Master thesis Technical Medicine

Medical Sensing and Stimulation

Observing Electrical Brain Responses during Processing of Nociceptive Stimuli around the Detection Threshold combined with a Cold Pressor Test

An Explorative Study

R. (Ruben) Dollen, BSc.

3-12-2020

UNIVERSITY OF TWENTE.

St Antonius
een santeon ziekenhuis

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PREFACE

This thesis is the major end product after one year of hard work to obtain my master's degree as a Technical Physician. In the past year, I worked on the validation and implementation of the NDT-EP method in the St. Antonius Hospital in Nieuwegein. I was happy to be able to do my M3 internship at the Department of Anesthesiology, Intensive Care and Pain Medicine, which offered me to develop personally and professionally. My graduation research would not have been possible without the collaboration and support of many people. Therefore, I would like to take the opportunity to thank them.

First of all, I would like to thank my technological, medical and process supervisor. Jan and Imre, thank you for offering me the opportunity to conduct my graduation research in your research group. Thanks for sharing your knowledge, suggestions and critical thinking. I really enjoyed the enthusiasm and our discussions. Thanks for being part of the team. Marleen, thank you for having been my process supervisor for the past two years. I really enjoyed our conversations at the University at the end of each internship. You really helped me to realize the importance of reflection by asking questions that made me think about myself and the things I do.

As daily colleagues, I would like to thank Tom, Silvano, Jelle, Eva, Marloe, Manon, Myrthe and Judith. Many thanks for providing me valuable input on my study throughout the year, for thinking together and exchanging ideas. Thanks for letting me feel part of the team, and the fun times we had.

I would like to thank Myrthe for conducting a pilot study regarding CPM. It really improved the protocol as it was used in this study. Also, I would like to thank CHDR in Leiden for their hospitality. Robert-Jan Doll and Wim Oomen, the visit you provided me and your professionalism improved my test protocol considerably.

Also from Enschede, Boudewijn, without your help and support my graduation would be nothing. Thank you for all the instances you took the time to help me debugging in Matlab. Without your quick responses and bright ideas, I would never have been able to rapidly resume my (programming) activities. Niels, thank you for helping me writing on the study protocol. It really speeded up granting study approval. I'm really looking forward to collaborate on writing scientific papers.

Lastly, my parents and brother. Despite the ups and downs I experienced during my time as a student, you always supported me and believed in me. Thank you for all your unconditional love and support.

SUMMARY

Introduction: Conditioned pain modulation (CPM) evaluates the effect of a painful conditioning stimulus on a test stimulus. In this study, the technical feasibility of the NDT-EP method combined with a painful conditioning stimulus, chosen as the cold pressor test (CPT), was explored in a clinical environment. Also, the effect of CPT on nociceptive detection thresholds (NDTs) and evoked potentials (EPs) was investigated.

Methods: Two groups of subjects were included in this study. The first group involved healthy pain-free subjects and the second group comprised chronic low back pain patients diagnosed with failed back surgery syndrome (FBSS). Healthy subjects underwent two measurement sessions to perform a test-retest analysis. Intra-epidermal stimuli were applied as a test stimulus, while CPT was used as a conditioning stimulus (1°C for 7 minutes). A test-retest reliability analysis was performed on healthy subjects by comparing NDTs of test measurements to retest measurements. Also, EP amplitudes of test measurements were compared to retest measurements for each CPT phase (i.e. pre-, per-, and postCPT), separately. Additionally, the effect of CPT on NDTs and EPs for test, retest and FBSS measurements was explored. Also the contribution of age to the effect of CPT on NDTs and EPs was investigated in healthy subjects.

Results: Twenty healthy subjects (median age: 40.5 years, eleven females) and six FBSS patients (median age 61.5 years, four females) were included in this study. No difference was found in detection probability for retest measurements following single pulse stimulation compared to test measurements of healthy subjects. However, detection probabilities of retest measurements following double pulse stimulation differed from test measurements ($P = 0.048$). EP amplitudes perCPT and postCPT of test measurements for double pulse stimuli differed from retest measurements ($P = 0.022$ and $P = 0.036$, respectively). No differences were found for EP amplitudes preCPT and all CPT phases for single pulse stimuli between test and retest measurements. Detection probabilities of test and retest measurements were significantly modulated by condition and interactions where condition was involved. The effect of CPT on NDTs obtained during application of CPT was moderate, while NDTs obtained after termination of CPT were significantly modulated compared to pre- and perCPT NDTs ($P < 0.010$). Moderate to poor effect of CPT was found in FBSS patients and age analysis.

Conclusion: Results demonstrated that both healthy subjects and FBSS patients seem to be able to properly execute the experiment and tolerate the intensity of the conditioning stimulus. Good reliability of single pulse stimulation in detection probabilities, NDTs and EP amplitudes was observed. Detection probabilities and average group NDTs in both stimulus properties were modulated by CPT in healthy subjects. Average group NDTs were moderately affected by CPT in FBSS patients and age analysis. EP amplitudes were poorly affected by CPT in all study groups. Effects of psychological factors, such as expectancies and distraction, in pain modulation cannot be excluded. Additional research, including subject training and application of control CPT experiment, is recommended to investigate the influence of psychological factors on pain modulation.

Keywords: *Evoked potential, failed back surgery syndrome, conditioned pain modulation, cold pressor test, linear mixed regression, nociceptive threshold, conditioning stimulus, descending modulatory pathways, pain inhibition.*

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BMI	Body mass index
CNS	Central nervous system
CPM	Conditioned pain modulation
CPT	Cold pressor test
CSI	Central sensitization inventory
DCML	Dorsal column-medial lemniscal
DNIC	Diffuse noxious inhibitory control
DP	Double pulse
EEG	Electroencephalography
EP	Evoked potential
FBSS	Failed back surgery syndrome
GCT	Gate control theory
GLMM	Generalized linear mixed model
HC	Healthy control
IES	Intra-epidermal stimulation
IPI	Inter-pulse interval
LMM	Linear mixed model
MTT	Multiple threshold tracking
NDT	Nociceptive detection threshold
NoP	Number of pulses
NRS	Numeric rating scale
PNS	Peripheral nervous system
PW	Pulse-width
SP	Single pulse

1. INTRODUCTION

1.1. GENERAL INTRODUCTION

Nineteen percent of adults suffer from moderate to severe chronic pain and many of them cannot maintain an independent lifestyle because their pain interferes substantially with their daily activities (Edwards *et al.*, 2003; Thomson & Jacques, 2009). Forty percent of chronic pain patients does not receive adequate treatment for the pain (Breivik *et al.*, 2006). This emphasizes the importance of improving treatments for chronic pain. Pain itself often modifies the way the central nervous system works, so that a patient actually becomes more sensitive and gets more pain with less provocation (Kamen, 2018). This is called central sensitization and plays an important role in chronic pain syndromes, such as failed back surgery syndrome (FBSS). FBSS is characterized by chronic back and/or leg pain and functional impairment following back surgery and has a prevalence of 10-40% (Sahin *et al.*, 2009). Patients suffering from FBSS are supposed to have an altered central sensitization forced by constant stimulation of nociceptive circuits (Blond *et al.*, 2015; Bordoni & Marelli, 2016; Sahin *et al.*, 2009; Shapiro, 2014; C. J. Woolf, 2011; Yalbuzdag *et al.*, 2016). Although the complex pathophysiology is poorly understood, it involves both nociceptive and neuropathic factors (Rigoard *et al.*, 2015).

It is important to study these underlying mechanisms (central and peripheral), and how they are altered in chronic pain patients compared to healthy controls. One major obstacle is the lack of an objective measure of central and peripheral sensitivity. Tracking psychophysical thresholds can facilitate the investigation of the underlying mechanisms of sensitization. Recently, a method has been developed for measuring nociceptive detection thresholds (NDTs), which are stimulus amplitude thresholds for a detectable sensation, using intra-epidermal electrocutaneous stimulation (IES) of the skin. In earlier studies, a multiple threshold tracking (MTT) algorithm for measuring NDTs of IES stimuli with single and multiple pulses was developed (Doll *et al.*, 2015, 2016b). Changes of the NDT related to stimulus parameters were demonstrated (Doll *et al.*, 2016a). Additionally, multiple-trial averages of the electroencephalographic (EEG) signal, referred to as evoked potentials (EPs), have been shown sensitive to changes in stimulus parameters (André Mouraux *et al.*, 2014; Esther M. van der Heide *et al.*, 2009; Catherine J. Vossen *et al.*, 2015). Since MTT has been shown to be effective in measuring the effect of stimulus parameters on stimulus detection, while the EP has been shown to reflect neurophysiological activity related to stimulus processing, a combination of both techniques might provide insight into the relation between neurophysiological activity and nociceptive stimuli.

The assessment of the observability of changes in the nociceptive function are hypothesized to play a key role in the development and maintenance of chronic pain, including FBSS. Le Bars *et al.* reported that activity in the dorsal horn and trigeminal nuclei is inhibited by the application of noxious electrical stimuli to distal body areas in rats (Daniel Le Bars *et al.*, 1979; D. Le Bars *et al.*, 1979). This phenomenon was initially termed 'diffuse noxious inhibitory control' (DNIC). Conditioned pain modulation (CPM), the human counterpart of DNIC, has been identified as an advanced psychophysical measure with high clinical relevancy in the characterization of one's capacity to modulate pain and consequently one's susceptibility to acquire pain disorders (Yarnitsky *et al.*, 2015). CPM inhibits pain through cerebral/ supraspinal and cerebrospinal mechanisms, reflecting activation of the endogenous analgesia system, where ascending pain-induced activity evokes descending pathways, which subsequently elicit inhibitory effects on spinal

nociceptive inputs. Although knowledge on pain modulation has improved significantly over the years, the relation between CPM and chronic pain in humans is still incompletely understood. Additionally, age-associated decrements in CPM were observed (Edwards *et al.*, 2003; Naugle *et al.*, 2015; Washington *et al.*, 2000). Therefore, additional research is needed to further understand (differences in) the endogenous pain inhibition ability of healthy subjects and chronic pain patients and its sensitivity to subject characteristics.

Various research groups have been used the Cold Pressor Test (CPT, immersing an extremity (hand or foot) into ice-cold water) extensively and consistently as a conditioning stimulus for the activation of CPM (Pud *et al.*, 2009; van Wijk & Veldhuijzen, 2010). They demonstrated that CPT is able to activate central mechanisms such as CPM and induces a well characterized and demonstrated centrally mediated change in the nociceptive system. The activation of CPM can be observed by a lower sensitivity to nociceptive stimuli during and shortly after the CPT. Other results show that activation of CPM also modulates EPs obtained by (electro)cutaneous stimulation (Eitner *et al.*, 2018; Hoffken *et al.*, 2017; Torta *et al.*, 2015).

CPT can be used to induce the change in pain perception, referred to as conditioning stimulus (CS), and has been identified and tested in technical feasibility studies at the University of Twente wherein the NDT-EP method was combined with CPT baths of different temperatures. It was shown that it is technically feasible to combine the NDT-EP method with a CPT and the immersion times (and consequently the number of Stimulus-Response Pairs (SRPs)) of the subjects were markedly higher than expected. Therefore, the conclusion of the technical pilot was that the present setup and protocol could be used for follow-up research in a clinical environment. Here, the integrity of the endogenous modulating mechanism might be investigated by analyzing the nociceptive detection thresholds and evoked potentials obtained using the NDT-EP method as a result of application of the cold pressor test (CPT) in both pain-free subjects and chronic pain patients.

For the above-mentioned reasons, CPT is a promising technique for the validation of the NDT-EP method using results of nociceptive detection thresholds and evoked potentials of pain-free subjects. Results might contribute to the knowledge in mechanisms involved in both ascending and descending pathways of the nociceptive system and consequently, might provide insight into the underlying pathophysiology of chronic pain.

However, it is still unknown whether the NDT-EP method can be combined with CPT in a clinical environment. This leads to the following central aim:

Central aim:

Explore the feasibility of the NDT-EP measurement method combined with a cold pressor test in (1) healthy pain-free subjects and (2) chronic low back pain patients diagnosed with failed back surgery syndrome (FBSS).

1.2. THESIS OUTLINE

This thesis comprises of six chapters. Chapter 2. Background elaborates on signal processing in the peripheral and central nervous system and provides knowledge about mechanisms involved in descending pain modulation. Chapter 3. Methods is focused on the methodology employed in this study, including different statistical models used for data analysis. Subsequently, chapter 4. Results describes measurement outcomes, followed by chapter 5. Discussion, in which the results are discussed, strengths and limitations are provided along with recommendations for further research. Conclusion are drawn in the last chapter, 6. Conclusion.

2. BACKGROUND

2.1. PAIN

The International Association of the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” (IASP, 2020a). Pain encourages to adjust behavior to reduce the risk of tissue damage and, after injury, helps the healing process by fostering resting the painful area. Pain is a subjective experience and cannot be measured easily. Its severity depends on individual differences in physiological, emotional and cognitive condition.

Pain can be divided in acute pain and chronic pain. Acute pain is caused by an internal or external stimulus such as an infection or stab wound and typically has a specific, treatable cause. While chronic pain is caused by, for instance, an ongoing disease process (i.e. cancer or arthritis) or dysfunction or damage of the nervous system (painful diabetic neuropathy). Chronic pain is defined as pain that lasts or recurs for longer than three months (IASP, 2020b).

In order to be able to alleviate pain, it is pivotal to understand the complex mechanisms by which pain is triggered and processed in higher brain areas. These signals are transmitted through a complex system of neural pathways from the peripheral nervous system (PNS) to the central nervous system (CNS). The next chapter will focus on how these pathways are initiated and how signals are processed from the PNS through the spinal cord to higher brain centers.

2.2. SIGNAL PROCESSING IN THE NERVOUS SYSTEM

2.2.1. SENSORY NERVE FIBERS

Primary afferents are sensory neurons located in the periphery of the human body and convey pain, temperature and light touch modalities from the periphery to the CNS through thinly myelinated and unmyelinated nerve fibers (first-order nerve fibers). Afferents are pseudo-unipolar neurons that have one axon leaving the cell body dividing into two branches (one travelling to the periphery and one to the spinal cord), as opposing to multipolar neurons which consist of an axon and dendrite. Afferents can be divided in myelinated A-fibers and unmyelinated C-fibers (see Figure 1). A-fibers can be subdivided into Aα-, Aβ-, Aγ- and Aδ-fibers. Aα-fibers belong to the thickest A-fibers of about 13-20 µm in diameter, whereas Aβ- and Aγ-fibers are around 6-13 µm and 3-8 µm in diameter respectively. These fibers are involved in transmitting proprioception and touch stimuli. Aδ-fibers belong to the smallest myelinated fibers of about 2-5 µm in diameter and have a relatively fast conduction velocity of around 30 m/s. C-fibers belong to the thinnest fibers being unmyelinated, less than 2 µm in diameter and have a relatively slow conduction velocity of around 2 m/s. Both Aδ-fibers and C-fibers are involved in transmitting painful stimuli from free nerve endings, known as nociceptors, in the periphery to higher brain centers.(Hilgenberg-Sydney & Conti, 2011; Hughes & Appel, 2016; Salzer, 2015; Yam *et al.*, 2018)

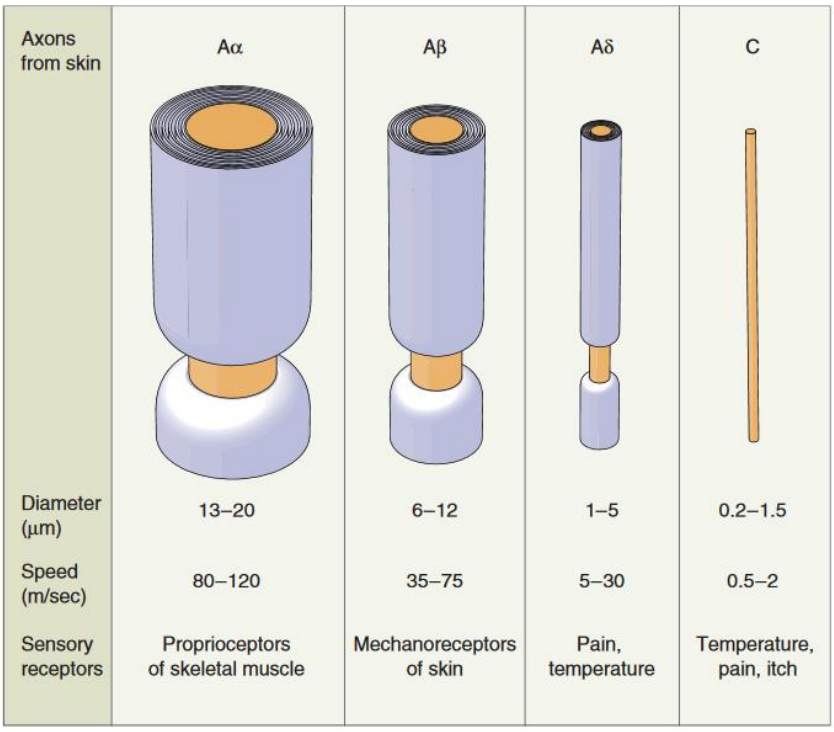

Figure 1: Various primary afferent fibers. Aα-, Aβ- and Aδ-fibers are surrounded by myelin (light-blue sheath), which speeds the conduction of nerve impulses. Adapted from: Bear, M. F., Connors, B. W., & Paradiso, M. A. (2016). Neuroscience: Exploring the Brain*: Wolters Kluwer.*

Pain can be divided into an early perception of sharp pain and a later perception of a duller, burning and longer-lasting pain sensation, which are also often referred to as first and second pain. Aδ-fibers are responsible for transmitting this first pain, whereas C-fibers are involved in transmission of this second pain (see Figure 2).

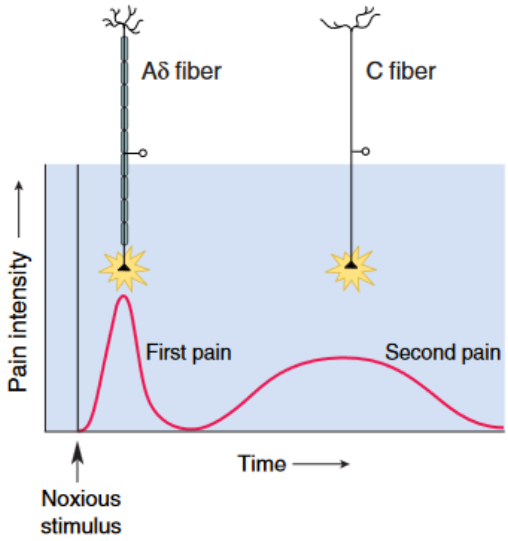

Figure 2: The first pain sensation after a noxious stimulus is mediated by fast Aδ-fibers. The second, longer-lasting pain sensation is mediated by slow C-fibers. Source: Bear, M. F., Connors, B. W., & Paradiso, M. A. (2016). Neuroscience: Exploring the Brain: Wolters Kluwer.

2.2.2. SYNAPTIC TRANSMISSION

Axons can be myelinated or unmyelinated. They conduct action potentials away from the nerve cell body. In unmyelinated axons action potentials travel along the axon by affecting its neighboring segment referred to as continuous propagation. The action potentials propagate in the upstream direction only, since the proximal segment of the axon that just generated an action potential is in refractory period. In myelinated axons the action potentials jump along the periodic gaps in the insulating myelin, also known as the nodes of Ranvier, enabling faster and more reliable signal propagation, which is known as saltatory conduction. Once the action potential reaches the presynaptic terminal at the end of the axonal branches the chemical synapse occurs in the synaptic cleft (see Figure 3). The action potential creates membrane depolarization causing opening of the voltage-gated sodium channels and eventually leading to further entry of calcium into the axon terminal. Here, calcium and the neurotransmitter form a vesicle which is fused with the presynaptic cell membrane, known as exocytosis, whereby the neurotransmitters are released into the synaptic cleft. Pain-associated neurotransmitters involved in (in)activation of receptors are eicosanoids (such as prostaglandin, prostacyclin and leukotriene B4), neuropeptides (such as nerve growth factor, bradykinin, substance P, neurokinin A and B and opioid peptides), amino acids (such as glutamate, glycine, GABA and CGRP), nitric oxide, norepinephrine, serotonin (5-HT), histamine and ATP. The neurotransmitters diffuse across the gap to bind to ionotropic or metabotropic receptors located on the postsynaptic cell membrane and act as agonists allowing directly or indirectly (through second messengers) opening of ion channels. The subsequent influx of ions results in either an excitatory postsynaptic potential allowing depolarization to promote action potential generation or an inhibitory postsynaptic potential resulting in hyperpolarization to inhibit action potential generation in the postsynaptic neuron. Once the postsynaptic membrane receptors are activated resulting in inhibition or generation of an action potential the neurotransmitters are either recycled, broken down or diffused.(Neishabouri & Faisal, 2014; Wei *et al.*, 2019)

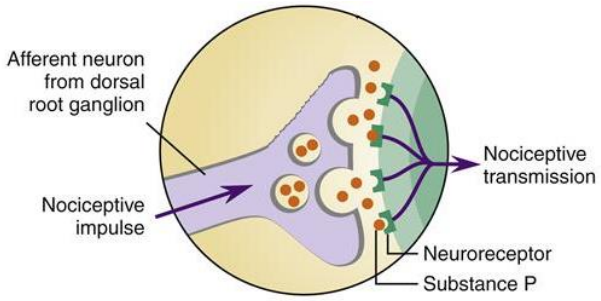

Figure 3: Example of signal transmission. Neurotransmitter substance P is released by afferent neurons into the synaptic cleft by nociceptive impulse. The neurotransmitters bind to the neuroreceptors located on the postsynaptic terminal causing signal transmission. Adapted from: Joni D. Marsh (2016). Basic Medical Key. Chapter 47: Pain - afferent sensory pain fibers.

2.2.3. DORSAL HORN

The neuronal cell bodies of afferents are located in the dorsal root ganglia, which is a cluster of neurons in the dorsal root of a spinal nerve. The dorsal root enters the spinal cord in the region of the dorsal horn located in the dorsal part of the gray matter of the spinal cord. (Todd, 2010)

The gray matter is divided into ten layers (Rexed lamina I-X) based on shape, size and function of the neurons found in these areas (see Figure 4). In the dorsal part of the gray matter (lamina I-VI), called the dorsal horn, sensory neurons are situated, while motor neurons are situated in the ventral part of the gray matter (lamina VII-IX), known as the ventral horn. In lamina X the central gray substance is located. The superficial layers of the dorsal horn (lamina I-III) consist of nociceptive-specific (NS) neurons, projection neurons and inhibitory and excitatory interneurons, while deep layers (lamina V and VI) contain wide

dynamic range (WDR) neurons. C-fibers terminate in laminae I and II; Aδ-fibers terminate in laminae I and V; Aβ-fibers terminate in laminae III, IV, and V.(Braz *et al.*, 2014; Todd, 2017)

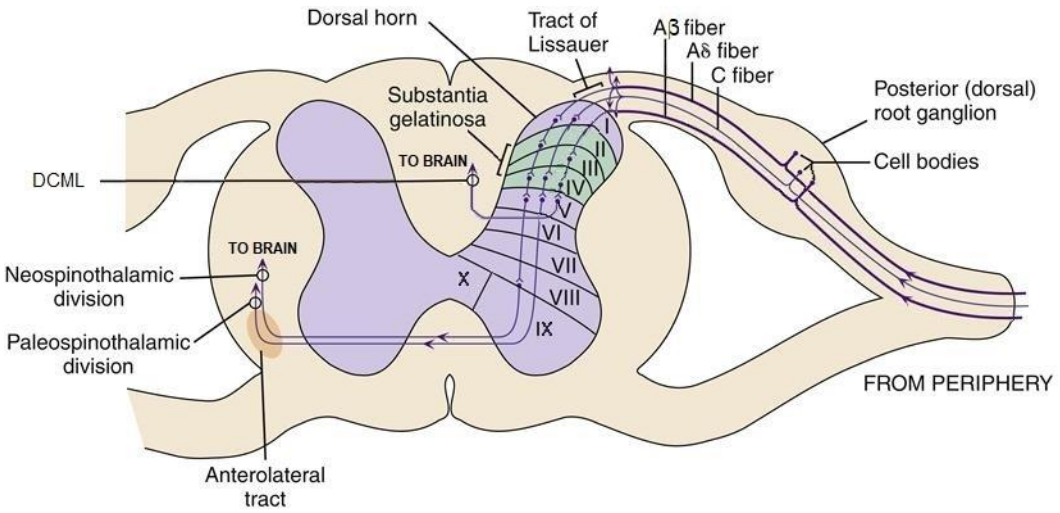

Figure 4: Spinal cord segment showing primary afferent fibers. Aβ-, Aδ-, and C-fibers from the periphery enter the spinal dorsal horn through the dorsal root. The cell bodies of the afferent fibers are located in the dorsal root ganglion. Depending on the type of fiber the neuron ascends ipsilaterally or contralaterally to the brain. Aβ-fibers ascend ipsilaterally through the Dorsal Column-Medial Lemniscal Pathway (DCML), while Aδ-, and C-fibers ascend contralaterally through the neospinothalamic and paleospinothalamic divisions which form the spinothalamic tract, as a part of the anterolateral tract. Decussation of DCML pathway occurs in the medulla oblongata. Adapted from: Joni D. Marsh (2016). Basic Medical Key. Chapter 47: Pain - afferent sensory pain fibers.

