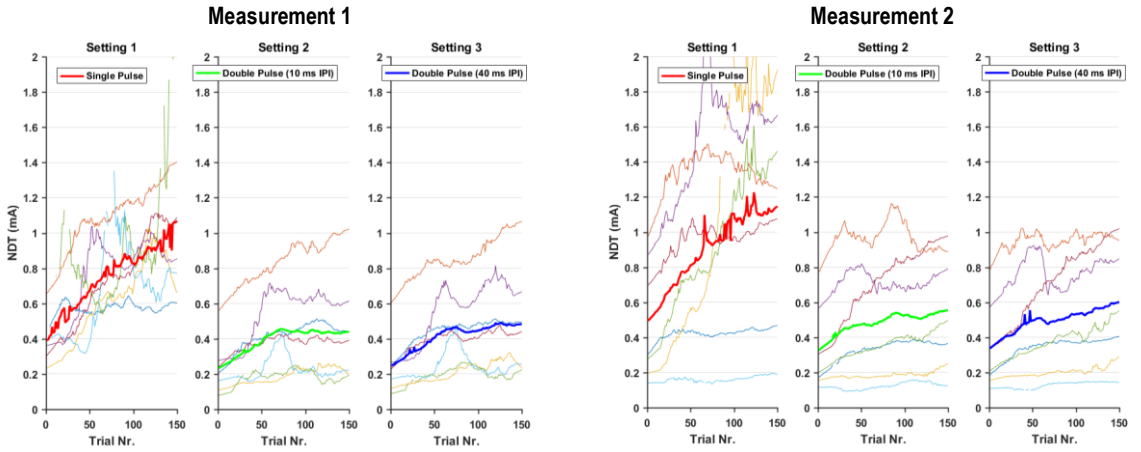

Figure 16. Individual NDTs without cleaning the data. Measurement 1 (left) and measurement 2 (right) from 7 FBSS patients at St. Antonius Hospital.

A4 Complex regional pain syndrome

CRPS is a regional, post-traumatic pain syndrome that is characterized by spontaneous or stimulus-induced pain in combination with sensory, autonomic, trophic, and motor disturbances[83–85]. The severe and often chronic pain condition is disproportionate to any preceding injury and is not restricted anatomically to the distribution of a specific peripheral nerve[84]. CRPS may develop after injury (limb trauma or peripheral nerve lesions) in up to 5% of all cases (and most often affects one limb)[86].

CRPS comprises a variety of symptoms, which cluster into four distinct categories: (1) abnormal pain, (2) vasomotor and temperature changes, (3) sudomotor changes and edema, and (4) motor dysfunction and tropic changes[87,88]. To address the problems of patient heterogeneity, IASP has proposed consensus-based diagnostic criteria[85]. The term CRPS is subdivided into CRPS-I and CRPS-II. Type I develops without obvious nerve lesion, whereas type II always follows nerve lesions.

Currently, the pathophysiology of CRPS is incompletely understood. It supposed to be multifactorial in nature, which is characterized by an aberrant (neurogenic) inflammatory response to tissue injury[83,84]. Although there is a marked reduction of nociceptive fibers (C and Að fibers) suggesting that CRPS-I is a true neuropathic pain condition with an altered peripheral nervous system[85,88–90], also there are signs of central changes in pain processing[85,88]. Possibly, central sensitization contributes to cortical reorganization, while maladaptive cortical reorganization, in turn, plays a role in chronification of pain[85,91]. There is no conclusive data whether cortical reorganization and altered brain processing are the cause or the result of CRPS[85].