The majority of the projection neurons located in laminae I and III terminate in multiple brain regions. Many of the projection neurons are interconnected in the dorsal horn through interneurons, which facilitate indirect nociceptive inputs. Projection neurons target the caudal ventrolateral medulla (CVLM), the nucleus of the solitary tracts (NTS), the lateral parabrachial area (LPb), the periaqueductal gray matter (PAG) and several nuclei located in the thalamus, including the ventral posterolateral nucleus (VPL), the intralaminar nuclei and the posterior thalamic nucleus. Ventral posteromedial nucleus receives cutaneous inputs from the face and is therefore outside the scope of this report.(Braz *et al.*, 2014; Todd, 2017)

2.2.4. Signal transmission through the spinal cord to higher brain centers

Nociceptive inputs are delivered to the somatosensory cortex through two major somatosensory pathways, known as the dorsal column-medial lemniscal pathway and the spinothalamic pathway. All sensations transmitted through both pathways are projected to the somatosensory cortex contralaterally due to decussation of the pathways at multiple levels. However, note that decussation occurs at different locations for each pathway. The dorsal column-medial lemniscal pathway decussates at the medulla oblongata, whereas the spinothalamic pathway decussates in the spinal cord, so that inputs originating from the left side of the body are represented in the right hemisphere of the brain and inputs from the right side of the body are represented in the left hemisphere. Both pathways are shown in Figure 5 and are elaborated in the next two chapters.

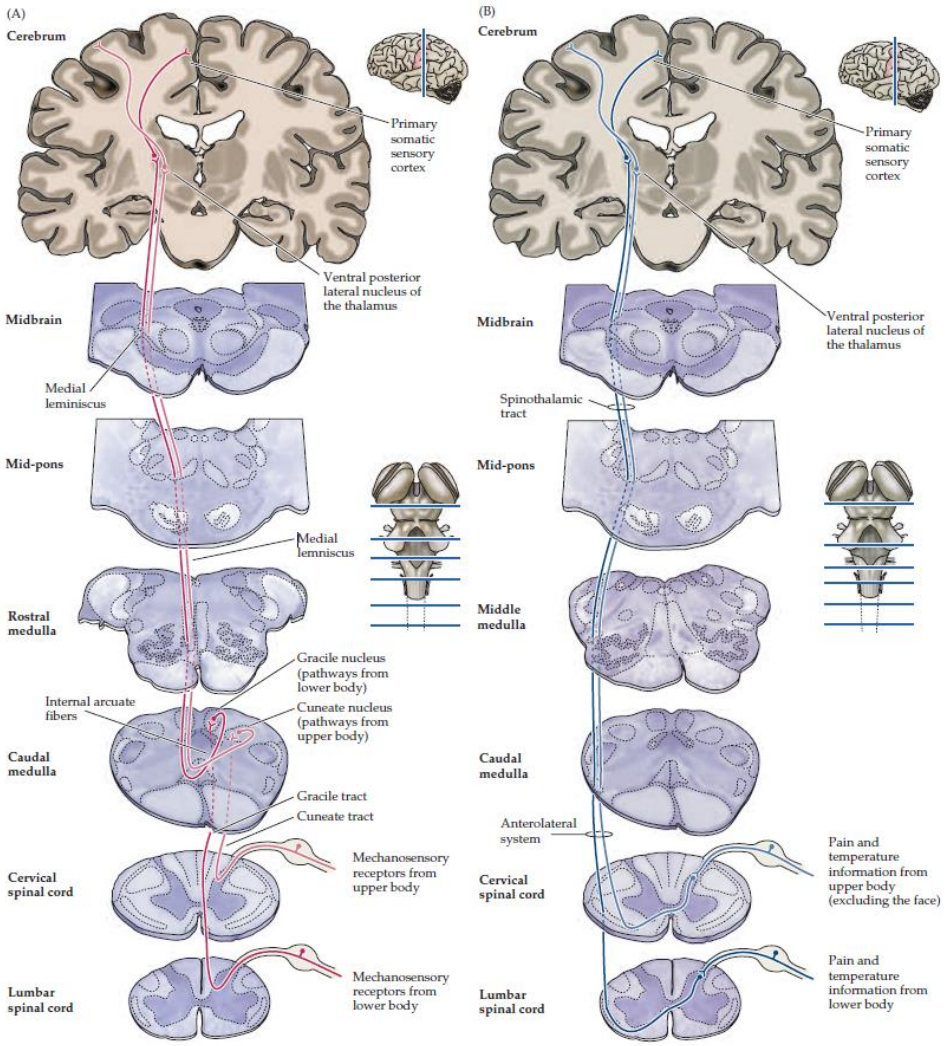

Figure 5: Schematic representation of the main somatosensory pathways. (A) The dorsal column–medial lemniscus pathway carries information from the mechanoreceptors, that mediate tactile discrimination and proprioception, to the ventral posterolateral nucleus in the thalamus. (B) Spinothalamic pathway carries pain and temperature from the spinal cord to the ventral posterolateral nucleus in the thalamus. Note that for each pathway decussation occurs at different locations. Adapted from: Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W. C., & LaMantia, A. S. (2018). Neuroscience: Oxford University Press.

2.2.4.1. Dorsal Column-Medial Lemniscal Pathway

Gnostic sensibility refers to the sensation of fine touch, vibration and proprioception. Information originating from mechanosensory receptors and proprioceptors located in the lower part of the body (below T6) enters the lumbar spinal cord and is transmitted through medial pathways of the spinal cord, known as gracile fasciculus. Other than this, information from the mechanosensory receptors and proprioceptors positioned in the upper part of the body (T6 and above) enters the cervical spinal cord and is transmitted through the lateral pathways of the spinal cord, known as cuneate fasciculus. The first-order neurons ascend ipsilateral through the mechanosensory tract located in the dorsal columns. The first-order neurons synapse with second-order neurons in the gracile nucleus and cuneate nucleus, also referred to as the dorsal column nuclei, located in the medulla oblongata, which is along with the pons and midbrain referred to as the brainstem.(Darian-Smith, 2009)

The second-order neurons, also known as the internal arcuate fibers, cross over at the midline of the medulla oblongata and travel up to the medial lemniscal pathway of the brainstem towards the contralateral ventral posterolateral nucleus of the thalamus, which serves as an intermediate relay. The second-order neurons synapse with the third-order neurons in the VPL, which carry the information to the primary somatosensory cortex (S1). The dorsal columns and medial lemniscus is often referred to as the dorsal column-medial lemniscal pathway (DCML).(Navarro-Orozco & Bollu, 2020; Purves *et al.*, 2018)

2.2.4.2. SPINOTHALAMIC PATHWAY

The vital sensibility refers to sensation of pain, temperature and crude touch. Sensations are transmitted from thermoreceptors and nociceptors through the PNS to the spinal cord. These sensations are conveyed from the spinal cord to the brain by synapsing the first-order neurons with the second-order neurons in the substantia gelatinosa of Rolando (lamina II) or the nucleus proprius (laminae III and IV). Unlike the dorsal column-media lemniscal pathway, one or two segments above or below the level of dorsal entry via Lissauer's tract, the second-order neurons decussate directly via the anterior white commissure to the contralateral side of the spinal cord. Through the spinothalamic pathway, which is composed of the ventral paleospinothalamic and lateral neospinothalamic divisions (see Figure 4), the second-order neurons ascend to the higher brain centers. The ventral pathway is involved in carrying sensory input about crude touch, while the lateral pathway transmits information about pain and temperature. Together with the spinoreticulothalamic pathway and the spinotectal pathway, the spinothalamic pathway forms the anterolateral system. The second-order neurons ascend via the vental posterolateral nucleus of the thalamus by synapsing with third-order neurons, which project to the primary somatosensory cortex.(Al-Chalabi *et al.*, 2020; Lipshetz *et al.*, 2018; Purves *et al.*, 2018; Todd, 2010)

2.2.5. SOMATOSENSORY CORTEX

Neurons in the thalamus project to cortical neurons located in the layers of the somatosensory cortex. The somatosensory system can be subdivided into the primary somatosensory cortex of the brain (S1), located in the postcentral gyrus of the parietal lobe, and the secondary somatosensory cortex. The primary somatosensory cortex is made up by Brodmann areas 3 (3a and 3b), 1 and 2 (see Figure 6). Nociceptive information transmitted to these areas is thought to be responsible for discrimination of pain. Research carried out that areas 3b and 1 respond primarily to cutaneous stimulation, area 3a mainly receives proprioceptive information, and area 2 processes both tactile and proprioceptive information. Each of the four areas of the somatosensory cortex has a complete representation of the body.

A topographic representation of all body parts of which the somatosensory cortex receives sensations is formed by the cortical sensory homunculus, which means 'little man' in Latin. Representations are medial-to-lateral organized with the inferior limb in the medial part, the face at the bottom of gyrus and the hand area in between (see Figure 7). Note that the area of the somatosensory cortex devoted to the face and hands is much larger than the relative body area in these regions. Thus, the sensory input that is particularly significant gets relatively more cortical representation.(Purves *et al.*, 2018)

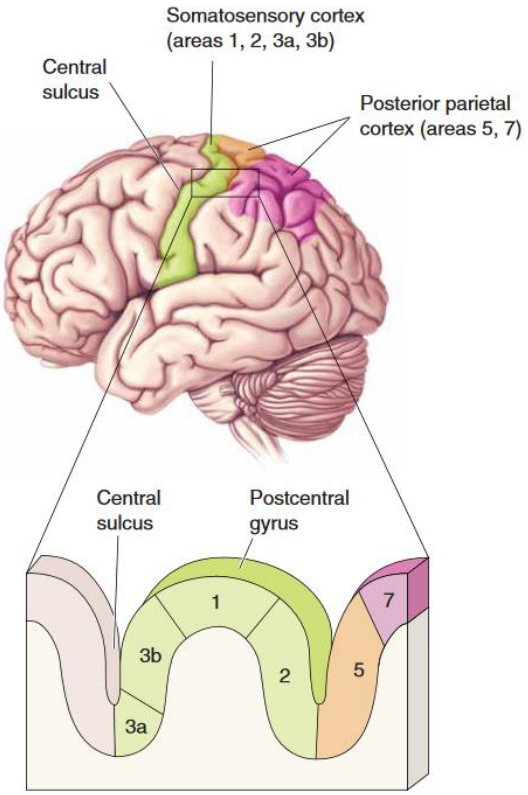

Figure 6: Somatosensory divided into multiple Brodmann areas. Adapted from: Reed, C., & Ziat, M. (2018). Haptic Perception: From the Skin to the Brain.

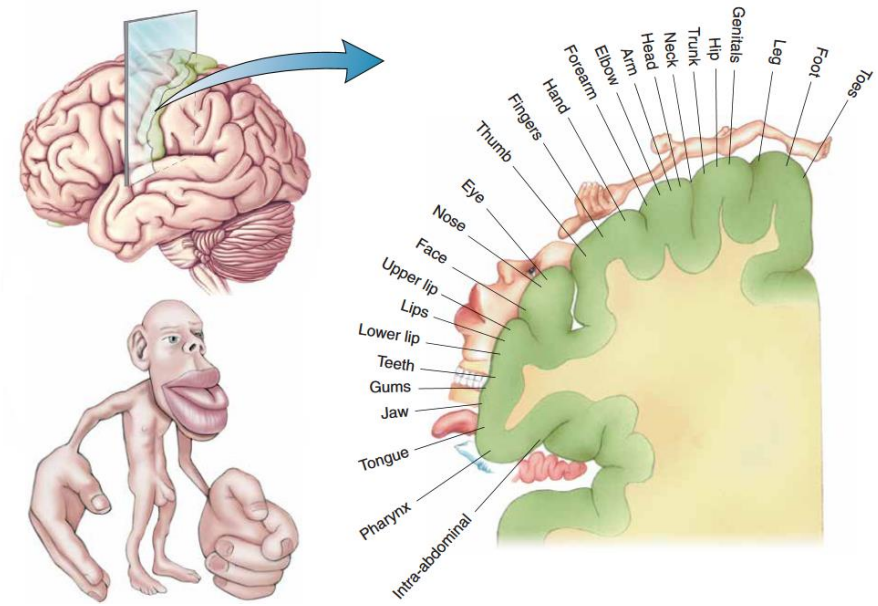

Figure 7: Homunculus on the somatosensory cortex. Body parts with higher sensitivity occupy larger areas on the somatosensory cortex. Adapted from: Reed, C., & Ziat, M. (2018). Haptic Perception: From the Skin to the Brain.

2.3. PAIN MODULATORY MECHANISMS

For many years it was suggested that endogenous mechanisms, located somewhere in the CNS, can modulate nociceptive information. Evidence for such pain modulatory mechanisms was first described by Beecher during the Second World War (Beecher, 1946). He reported that three-quarters of badly wounded men experienced none to only moderate pain and did not need pain relief medication. He concluded that multiple emotional factors, such as stress and anxiety, contributed in blocking nociceptive signals received from the periphery, causing hypoalgesia. These results suggest that pain sensation does not solely rely on noxious inputs, but also on interplay between multiple variables allowing pain modulation. The gate control theory, which is considered as a segmental spinal inhibition system, and the descending modulatory pathways, which modulate the ascending pain transmission system, are two circuits that can modulate pain. In the next chapters these modulatory circuits will be elaborated.

2.3.1. GATE CONTROL THEORY

The first pain modulatory mechanism, known as the 'Gate Control Theory (GCT)', was proposed by Melzack and Wall in 1965 (Melzack & Wall, 1965). In short, their theory propose that pain is determined by interactions between three systems which could result in pain inhibition by innocuous inputs. The first system comprises interaction of the substantia gelatinosa (SG), acting as a gate control system, with afferent fibers before (in)activation of projection cells (T). The second system comprises afferents, located within the dorsal column, which activates brain processes allowing modulatory alterations of the gate control system. The last system comprises activation of the 'action system' by projection cells which is responsible for pain perception and response.(Melzack & Wall, 1965)

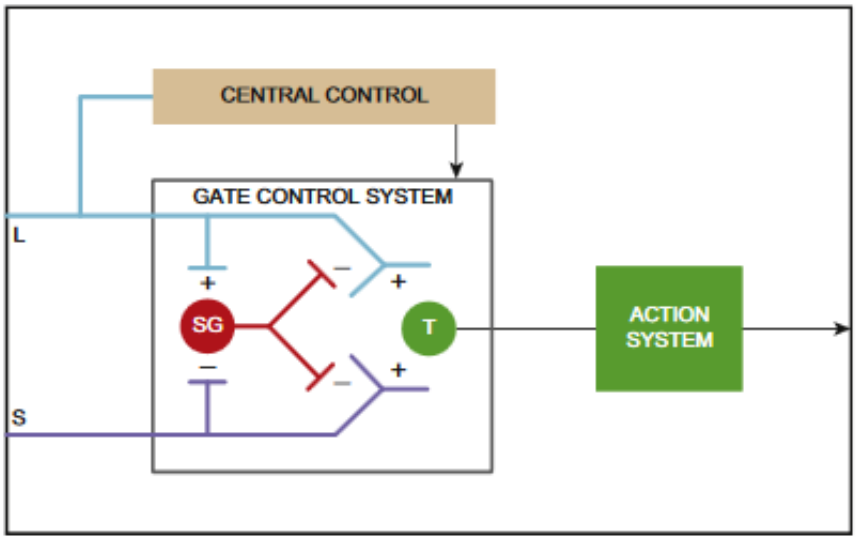

Figure 8: Schematic representation of the gate control theory. Activity of projection cells (T) is increased directly by activation of small-diameter fibers (S), such as C-fibers, and indirectly by inhibition of the substantia gelatinosa (SG), resulting in signal transmission to higher brain centers (the 'Action System'). Pain is modulated by stimulation of large fibers (L), such as Aβ-fibers, causing inhibition of synapsing of first-order neurons with projection cells (T) by increased activity of the substantia gelatinosa (SG). Also, output of the dorsal horn can be regulated by supraspinal control systems, indicated by 'Central Control'. Excitation and inhibition is indicated by + and -, respectively. Adapted from: Braz, J., Solorzano, C., Wang, X., & Basbaum, A. I. (2014). Transmitting pain and itch messages: a contemporary view of the spinal cord circuits that generate gate control. Neuron, 82(3), 522-536. doi:10.1016/j.neuron.2014.01.018

The principal of the gate control theory comprises signaling from the spinal cord to the brain, which depends on the balance of large-diameter (Aβ-fibers) and small-diameter (C-fibers) primary afferent fibers synapsing on projection cells through inhibitory interneurons (see Figure 8). Local interactions between afferents and neural circuits within the dorsal horn can modulate input to higher brain centers. The ability to reduce the sensation of pain relies on these interactions by activating low-threshold mechanoreceptors (large-diameter primary afferent Aβ-fibers).

When no input comes in and both large afferents (L) and small afferents (S) are deactivated, the inhibitory interneurons located in the substantia gelatinosa (corresponding to lamina II of the superficial dorsal horn), which act as a gate control system, prevent the projection cells from transmitting signals to the CNS. In this case the gate is closed.

When large afferents are stimulated both inhibitory interneurons and projection cells are stimulated. However, the inhibitory interneurons prevent the projection cells from transmitting signals to the CNS. The net effect of the large afferents is to close the gate and prevent nociceptive pain signals to travel along the spinothalamic pathways to the somatosensory cortex.

In case small afferents are stimulated, the inhibitory interneurons are inhibited and the projection cells are excited. This means that the gate is open resulting in transmission of the nociceptive pain signals to the somatosensory cortex (action system).(Braz *et al.*, 2014; Melzack & Wall, 1965; Purves *et al.*, 2018)

When stimulation is prolonged, the small-diameter fibers become more dominant than the large-diameter fibers resulting in increased sensation (Debbag & Khidhir, 2016).

2.3.2. DESCENDING PAIN MODULATION

It is widely accepted that endogenous analgesic systems, comprising descending inhibitory (antinociceptive) and facilitatory (pronociceptive) circuits, are involved in pain modulation (H. Fields *et al.*, 2006; Millan, 2002). Modulatory effects arise from activation of descending pain modulation pathways projecting to the dorsal horn of the spinal cord regulating nociceptive signals to higher brain centers, such as the brainstem (Chichorro *et al.*, 2017; Goadsby *et al.*, 2017). In 1969, Reynolds successfully performed surgery on rats during analgesia induced by electrical focal brain stimulation (Reynolds, 1969). Research has shown that electrical stimulation and microinjection of opioids of the periaqueductal gray (PAG) area in the midbrain and the rostral ventromedial medulla (RVM) in the medulla, acting as a final relay in the control of descending pain facilitation, produce these analgesic effects, confirming involvement of both areas in descending spinal nociceptive processing (Behbehani, 1995; H. L. Fields, 2000; Lei *et al.*, 2014; Millan, 2002; Morgan *et al.*, 2008; Ossipov *et al.*, 2014).

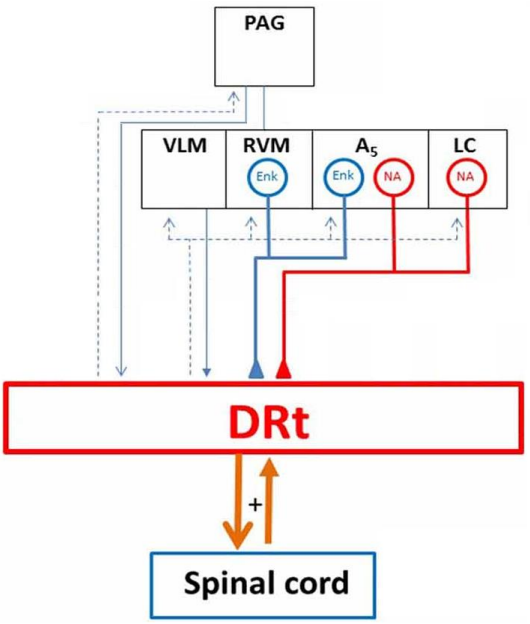

Figure 9: Schematic representation of supraspinal modulatory systems. The periaqueductal gray (PAG) receives input from forebrain areas and relays this information to the RVM, which projects to the dorsal reticular nucleus (DRt) cord by releasing enkephalins (Enk). Also, the DRt receives afferent inputs from caudal ventrolateral medulla (VLM), a noradrenergic group (A_5) and locus coeruleus (LC) by the release of neurotransmitters noradrenaline (NA) and Enk. The DRt serves as a major relay for descending pain inhibitory and facilitatory inputs from higher brain centers to the dorsal horn located in the spinal cord. Adapted from: Martins, I., & Tavares, I. (2017). Reticular Formation and Pain: The Past and the Future. Front Neuroanat, 11, 51.

It is now well established that the PAG, RVM, dorsal reticular nucleus (DRt) and all their interconnections play a key role in top-down pain modulation (see Figure 9) (Millan, 2002). The PAG receives input from cortical and subcortical forebrain sites and projects directly to the RVM (Averitt & Murphy, 2013). The RVM projects directly to the DRt and can either facilitate (ON cells) or inhibit (OFF cells) nociceptive input (De Felice *et al.*, 2011). This descending system is often referred to as the periaqueductal gray-rostral ventromedial medulla system (PAG-RVM system). The DRt serves as a major relay in facilitation or inhibition of signals from the RVM to the dorsal horn, located in the spinal cord (Amorim *et al.*, 2015; Leiras *et al.*, 2016; Martins *et al.*, 2013; Velo *et al.*, 2013; L. Zhang *et al.*, 2005). Also, DRt receives direct inputs from cortical areas, such as the primary somatosensory cortex with involvement of GABA (Almeida *et al.*, 2002; Desbois *et al.*, 1999).

Further studies have shown involvement of the nucleus cuneiformis (NCF) in the midbrain, the parabrachial nucleus, the locus coeruleus (LC), the dorsal and medial raphe nuclei in the pons and the reticular formation in the medulla releasing neurotransmitters, such as noradrenaline (NA), serotonin, dopamine, histamine and acetylcholine. These neurotransmitters can exert a bidirectional pain modulatory effect, both facilitatory and inhibitory, on the activity of inhibitory interneurons and subsequently projections neurons located in the dorsal horn of the spinal cord (see Figure 10) (Bannister *et al.*, 2009; Bannister & Dickenson, 2017; Brenchat *et al.*, 2010; H. L. Fields *et al.*, 1991; Purves *et al.*, 2018). The structures and neurotransmitters are involved in the serotonergic and noradrenergic systems (Todd, 2010). Additionally, during the neonatal period the facilitatory effect is predominant, while it shifts towards a more inhibitory effect in a later phase (Hathway *et al.*, 2009). Early exposure to noxious inputs can alter the development of endogenous descending modulatory pathways (Walker *et al.*, 2015).

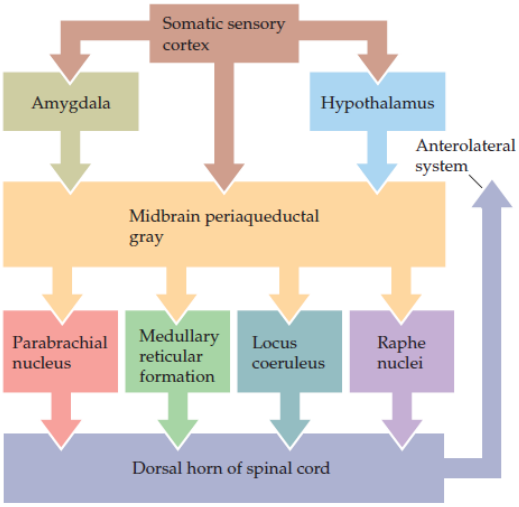

Figure 10: Schematic representation of the descending pathways that can modulate the transmission of ascending nociceptive signals. Adapted from: Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W. C., & LaMantia, A. S. (2018). Neuroscience: Oxford University Press.

Endogenous opiate neurotransmitters, including enkephalins, dynorphins and endorphins, are involved in pain management by initiating a cascade of interactions resulting in inhibition of the release of tachykinins, such as substance P, a key protein involved in the transmission of pain (see Figure 11) (Arcaya *et al.*, 1999; Goodman, 1996; Miller *et al.*, 2010; Mudge *et al.*, 1979; Stein, 1995; Zachariou & Goldstein, 1997). Also, inhibition of GABA release results in production of dopamine which can modulate nociceptive signals by acting on dopamine receptors located in different laminae in the dorsal horn of the spinal cord (Goodman, 1996; Miller *et al.*, 2010; Puopolo, 2019).

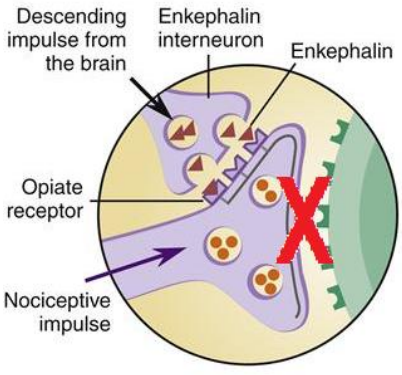

Figure 11: Example of descending signal inhibition by endogenous opiate neurotransmitters. Enkephalins are released by interneurons by descending impulses from higher brain centers. The enkephalins bind to the opiate receptors to prevent release of substance P resulting in inhibition of signal transmission (denoted by the red X). Adapted from: Joni D. Marsh (2016). Basic Medical Key. Chapter 47: Pain - afferent sensory pain fibers.

Originally, it was assumed that these modulatory circuits primarily served as an inhibitory mechanism, however, it is now clear that these pathways are dynamic, with the capacity to facilitate and inhibit pain, and can be modified under different behavioral, emotional and pathological conditions (Heinricher *et al.*, 2009). They play a crucial role in maintaining balance of inhibitory and facilitatory effects determining efficacy of nociceptive signal transmission (Purves *et al.*, 2018).

2.4. PERIPHERAL AND CENTRAL SENSITIZATION

Peripheral sensitization occurs after peripheral tissue injury and manifests as a reduced threshold of nociceptor afferent peripheral terminals (Clifford J. Woolf & Chong, 1993). It is initiated when the peripheral nociceptors are exposed to noxious stimulus (Hucho & Levine, 2007). Peripheral sensitization is described by the IASP as "increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields" (IASP, 2020c). The increased sensitivity to stimuli in the surrounding undamaged tissue after injury is referred to as primary hyperalgesia or primary allodynia (Bolay & Moskowitz, 2002; Graven-Nielsen & Arendt-Nielsen, 2002).

IASP defines central sensitization as "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input" (IASP, 2020d). Clinically, central sensitization manifests as increased neuronal excitability, which results in reversible pain hypersensitivity and a decreased pain threshold producing painful sensory response elicited by innocuous inputs (Latremoliere & Woolf, 2009; C. J. Woolf, 2011). Central sensitization is characterized by secondary hyperalgesia and secondary allodynia (see Figure 12) (Purves *et al.*, 2018). Projection neurons in the dorsal horn show an increased excitability, known as 'wind-up', which is induced by prolonged, long-lasting nociceptive exposure and/or decreased pain inhibition (descending facilitation) (Fernandez-de-las-Penas, 2018; Herrero *et al.*, 2000; Steeds, 2009).

Also, animal studies revealed that descending facilitation from reticular nuclei is involved in the maintenance of spinal excitability (Suzuki *et al.*, 2004). Neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), showed that mechanical hyperalgesia, induced by capsaicin exposure, causes increased supraspinal activity found in the brainstem, thalami, cerebellum, primary and secondary somatosensory cortices, insula and cingulate cortex (Mainero *et al.*, 2007; Zambreanu *et al.*, 2005). However, other studies found that central sensitization is initiated by capsaicin exposure and is not solely maintained by peripheral nociceptive input (Ji *et al.*, 2003).

Central sensitization can occur within minutes after nociceptive stimulation and is reversible within a few minutes, while long-lasting nociceptive stimulation is able to generate enduring plastic changes in the CNS (Fernandez-de-las-Penas, 2018). Central sensitization has been documented in various pain conditions such as fibromyalgia, osteoarthritis and failed back surgery syndrome. It is thought that central sensitization plays a fundamental role in the development and maintenance of these chronic pain conditions (Harte *et al.*, 2018). Central sensitization can be evaluated using the central sensitization inventory (CSI) (Mayer *et al.*, 2012).

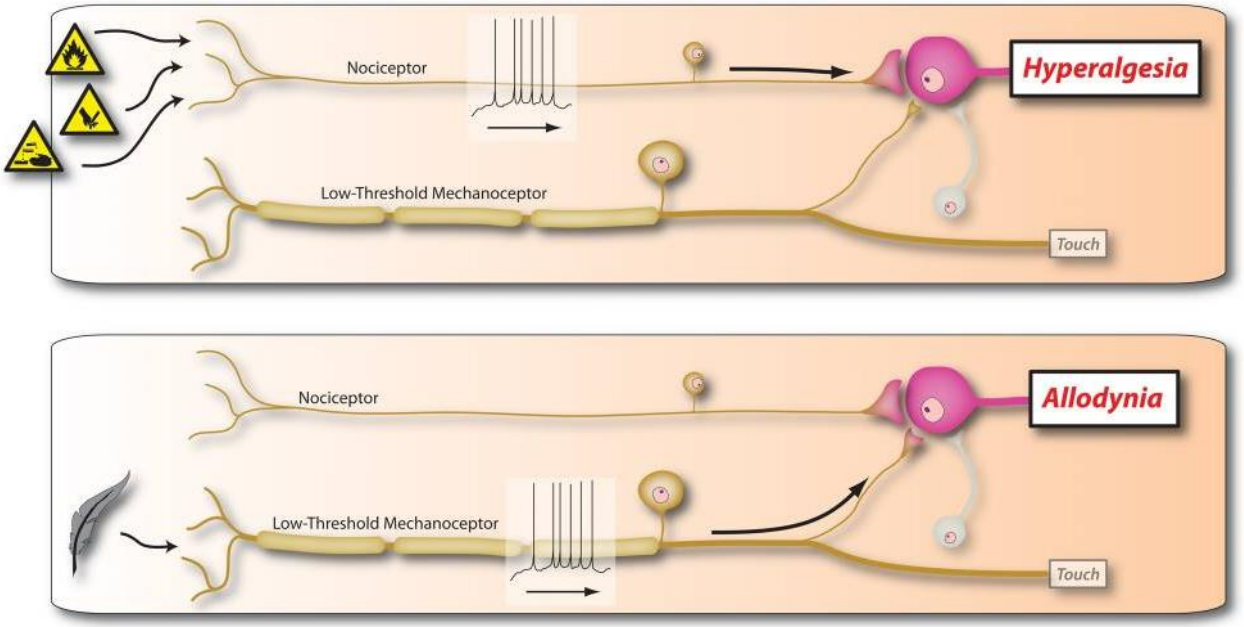

Figure 12: Schematic overview of central sensitization. Aδ- and C-nociceptive fibers are involved in central sensitization and elicit hyperalgesia (upper panel). In the lower panel, Aα- and Aβ- fibers are activated by low-threshold mechanoreceptors. In normal conditions this activation results in touch sensation. However, when centrally sensitized these fibers intersect with the synapses located in the 'pain pathway' causing nociceptive sensation (allodynia). Adapted from Woolf, C. J. (2011). Central sensitization: implications for the diagnosis and treatment of pain. Pain, 152*(3 Suppl), S2-15.*

2.5. CHRONIC PAIN PATIENTS – FAILED BACK SURGERY SYNDROME (FBSS)

Low back pain presents a tremendous burden on people and is with an estimated 9.4% global incidence the leading cause of disability worldwide (Hoy *et al.*, 2014). In 2015, low back pain was responsible for almost 60.1 million disability-adjusted life years (DALYs) (Vos *et al.*, 2016). To relieve patients from low back pain with radiating leg pain, surgical treatments, such as laminectomy or discectomy, can be considered. Over the past decades, utilization of spinal surgery has grown considerably in Western countries due to an ageing population (Grotle *et al.*, 2019). Despite spinal surgery, approximately 30% of patients lack improvement and are experiencing post-operative persistence or recurrence of low back pain with or without radiating leg pain, due to a variety of causes including recurrence of disc herniation, new disc herniation at other spinal levels, foraminal stenosis, epidural fibrosis or scarring (Fritsch *et al.*, 1996). A systemic literature review shows recurrence of back or leg pain in 5%-36% of patients within 2 years after discectomies for lumbar disc herniation (Parker *et al.*, 2015). Another study demonstrated that 29.2% of patients had either no change or even increased pain within 1 year after laminectomy (Skolasky *et al.*, 2014). Also, many preoperative factors have shown to affect postoperative outcomes after spinal surgery negatively which could ultimately lead to FBSS, such as inaccurate diagnosis, depression, smoking or excessive preoperative analgesics intake (Baber & Erdek, 2016).

Failed back surgery syndrome (FBSS) refers to patients experiencing such persistent or recurrent low back pain with or without radiation to the leg (Ganty & Sharma, 2012). The International Association for the Study of Pain defines FBSS as "lumbar spinal pain of unknown origin either persisting despite surgical intervention or appearing after surgical intervention for spinal pain originally in the same topographical location" (Harvey, 1995). It is a highly prevalent condition and patients are often dealing with depression,

financial stress, unemployment and loss of self-esteem (Taylor & Taylor, 2012; Thomson & Jacques, 2009). FBSS is a complex pain problem and pain may arise from viscera, blood vessels and nerves or from muscles or joints of the spine and pelvis (Leveque *et al.*, 2001). Treatment of FBSS is comprehensive and physicians are often faced with challenges in care management (Van Buyten & Linderoth, 2010). Multidisciplinary pain management strategies, such as pharmacologic, rehabilitation and behavioral therapies, are employed with varying degrees of success. Also, re-operation might be an option but is rarely successful (Assaker & Zairi, 2015; North *et al.*, 2005).

Due to treatment complexity and when patients are refractory to conservative therapies, a multidisciplinary approach is often recommended in which neuromodulation is indicated for FBSS patients with predominant radiating leg pain (Van Buyten & Linderoth, 2010). Its potential has been described for the first time in 1967 in a case study published by Shealy *et al. (Shealy et al., 1967)*. They were capable of complete elimination of pain in a 70-year-old male using electrical stimulation of the dorsal columns.

After decades of improvement and refining, recent research shows that neuromodulation results in higher rate of success measured in terms of pain rating, neurological function, quality of life and ability to restore functioning (North *et al.*, 1991; North *et al.*, 1993). Research shows that neuromodulation is frequently considered to be used as a last resort therapy in the treatment of FBSS, while others state that neuromodulation should be offered early in the pain management plan (Ganty & Sharma, 2012; Palmer *et al.*, 2019). Also, research has shown that early implementation of neurostimulators increases its success in FBSS patients (Kumar *et al.*, 2006; T. C. Zhang *et al.*, 2014).

A dedicated multidisciplinary team of trained professionals experienced in pain management and specialized in assessing a patient's eligibility for neuromodulation is located in the St. Antonius Hospital. This team consists of anesthesiologists, neurologists and psychiatrists. Meetings are scheduled once a month in which a patient's eligibility is evaluated. Depending on the decision, a non-invasive trial stimulator is placed prior to the permanent implantation, which could provide enhanced predictive value for long-term efficacy of the neurostimulator.

A systemic review demonstrates the benefits of neuromodulation in patients suffering from chronic low back and leg pain. 62% of those patients experienced 50% pain relief, 53% of the patients reported termination of analgesic consumption and 40% of the patients were able to resume working (Taylor *et al.*, 2005). These results also indicate that a considerable number of patients suffers from recurrent pain even after implantation of a neurostimulator. This emphasizes the complexity of chronic low back pain and highlights the need to investigate the underlying pain mechanisms.

2.6. CONDITIONED PAIN MODULATION

2.6.1. ASSESSMENT OF DESCENDING PAIN INHIBITORY PATHWAYS

There is growing evidence suggesting that chronic pain conditions are associated with disturbance of endogenous modulatory mechanisms. It is believed that dysfunction of these mechanisms is involved in chronic pain and is of interest because it can inhibit or facilitate transmission of noxious inputs and hence, it may promote and maintain pain (Ossipov *et al.*, 2014). It is thought that patients failing in recruiting modulatory mechanisms may be more likely to develop a chronic pain condition (Ossipov *et al.*, 2014). Additionally, Baliki *et al.* investigated interactions between higher brain areas by activation those using acute noxious thermal stimuli in controls and chronic back pain (CBP) patients (Baliki *et al.*, 2010). Results

showed that they were able to predict development of chronic low back pain with high accuracy by studying functional connectivity between the prefrontal cortex and the nucleus accumbens. Dysfunction of these endogenous mechanisms has also been demonstrated in patients suffering from fibromyalgia, irritable bowel syndrome and osteoarthritis (Ablin & Buskila, 2013; Arendt-Nielsen *et al.*, 2010; Williams *et al.*, 2013).

Le Bars *et al*. first demonstrated assessment of central pain modulatory systems with electrophysiological studies in rats by application of a noxious stimulus (D. Le Bars *et al.*, 1979). Activity of dorsal horn neurons was strongly inhibited by application of noxious stimuli to multiple parts of the body. They also found that innocuous stimuli were ineffective and therefore the modulatory system was proposed to be termed as 'diffuse noxious inhibitory control' (DNIC). Additional studies showed reduced DNIC in rats due to persistent morphine exposure, which resulted in hypersensitivity to noxious stimuli (Okada-Ogawa *et al.*, 2009).

The human counterpart of DNIC is known as conditioned pain modulation (CPM). Assessment of pain modulatory pathways, using conditioned pain modulation paradigms, provides insights into efficiency and functioning of the central descending modulatory system (Neogi, 2016). Its assessment is based on 'pain inhibits pain' by the application of a noxious 'conditioning stimulus' to one part of the body which results in decreased pain perception from another noxious 'test stimulus' applied to an extra-segmental body region.

Patients with chronic pain conditions appear to show reduced CPM efficacy, reflecting an impairment in pain inhibitory mechanisms (Daenen *et al.*, 2013; Vaegter *et al.*, 2016). However, its etiology remains unclear. The finding of less efficient CPM could be the result of long-standing chronic pain resulting in exhausting their pain inhibition capacity, which indicates that those chronic pain patients had a normal CPM efficacy at baseline. However, chronic pain patients may already had reduced CPM before their chronic pain condition manifested, suggesting that their chronic pain condition may be caused by their reduced CPM efficacy (Yarnitsky *et al.*, 2010). Yarnitsky *et al.* investigated CPM efficacy in pre-thoracotomy pain-free patients and discovered a higher risk to develop chronic pain after surgery in patients with less efficient CPM (Yarnitsky *et al.*, 2008). Similar discoveries were found by Wilder-Smith *et al.* studying chronic pain after abdominal surgery (Wilder-Smith *et al.*, 2010).

Although the rapid expansion of knowledge about pain mechanisms increased over the past decades, mechanisms that initiate the development of chronic pain conditions remain not completely understood. Questions arise why some individuals fail in recruiting endogenous descending pathways and others don't, which emphasizes the need for further investigation of endogenous modulatory mechanisms and their role in pain chronification.

2.6.2. Cold pressor test

CPM is typically triggered by a painful, conditioning stimulus at a remote body area and induces inhibition of nociceptive sensation to a test stimulus applied to an extra-segmental body region. Test protocols vary in timing, modality, intensity, duration and location of the stimuli (Pud *et al.*, 2009). Electrical, thermal (heat or cold), mechanical, and chemical stimuli have been used as both test and conditioning stimuli in various paradigms to assess CPM. Research showed that the cold pressor test (CPT), as a conditioning stimulus, is one of the most frequently used and efficient method due to its consistent results in inducing

CPM (Lewis *et al.*, 2012; Petersen *et al.*, 2017). CPT requires immersion of an extremity into water and induces a nociceptive stimulation to trigger CPM, mediated by activation of thermoreceptors and nociceptors (see Figure 13) (Granovsky *et al.*, 2016; Marchand & Arsenault, 2002).

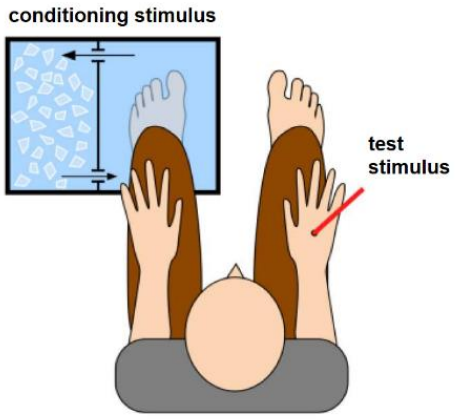

Figure 13: Example of conditioning pain modulation (CPM) induced by application of the cold pressor test (CPT) as conditioning stimulus. A test stimulus is applied on the right hand of the subject to assess CPM. The mesh screen separates the inner compartment from the outer compartment to prevent direct contact between the ice and the left foot of the subject. Source: Torta, D. M., Churyukanov, M. V., Plaghki, L., & Mouraux, A. (2015). The effect of heterotopic noxious conditioning stimulation on Adelta-, C- and Abeta-fibre brain responses in humans. Eur J Neurosci, 42(9), 2707-2715. doi:10.1111/ejn.13071

Consensus regarding application of noxious or innocuous stimulus as conditioning stimulus to induce CPM is lacking, however research shows that spatial summation is relevant in pain inhibition (Honigman *et al.*, 2016; Le Bars *et al.*, 1992; Villanueva & Bars, 1995). Others found that the magnitude of CPM activation was not affected by increased conditioning stimulus intensity, which suggests that pain inhibition reaches a ceiling effect and may be a saturable phenomenon (Nir & Yarnitsky, 2015). Standardization and control of water temperature are lacking, hindering the ability to compare data from various studies (Yarnitsky *et al.*, 2015). Therefore, comparability and reliability are questioned by some studies due to the lack of standardization, while others state excellent reliability and validity (Arendt-Nielsen *et al.*, 2012; Eccleston, 1995; Edens & Gil, 1995). Manresa *et al.* and Gehling *et al.* studied test-retest reliability of CPM paradigms (Biurrun Manresa *et al.*, 2014; Biurrun Manresa *et al.*, 2011; Gehling *et al.*, 2016). Results demonstrated good reliability with CPT as conditioning stimulus and electrical simulation as test stimulus. Kennedy *et al.* investigated intersession reliability across multiple studies and found intraclass correlation coefficients (ICCs) up to 80 (Kennedy *et al.*, 2016). Koenig *et al.* showed excellent 2-week test–retest reliability using the CPT as conditioning stimulus in healthy subjects (Koenig *et al.*, 2014). Note that results are based on assessment of pain thresholds and pain tolerance thresholds.

Research recommends usage of one upper and one lower limb for application of both stimuli to ensure descending pain inhibition rather than segmental spinal inhibition (Granovsky *et al.*, 2013). It has been observed that the pain sensation during CPT is time-dependent, fluctuating and maximal after 60 seconds of onset of immersion (Wolf & Hardy, 1941). Other results show that pain inhibition is maximal during application of the conditioning stimulus and persists for several minutes after termination of the conditioning stimulus (Lewis *et al.*, 2012). The duration of CPM effects is short, however, the exact duration of pain inhibition remains unknown and is known to be paradigm-dependent lasting 5 minutes to up to 60 minutes after application of the conditioning stimulus (Fujii *et al.*, 2006; Graven-Nielsen *et al.*, 1998; Graven-Nielsen & Mense, 2001; Tuveson *et al.*, 2006; Yarnitsky *et al.*, 2015). Also, CPM effects seems to be prone to psychological mediators such as distraction, expectancies, pain catastrophizing and adaptation and efficacy seems to be age- and gender-related (Edwards *et al.*, 2003; H. L. Fields, 2000;

Goffaux *et al.*, 2007; Hermans *et al.*, 2016; Kakigi, 1994; Lariviere *et al.*, 2007; Lautenbacher *et al.*, 2007; Washington *et al.*, 2000).

Also, pain intensity was not as great with slow cooling (from 20°C to 0°C in 60 minutes) compared to rapid cooling at comparable water temperatures. Studies showed that maximum tolerance time of immersion varied considerably at minimal temperature variations, which emphasizes the demand of temperature stability of the conditioning stimulus (Mitchell *et al.*, 2004).

2.7. Assessment of sensory functioning

2.7.1. Quantitative Sensory Testing

Assessment of pain and quantitative assessment of somatosensory functioning to identify mechanisms underlying pathologic pain conditions is not straightforward and a gold standard is lacking (Jespersen *et al.*, 2013). Nevertheless, clinicians rely on pain characteristics and other possible signs for hypersensitivity, indicating central sensitization. Methods to assess pain and changes in somatosensory functioning include quantitative sensory testing (QST) and patient reported outcome measures (PROMs), such as numerical rating score (NRS) and the McGill Pain Questionnaire (Fillingim *et al.*, 2016). It is showed that pressure algometry is a useful tool in pain threshold quantification as it assesses pressure-pain sensitivity and central mechanisms related to spatial and temporal summation of pressure-pain (Pelfort *et al.*, 2015; Rolke *et al.*, 2005). Next to this, a laboratory-based QST, using a standardized test protocol (such as the DFNS protocol) has been proposed as a stratification tool allowing assessment of somatosensory function of skin and deep somatosensory afferents (Rolke *et al.*, 2006). The DFNS protocol includes 13 parameters, assessed by 7 different test devices (Magerl *et al.*, 2010; Pfau *et al.*, 2012; Rolke *et al.*, 2006). However, these methods are mainly based on supra-threshold and tolerance as the subject needs to give a pain rating on a scale and/or indicate the stimulation as painful and are therefore prone to subjectivity.

2.7.2. Observing nociceptive processing

Tracking psychophysical thresholds can facilitate the investigation of the underlying mechanisms of sensitization and the (dys)functioning of endogenous descending pathways by describing the relationship between physical stimuli and the corresponding subjective responses. To investigate these thresholds, multiple methods have been developed which rely on application of a stimulus with varying amplitude while measuring the subject's response to the stimulus. Assessment of the nociceptive system relies on selective stimulation of nociceptive-free nerve endings, which are known to be located mainly in the epidermis (Kruger *et al.*, 1985). Research has shown that electrocutaneous stimulation seems to be a promising technique to investigate the nociceptive system by measuring various detection and/or pain thresholds, such as the electrical sensory threshold (EST) and the electrical pain threshold (EPT) (Lund *et al.*, 2005). However, ESTs and EPTs do not offer specific information about the individual's nociceptive system and are highly influenced by instructions provided by the observer. Several investigators have suggested the use of intra-epidermal electrical stimulation (IES) to selectively activate nociceptors (i.e. Aδ-fibers and C-fibers) without co-activation of tactile nerve fibers, which seems promising in studying the nociceptive system (Inui & Kakigi, 2012; Mouraux *et al.*, 2010). Currently used methods to quantify pain are based on pain experiences, which is a difficult outcome to measure due to its subjective nature.

2.7.2.1. STIMULUS SELECTION PROCEDURES AND THRESHOLDS ESTIMATION METHODS

Doll *et al.* investigated the performances of three different adaptive stimulus detection procedures and two estimation methods in determining non-stationary thresholds over longer periods of time (Doll *et al.*, 2014). Stimulus procedures comprised the simple staircase stimulus selection, the random staircase stimulus selection and the minimum entropy stimulus selection. Estimation methods comprised logistic regression and Bayesian estimation. Monte Carlo simulations were used to compare bias and precision of the different procedures and methods in selection of stimulus amplitudes and estimating thresholds. Based on simulations and a human subject experiment, it was recommended to combine the random staircase stimulus selection with logistic regression over a shifting window, as it provides robust non-stationary threshold estimations which is supported by their high precision and small bias.

In the random staircase stimulus selection procedure, stimuli are randomly selected from a small, predefined set of stimulus amplitudes ranging from 0 mA to 1.5 mA. The number of amplitudes (NoA) in the set equals 256, which means that stimulus amplitudes are separated by $5.88 \cdot 10^{-3}$ mA. After a non-detected or detected stimulus, the stimulus amplitudes in the set increases or decreases respectively by a fixed step size (0.05 mA, 0.1 mA or 0.2 mA) (see Figure 14).

Simultaneous application of stimuli with different properties could allow observation of multiple thresholds, which is referred to as Multiple Threshold Tracking (MTT). The performance of estimation of multiple non-stationary thresholds has also been investigated by Doll *et al.* (Doll *et al.*, 2016b). In later studies, these thresholds are referred to as nociceptive detection thresholds (NDTs).

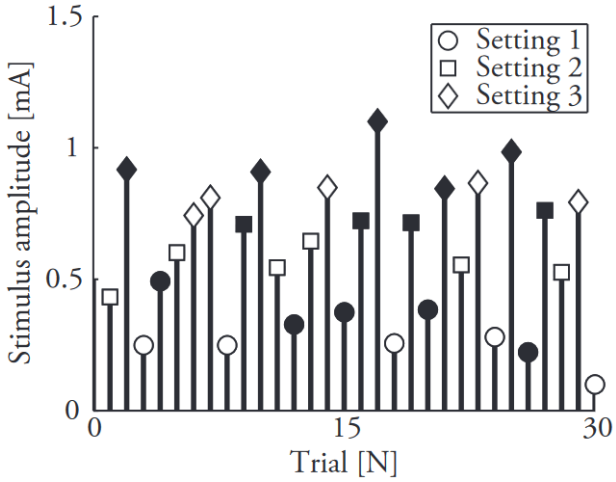

Figure 14: Example of multiple stimulus properties within a single experiment. During the experiment the different stimulus properties are presented in a random intermingled order. Using logistic regression multiple thresholds can be observed simultaneously (not shown). Non-detected and detected stimuli are presented by the open and close markers, respectively. Adapted from: Doll, R. J., Veltink, P. H., & Buitenweg, J. R. (2016b). Psychophysical methods for improved observation of nociceptive processing. Characterization of a psychophysical method for simultaneous tracking of multiple non-stationary thresholds, 58-75. doi:10.3990/1.9789036540377

2.7.2.2. PSYCHOPHYSICAL CURVE

The relation between the physical stimuli and the subject's response can be explored with psychophysics (Klein, 2001). The psychophysical curve is a sigmoidal function and relates the subject's performance in detecting applied electrical stimuli to the stimulus amplitudes, shown in Figure 15. Various psychophysical paradigms, such as simple forced-choice and two-alternative forced choice (2AFC), contribute in the generation of such a psychophysical curve. The detection threshold of each stimulus setting is defined as the stimulus amplitude resulting in a 50% detection probability. Also, research shows that the slope of the psychophysical curve provides information about the reliability of stimulus detection probability and, hence, the estimated detection threshold (Gold & Ding, 2013).

Doll *et al.* described the effect of pure probability summation on the expected detection threshold for double-pulse stimuli (Doll *et al.*, 2016a). Their results showed that the obtained estimated detection threshold for double-pulse stimuli was lower compared to the expected threshold for double-pulse stimuli based on pure probability summation (see Figure 15). This indicates that processes other than pure probability summation, such as paired-pulse facilitation or augmentation, are involved causing a shift in the psychophysical curve resulting in lowering the detection threshold when double-pulse stimuli are applied.

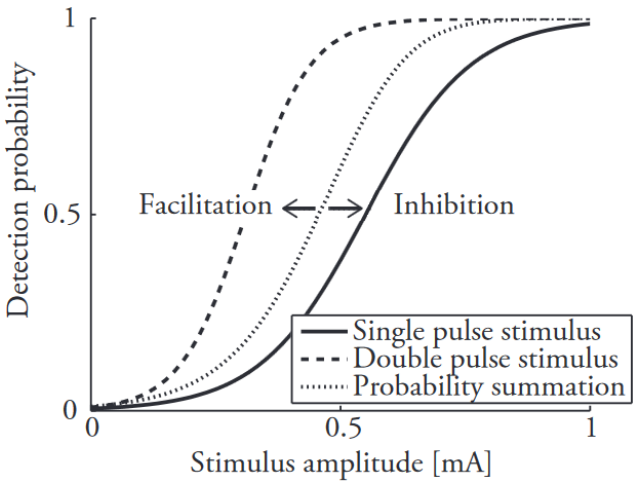

Figure 15: Psychophysical curves for single pulse and double pulse stimulation. The solid line presents the curve for single pulse stimulation, the dashed line presents the curve for double pulse stimulation and the dotted line presents the expected curve for double pulse stimulation based on pure probability summation. The expected curve can shift due to facilitation or inhibition. Adapted from: Doll, R. J., Veltink, P. H., & Buitenweg, J. R. (2016b). Psychophysical methods for improved observation of nociceptive processing. Characterization of a psychophysical method for simultaneous tracking of multiple non-stationary thresholds, 58-75. doi:10.3990/1.9789036540377

2.7.2.3. NDT-EP METHOD

Recently, a method has been developed for measuring NDTs by combining the random staircase procedure with logistic regression (Doll *et al.*, 2014). In later studies, a multiple threshold tracking algorithm was used to estimate multiple thresholds for single and multiple pulse stimuli within a single experiment (Doll *et al.*, 2016b). Also, estimation of the psychophysical thresholds and slopes were studied for non-stationary processes, reflecting the sensitivity of multiple threshold tracking (Doll *et al.*, 2015). NDTs were determined by application of different stimulus properties using intra-epidermal electrocutanous stimulation (IES) of the skin with varying intensities at detection levels. Measuring NDTs at detection levels using intra-epidermal electrocutaneous stimulation is less prone to instructions and lacks the subjectivity, as is the case for measuring pain tolerance thresholds. It is assumed that detection thresholds reflect underlying sensory processes, whereas pain tolerance thresholds are prone to a subject's willingness to endure noxious stimulation resulted by motivation or attitude (Edens & Gil, 1995). Next to this, intra-epidermal electrocutanous stimulation could serve as the test stimulus in CPM

paradigms as electrical stimulus showed good reliability in CPM paradigms (Biurrun Manresa *et al.*, 2014; Biurrun Manresa *et al.*, 2011; Gehling *et al.*, 2016).

Different temporal stimulus properties comprise variations in inter-pulse-interval (IPI) and number of pulses (NoP), while the pulse width (PW) was fixed at 0.21 ms (see Figure 16). By varying temporal stimulus properties contributions of nociceptive mechanisms, i.e. peripheral and central nociceptive processes, can be investigated and might be dissected. Activation of nociceptors, reflecting peripheral processes, can be modulated by varying stimulus intensities, while signal transmission through the central nervous system can be modulated by varying NoP and IPI, introducing temporal summation of the post-synaptic neuronal activity (E. M. van der Heide *et al.*, 2009). Doll *et al.* investigated the effect of different temporal stimulus properties on the psychophysical functions and, hence, the estimated thresholds and slopes (Doll *et al.*, 2016a). Results showed that increasing NoP resulted in lower NDTs and steeper slopes. For double pulse stimuli, however, NDTs increased with increasing IPI.

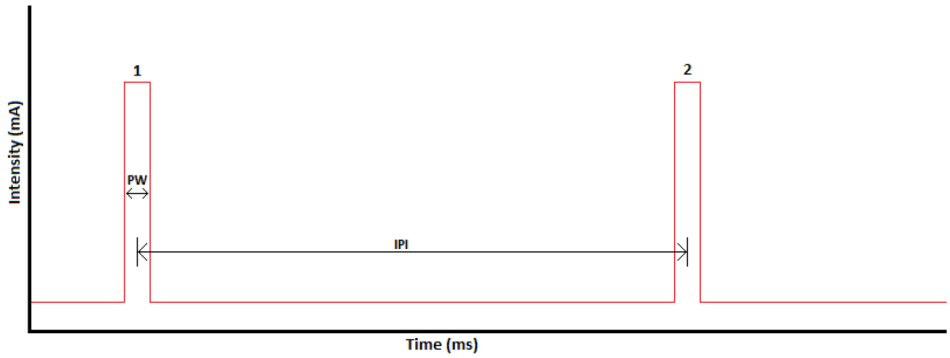

Figure 16: Example of a train of pulses. Number of pulses (NoP) equals two with a predefined inter-pulse-interval (IPI) and pulse width (PW). Not drawn to scale.

In later studies, multiple threshold tracking was combined with electroencephalographic (EEG) measurements, as an objective measure of nociception related activity in the central nervous system (van Berg & Buitenweg, 2018). Nociceptive detection threshold measurements combined with electroencephalographic measurements are referred to as the NDT-EP measurement method. Neuro-electric responses to randomly interchanging sensory stimuli, referred to as evoked potentials (EPs), have been analyzed using mixed-effects models in a laboratory environment by Van den Berg (van Berg & Buitenweg, 2018). Multiple-trial averages of EPs, referred to as grand average EPs, were altered by changes in temporal stimulus properties, such as NoP or number of trials (A. Mouraux *et al.*, 2014; E. M. van der Heide *et al.*, 2009; C. J. Vossen *et al.*, 2015).

Berfelo was the first who applied the NDT-EP measurement method in a clinical environment by studying healthy subjects and chronic pain patients due to FBSS (Berfelo, 2019). Results were promising and demonstrated that initial nociceptive detection values and NDTs observed in FBSS patients were altered compared to those in healthy subjects. Also, EPs observed in FBSS patients were differently modulated by stimulus properties compared to EPs observed in healthy subjects. These findings encouraged to proceed with studying NDTs and EPs in chronic pain patients. Later, Gefferie explored the feasibility of the NDT-EP method in diabetes mellitus (DM) patients and in a lidocaine model of small fiber neuropathy (SFN)

applied to healthy pain-free subjects (Gefferie, 2020). DM patients comprised 2 groups: patients with chronic painful diabetic peripheral neuropathy (PDPN) and patients without pain complaints. As expected for the lidocaine experiment, EP amplitudes were significantly reduced for those exposed to lidocaine, compared to placebo and control measurements. Also, detection probabilities were altered in chronic PDPN patients compared to the patients without pain. Reduced EP amplitudes were found in chronic PDPN patients compared to non-painful DM patients.

Thus, in both laboratory and clinical environment, the NDT-EP method combined with CPT might provide insight into the relation between neurophysiological activity and nociceptive stimuli. Results of clinical research suggest that the NDT-EP method seems to be promising in discriminating between multiple chronic pain conditions.

2.8. STATISTICAL ANALYSES

Linear mixed-effects models (LMMs) describe the relation between a response variable (e.g. average EPs and average NDTs) and other variables that might explain the variations in the response variable, such as stimulus amplitude, stimulus properties or response. LMM is an extension of the simple linear model and allows analysis of both fixed and random effects, hence the name mixed-effects. Variables are considered to be fixed effects when the samples exhaust the regression parameter and is constant across all levels of the entire model parameter. Random effects are variables comprising a small or negligible subset of all levels of the regression parameter and account for unwanted sources of variation. In EEG data and detection probability analysis it is important to distinguish between categorical fixed effects and continuous fixed effects, which are also known as covariates.(D. M. Bates, 2010; Magezi, 2015; Pinheiro & Bates, 2000)

The probability of the response variable is often modeled as a logit function as a function of multiple regression coefficients (Winter, 2013). An example of such a model with random effect A could be as follows:

$$\begin{aligned} logit\left(P(R=1|A_i)\right) &:= log\frac{P(R=1|A_i)}{1-P(R=1|A_i)} \\ &= \beta_0 + \beta_1 x_{1,i} + \beta_2 x_{2,i} + \beta_3 x_{3,i} + \beta_4 x_{1,i} x_{2,i} + u_{0,i} + u_{1,i} x_{1,i} + \varepsilon \end{aligned} \quad (1)$$

Where P is the probability, β_0 is the actual parameter of interest, known as the global mean or intercept, $\beta_1 x_1$, $\beta_2 x_2$ and $\beta_3 x_3$ are regression parameters classified as fixed effects, $\beta_4 x_1 x_2$ is the interaction between two fixed regression parameters, u_0 is the random intercept for the i-th A, $u_1 x_1$ is the random slope for the i-th A and ε is the residual of the errors.

In Wilkinson-Rogers notation the function would be as follows:

$$y \sim 1 + x1 + x2 + x3 + x1 * x2 + (1 + x1|A) \quad (2)$$

Where y is the response variable, 1 is the intercept, $x1$, $x2$ and $x3$ are fixed regression coefficients, $x1 * x2$ is the interaction between two fixed regression coefficients and $1 + x1$ are the random intercept and random slope, respectively, which are assumed to differ for each A.

2.8.1. Detection probabilities and nociceptive detection thresholds

In psychophysical studies, logistic regression is often considered to investigate stimulus-response relations (Treutwein, 1995). Doll *et al.* investigated thresholds and slopes of detection probabilities and to what extent those parameters were affected by different temporal stimulus properties (Doll *et al.*, 2016a). To study these contributions, a generalized linear mixed model (GLMM) was built to estimate the detection probability. GLMMs are an extension of LMMs to allow binary response variables from different distributions. Results demonstrated that multiple fixed affects, such as intercept, stimulus amplitude, stimulation time, and interaction between setting and stimulus amplitude, significantly affected detection probability. These findings suggest that using GLMM enables proper analysis of the effects of temporal stimulus properties on the estimated nociceptive detection probabilities.

2.8.2. Evoked potentials

Proper estimation of evoked potentials relies on the sufficient number of trials. However, conventional averaging of stimulus-related signals leads to poor estimation of the evoked potentials. Therefore, an analysis method, which is robust for variations in the number of trials, is preferred. Linear mixed-effects models (LMM) are considered robust and powerful models in analyzing complex and correlated data while accounting for multiple sources of variations (Oberg & Mahoney, 2007). Van den Berg investigated whether LMM could be used for analysis of variations within EEG data and its relation with stimulus intensity and temporal stimulus properties (van Berg & Buitenweg, 2018). Results showed that LMM was able to reduce background activity and enables proper analysis of EEG data obtained with NDT estimation measurements.

2.9. Prior work

Consensus regarding application of noxious or innocuous stimulus as conditioning stimulus to induce CPM is lacking, however research shows that spatial summation is relevant in pain inhibition (Honigman *et al.*, 2016; Le Bars *et al.*, 1992; Villanueva & Bars, 1995). Others found that the magnitude of CPM activation was not affected by increased conditioning stimulus intensity, which suggests that pain inhibition reaches a ceiling effect and may be a saturable phenomenon (Nir & Yarnitsky, 2015). Standardization and control of water temperature are lacking, hindering the ability to compare data from various studies (Yarnitsky *et*

al., 2015). Therefore, comparability and reliability are questioned by some studies due to the lack of standardization, while others state excellent reliability and validity (Arendt-Nielsen *et al.*, 2012; Eccleston, 1995; Edens & Gil, 1995). Manresa *et al.* and Gehling *et al.* studied test-retest reliability of CPM paradigms (Biurrun Manresa *et al.*, 2014; Biurrun Manresa *et al.*, 2011; Gehling *et al.*, 2016). Results demonstrated good reliability with CPT as conditioning stimulus and electrical simulation as test stimulus. Kennedy *et al.* investigated intersession reliability across multiple studies and found intraclass correlation coefficients (ICCs) up to 80 (Kennedy *et al.*, 2016). Koenig *et al.* showed excellent 2-week test–retest reliability using the CPT as conditioning stimulus in healthy subjects (Koenig *et al.*, 2014). Note that results are based on assessment of pain thresholds and pain tolerance thresholds.

Research recommends usage of one upper and one lower limb for application of both stimuli to ensure descending pain inhibition rather than segmental spinal inhibition (Granovsky *et al.*, 2013). It has been observed that the pain sensation during CPT is time-dependent, fluctuating and maximal after 60 seconds of onset of immersion (Wolf & Hardy, 1941). Other results show that pain inhibition is maximal during application of the conditioning stimulus and persists for several minutes after termination of the conditioning stimulus (Lewis *et al.*, 2012). The duration of CPM effects is short, however, the exact duration of pain inhibition remains unknown and is known to be paradigm-dependent lasting 5 minutes to up to 60 minutes after application of the conditioning stimulus (Fujii *et al.*, 2006; Graven-Nielsen *et al.*, 1998; Graven-Nielsen & Mense, 2001; Tuveson *et al.*, 2006; Yarnitsky *et al.*, 2015). Also, CPM effects seems to be prone to psychological mediators such as distraction, expectancies, pain catastrophizing and adaptation and efficacy seems to be age- and gender-related (Edwards *et al.*, 2003; H. L. Fields, 2000; Goffaux *et al.*, 2007; Hermans *et al.*, 2016; Kakigi, 1994; Lariviere *et al.*, 2007; Lautenbacher *et al.*, 2007; Washington *et al.*, 2000).

Also, pain intensity was not as great with slow cooling (from 20°C to 0°C in 60 minutes) compared to rapid cooling at comparable water temperatures. Studies showed that maximum tolerance time of immersion varied considerably at minimal temperature variations, which emphasizes the demand of temperature stability of the conditioning stimulus (Mitchell *et al.*, 2004).

Doll *et al.* were the first who explored the feasibility of the NDT-EP method combined with CPT. They explored temporary changes in NDTs by an experimental perturbation (Doll *et al.*, 2016b). Two combinations of stimulus properties were used for NDT estimation. Five minutes after the onset of the experiment, the cold pressor test was applied to the right hand for two minutes. After two minutes, the cold pressor test was terminated and subjects were asked to continue the experiment for about thirteen minutes. Results are shown in Figure 17. As shown in the figure, NDTs of both stimuli settings increased during CPT. Also, a sustained CPT effect can be observed after terminating CPT. However, at that time it was not clear whether these increases were caused by the perturbation or by technical limitations in tracking methodology.

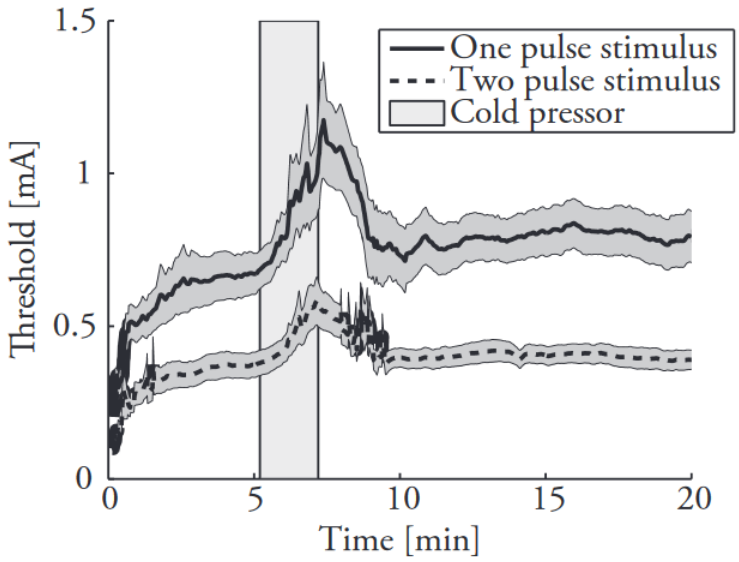

Figure 17: Estimated nociceptive detection thresholds for two combinations of stimuli. Perturbation by application of CPT between five and seven minutes after onset of the experiment (indicated by the light grey area). Standard errors are indicated by the light grey area around the detection thresholds. Source: Doll, R. J., Veltink, P. H., & Buitenweg, J. R. (2016b). Psychophysical methods for improved observation of nociceptive processing. Characterization of a psychophysical method for simultaneous tracking of multiple non-stationary thresholds, 58-75. doi:10.3990/1.9789036540377

Later, a similar experiment was conducted in a laboratory environment by Mammadli (Mammadli, 2019). In addition to the experiment conducted by Doll et al., EPs were evaluated to improve objectivity as well. Due to the lack of standardization and control of water temperature, technical feasibility was explored for two different water temperatures (1°C and 6°C). Results were surprising as almost all participant were able to tolerate both water temperatures for the maximum time (7 and 10 minutes respectively). Increases in NDTs of both settings along with a decrease of amplitudes of EPs for both central and contralateral components were observed, which suggests that combining the NDT-EP method with CPT might be interesting to investigate in a clinical environment.

Van Merkerk was the first who explored the combination of CPT with the NDT-EP method in a clinical environment (van Merkerk, 2019). Five subjects were asked to immerse their foot into a 1°C bucket for 7 minutes. One week later, the same subjects were asked to immerse their foot into a 4°C bucket for 10 minutes. The general feasibility and the effect of CPT on NDTs and EPs were assessed for both measurement sessions. Based on findings of Van Merkerk and Mammadli, it was concluded that it might be interesting to further explore the NDT-EP method combined with CPT at 1°C for 7 minutes as a potential CPM paradigm.

2.10. IMPLICATIONS

CPM inhibits pain through supraspinal inhibitory pathways, which reflect the activation of endogenous analgesia mechanisms, and induce inhibitory effects on spinal nociceptive inputs. Currently, there is great

interest in testing CPM since there is a growing body of evidence suggesting that CPM may be an important biomarker of chronic pain and a predictor of treatment response (e.g. efficacy of pain medication) based on individuals' endogenous analgesia capacity (Damien *et al.*, 2018; Yarnitsky *et al.*, 2012). Additionally, CPM paradigms can assist in characterizing pain syndromes and predict pain acquisition (Yarnitsky, 2015). This suggests a pivotal role for CPM assessment in a clinical environment. Although knowledge on CPM has improved significantly over the years, its relation with chronic pain is still incompletely understood. However, research suggests that imbalanced modulatory pathways may facilitate pain and promote pain chronification (Damien *et al.*, 2018). Also, age-associated decrements have been observed in healthy subjects, which highlight the specificity of pain inhibition ability and its sensitivity to subject characteristics (Edwards *et al.*, 2003). Additional research is needed to further understand (differences in) the endogenous pain inhibition ability of healthy subjects and FBSS patients.

Traditional CPM paradigms rely on one test stimulus applied as a stand-alone and one test stimulus during (parallel paradigm) or immediately after applying the conditioning stimulus (sequential paradigm) (Yarnitsky, 2015). The effects of these paradigms on the test stimulus and, hence, nociceptive processing, is frequently observed with Quantitative Sensory Testing (QST) methods (Arendt-Nielsen & Yarnitsky, 2009; Rolke *et al.*, 2006). However, these methods rely on a single threshold estimate. The integrity of endogenous modulating mechanisms and its dynamics might be investigated by analyzing evoked potentials and nociceptive detection thresholds over time, obtained using the NDT-EP method, which serves as the test stimulus, as a result of application of CPT, which serves as the conditioning stimulus. Results from the NDT-EP method combined with CPT are needed to validate the measurement technique in pain-free subjects. Next, it is important to explore the feasibility of the NDT-EP method combined with CPT in FBSS patients. Subsequently, results may contribute to provide insight into the underlying pathophysiology of chronic pain.

2.10.1 Approach

2.10.1.1. Primary research objectives

The primary objective is to investigate the feasibility of measurements of the NDT and EEG recordings with simultaneous application of nociceptive stimuli combined with CPT in a clinical environment. Next to this, combining the presented methodology with CPT could assist in validation of the NDT-EP method by studying the responsiveness of NDTs and EPs to CPT in healthy subjects and FBSS patients. Also, as part of validation, reproducibility could be investigated by conducting a test-retest analysis.

Primary research questions:

- (How) can NDTs and EPs be measured with the NDT-EP method combined with CPT?
- Are results of the NDT-EP experiment combined with CPT in healthy controls reproducible by performing a test-retest at St. Antonius Hospital?
- How do NDTs and EPs of healthy controls and FBSS patients behave pre-, during and post-CPT-induced CPM activation with simultaneously applied electrical stimuli using a multiple threshold tracking paradigm at St. Antonius Hospital?
- How are neurophysiological responses (average EPs) pre-, during and post-CPT related to the delivered electrocutaneous stimulus properties in healthy controls and FBSS patients?

2.10.1.2. SECONDARY RESEARCH OBJECTIVES

The secondary objective is to investigate whether NDTs and EPs manifest different behavior between healthy controls and FBSS patients across different CPT phases. Other objectives are to investigate how NDTs and EPs are related to central sensitization in FBSS patients and to investigate whether differences can be found between different age groups in healthy controls.

Secondary research questions:

- How do NDTs and EPs before, during and after CPT behave in FBSS patients compared to healthy controls?
- How are these NDTs and EPs related to central sensitization in FBSS patients?
- To what extent do NDTs and EPs of healthy controls differ across age groups?

3. METHODS

3.1. STUDY POPULATION

Healthy subjects were recruited using recruitment posters (see Appendix A). Next to this, elderly people involved in intra-hospital transfer service were approached to participate as well. FBSS patients were asked for participation during multidisciplinary meetings in which the patient's eligibility for neuromodulation was discussed. Also, FBSS patients were recruited at the outpatient clinic prior to or after a pain specialist appointment. Measurements took place at the St. Antonius Hospital Nieuwegein between January 2020 and September 2020. Prior to each measurement session, a screening list for COVID-19 was filled in and subsequently a written informed consent was provided by the subjects (see Appendix B and Appendix C). Inclusion- and exclusion criteria are shown in Table 1. Chronic pain patients were allowed to continue the use of pain medication. Early withdrawal of subjects and subjects of which impedances of EEG electrodes CPz, M1, M2, F3, F4, T7 or T8 were higher than 5 kOhm were exclusion criteria as well.

Table 1: General inclusion and exclusion criteria for participation.

Inclusion criteria	Healthy subjects	A signed, written informed consent
		Aged from 18 to 75 years
		No history of pathological pain
	FBSS patients	A signed, written informed consent
		Aged from 18 to 75 years
		Recurrent low back pain after undergoing low back spinal surgery (>3 months of recurrent pain)
		Chronic pain predominantly in the leg(s) (i.e. leg pain > back pain)
		No implanted neurostimulation device
		No transcutaneous electrical nerve stimulation device (TENS)
		No implanted intrathecal pain pump
		Post laminectomy syndrome (at level L4, L5 or S1) and diagnosed with FBSS
Exclusion criteria	Healthy subjects and FBSS patients	Subject's refusal during the study
		Recent pain treatment other than current medication intake (<3 months)
		Skin problems at the site of the pain sensitivity measurement
		Communication problems or incapable of following the instructions
		Pregnancy
		Consumption of alcohol or drugs[1] within 24 hours before the experiment
		Implanted stimulating device (e.g. pacemaker, ICD)
		Disorders that could affect the nervous system (e.g. fibromyalgia, multiple sclerosis, carpal tunnel syndrome, diabetes mellitus, CRPS, osteoarthritis)
		Unable to undergo pain sensitivity measurement
		Cardiac diseases (arrhythmias, heart valve defects, heart muscle diseases)
		Open wound on the foot to be immersed
		Frequent caffeine use (>8 units/day)
		BMI > 30 $kg \cdot m^{-2}$

[1] This study did not restrict any medication intake for pain relief. If so, however, this was noted.

3.2. STUDY DESIGN

3.2.1. OVERALL STUDY DESIGN

This study was a mono-center, explorative, cross-sectional study. The study was carried out in the Department of Anesthesiology-Pain Medicine at St. Antonius Hospital Nieuwegein, The Netherlands. During each measurement session electrical brain responses were monitored during the processing of nociceptive stimuli around the detection threshold. The number of measurements varied depending on their assigned condition. For test-retest analyses healthy subjects were asked to undergo two measurements. The test-retest time interval was chosen to be at least 7 days to preclude any influence of CPT on the second measurement session. Time-of-day was kept consistent across measurements for each subject. Chronic pain patients were asked to undergo a single measurement prior to a hospital visit due to COVID-19 restrictions. Study approval was granted by the Medical Research Ethics Committees United (MEC-U, file number: NL71927.100.19) on December 19th 2019.

3.2.2. STUDY PARAMETERS

3.2.2.1. PRIMARY STUDY PARAMETERS

Four primary study parameters were considered in this study. Firstly, effects of coefficient estimates, comprising different temporal stimulus properties, on detection probability were investigated using a generalized linear mixed regression model. Secondly and thirdly, average group NDTs and average group slopes were determined by measuring the subject's response (detected or not detected) to multiple stimuli with different stimulus intensities. Average group NDTs were defined as the stimulus amplitude resulting in a 50% detection probability. Analysis was done separately for single pulse stimuli and double pulse stimuli. Lastly, subject's neurophysiological activity in response to multiple stimuli were studied as well. Using a linear regression model, effects of coefficient estimates on EP amplitude were examined.

3.2.2.2. SECONDARY STUDY PARAMETERS

Secondary study parameters for both study groups included age, gender, handedness and BMI as demographic characteristics. Also, outcomes of neurological evaluation and CSI were obtained prior to the measurement session. Water temperature of the warm container used prior to CPT was noted. During CPT, immersion time and water temperature was noted as well.

Pain evaluation outcomes comprised current NRS score, average NRS score of last week, NRS scores right after application of CPT and after termination of the experiment and pQST outcomes on both feet and hands. Disease-related characteristics for FBSS patients comprised present pain medication intake, usage of pain medication before the experiment, usage of non-pain related medication, recent pain treatment (<3 months), date of diagnosis and time since diagnosis. Parameters and outcomes were noted on dedicated documentation forms (see Appendix D).

3.3. MATERIALS AND PROCEDURES

3.3.1. GENERAL MATERIALS AND METHODS

3.3.1.1. INTRA-EPIDERMAL STIMULATION ELECTRODES

An IES-5 electrode was used to deliver intra-epidermal electrocutaneous stimuli. This electrode contains an array of 5 micro-needles. Each electrode protrudes 0.2mm through the stratum corneum of the skin and is therefore considered to be non-invasive. Superficial intrusion of the epidermis allows stimulation of superficial nociceptive skin fibers specifically, such as Aδ-fibers. The electrodes are sterilized prior to each measurement session at the University of Twente using a monitored autoclave at 121°C for at least 17 minutes.

3.3.1.2. STIMULATION DEVICE

During this study, the AmbuStim device is used for stimulation. The AmbuStim is an electrical 1-channel stimulator which is developed by the BSS group at the University of Twente. LabView (2013, SP1) is used to control the AmbuStim by providing specific stimuli. The applied stimulus amplitudes (in mA) and their trigger codes, along with the responses to stimuli and the stimulus response time in milliseconds are registered by the dedicated program. All communication between LabView and the AmbuStim is logged.

3.3.1.3. STIMULI

Two different settings of temporal electrical properties were randomly applied during each measurement session. Properties comprises number of peaks (NoP), inter-pulse interval (IPI) and pulse-width (PW). PW was equal for both settings while NoP, and subsequently IPI, varied. During each measurement session, the two stimulus protocols were executed, which are elaborated in Table 2.

Table 2: Stimulus settings and their temporal stimulus properties used in this study.

Stimulus type	Stimulus property	NoP	IPI (ms)	PW (ms)
1	Single Pulse (SP)	1	-	0.21
2	Double Pulse (DP)	2	10	0.21

Abbreviations: NoP = Number of Pulses, IPI = Inter-Pulse-Interval, PW = Pulse-Width. IPI for single pulse stimulation is not applicable.

3.3.1.4. MULTIPLE THRESHOLD TRACKING

During each measurement session a total of 450 stimuli were applied. Since the stimulus protocol consisted of single pulse and double pulse stimuli, 225 stimuli of each stimulus type were applied to the subject. Each stimulus was randomly selected according to the MTT procedure to decrease observer and subject bias. During the measurements the subject's response (detected vs non-detected) to each randomized stimulus was used to estimate two simultaneously tracked nociceptive detection thresholds (NDTs).

3.3.1.5. EEG RECORDING

Evoked potentials (EPs) were registered simultaneously to the electrical stimulation by recording scalp EEG continuously with a sample frequency of 1 kHz using an ANT Neuro Waveguard EEG cap containing 64 Ag/AgCl electrodes in combination with a TMSi 72-channel Refa EEG amplifier. A dedicated laptop running TMSi Polybench software (Polybench Designer 1.30.0) was used to record EEG and trigger codes. Prior to the measurement the EEG cap was adjusted accordingly to the subject after the circumference of their head. Distances between multiple craniometrics points were measured to ensure Cz electrode was positioned in the middle, between nasion and inion and between both mastoid process of the temporal bone, all located with mild palpation. The ground electrode was located on the forehead and on both earlobes were electrodes applied as well. Conductive gel was applied using a needle and syringe to enable better electrical conductivity measured as impedance which was aimed to be below 5 kOhm. Prior to each measurement, subjects were instructed to maintain eye fixation and to avoid movements during pressing the response button on the stimulation device to reduce ocular and muscle artifacts.

3.3.1.6. CENTRAL SENSITIZATION INVENTORY

A Dutch version of the central sensitization inventory (CSI) was filled in by the participants prior to each measurement (see Appendix E). The CSI is a questionnaire that quantifies the main symptoms that are associated with central sensitivity syndrome (CSS). The questionnaire consists of two parts. The first part assesses key symptoms associated with central sensitivity syndrome. The second part determines possible comorbidities associated with central sensitivity syndrome. The questionnaire is widely used to measure somatic and emotional symptoms related to central sensitization and has been validated by Mayer *et al.* (Mayer *et al.*, 2012).

3.3.1.7. NEUROLOGICAL EVALUATION

A neurological evaluation of the dorsal hands, containing sensory anamnesis and two exploratory neurological assessments, was done to obtain a rough estimate of cutaneous sensory function. The sensory anamnesis was performed by asking the subject if he or she has been (self-reported) diagnosed with a sensory disorder of the hands. Next, an exploratory test of light touch sensitivity was performed by using a piece of medical cloth. Finally, a pin-prick test for small fiber function was performed using the dull and sharp end of a bisected cotton bud. During both exploratory neurological assessments subjects were asked to close their eyes. Attributes were pressed against the dorsum of both hands in random order to assess cutaneous sensory function. After each session, subjects were asked to express what was felt.

3.3.1.8. QUANTITATIVE SENSORY TESTING

Prior to each measurement, pQST measurements were performed by obtaining the pressure pain detection threshold (PPDT). PPDT was measured using the Wagner FPX® Algometer (Wagner Instruments, Greenwich, USA), of which its reliability has been investigated thoroughly (Jensen *et al.*, 1986; Kosek *et al.*, 1993). The 1 cm^2 rubber probe (at the tip of the Algometer) was placed at the midpoint of the muscle belly of the third dorsal interosseous muscle of both hands. The algometer probe was pressed against the muscle at a constant rate of approximately 5 N/s until PPDT was reached. PPDT was taken as the amount

of pressure required to elicit a sensation of pain distinct from pressure or discomfort. Subjects were instructed to express pain by saying “stop” as soon as a sensation of pain was felt. At this point, the algometer was immediately released and retracted from the measurement site by the observer. Subjects and observer were not able to see the algometer display during the measurement to avoid awareness of their own PPDT measurement.

3.3.1.9. COLD PRESSOR TEST

During the measurements, subjects were comfortably positioned in a chair in a quiet room with an ambient temperature of approximately 22°C. Prior to the cold pressor test (CPT), subjects were asked to immerse their right foot into a polystyrene container with hot water of 35°C for 120 seconds, similar to other studies (Siebenga *et al.*, 2020). During this phase, subjects were instructed to relax and release the response button on the stimulation device. Next, the CPT subjects were asked to immerse their foot into a polystyrene container with cold water of 1°C. Subjects were instructed to continue the measurement by concentrating and focusing their attention and subsequently by pressing the response button as soon as possible after foot immersion. The water temperature was aimed to be constant during immersion by ensuring the presence of ice on top of the water surface. Any increase in water temperature caused by immersion of the foot and the ambient temperature was counteracted by melting of ice.

During immersion the water was slowly recirculated manually by the observer every 3 minutes (t = 3.00 min. and t = 6.00 min.) to prevent the forming of a film of warm water surrounding the foot. Water temperatures were verified using thermometers and were reported every minute during immersion in both water containers. Withdrawal of the foot by the subject is allowed at any time (i.e. whenever the pain caused by the CPT is felt as intolerable). The duration of foot immersion was noted. After 7 minutes of immersion, the subject was instructed to remove the foot from the water. After removal, the foot was dried by the observer. Plastic bags were used to cover the inside of both polystyrene containers to prevent leakage of the containers which could cause grounding issues (i.e. 50 Hz noise on EEG data). During CPT a blood pressure cuff was applied around the lower leg and inflated 20 mmHg below diastolic blood pressure to limit blood flow warming the foot.

3.3.2. GENERAL PROCEDURES

Each measurement session consisted of multiple phases (see Figure 18). Prior to the experiment, preparations were made along with the familiarization to familiarize the subject with the stimulus protocols. Next, a baseline measurement was taken (preCPT) followed by the perCPT phase in which the cold pressor test was applied. After 7 minutes, the cold pressor test was terminated and the NDT-EP measurement was resumed (postCPT). PostCPT phase was terminated after 225 SRPs per setting had been collected and subsequently, the subject was disconnected from the equipment and debriefed. Each phase is elaborated in the next sections.

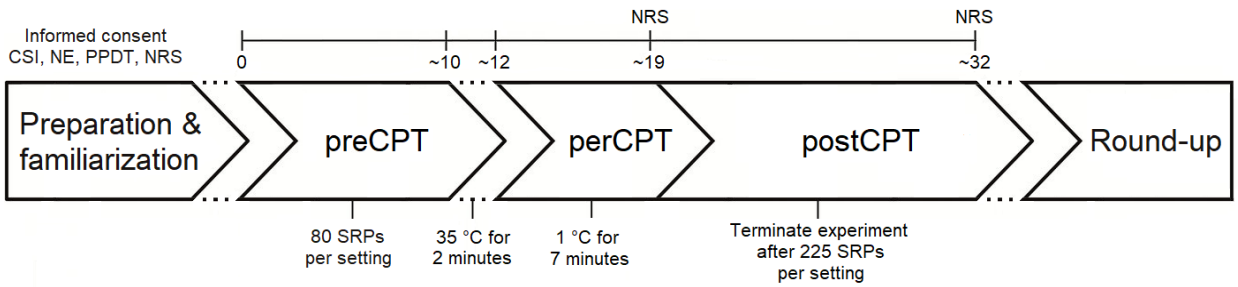

Figure 18: Timeline of a single measurement session. Prior to the experiment, informed consent, central sensitization inventory (CSI), neurological evaluation (NE), pressure pain detection thresholds (PPDTs) and numeric rating scale outcomes were filled in and collected. During pre-, per-, and postCPT, nociceptive detection thresholds were tracked over time and evoked potentials were obtained simultaneously. Between pre-, and perCPT, tracking was terminated temporarily and subjects were instructed to immerse their foot into a hot water container for 2 minutes. At the end of cold pressor test application and at the end of the experiment, NRS scores were obtained.

3.3.2.1. PREPARATION

Subjects were asked to eat sufficiently prior to the measurements to reduce cognitive distraction caused by increased appetite. Next to this, instructions were provided to avoid heavy meals and consumption of caffeine, alcohol and non-pharmaceutical drugs 24 hours prior to the measurements. Prior to the start of each measurement session subjects were asked to read and sign the informed consent form. A questionnaire, including CSI, the standard Numeric Rating Scale (NRS), and a case report form (CRF) were filled in by all subjects. NRS is used to measure present and past pain. The subject can grade pain intensity on a scale of 0-10. Where 0 means 'no pain' and 10 means 'worst imaginable pain'. PPDT was obtained using the method described in *2.3.1.7 Quantitative Sensory Testing.* Next, neurological evaluation to assess sensory function was performed and blood pressure was measured. To reduce artifacts in the EEG data, subjects were asked to turn off their mobile devices. Also, subjects were instructed to remove their earrings to ease the application of electrodes on both ear lobes. After adjusting the best sized EEG cap, subjects were connected to the EEG amplifier. All EEG electrodes were filled with conductive gel. EEG topographies were used to assure scalp electrodes impedances below 5 kOhm. For stimulation an electrode was positioned and fixed gently with medical tape on the dorsum of the dominant hand. The stimulation device was connected with the stimulation electrode and was held by the subject with the non-dominant hand. Using a Bluetooth connection, the stimulation device was wirelessly connected with LabView installed on a dedicated laptop.

3.3.2.2. FAMILIARIZATION

Multiple stimuli were applied to the subjects to familiarize the subject with the two stimulus protocols. The subject was asked to press and hold the response button on the stimulation device to trigger three series of stimuli. Stimuli were administered at 1 Hz with slowly increasing stimulus intensity of 0.05 mA/s from 0 mA up to a maximum stimulus intensity of 1.0 mA. In the first series the subject was instructed to release the response button after feeling the stimulus-related sensation clearly (3 or 4 stimulus-related sensations). During the second and third series, the subject was instructed to release the response button as soon as they felt any sensation assigned to the applied electrical stimuli (setting 1 and 2 respectively). Estimates of detection threshold in the second and third series were used as initial detection thresholds at the onset of the NDT-EP experiment.

3.3.2.3. NDT-EP EXPERIMENT COMBINED WITH CPT

At the onset of the experiment, subjects were instructed to limit eye blinking and facial movements to reduce EEG artifacts. Next, subjects were instructed to press the response button to start the application of electrical stimuli and to release the response button every time a stimulus-related sensation was felt. Every time an electrical stimulus was applied the subject the subject had to release the response button within 1000 ms in order to label the applied stimulus as "detected". The applied stimulus was labeled as "undetected" when the response time exceeded 1000 ms or when the subject did not release the response button at all. One second after releasing the response button the subject was instructed to repress the response button to proceed the experiment. After application of 80 stimuli of each setting the subject was asked to release the response button to terminate the application of stimuli temporarily.

Next, the subject was asked to immerse their right foot into the polystyrene container with hot water. A blood pressure cuff was applied loosely around the lower leg to be inflated during the CPT. After 120 seconds, the foot was removed from the hot water and immersed into the container with ice cold water. The blood pressure cuff was inflated 20 mmHg below diastolic blood pressure and subjects were instructed to concentrate and focus as soon as possible. To proceed the experiment, subjects were asked to repress the response button.

Seven minutes after immersion into the cold water container, subjects were instructed to release the response button and to remove the foot from the cold water, regardless the number of applied electrical stimuli. The blood pressure cuff was removed from the lower leg and the foot was dried using towels by the observer. NRS score was obtained and the lid of the polystyrene container was used to prevent grounding issues by placing it between the floor and right foot.

Next, the subject was instructed to proceed the experiment as soon as possible by repressing the response button. After approximately 230 stimuli of each setting the subject was asked to release the button to terminate the measurement. NRS score was obtained again and the subject was disconnected from the measurement set-up.

3.4. DATA PREPARATION

3.4.1. DETECTION PROBABILITIES AND NOCICEPTIVE DETECTION THRESHOLDS

During each measurement session, NDTs were tracked and estimated over time, using a window of 30 trials, in response to intra-epidermal stimulation with known intensity. NDTs were real-time estimated, which allowed preliminary visualizations. During data analyses, NDTs were re-estimated using generalized linear mixed regression allowing statistical analysis of the relation between stimulus properties and the subject's response.

3.4.2. EVOKED POTENTIALS

EEG signals are prone to artifacts which makes denoising a crucial step (Barthelemy *et al.*, 2017). These artifacts include contamination from the heart, eye and muscle movements (Matiko *et al.*, 2013). EEG data were preprocessed using MATLAB R2015b (Math-Works, Natick, MA, USA) and the Fieldtrip toolbox (Oostenveld *et al.*, 2011). Widely used decomposition methods are based on regression, principal

component analysis (PCA) and independent component analysis (ICA). In the present study electro-oculogram (EOG) components generated by the eye were removed using ICA. In addition, a band-pass filter was applied to remove frequencies outside the 0.1 Hz and 40 Hz frequency band, which means that undesired frequencies such as frequencies produced by the AC power line were filtered out. Subsequently, variances of trials and channels of each subject were inspected visually and outliers were rejected manually. The window of EEG analysis ranges from 500 ms before to 1000 ms after the stimulus onset. The period before the onset of the stimulus was used to apply baseline corrections.

Butterfly plots consisting of global field power (GFP) were constructed for each data set to be analyzed. The highest peaks of GFP for test-retest dataset and test and FBSS datasets were selected to determine latencies for central derivations (CPz-A1A2) and contralateral derivations (T7-F4 or T8-F3, depending on the side of electrical stimulation). These latencies were later used for statistical analyses. Plots of grand average EPs were constructed for each study group to assess the outcomes visually.

3.5. DATA ANALYSIS

3.5.1. SUBJECT CHARACTERISTICS

Statistical comparison of subject characteristics between both groups was made using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, N.Y., USA). Assessment of sample distribution was performed twofold. Firstly, using Q-Q plots (quantile-quantile plots) quantiles of two probability distributions were visually inspected. Secondly, a mathematical approach by means of Shapiro-Wilk tests were used to determine sample distribution. Non-categorical variables were evaluated using two-tailed independent samples t-tests if variables were normally distributed and using Mann-Whitney U tests if not. Differences between categorical variables (i.e. gender, outcomes of neurological evaluation and handedness) were evaluated by Fisher's Exact Test.

3.5.2. DETECTION PROBABILITIES AND NOCICEPTIVE DETECTION THRESHOLDS

Generalized linear mixed model effects, along with average NDTs and slopes, were statistically analyzed using R-4.0.2. (R Foundation for Statistical Computing, Vienna, Austria) and the lme4 library (D. Bates *et al.*, 2015).Type III ANOVA tables were used to test the fixed model effects statistically. Average NDTs and slopes were evaluated using post hoc statistical testing (Z-statistics). To accomplish the objectives described in 2.10.1.1. Primary research objectives and 2.10.1.2. Secondary research objectives, multiple GLMMs were fitted to the data. Properties of these models are elaborated in the next sections.

3.5.2.1. REPRODUCIBILITY OF MEASURING PSYCHOPHYSICAL DETECTION THRESHOLD

To evaluate the reproducibility of measuring psychophysical detection thresholds, a GLMM was constructed for test-retest results of the healthy subjects. The effects of stimulus properties and measurement session on the psychophysical detection probability were evaluated using generalized linear mixed model (GLMM) using a logit link function. The intercept and interactions between measurement session and amplitude of single and double pulse and between trial number and

measurement session were included as fixed effects. All fixed effects were included as random effects, grouped by subject number. The corresponding equation of the GLMM is presented as:

$$SR \sim 1 + A\_SP * RT + A\_DP * RT + TRL * RT + (1 + A\_SP * RT + A\_DP * RT + TRL * RT \mid subjectNR) \quad (3)$$

3.5.2.2. Effect of CPT on NDTs in healthy subjects and FBSS patients

The relationships between delivered electrocutaneous stimulus properties and psychophysical detection probability and between CPT phase and psychophysical detection probability were evaluated in healthy controls (both test and retest results) and FBSS patients. The effects of stimulus properties and CPT phase on the psychophysical detection probability were evaluated using generalized linear mixed model (GLMM) using a logit link function. The intercept and interactions between CPT phase and amplitude of single and double pulse and between trial number and CPT phase were included as fixed effects. All fixed effects were included as random effects, grouped by subject number. The corresponding equation of the GLMM is presented as:

$$SR \sim 1 + A\_SP * CPT + A\_DP * CPT + TRL * CPT + (1 + A\_SP * CPT + A\_DP * CPT + TRL * CPT \mid subjectNR) \quad (4)$$

GLMM coefficients:

SR	= subject's response
A_SP	= amplitude of single pulse
RT	= study group test results or retest results of healthy subjects
CPT	= cold pressor test phase (pre-, per- or postCPT)
A_DP	= amplitude of double pulse
TRL	= stimulus trial number
subjectNR	= subject number

3.5.3. Evoked potentials

Contributions of fixed effects on EP amplitudes were evaluated using MATLAB R2017a (Math-Works, Natick, MA, USA). T-statistics was used to test the significance of fixed effects on EP amplitudes at fixed group latencies based on global field power waveforms. To accomplish the objectives described in 2.10.1.1. Primary research objectives and 2.10.1.2. Secondary research objectives, multiple LMMs were fitted to the data. Properties of these models are elaborated in the next sections.

3.5.3.1. Reproducibility of measuring neurophysiological brain responses (EPs)

To evaluate the reproducibility of measuring EPs, a LMM was constructed for test-retest results of the healthy subjects. The effects of stimulus properties, retest, number of trials and the subject's response on EPs were evaluated using linear mixed model (LMM) using a logit link function. The intercept and the interaction between number of trials, subject's response and retest were included as fixed effects. Also, interaction between retest and amplitude of single and double pulse were included as fixed effects. All fixed effects were included as random effects, grouped by subject number. Note that the effect CPT was not added to the LMM as test-retest analyses were performed within CPT phases for each CPT phase (pre-, per- and postCPT). The corresponding equation of the GLMM is presented as:

$$EP \sim 1 + A\_SP * RT + A\_DP * RT + TRL * SR * RT + (1 + A\_SP * RT + A\_DP * RT + TRL * SR * RT \mid subjectNR) \quad (5)$$

3.5.3.2. EPs in healthy subjects and FBSS patients pre-, during and post-CPT

The relationships between delivered electrocutaneous stimulus properties and EPs, reflecting neurophysiological brain responses, and between CPT phase and EPs were evaluated in healthy controls and FBSS patients. The effects of stimulus properties and CPT phase on EPs were evaluated using three linear mixed models (LMMs) using a logit link function. The intercept and interaction between number of trials, subject's response and CPT phase were included as fixed effects. Also, interaction between CPT phase and amplitude of single and double pulse were included as fixed effects. All fixed effects were included as random effects, grouped by subject number. Note that the effect diagnose is missing as LMM is fitted to the datasets separately. The corresponding equation of the LMM is presented as:

$$EP \sim 1 + A\_SP * CPT + A\_DP * CPT + TRL * SR * CPT + (1 + A\_SP * CPT + A\_DP * CPT + TRL * SR * CPT \mid subjectNR) \quad (6)$$

3.5.3.3. EP differences across age groups in healthy subjects

To evaluate the influence of age on the CPT effect on EPs, a third LMM was modulated. The effects of stimulus properties and CPT phase on EPs were evaluated using a linear mixed model (LMM) using a logit link function. Results of the first measurement session of the healthy subjects were used in the model. Subjects were divided into age groups of between 18 and 35 years, 36 to 55 years and 56 to 75 years. Each dataset was fitted to the LMM separately. The intercept and interaction between number of trials, subject's response and CPT phase were included as fixed effects. Also, interaction between CPT phase and amplitude of single and double pulse were included as fixed effects. All fixed effects were included as random effects, grouped by subject number. The corresponding equation of the LMM is presented as:

$$EP \sim 1 + A\_SP * CPT + A\_DP * CPT + TRL * SR * CPT + (1 + A\_SP * CPT + A\_DP * CPT + TRL * SR * CPT \mid subjectNR) \quad (7)$$

LMM coefficients:

EP	= evoked potential
A_SP	= amplitude of single pulse
RT	= study group test results or retest results of healthy subjects
CPT	= cold pressor test phase (pre-, per- or postCPT)
A_DP	= amplitude of double pulse
TRL	= stimulus trial number
SR	= subject's response
subjectNR	= subject number

4. RESULTS

4.1. STUDY POPULATION

4.1.1. HEALTHY SUBJECTS

After screening for in- and exclusion criteria, 22 healthy subjects were eligible for participating in the study. One healthy subject was excluded due to technical failure and one healthy subjects was not able to continue the experiment due to physical issues. This resulted in a group of twenty healthy subjects (eleven females, age 19-72 years, average 45.6 years) measured between January 22nd 2020 and September 8th 2020.

4.1.2. FBSS PATIENTS

Between July 10th 2020 and September 17th 2020 seven chronic pain patients were included for participation. One chronic pain patient was excluded from the data set, due to not fully meeting the inclusion criteria (wrong diagnosis). This resulted in a group of six FBSS patients (four females, age 48-73 years, average 61.5 years) eligible for analysis.

4.1.3. GENERAL DEMOGRAPHIC CHARACTERISTICS

An overview of demographic characteristics for healthy subjects and FBSS patients is shown in Table 3. Age, gender, BMI and handedness were not significant between both groups.

Table 3: Demographic characteristics for healthy subjects and failed back surgery syndrome (FBSS) patients.

Characteristic	HC group (n = 20)	FBSS group (n = 6)	*P*-value
Age (years)	40.5 (44.0)	61.5 (14.5)	0.113
Gender (M/F)	9/11	2/4	1.00
BMI (kg/m^2)	24.8 ± 2.8	27.3 ± 2.6	0.067
Handedness (left/right)	5/15	0/6	0.298

Normal distributed numeric variables are displayed as mean ± standard deviation. Non-normal distributed numeric variables are displayed as median (interquartile range). Significance levels: p = 0.05. Abbreviations: HC = healthy controls, FBSS = failed back surgery syndrome, F = female, M = male, BMI = body mass index.

4.2. TEST-RETEST ANALYSIS

4.2.1. GROUP CHARACTERISTICS

Healthy subjects measured for the first time did not significantly differ in NRS score in the past week and the NRS score at the onset of the experiment from healthy subjects measured for the second time (Table 4). Average water temperature of the hot container, which was used prior to the CPT, did not significantly

differ between test and retest measurements. Average water temperature during CPT was significantly lower during retest measurements. All PPDT measurements did not significantly differ between both groups. Both groups did not significantly differ in average post CPT NRS score, post experiment NRS score, CSI score and neurological evaluation. The median (interquartile range) of time between test and retest measurements was 14.0 (14.75) and ranged from 7 to 198 days.

Table 4: Measurement outcomes for healthy subjects' test and healthy subjects' retest.

	HC test (n = 20)	**HC retest (n = 20)**	***P*-value**
PPDT left hand (N)	69.9 ± 16.4	69.1 ± 13.9	0.872
PPDT right hand (N)	67.9 ± 13.3	64.9 ± 12.2	0.448
PPDT left foot (N)	79.8 ± 23.7	72.7 ± 17.5	0.288
PPDT right foot (N)	74.0 ± 23.4	73.1 ± 12.3	0.880
Temperature hot water container (°C)	35.1 (0.7)	35.0 (1.3)	0.973
Temperature during CPT (°C)	0.5 (0.8)	0.4 (0.8)	0.012*
NRS score past week	0.0 ± 0.0	0.0 ± 0.0	1.00
NRS score current	0.0 ± 0.0	0.0 ± 0.0	1.00
NRS score post CPT	6.0 (2.0)	6.0 (4.0)	0.901
NRS score post experiment	2.0 (1.75)	2.0 (1.0)	0.325
CSI score	12.5 (14.5)	11.5 (15.0)	0.524
NE soft-cloth (altered/unaltered)	0/20	0/20	1.00
NE pin-prick (altered/unaltered)	0/20	0/20	1.00

*Normal distributed numeric variables are displayed as mean ± standard deviation. Non-normal distributed numeric variables are displayed as median (interquartile range). Significant differences tested using Mann-Whitney U tests are indicated with *. Significance levels: p = 0.05. Outcomes of neurological evaluation are provided for one hand as outcomes were consistent for both hand sides. Abbreviations: HC = healthy controls, PPDT = pressure pain detection threshold, N = Newton, CPT = cold pressor test, NRS = numeric rating scale, CSI = central sensitization inventory, NE = neurological evaluation.*

4.2.2. Detection probabilities and NDTs

Average percentages of detection rate for single and double pulse stimulation are shown in Table 5.

Table 5: Average percentages of detection rate for single pulse (SP) and double pulse (DP) stimulation. Data are shown for test and retest measurements as average percentages per CPT phase and as general average percentages.

	preCPT		*perCPT*		*postCPT*		*Total*	
Study group	**SP**	**DP**	**SP**	**DP**	**SP**	**DP**	**SP**	**DP**
HC test	36.1%	48.5%	32.5%	45.9%	35.7%	47.2%	35.1%	47.3%
HC retest	38.5%	45.2%	33.3%	44.7%	39.2%	50.7%	37.5%	47.3%

Estimated NDTs for test and retest results are shown in Figure 19. Results of GLMM statistics are shown in Table 6. Average NDTs and slopes are displayed in Table 7. Note that GLMMs used to generate the estimated NDTS differ from the GLMMs used to perform statistical analysis and are therefore for illustrative purposes only.

Results of test-retest analysis showed that retest was no significant determinant of detection probabilities (Table 6). Interestingly, retest measurements showed altered detection probabilities following double pulse stimulation compared to test measurements, whereas interaction between retest and single pulse stimulation demonstrated no significant difference compared to test measurements. Average group slopes following double pulse stimulation were significantly steeper for test measurements compared to retest measurements (Table 7). Also, average NDTs following double pulse stimulation were significantly higher for retest measurements. No significant differences were found of average NDTs and slopes between test and retest measurements following single pulse stimulation.

Table 6: Regression parameter estimates and fixed effects for generalized linear mixed model of detection probabilities. Model was fitted to results of test and retest measurements of healthy subjects. Test results were used as reference level.

Parameter	**Parameter estimate**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-1.92	40.83 (1)	<0.001
Retest	-0.24	0.23 (1)	0.634
Retest x amplitude SP	-0.63	0.07 (1)	0.785
Retest x amplitude DP	-3.94	3.92 (1)	0.048*
Retest x trial number	0.16	1.59 (1)	0.207

*Significant differences tested using Type III Wald chi-square tests are indicated with *. Significance level: p = 0.05. Abbreviation: df = degrees of freedom.*

Table 7: Average nociceptive detection thresholds (NDTs) and slopes of psychophysical functions. Averages were derived from a generalized linear mixed model fitted to results of test and retest measurements of healthy subjects. Values between brackets represent 95% confidence intervals.

	Single pulse		*Double pulse*	
Study group	**Mean NDT (mA)**	**Mean slope (mA^{-1})**	**Mean NDT (mA)**	**Mean slope (mA^{-1})**
HC test	0.46 [0.28 0.64]	4.91 [1.98 7.83]	0.17 [0.12 0.22]	13.13 [6.89 19.38]
HC retest	0.54 [0.28 0.80]	4.28 [0.88 7.68]	0.27 [0.20 0.35]	8.57 [3.76 13.37]
P-value	0.584	0.785	0.031*	0.048*

*Significant differences tested using two-tailed independent samples Z-test are indicated with *. Significance levels: p = 0.05. Abbreviations: NDT = nociceptive detection threshold, mA = milliampere.*

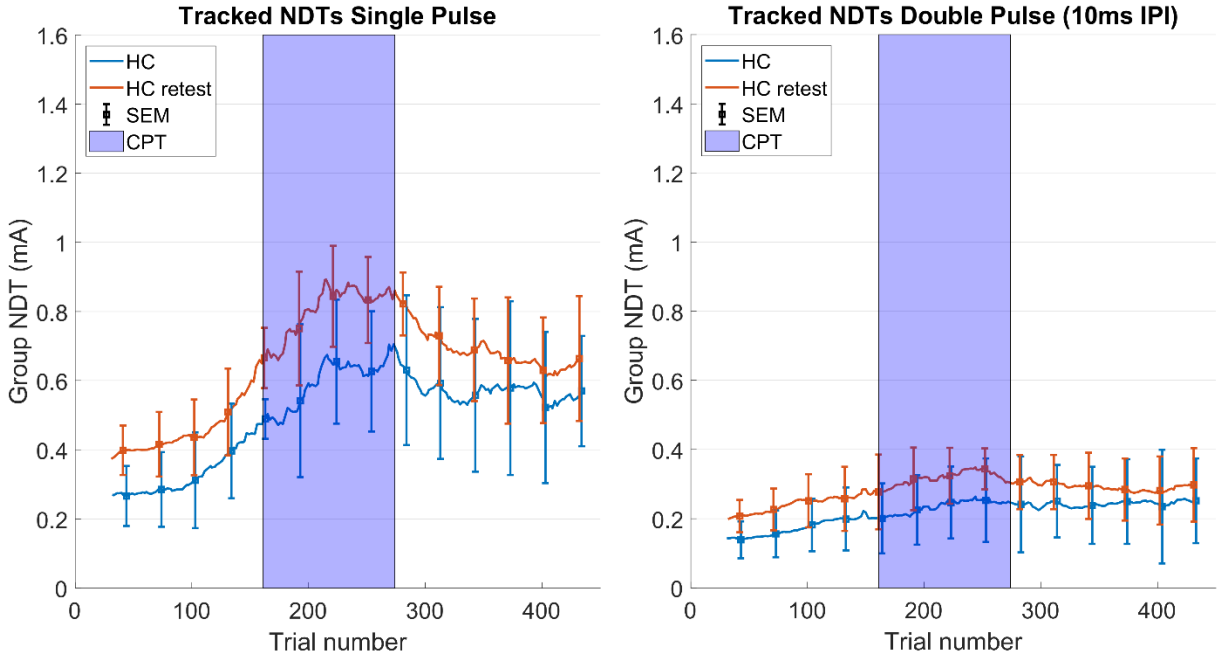

Figure 19: Estimated nociceptive detection thresholds (NDTs) for test and retest measurements tracked over trial number. Left panels show average NDTs for single pulse intra-epidermal stimulation. Right panel shows average NDTs for double pulse (IPI = 10 ms) intra-epidermal stimulation. Application of CPT for seven minutes is indicated by the light blue area.

4.2.3. Evoked potentials

Three LMMs were fitted to the EEG data. Each model was fitted to the EEG data for each CPT phase for both study groups (test and retest). EP latencies on which statistical analysis was performed, were based on global field power (GFP) maxima of butterfly plots. For test-retest comparison datasets of first and second measurement were combined (see Appendix F, Figure 27). Maxima were determined to be 197 ms and 428 ms for contralateral and central derivations, respectively. However, EP latencies used for central derivation of preCPT analysis and contralateral derivation of postCPT analysis were replaced by 422 ms and 192 ms, respectively, due to software-related issues retaining statistical analysis.

Note that only main results involving the effects retest and stimulus amplitudes are shown for central derivations (see Table 8). For full tables and results of contralateral derivations refer to Appendix G, chapter 1, 2 and 3.

Results show that retest was not significant for each CPT phase, which implicates retest measurements did not result in altered EP amplitudes compared to test measurements for both single pulse and double pulse stimuli. Also, retest did not significantly differ from test measurements following single pulse stimulation. However, interaction between retest and double pulse stimulation were significantly different for per- and postCPT compared to test measurements. This implicates that during and after application of CPT, EP amplitudes were significantly modulated compared to test measurements (see Table 8, Figure 20).

*Table 8: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) and corresponding P-values evaluated at P422 for preCPT and P428 for perCPT and postCPT. The models were fitted to test and retest results of healthy subjects for each CPT phase. Test results were used as reference level. Significant differences are indicated with *. Significance level: p = 0.05.*

	preCPT		*perCPT*		*postCPT*	
Parameter	**Parameter estimate**	***P*-value**	**Parameter estimate**	***P*-value**	**Parameter estimate**	***P*-value**
Retest	-0.86	0.140	-1.48	0.082	-1.50	0.144
Retest x amplitude SP	-4.26	0.054	0.25	0.835	2.21	0.060
Retest x response	-1.69	0.356	1.01	0.233	0.77	0.365
Retest x trial number	-0.01	0.877	-0.004	0.768	-0.01	0.320
Retest x amplitude DP	3.27	0.061	3.25	0.022*	2.21	0.036*
Retest x response x trial number	0.05	0.003*	-0.04	0.097	-0.01	0.590

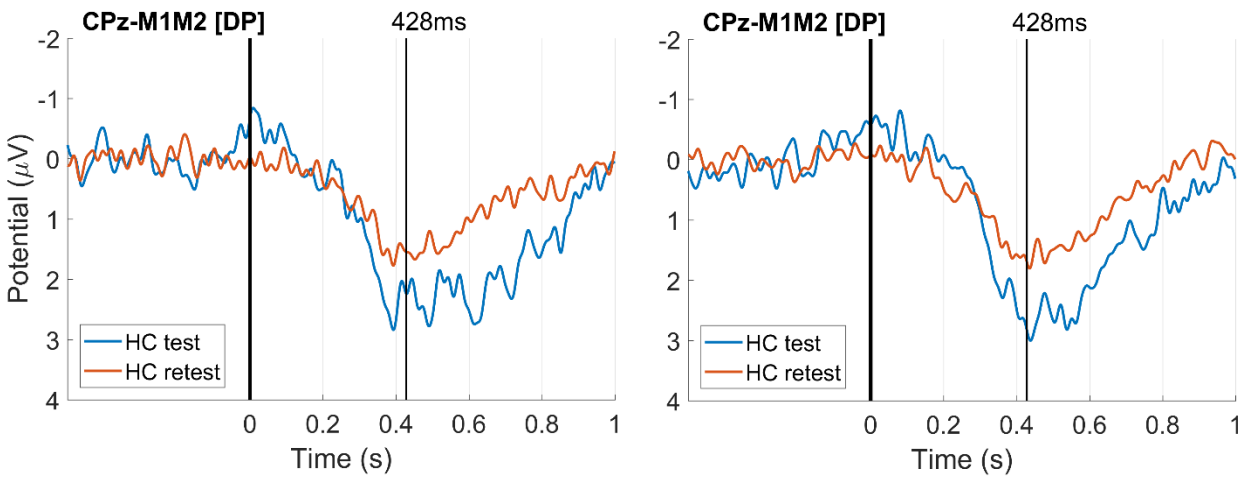

Figure 20: Grand average evoked potentials of central derivation (CPz-A1A2) following double pulse stimulation (DP) with inter-pulse interval of 10 ms for test and retest measurements. Left panel shows grand average evoked potential during cold pressor test (perCPT) and right panels shows grand average evoked potentials after cold pressor test (postCPT). Statistical analyses were performed at P428.

4.2.4. Cold pressor test and NDTs

To explore the effect of CPT on detection probabilities and NDTs, two generalized linear mixed models were fitted to the data. One model was fitted to the test results, while the other was fitted to the retest results.

4.2.4.1. Effect of CPT on detection probabilities and NDTs – test results

Results of test analysis showed that a significant difference in detection probability was found with respect to condition and interaction between condition and stimulus properties (see Table 9). Also, the detection probability was significantly altered with respect to the interaction between condition and trial number.

Average group slopes following both single pulse and double pulse stimulation were significantly steeper prior to CPT compared to during CPT and after CPT. Average group NDTs were not significant between preCPT and perCPT following both single pulse and double pulse stimulation. Interestingly, average group NDTs obtained after CPT were significantly lower compared to preCPT and perCPT for both stimulus properties.

Table 9: Regression parameter estimates of the fixed effects of the generalized linear mixed model fitted to results of test measurements of healthy subjects. PreCPT results were used as reference level.

Parameter	Parameter estimate	Effect χ^2 (df)	*P*-value
(Intercept)	-6.03	173.62 (1)	<0.001*
Condition		94.61 (2)	<0.001*
perCPT	3.01		
postCPT	3.52		
Condition x amplitude SP		14.57 (2)	<0.001*
perCPT	-6.24		
postCPT	-5.29		
Condition x amplitude DP		18.72 (2)	<0.001*
perCPT	-5.91		
postCPT	-5.40		
Condition x trial number		13.13 (2)	<0.001*
perCPT	0.75		
postCPT	1.53		

*Significant differences tested using Type III Wald chi-square tests are indicated with *. Significance level: p = 0.05. Abbreviation: df = degrees of freedom.*

Table 10: Average nociceptive detection thresholds (NDTs) and slopes of psychophysical functions. Averages were derived from a generalized linear mixed model fitted to results of test measurements of healthy subjects. Values between brackets represent 95% confidence intervals.

	Single pulse		*Double pulse*	
Condition	**Mean NDT (mA)**	**Mean slope (mA^{-1})**	**Mean NDT (mA)**	**Mean slope (mA^{-1})**
preCPT	0.66 [0.39 0.93]	12.17 [6.55 17.79]	0.29 [0.19 0.38]	28.10 [17.31 38.89]
perCPT	0.72 [0.43 1.00]	5.93 [2.98 8.87]	0.27 [0.18 0.36]	15.94 [9.14 22.74]
P-value	0.376	<0.001*	0.411	<0.001*
preCPT	0.66 [0.39 0.93]	12.17 [6.55 17.79]	0.29 [0.19 0.38]	28.10 [17.31 38.89]
postCPT	0.43 [0.29 0.57]	6.88 [3.20 10.57]	0.17 [0.13 0.21]	17.42 [9.59 25.24]
P-value	0.010*	<0.001*	0.001*	<0.001*
perCPT	0.72 [0.43 1.00]	5.93 [2.98 8.87]	0.27 [0.18 0.36]	15.94 [9.14 22.74]
postCPT	0.43 [0.29 0.57]	6.88 [3.20 10.57]	0.17 [0.13 0.21]	17.42 [9.59 25.24]
P-value	0.001*	0.089	0.004*	0.067

*Significant differences tested using two-tailed independent samples Z-test are indicated with *. Significance levels: p = 0.05. Abbreviations: NDT = nociceptive detection threshold, mA = mili ampere.*

4.2.4.2. Effect of CPT on detection probabilities and NDTs – retest results

Similar to test results, results of retest analysis demonstrated that a significant difference in detection probability was found with respect to condition and interaction between condition and stimulus properties. Also, the detection probability was significantly altered with respect to the interaction between condition and trial number.

Also, average group slopes following both single pulse and double pulse stimulation were significantly steeper prior to CPT compared to during CPT and after CPT. Average group NDTs were not significant between preCPT and perCPT following both single pulse and double pulse stimulation. Interestingly, average group NDTs obtained after CPT were significantly lower compared to preCPT and perCPT for both stimulus properties.

Table 11: Regression parameter estimates of the fixed effects of the generalized linear mixed model fitted to results of retest measurements of healthy subjects. PreCPT results were used as reference level.

Parameter	**Parameter estimate**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-6.22	68.44 (1)	<0.001*
Condition		47.48 (2)	<0.001*
perCPT	1.95		
postCPT	2.95		
Condition x amplitude SP		24.11 (2)	<0.001*
perCPT	-4.33		
postCPT	-4.94		
Condition x amplitude DP		17.10 (2)	<0.001*
perCPT	-2.31		
postCPT	-3.61		
Condition x trial number		14.43 (2)	<0.001*
perCPT	0.77		
postCPT	1.73		

*Significant differences tested using Type III Wald chi-square tests are indicated with *. Significance level: p = 0.05. Abbreviation: df = degrees of freedom.*

Table 12: Average nociceptive detection thresholds (NDTs) and slopes of psychophysical functions. Averages were derived from a generalized linear mixed model fitted to results of retest measurements of healthy subjects. Values between brackets represent 95% confidence intervals.

	Single pulse		*Double pulse*	
Condition	**Mean NDT (mA)**	**Mean slope (mA^{-1})**	**Mean NDT (mA)**	**Mean slope (mA^{-1})**
preCPT	0.71 [0.44 0.99]	11.20 [5.66 16.74]	0.38 [0.27 0.49]	20.85 [13.39 28.31]
perCPT	0.77 [0.43 1.10]	6.87 [2.77 10.97]	0.37 [0.22 0.52]	14.22 [7.59 20.84]
P-value	0.549	<0.001*	0.813	0.004*
preCPT	0.71 [0.44 0.99]	11.20 [5.66 16.74]	0.38 [0.27 0.49]	20.85 [13.39 28.31]
postCPT	0.53 [0.33 0.73]	6.26 [2.20 10.32]	0.27 [0.20 0.34]	12.31 [6.14 18.49]
P-value	0.010*	<0.001*	0.007*	<0.001*
perCPT	0.77 [0.43 1.10]	6.87 [2.77 10.97]	0.37 [0.22 0.52]	14.22 [7.59 20.84]
postCPT	0.53 [0.33 0.73]	6.26 [2.20 10.32]	0.27 [0.20 0.34]	12.31 [6.14 18.49]
P-value	0.005*	0.079	0.019*	0.001*

*Significant differences tested using two-tailed independent samples Z-test are indicated with *. Significance levels: p = 0.05. Abbreviations: NDT = nociceptive detection threshold, mA = miliampere.*

4.2.5. COLD PRESSOR TEST AND EVOKED POTENTIALS

To explore the effect of CPT on amplitudes of EPs for test-retest results, datasets were analyzed separately by constructing two linear mixed models. EP latencies for test results were determined to be 192 ms and 426 ms for contralateral and central derivations, respectively, and for retest results, EP latencies were determined to be 175 ms and 492 ms for contralateral and central derivations, respectively (see Appendix F, Figure 28). EP latency for contralateral derivation analysis of test results was replaced by 193 ms due to software-related issues.

4.2.5.1. EFFECT OF CPT ON EVOKED POTENTIALS – TEST RESULTS

Test results are shown in Table 13. Note that only statistics and grand average EPs of central derivation are shown, as none of the main effects and their interactions for contralateral derivations of test and retest results were significantly different. For full tables and results of contralateral derivations refer to Appendix G (*4.1 Test measurements*).

Results show that for test measurements, interaction between condition 1 (perCPT) and single pulse amplitude and between condition 2 (postCPT) and single pulse amplitude significantly differ from condition 0 (preCPT) (see Table 13, Figure 21). Condition 1 and condition 2 and interaction between condition 1 and double pulse amplitude and between condition 2 and double pulse amplitude were not significantly different compared to condition 0. This implicates for test measurements that CPT modulated EP amplitudes significantly, compared to EP amplitudes prior to application of CPT following single pulse stimulation only.

*Table 13: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test measurements of healthy subjects. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P426. PreCPT test results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
Condition 1	0.12	[-2.85 3.09]	0.08 (921)	0.938
Condition 2	-0.54	[-3.89 2.80]	-0.32 (655)	0.750
Condition 1 x amplitude SP	-3.25	[-5.75 -0.75]	-2.57 (183)	0.011*
Condition 2 x amplitude SP	-3.32	[-5.91 -0.73]	-2.53 (193)	0.012*
Condition 1 x amplitude DP	0.33	[-3.13 3.80]	0.19 (639)	0.850
Condition 2 x amplitude DP	1.33	[-1.92 4.57]	0.80 (1146)	0.422

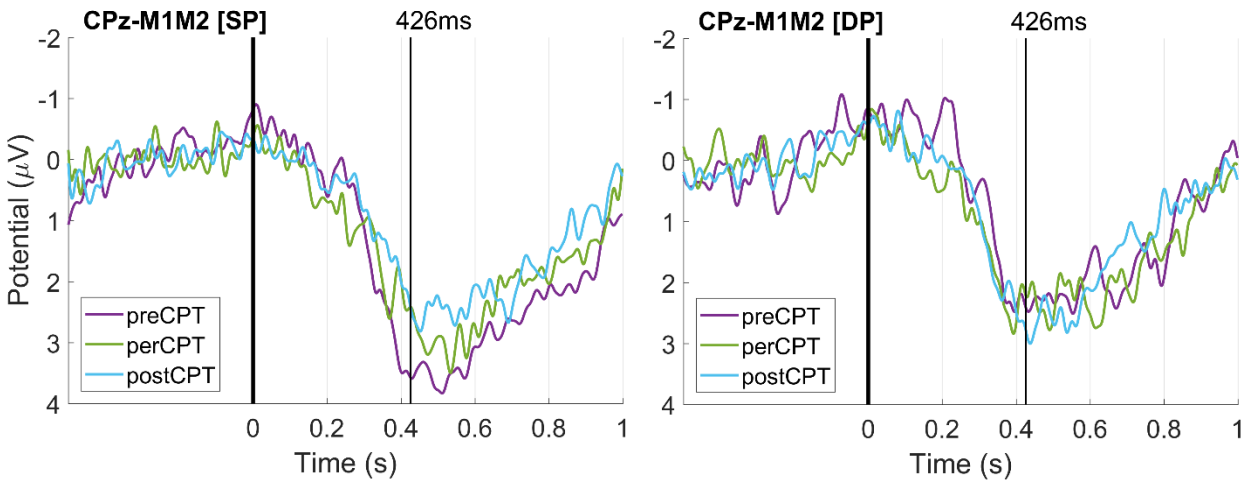

Figure 21: Grand average evoked potentials of central derivation (CPz-A1A2) of test measurements following single pulse (SP) stimulation (left panel) and double pulse stimulation (DP) with inter-pulse interval of 10 ms (right panel). EEG data were split into pre-, per and postCPT datasets indicated by purple, green and blue plots. Statistical analyses were performed at P426.

4.2.5.2. Effect of CPT on evoked potentials – retest results

Retest results are shown in Table 14. Note that only statistics and grand average EPs of central derivation are shown, as none of the main effects and their interactions for contralateral derivations of test and retest results were significantly different. For full tables and results of contralateral derivations refer to Appendix G (*4.2 Retest measurements*).

Results of retest measurements show that none of the effects and the interaction between stimulus amplitudes and conditions were significantly different from preCPT EP amplitudes (see Table 14, Figure 22). This implicates for retest measurements that CPT did not modulate EP amplitudes significantly, compared to EP amplitudes prior to application of CPT following both single pulse and double pulse stimulation.

*Table 14: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to retest measurements of healthy subjects. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P492. PreCPT retest results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
Condition 1	0.63	[-1.05 2.31]	0.73 (8024)	0.464
Condition 2	0.40	[-1.46 2.26]	0.42 (7079)	0.673
Condition 1 x amplitude SP	0.15	[-1.29 1.59]	0.21 (1244)	0.835
Condition 2 x amplitude SP	0.54	[-0.86 1.95]	0.77 (76)	0.442
Condition 1 x amplitude DP	1.46	[-0.68 3.61]	1.40 (26)	0.173
Condition 2 x amplitude DP	0.48	[-1.55 2.50]	0.48 (32)	0.636

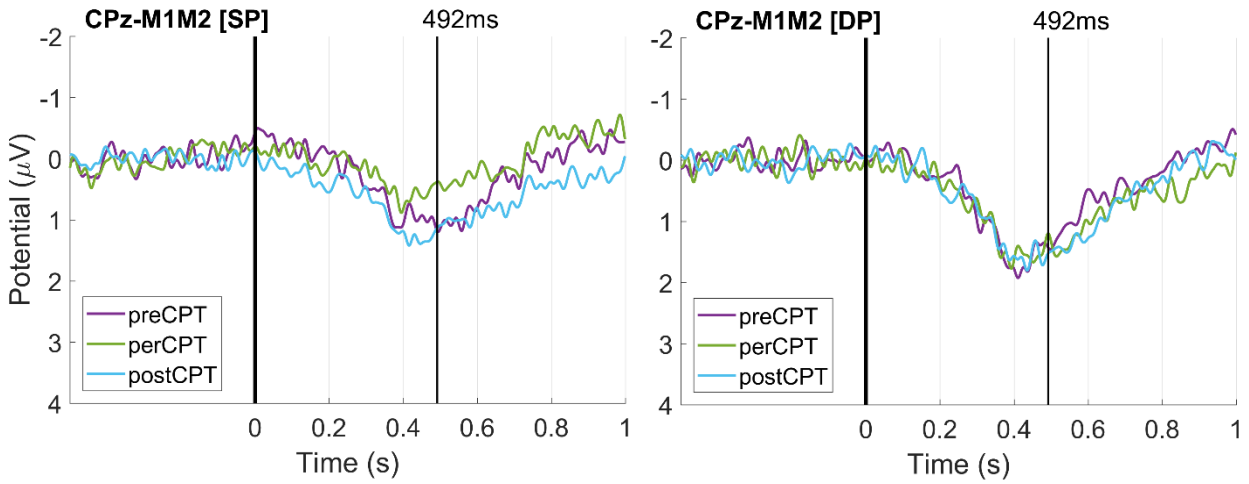

Figure 22: Grand average evoked potentials of central derivation (CPz-A1A2) of retest measurements following single pulse (SP) stimulation (left panel) and double pulse stimulation (DP) with inter-pulse interval of 10 ms (right panel). EEG data were split into pre-, per and postCPT datasets indicated by purple, green and blue plots. Statistical analyses were performed at P492.

4.3. TEST-FBSS ANALYSIS

4.3.1. GROUP CHARACTERISTICS

Both groups did not significantly differ in all average PPDT outcomes (Table 15). Average NRS score in the past week, NRS score at the onset of the experiment and NRS score at the end of the experiment were significantly higher for FBSS patient compared to the healthy controls. NRS score after CPT, average water temperature during CPT and outcomes of neurological evaluation did not significantly differ between both groups. Average water temperature of the hot container which was used prior to the CPT was significantly lower for FBSS patients compare to healthy controls. CSI score was significantly higher for FBSS patients.

Table 15: Measurement outcomes for healthy controls and failed back surgery syndrome patients. Disease-related characteristics are only shown for FBSS.

	HC test (n = 20)	**FBSS (n = 6)**	***P*-value**
PPDT left hand (N)	69.9 ± 16.4	69.8 ± 7.3	0.960
PPDT right hand (N)	67.9 ± 13.3	70.2 ± 7.3	0.714
PPDT left foot (N)	79.8 ± 23.7	72.8 ± 5.0	0.217
PPDT right foot (N)	74.0 ± 23.4	70.5 ± 5.6	0.472
Temperature hot water container (°C)	35.1 (0.7)	35.0 (0.8)	0.05*
Temperature during CPT (°C)	0.5 (0.8)	0.6 (0.4)	0.871
NRS score past week	0.0 ± 0.0	7.0 ± 1.9	<0.001**
NRS score current	0.0 ± 0.0	5.8 ± 2.4	0.002**
NRS score post CPT	6.0 (2.0)	5.0 (6.5)	0.534
NRS score post experiment	2.0 (1.75)	5.0 (5.0)	0.001*
CSI score	12.5 (14.5)	32.0 (14.5)	0.001*
NE soft-cloth (altered/unaltered)	0/20	0/20	1.00
NE pin-prick (altered/unaltered)	0/20	0/20	1.00
Duration of disease (years)	N.A.	4.2 ± 1.1	N.A.
Use of pain medication (yes/no)	N.A.	5/1	N.A.
Use of medication 24 hours before experiment (yes/no)	N.A.	2/4	N.A.
Recent pain treatment (<3 months) (yes/no)	N.A.	0/6	N.A.

*Normal distributed numeric variables are displayed as mean ± standard deviation. Non-normal distributed numeric variables are displayed as median (interquartile range). Significant differences tested using Mann-Whitney U tests are indicated with *. Significant differences tested using two-tailed independent samples t-tests are indicated with **. Outcomes of neurological evaluation are provided for one hand as outcomes were consistent for both hand sides. Significance levels: p = 0.05. Abbreviations: HC = healthy controls, FBSS = failed back surgery syndrome, PPDT = pressure pain detection threshold, N = Newton, CPT = cold pressor test, NRS = numeric rating scale, CSI = central sensitization inventory, NE = neurological evaluation.*

4.3.2. Effect of CPT on detection probabilities and NDTs

Average percentages of detection rate for single and double pulse stimulation are shown in Table 16.

Table 16: Average percentages of detection rate for single pulse (SP) and double pulse (DP) stimulation. Data are shown for test measurements of healthy controls and FBSS patients as average percentages per CPT phase and as general average percentages.

	preCPT		*perCPT*		*postCPT*		*Total*	
Study group	**SP**	**DP**	**SP**	**DP**	**SP**	**DP**	**SP**	**DP**
HC test	36.1%	48.5%	32.5%	45.9%	35.7%	47.2%	35.1%	47.3%
FBSS	38.3%	44.5%	47.2%	49.0%	36.9%	43.8%	39.9%	45.3%

Estimated NDTs for test results of healthy controls and FBSS results are shown in Figure 23. Note that GLMMs used to generate these NDTs differ from the GLMMs used to perform statistical analysis (see Table 17 and Table 18) and are therefore for illustrative purposes only.

Results of test measurements of healthy subjects are elaborated in *4.2.4.1. Effect of CPT on detection probabilities and NDTs – test results*. Table 17 and Table 18 show results of FBSS patients. In contrast to test results of healthy subjects, no significant difference in detection probability was found with respect to condition and interaction between condition and stimulus properties in FBSS patients. Also, the detection probability was not significantly altered with respect to the interaction between condition and trial number in FBSS patients.

Similar to test results of healthy subjects, average group slopes of FBSS patients following both single pulse and double pulse stimulation were significant between preCPT and postCPT and not significant between perCPT and postCPT. In contrast to test results of healthy subjects, average group slopes of FBSS patients demonstrated no significant difference between preCPT and perCPT for both stimulus properties.

Average group NDTs were not significant between preCPT and perCPT following both single pulse and double pulse stimulation which is similar to results of test measurements of healthy subjects. Also, similar to test results of healthy subjects, average group NDTs were significant between perCPT and postCPT in FBSS patients. Interestingly, average group NDTs obtained between preCPT and postCPT following single pulse stimulation were not significant, while these NDTs were significant following double pulse stimulation in FBSS patients compared to test results of healthy subjects. More strikingly, results demonstrated that average group NDTs of healthy subjects decreased after CPT compared to preCPT and perCPT, while average group NDTs of FBSS patients increased after CPT for both stimulus properties.

Table 17: Regression parameter estimates of the fixed effects of the generalized linear mixed model fitted to results of FBSS patients. PreCPT results were used as reference level.

Parameter	**Parameter estimate**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-7.48	38.70 (1)	<0.001*
Condition		7.63 (2)	0.022
perCPT	3.69		
postCPT	2.82		
Condition x amplitude SP		4.63 (2)	0.099
perCPT	-3.92		
postCPT	-7.28		
Condition x amplitude DP		5.40 (2)	0.067
perCPT	-5.83		
postCPT	-10.70		
Condition x trial number		1.36 (2)	0.507
perCPT	0.78		
postCPT	2.19		

*Significant differences tested using Type III Wald chi-square tests are indicated with *. Significance level: p = 0.05. Abbreviation: df = degrees of freedom.*

Table 18: Average nociceptive detection thresholds (NDTs) and slopes of psychophysical functions. Averages were derived from a generalized linear mixed model fitted to results of FBSS patients. Values between brackets represent 95% confidence intervals.

	Single pulse		*Double pulse*	
Condition	**Mean NDT (mA)**	**Mean slope (mA^{-1})**	**Mean NDT (mA)**	**Mean slope (mA^{-1})**
preCPT	0.90 [0.61 1.19]	10.32 [4.665 15.99]	0.40 [0.25 0.55]	23.22 [8.21 38.23]
perCPT	0.75 [0.49 1.01]	6.40 [1.43 11.37]	0.36 [0.15 0.56]	13.46 [3.55 23.37]
P-value	0.416	0.211	0.693	0.180
preCPT	0.90 [0.61 1.19]	10.32 [4.665 15.99]	0.40 [0.25 0.55]	23.22 [8.21 38.23]
postCPT	1.41 [1.07 1.74]	3.04 [1.57 4.51]	0.82 [0.53 1.10]	5.23 [3.17 7.29]
P-value	0.073	0.032*	0.027*	0.031*
perCPT	0.75 [0.49 1.01]	6.40 [1.43 11.37]	0.36 [0.15 0.56]	13.46 [3.55 23.37]
postCPT	1.41 [1.07 1.74]	3.04 [1.57 4.51]	0.82 [0.53 1.10]	5.23 [3.17 7.29]
P-value	0.001*	0.246	0.011*	0.097

*Significant differences tested using two-tailed independent samples Z-test are indicated with *. Significance levels: p = 0.05. Abbreviations: NDT = nociceptive detection threshold, mA = miliampere.*

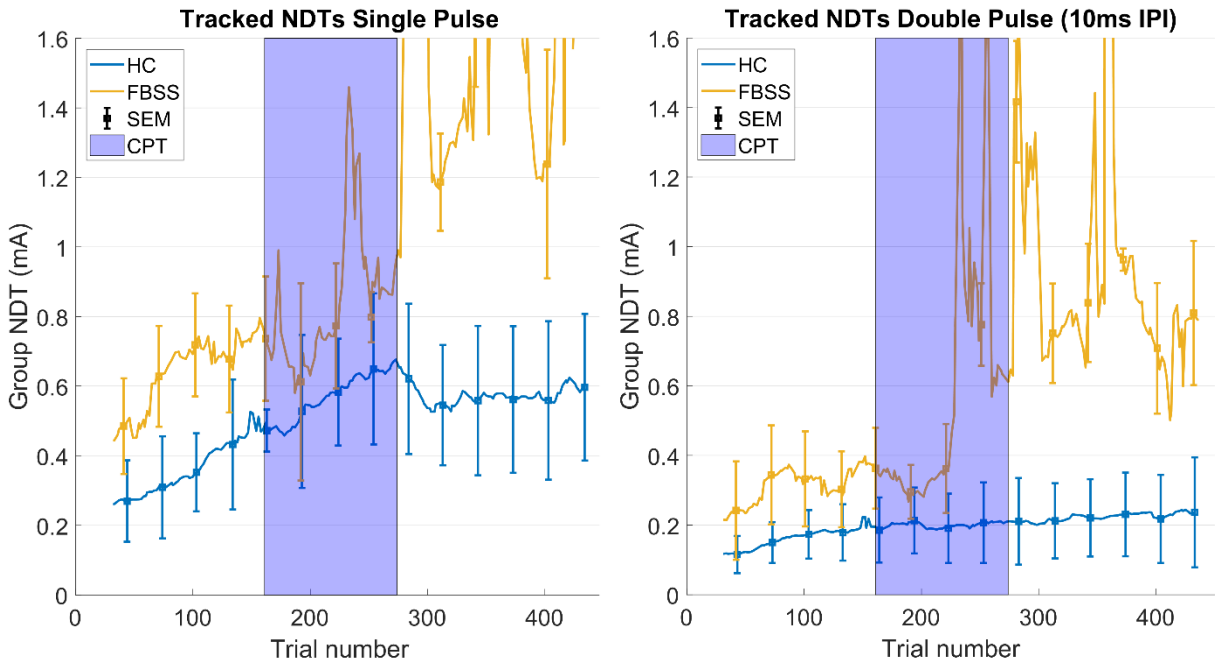

Figure 23: Estimated nociceptive detection thresholds (NDTs) for healthy controls (first measurement) and failed back surgery syndrome patients (FBSS) tracked over trial number. Left panels shows average NDTs for single pulse intra-epidermal stimulation. Right panel shows average NDTs for double pulse (IPI = 10 ms) intra-epidermal stimulation. Application of CPT for seven minutes is indicated by the light blue area.

4.3.3. Effect of CPT on evoked potentials

EP latencies for FBSS results were determined to be 211 ms and 490 ms for contralateral and central derivations (see Appendix F, Figure 29). EP latency for contralateral derivation analysis was replaced by 218 ms due to software-related issues. FBSS results are shown in Table 19 and Figure 24. Note that only statistics and grand average EPs of central derivation are shown. For full tables and results of contralateral derivations refer to Appendix G (*4.3 FBSS measurements*). Results of test measurements of healthy subjects are elaborated in 4.2.5.1. Effect of CPT on evoked potentials – test results.

Results show that both condition 1 (perCPT) and condition 2 (postCPT) and their interaction with both stimulus properties did not significantly differ from condition 0 (preCPT). This implicates that CPT did not modulate EP amplitudes significantly, compared to EP amplitudes prior to application of CPT following both single pulse and double pulse stimulation.

*Table 19 Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to FBSS measurements. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P490. PreCPT FBSS results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	Parameter estimate	95% confidence interval	Effect χ^2 (df)	*P*-value
Condition 1	-0.92	[-2.87 1.04]	-0.92 (2242)	0.357
Condition 2	-0.51	[-2.99 1.97]	-0.41 (80)	0.684
Condition 1 x amplitude SP	0.03	[-1.85 1.91]	0.03 (461)	0.975
Condition 2 x amplitude SP	-0.21	[-2.58 2.16]	-0.18 (34)	0.857
Condition 1 x amplitude DP	0.76	[-1.43 2.96]	0.68 (1327)	0.495
Condition 2 x amplitude DP	2.07	[-3.24 7.38]	1.16 (3)	0.321

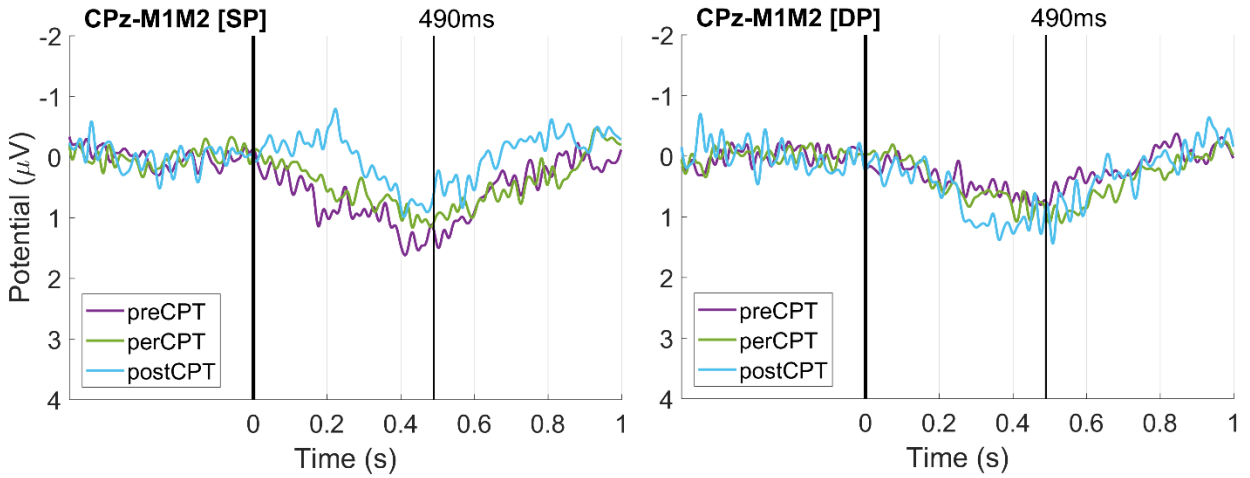

Figure 24: Grand average evoked potentials of central derivation (CPz-A1A2) of test measurements following single pulse (SP) stimulation (left panel) and double pulse stimulation (DP) with inter-pulse interval of 10 ms (right panel). EEG data were split into pre-, per and postCPT datasets indicated by purple, green and blue plots. Statistical analyses were performed at P490.

4.4. AGE ANALYSIS

For age analysis, test results of healthy subjects were divided into three age groups, i.e. lower age (LA), middle age (MA) and higher age (HA). Subjects aged between 18 and 35 years were assigned to the lower age group, subjects aged between 35 and 55 years were assigned to the middle age group and subjects aged between 56 and 75 years were assigned to the higher age group.

An overview of demographic characteristics for healthy subjects grouped by age is shown in Table 20. As healthy subjects were divided into age group, age between lower age and middle age group and between lower age and higher age group were significant. Gender was not significant between lower age and middle age group and between lower age and higher age group. However, handedness was significant between lower age and middle age group and between lower age and higher age group. BMI and average water temperature of the hot container which was used prior to the CPT were significantly higher in middle age and higher age groups compared to lower age group.

Table 20: Demographic characteristics for healthy subjects grouped by age. Lower age group was used as reference level.

	Lower age (n = 6)	**Middle age (n = 7)**		**Higher age (n = 7)**	
Characteristic			***P*-value**		***P*-value**
Age (years)	24.0 (5.0)	40.0 (6.0)	0.003*	70.0 (3.0)	0.002*
Gender (M/F)	3/3	3/4	1.00	3/4	1.00
BMI (kg/m^2)	22.1 ± 1.7	25.9 ± 1.5	0.001**	26.0 ± 3.2	0.026**
Handedness (left/right)	5/1	0/7	0.005***	0/7	0.005***

*Normal distributed numeric variables are displayed as mean ± standard deviation. Non-normal distributed numeric variables are displayed as median (interquartile range). Significant differences tested using Mann-Whitney U tests are indicated with *. Significant differences tested using two-tailed independent samples t-tests are indicated with **. Significant differences tested using Fisher's Exact Test are indicated with ***. Significance levels: p = 0.05. Abbreviations: F= female, M = male, BMI = body mass index.*

4.4.1. GROUP CHARACTERISTICS

Results of measurement outcomes are shown in Table 21. The lower age group was used as the reference level of analysis. NRS score in the past week, NRS score at the onset of the experiment, post CPT and post experiment NRS scores were not significant between lower age and middle age group and between lower age and higher age group. Also, CPT temperatures, pQST measurements, and CSI and NE outcomes demonstrated no significant differences between the groups. Average water temperature of the hot container, which was used prior to the CPT, was significantly higher in middle age and higher age groups compared to lower age group.

Table 21: Measurement outcomes for test results of healthy subjects grouped by age. Age group include lower age (18-35 years), middle age (36-55 years) and higher age (56-75 years). Lower age group was used as reference level.

	Lower age (n = 6)	**Middle age (n = 7)**		**Higher age (n = 7)**	
			***P*-value**		***P*-value**
PPDT left hand (N)	63.9 ± 8.2	76.3 ± 15.1	0.103	69.3 ± 21.0	0.550
PPDT right hand (N)	64.4 ± 7.3	73.3 ± 14.1	0.193	66.1 ± 15.4	0.818
PPDT left foot (N)	67.1 ± 19.5	92.5 ± 25.1	0.069	78.0 ± 19.7	0.340
PPDT right foot (N)	62.5 ± 21.4	83.1 ± 27.2	0.163	76.4 ± 17.4	0.223
Temperature hot water container (°C)	34.5 (0.7)	35.2 (1.2)	0.003*	35.5 (1.1)	<0.001*
Temperature during CPT (°C)	0.4 (0.7)	0.3 (0.8)	0.089	0.4 (0.9)	0.256
NRS score past week	0.0 ± 0.0	0.0 ± 0.0	1.00	0.0 ± 0.0	1.00
NRS score current	0.0 ± 0.0	0.0 ± 0.0	1.00	0.0 ± 0.0	1.00
NRS score post CPT	6.2 ± 1.2	6.4 ± 1.5	0.737	6.0 ± 1.2	0.801
NRS score post experiment	1.8 ± 0.8	1.9 ± 0.9	0.960	2.1 ± 0.7	0.456
CSI score	11.8 ± 6.7	12.7 ± 7.2	0.824	14.7 ± 9.8	0.556
NE soft-cloth (altered/unaltered)	0/6	0/7	1.00	0/7	1.00
NE pin-prick (altered/unaltered)	0/6	0/7	1.00	0/7	1.00

*Normal distributed numeric variables are displayed as mean ± standard deviation. Non-normal distributed numeric variables are displayed as median (interquartile range). Significant differences tested using Mann-Whitney U tests are indicated with *. Significance levels: p = 0.05. Outcomes of neurological evaluation are provided for one hand as outcomes were consistent for both hand sides. Abbreviations: PPDT = pressure pain detection threshold, CPT = cold pressor test, NRS = numeric rating scale, CSI = central sensitization inventory, NE = neurological evaluation.*

4.4.2. Effect of CPT on detection probabilities and NDTS

Average percentages of detection rate for single and double pulse stimulation are shown in Table 22.

Table 22: Average percentages of detection rate for single pulse (SP) and double pulse (DP) stimulation grouped per age group. Data are shown as average percentages per CPT phase and as general average percentages.

	preCPT		*perCPT*		*postCPT*		*Total*	
Age group	**SP**	**DP**	**SP**	**DP**	**SP**	**DP**	**SP**	**DP**
18-35 years	47.5%	53.7%	49.7%	51.8%	49.9%	49.6%	49.0%	51.6%
36-55 years	30.8%	45.3%	32.0%	46.7%	37.4%	49.6%	33.8%	47.4%
56-75 years	31.7%	40.0%	18.1%	40.1%	21.9%	42.9%	24.4%	43.7%

Estimated NDTs grouped by age for test results of healthy controls are shown in Figure 25. Note that these NDTs are for illustrative purposes only, as the actual statistical analysis was performed in R using a different GLMM as those used to generate NDTs plots. However, statistical analysis of NDTs across age groups could not be performed due to a computational error.

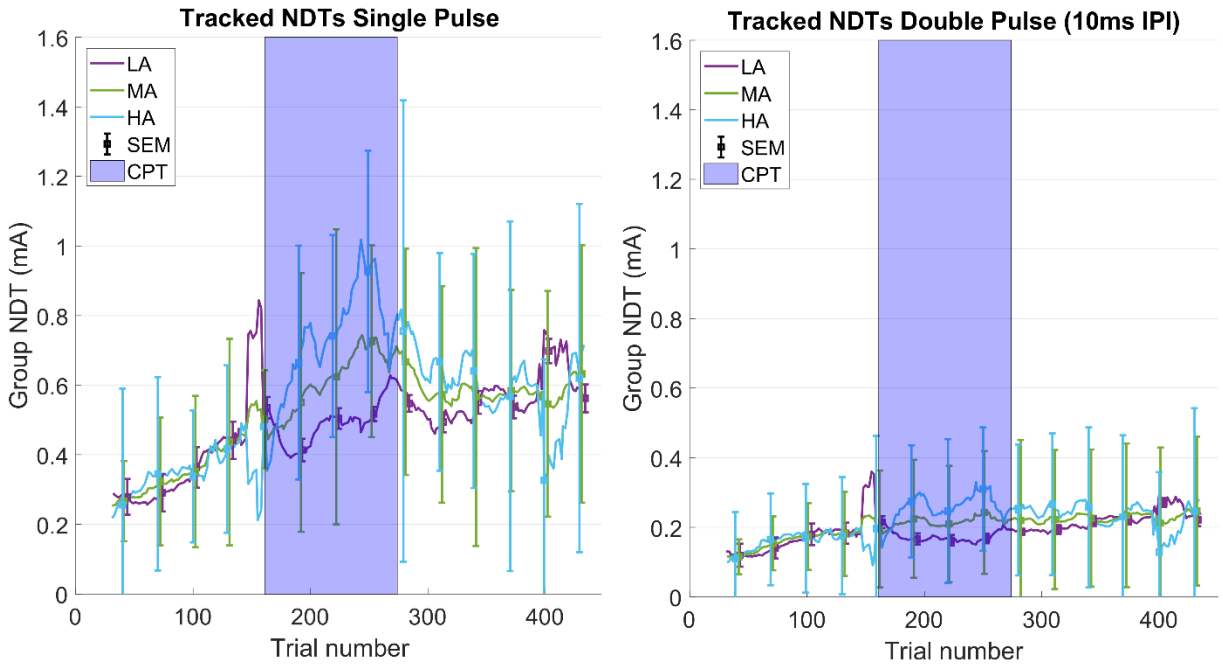

Figure 25: Estimated nociceptive detection thresholds (NDTs) for healthy controls (first measurement) tracked over trial number. Healthy subjects were over three age groups. 18-35 years (lower age = LA), 36-55 years (middle age = MA) and 56-75 years (higher age = HA). Left panels shows average NDTs for single pulse intra-epidermal stimulation. Right panel shows average NDTs for double pulse (IPI = 10 ms) intra-epidermal stimulation. Application of CPT for seven minutes is indicated by the light blue area.

4.4.3. Effect of CPT on evoked potentials

EP latencies based on butterfly plots of test results were used for statistical analysis of age groups (see Appendix F, Figure 28 (left panel)). However, due to software-related issues restraining statistical analysis, EP latency for central derivation analysis was replaced by 428 ms for the lower age group. Also, EP latency used for contralateral derivation analysis was replaced by 193 ms for lower and middle age groups. Age results are shown in Table 23 and Figure 26. Note that only statistics regarding (interaction with) CPT of central derivation are shown. Grand average EPs are shown for significant parameters only. For full tables and results of contralateral derivations refer to Appendix G (*4.3 FBSS measurements*).

Results show that across age groups, interaction between condition 1 (perCPT) and single pulse amplitude significantly differ from condition 0 (preCPT). This implicates that CPT modulates EP amplitudes significantly, compared to EP amplitudes prior to application of CPT following single pulse stimulation only.

*Table 23: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) and corresponding P-value. The models were fitted to test results of healthy subjects for each age group. Lower age group was evaluated at P428 and middle and higher age groups were evaluated at P426. PreCPT test results were used as reference level (condition 0). Significant differences are indicated with *. Significance level: p = 0.05.*

	Lower age		*Middle age*		*Higher age*	
Parameter	**Parameter estimate**	***P*-value**	**Parameter estimate**	***P*-value**	**Parameter estimate**	***P*-value**
Condition 1	-0.26	0.941	-1.78	0.479	1.86	0.385
Condition 2	-0.60	0.876	-0.34	0.918	-0.27	0.914
Condition 1 x amplitude SP	-5.78	0.538	-5.98	0.005*	-1.67	0.322
Condition 2 x amplitude SP	8.40	0.350	-2.40	0.359	-3.01	0.103
Condition 1 x amplitude DP	-16.77	0.192	-3.67	0.251	1.92	0.326
Condition 2 x amplitude DP	13.71	0.247	5.92	0.322	1.92	0.282

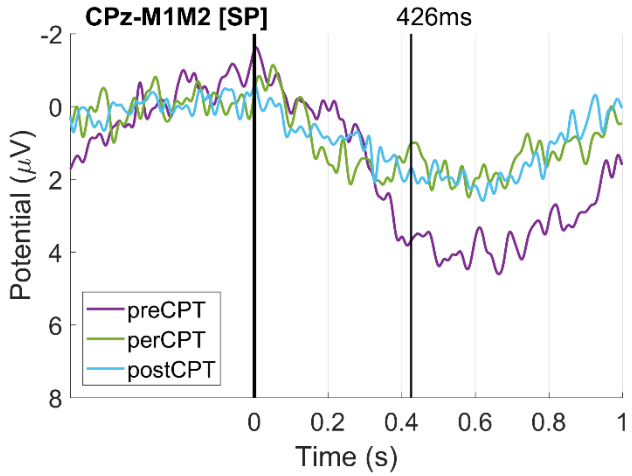

Figure 26: Grand average evoked potentials of central derivation (CPz-A1A2) of middle aged healthy subjects following single pulse (SP) stimulation. EEG data were split into pre-, per and postCPT datasets indicated by purple, green and blue plots. A significant difference between EP amplitudes for pre- and perCPT following single pulse stimulation was found in middle age group. Statistical analyses were performed at P426.

5. DISCUSSION

This thesis addressed the question whether the NDT-EP method combined with the cold pressor test (CPT) is feasible in a clinical environment. Also, the effect of application of a painful conditioning stimulus on a test stimulus was observed. An explorative, prospective study was conducted which consists of three parts.

The first part revealed that double pulse stimulation statistically affected detection probabilities in retest results compared to test results, resulting in higher NDTs for retest results, whereas single pulse stimulation did not affect detection probabilities and NDTs. Furthermore, amplitudes of EP were significantly modulated for retest results during and after application of CPT with respect to double pulse stimulation, whereas single pulse stimulation did not demonstrate any significant differences between test and retest results of each CPT phase. Also, detection probabilities and average group NDTs were similarly modulated across the different CPT conditions within each experiment, following single pulse and double pulse stimulation for both test and retest measurements. Test results showed that amplitudes of EPs were significantly modulated during CPT and after CPT compared to preCPT following single pulse stimulation. However, significant modulation of amplitude of EPs was not found for double pulse stimulation. In retest results none of the amplitudes of EPs were significantly modulated by CPT.

Results of the second part showed detection probability was not statistically affected by CPT in FBSS patients compared to healthy subjects. A significant decrease of average group NDTs was observed in test results of healthy subjects after CPT, while a significant increase was observed in FBSS patients. Amplitudes of EPs were significantly modulated with respect to single pulse stimulation in healthy subjects during and after CPT compared to prior to CPT. In FBSS patients, none of the amplitudes of EPs was statistically affected with respect to stimulus properties.

In the last part, only amplitudes of EPs were statistically affected during CPT with respect to single pulse stimulation in middle aged subjects compared to other condition, stimulus properties and age groups.

5.1. GENERAL FEASIBILITY

The main objective of this study was to explore the general feasibility of the NDT-EP method combined with CPT in a clinical environment in healthy subjects and, also, a first insight was provided into FBSS patients. In general, it can be concluded that both healthy controls and FBSS patients were able to tolerate CPT for the maximum time of 7 minutes. Based on average NRS scores, it can also be concluded that CPT was not considered as extremely painful. This can be substantiated by some subjects' experiences, reported after the experiment, which indicated that pain intensity caused by CPT felt uncomfortable rather than painful. These experiences can also be supported by other studies (Edens & Gil, 1995). Next to this, a substantial number of subjects noted that the pain intensity of CPT decreased over time. This could be caused by the formation of a hot water film around the immersed foot. However, other studies suggest that this phenomenon could be the result of adaption, which is thought to influence CPM effects, regardless any possible methodological limitations (Lautenbacher *et al.*, 2007; Wolf & Hardy, 1941).

In previous studies conducted by Berfelo and Gefferie (Berfelo, 2019; Gefferie, 2020), it was observed that some subjects had difficulties staying awake and focusing on stimulus detection during the experiment. This phenomenon was mostly observed in older subjects. In the present study, the experiment was

divided into three different phases, due to the application of CPT. Therefore, the experiment was not considered as time consuming by subjects, before the onset of the experiment, allowing subjects to maintain their focus during the experiment. In addition, due to the presence of pain induced by CPT, less subjects had difficulties with staying awake.

Next to this, as suggested by the results, the presence of ice cubes on top of the water surface enabled maintaining the temperature of the cold water container at 1°C and therefore fostered repeatability of the CPT. Also, during CPT a blood pressure cuff was used to limit blood flow warming the foot. It was observed that the used cuff was unable to maintain constant pressure. This could have affected the limitation of blood flow reduction. Therefore, in further research it is recommended to replace the used blood pressure cuff with a device which induces constant venous stasis such as an automatic blood pressure cuff.

Formation of a film of hot water surrounding the foot should be prevented. During foot immersion, the water was slowly recirculated manually by the observer every 3 minutes (t = 3.00 min. and t = 6.00 min.). Based on subjects' experiences, it was noticed that recirculation reinduced a temporary painful stimulus. This could indicate the added value of utilizing a circulation container for application of CPT. However, it should be noted that the used study protocol with non-circulating water container, already induced a strong painful sensation required to activate pain inhibitory pathways. By implementing circulating containers, a relatively stronger conditioning stimulus could be provided. However, it is expected that providing a stronger conditioning stimulus, could negatively affect the immersion time resulting in possible early withdrawal of subjects. As a result, less SPRs could be collected for threshold tracking, resulting in possible erroneous data analysis. Therefore, as the proposed protocol in this study enables simultaneous tracking of nociceptive detection threshold during application of a conditioning stimulus, utilizing non-circulation water containers is assumed to be sufficient as it provides a painful stimulus sensation which allows possible activation of descending pain inhibition pathways.

General detection rates for SP and DP indicate that during each phase of the experiment, sufficient SRPs were obtained to enable proper data analysis. However, in real-time estimated thresholds of older subjects during CPT, it was observed that maximum range of tracked NDT values (1.5mA) following single pulse stimulation was reached occasionally, which resulted in less detected stimuli. Therefore, pivotal information regarding the effect of CPT in older subjects might be missing for single pulse stimulation as the true detection thresholds were outside the range the predefined properties. This could be resolved increasing the maximum stimulus intensity, however this comes at the cost of a reduced stimulus intensity resolution which results in less accurate detection threshold estimation.

During CPT, different behavior of NDTs was observed among subjects. Compared to baseline measurements, multiple subjects showed an increase in NDT, whereas others showed a decrease in NDT. This might be the result of distraction caused by the conditioning stimulus resulting in an increased NDT or by increased attention, which could have resulted in increased focus towards the test stimulus, resulting in a lowered NDT. Similar behavior was observed and confirmed by other studies (Sandrini *et al.*, 2005). Next to this, a decreased NDT during CPT raises also the question of whether the true detection threshold was tracking before application of CPT. Further research in the contribution of attentional factors during the experiment is warranted. Individual training of subjects in familiarizing with stress and pain induced by CPT, might exclude the effect of distraction from the test stimulus and increased attention

towards the conditioning stimulus. Also, insights of the effect of distraction on stimulus detection can be provided by application of control water, in analogy with other studies (Veldhuijzen *et al.*, 2006).

5.2. INTERPRETATION OF THE RESULTS

5.2.1. DETECTION PROBABILITIES, NDTS AND EPS IN TEST-RETEST MEASUREMENTS

In the present study, findings of test-retest analyses showed that detection probabilities, average group NDTs and EP amplitudes were mainly altered in retest measurements following double pulse stimulation compared to test measurements. It should be noted that a significant difference was found between slopes of psychophysical curves in test and retest measurements for double pulse stimulation. In retest measurements this slope was found to be significantly lower and therefore, estimation of average group NDT might be less accurate for double pulse stimulation in retest measurements compared to the estimation in test measurements. Additionally, slopes of psychophysical curves between both test and retest measurements for single pulse stimulation were not significant, however, general slopes still remain shallow for single pulse stimulation, resulting in poor estimation of average NDTs.

Nevertheless, this means that single pulse stimulation showed a more stable behavior between both measurements. Deviated results between single pulse and double pulse stimulation might be due to the observed abrupt increase of stimulus amplitudes of single pulse stimulation evoked by CPT. This phenomenon was mainly observed in older subjects, which might have altered results substantially. Multiple older subjects showed decreased sensitivity to single pulse stimulation during and after CPT, which is supported by relatively low detection rates for single pulse stimulation during and after application of CPT. This resulted in receiving maximum amplitudes of single pulse stimulation (1.5mA) hampering proper estimation of NDT, which is supported by the relatively shallow slopes of psychophysical curves for test and retest measurements following single pulse stimulation. These results might have affected average group NDTs for both test and retest measurements, resulting in no significant difference between test and rest measurements for single pulse stimulation. By increasing the range of stimulus amplitude, allowing threshold estimation without saturation for single pulse stimulation, might exclude the possibility of this factor contributing to this discrepancy.

Initial thresholds of first measurements of healthy subjects are in line with results of a similar study conducted by Berfelo and Doll *et al.* (Berfelo, 2019; Doll *et al.*, 2016b). However, initial thresholds of test and retest measurements in healthy subject were found to differ, as the latter is substantially higher compared to the first measurement. Several factors might have contributed to this deviation. Time between test and retest measurements was set to be at least 7 days. As it is known that the CPT paradigm might have an inhibitory effect on pain, and therefore might result in increased NDTs, measurements might be influenced by an ongoing effect of CPT at the onset of the retest measurements.

Also, higher initial thresholds in retest measurements relative to test measurements could be due to psychological factors such as expectancies which might provoke stress induced hyperalgesia (SIH) (Jennings *et al.*, 2014). Moreover, test results of healthy subjects would have shown lowered initial thresholds as an effect of SIH compared to initial thresholds found in the study of Berfelo (Berfelo, 2019). Therefore, increased initial thresholds in retest measurements might be the result of multiple factors, in which psychological factors, including stress, expectancies and motivational aspects, possibly play a

pivotal role and thus, research regarding the influence of these factors on detection thresholds is clearly warranted.

5.2.2. Effect of CPT on detection probabilities, NDTs and EPs in test and retest measurements

In this study a significant effect of CPT on detection probabilities was observed in both test and retest results of healthy subjects. These results are consistent with statistical results of Doll *et al.* (Doll, 2012). However, estimated NDTs generated for illustrative purposes showed different behavior. Right after immersion into the water, an increase of threshold for single pulse stimulation was observed by Doll *et al*. (Doll *et al.*, 2016b). In later studies, similar behavior was observed for results of double pulse stimulation. In this study, for both test and retest measurements, this behavior of thresholds for both stimulus properties is less observed.

These differences in illustrative data might be the result of age differences between both studies. Doll *et al.* included 31 pain-free subjects with an average age of 24.4 years compared to 45.6 years in the present study. As reduced efficacy of endogenous analgesic mechanisms, resulting in less pain inhibition or even pain facilitation has been observed in elderly, inhibitory effects illustrated in estimated NDTs might be obscured (Edwards *et al.*, 2003).

Also, diverse models were used to visualize nociceptive detection thresholds, possibly resulting in deviated course of NDTs (Knafl *et al.*, 2012). Linear mixed models used in this study comprised of both fixed and random effects. All fixed effects were included as random effects and were grouped by subject number, while fixed and random effects of the generated models by Doll *et al.* were not consistent. Next to this, the time of immersion differed between studies. Doll *et al.* applied CPT for around 2-3 minutes, while in the present study an immersion time of 7 minutes was used, which resulted in a higher number of SRPs for threshold estimation. As a result, in the present study, thresholds might be estimated with higher precision, which should be taken into account when evaluating threshold estimation during CPT between the studies.

Furthermore, in this study, CPT was applied to the right foot rather than the right hand. Based on the cortical sensory homunculus, it can be assumed that the hand is more sensitive to CPT compared to the foot. Therefore, a stronger CPT effect on nociceptive thresholds might be expected when CPT is applied to the hands rather than the feet. However, research recommends usage of one upper and one lower limb for application of both stimuli to ensure descending pain inhibition rather than segmental spinal inhibition (Granovsky *et al.*, 2013). Moreover, divergent CPT effects were not observed between the studies based on outcomes of linear regression models.

Also, results of Doll *et al.* showed a prolonged elevation of thresholds for both stimulus properties, which suggests a sustained CPT effect for several minutes even after termination of CPT. However, this prolonged elevation might be the result of methodological differences, as the used tracking window for threshold estimation differed from the present study. This might have affected threshold estimation precision negatively for longer tracking window resulting in less clear CPT effect on NDTs in illustrative data.

Therefore, it is expected that due to methodological differences, divergent CPT effects on NDTs were observed between studies in illustrative data. However, results of statistical analysis were consistent and as tracking window length was not involved in statistical analysis, tracking window length does not affect these results.

Also, average group NDTs were altered during CPT compared to before CPT for both stimulus properties. However, this result was not found to be significant. Research suggests that delayed activation of pain inhibition pathways might be observed after application of CPT (Willer *et al.*, 1990). As a result, early stage tracked NDTs during CPT might be prone to attentional disturbances and, therefore, might not reflect true NDT affected by pain inhibition induced by CPT. It should be noted that average slopes of the psychophysical curve were significantly shallower during application of CPT compared to average preCPT slopes, possibly due to the relatively low SRPs compared to preCPT and application of CPT which impeded stimulus detection, and therefore average group NDTs during CPT were estimated with lower accuracy.

Average group NDTs of test and retest measurements obtained after termination of CPT were significantly lower compared to average NDTs obtained before and during application of CPT. These results indicate facilitation reflected by increased sensitivity to both stimulus properties after termination of CPT. It was expected that, due to an inhibitory effect evoked by CPT, NDTs of both stimulus properties would be elevated during and, shortly, after CPT, analogous to findings of Doll *et al. (Doll et al., 2016b)*. However, multiple subjects indicated experiencing increased stimulus perception immediately after CPT termination. It is hypothesized that CPT enhanced focus and alertness towards the test stimulus resulting in improved stimulus detection performance after CPT.

Findings of test-retest analyses showed that EP amplitudes were mainly modulated by CPT with respect to single pulse stimulation, while modulation of EP amplitudes due to CPT following double pulse stimulation is lacking. Research indicates that intra-epidermal electrical stimulation (IES) is preferred using current amplitudes below twice the detection threshold to selectively activate nociceptors without co-activation of other nerve fibers (Inui & Kakigi, 2012; Mouraux et al., 2010). Also, based on pain sensations and peak latencies, it is assumed that amplitudes generated by the dedicated stimulation device mainly activate Aδ-fibers and, hence, signals evoked by both single pulse and double pulse stimulation are assumed to be transmitted through identical nerve fibers. Pain modulatory effects arise from activation of descending pain modulation pathways projecting to the dorsal horn of the spinal cord regulating nociceptive signals to higher brain centers (Chichorro *et al.*, 2017; Goadsby *et al.*, 2017). It is thought that, if pain inhibitory pathways have been activated, detection probabilities, average group NDTs and EP amplitudes following both single and double pulse stimulation would have been affected by CPT. Therefore, other factors, such as distraction, may have contributed to different behavior between both stimulus properties during and after application of CPT (Sandrini *et al*., 2005; Yarnitsky, 2015).

Also, during the experiment, the test stimulus was administered to the dominant hand. CPT was applied to the right foot regardless of the side of the test stimulus. Various studies showed relatively large pain inhibition when conditioning stimulus was applied opposite to the side of the test stimulus (Klyne *et al.*, 2015; Svensson *et al.*, 2000; van Wijk & Veldhuijzen, 2010). However, no consensus can been found regarding the effect of CPT on the test stimulus in the presence of an ipsilaterally or contralaterally applied conditioning stimulus (Arendt-Nielsen & Gotliebsen, 1992; Lewis *et al.*, 2012; Pud *et al.*, 2005). Therefore, further research on the location of applied conditioning stimulus relative to the test stimulus and its influence on the magnitude of pain modulation is warranted.

5.2.3. Effect of CPT on detective probabilities, NDTs and EPs in FBSS patients

Statistics of detection probabilities and EPs of FBSS measurements showed no significance with respect to condition and interactions where condition was involved. These findings differ from results of test and retest measurements. Based on subjects' experiences regarding pain perception evoked by CPT, a less painful sensation of CPT was noticed in FBSS patients compared to healthy subjects. As a more intense conditioning stimulus is likely to yield larger hypoalgesic effects, this might clarify the absence of CPT effect on detection probabilities found in FBSS patients compared to results of healthy subjects (Svensson *et al.*, 2000). Difference in pain perception of CPT between both study groups is supported by the difference between NRS scores obtained prior to the experiment and after termination of CPT (NRS scores post CPT). In healthy subjects, a substantially increase of NRS scores was observed after termination of CPT compared to the baseline NRS scores. In FBSS patients, less deviated NRS scores were obtained after CPT compared to baseline NRS scores. Patients who have been undergoing chronic pain are used to painful situations, which could have made it easier to cope with pain allowing to shift their attention towards detection of test stimulus (Baliki & Apkarian, 2015). As a result, for chronic pain patients, application of CPT could have induced a less stressful and painful situation compared to healthy subjects resulting in unaltered detection probabilities and EP amplitudes. This emphasizes the subjectivity of pain perception, which might indicate the demand of an individualized temperature of the conditioning stimulus and, hence, a more intense conditioning stimulus in FBSS patients, to produce a CPT effect similar to those in healthy subjects.

Another explanation might be that patients suffering from chronic pain conditions appear to show reduced CPM efficacy, which reflects an impairment in pain mechanisms (Daenen *et al.*, 2013; Vaegter *et al.*, 2016). The finding of less efficient CPM could be the result of long-standing chronic pain resulting in exhausting their pain inhibition capacity (Yarnitsky *et al.*, 2010). Additionally, according to literature, expectancies and effects of pain inhibitory pathways are generally correlated (Bjorkedal & Flaten, 2012; Hermans *et al.*, 2016). It has been suggested that reduced pain inhibition can be due to induced cognitive and emotional factors and not necessarily to a dysfunctional pain inhibitory mechanism (Bjorkedal & Flaten, 2012). However, others observed that stress reactions were associated with decreased CPM magnitudes (Nir & Yarnitsky, 2015). However, this raises the assumption that CPM could be successfully activated by application of CPT and the NDT-EP method allows observation of the effects of inhibitory mechanisms. Next to this, the small number of included FBSS patients might be a contributory factor, hampering observation of significant contributions of conditions to detection probabilities. Therefore, it is recommended to enroll a larger number of FBSS patients before drawing any solid conclusions regarding the effect of CPT on detection probabilities in FBSS patients.

5.2.4. Effect of age on CPT

Illustrative data of NDTs between age groups showed increased NDTs during CPT with advancing age-group. This result was found in both single and double pulse stimulation. As mentioned earlier, increasing distraction from the test stimulus provoked by CPT was observed in older subjects. This is support by the observation of decline in inhibitory control, which limits the efficiency of selective attention (Madden & Langley, 2003). This possibly hindered the detection of stimuli at detection threshold, which could have resulted in elevated NDTs. Also, increased pain inhibition evoked by CPT with advancing age, resulting in

elevated NDTS, could be observed. However, as mainly contrary effects have been observed, increased pain inhibition with advancing age is unlikely (Edwards *et al.*, 2003; Naugle *et al.*, 2015; van Wijk & Veldhuijzen, 2010; Washington *et al.*, 2000). Additionally, it has been reported that the density of both unmyelinated and myelinated fibers has been found to decrease by the age of 60 years (Gibson & Farrell, 2004). As a consequence, older subjects are expected to require higher intensity stimuli in order to detection the applied stimulus at detection threshold. However, the number of subjects in each age group is relatively low, which leads to poor estimation of NDTs. Also, due to the relatively low number of subject in each age group, statistical analysis of NDTs could not be performed due to a computational error, presumably caused by overfitting (Hawkins, 2004). Therefore, it is recommended to enroll a larger number of healthy subjects before drawing any solid conclusions regarding the effect of distraction from the test stimulus.

Results of the effect of CPT on EP amplitudes showed that no significant effect of condition and fixed effect in which condition was involved was found in lower age and higher age group, while the interaction between CPT and single pulse stimulation was found to significantly modulate EP amplitude in middle age group. As described earlier, efficacy of pain inhibitory pathways seems to decrease with advancing age (Edwards *et al.*, 2003; Naugle *et al.*, 2015; van Wijk & Veldhuijzen, 2010; Washington *et al.*, 2000). As a result, it was expected that if CPT evoked a pain inhibitory effect through descending pain modulation pathways, it would have modulated EP amplitudes in lower age group relative to middle age and higher age groups. Next to this, older subjects seem to be more prone to distraction, which might modulate EP amplitudes as well (Blom *et al.*, 2012; Madden & Langley, 2003). However, analyses were performed at relatively small study groups and therefore, at this point, solid conclusions regarding the effect of age on pain modulation evoked by CPT should not be drawn yet. Again, enlarging all age groups would allow drawing more solid conclusions.

5.3. STRENGTH AND LIMITATIONS

A major strength of this study was the conduction of retest measurements of healthy subjects as a primary objective of this study was to explore the responsiveness of NDTs and EPs to CPT in healthy subjects to validate the NDT-EP method. Next to this, by extending the healthy, pain-free study group with FBSS patients, this study provided a first impression of the feasibility of the NDT-EP method combined with CPT in chronic low back pain patients.

An additional strength is that, prior to this study, multiple pilot studies had been conducted to improve the study design which resulted in a solid measurement protocol to investigate the effect of CPT on responsiveness of NDTs and EPs. By applying a blood pressure cuff around the lower leg, blood flow warming the foot was limited. Also, a polystyrene container with hot water of 35°C was used to acclimatize subjects and to ensure pain sensation was relatively consistent throughout all subjects. Next to this, for test-retest measurements of healthy subjects, time-of-day at which the experiment was conducted, was kept consistent across both measurements for each subject to minimize the effect of day rhythm on performance. An additional strength regarding the protocol comprised the use of one single observer for the entire experiment including pressure pain detection threshold measurements and neurological evaluations. In this way, observer bias was reduced and possible inter-observer variability was avoided.

Traditional CPM paradigms rely on one test stimulus applied as a stand-alone and one test stimulus during or immediately after applying the conditioning stimulus, known as parallel and sequential paradigms respectively. The effects of CPT on the test stimulus is frequently observed using methods which often rely on a single threshold estimate. In this study, conditioning stimulus and test stimulus were applied simultaneously, to observe the effect of conditioning stimulus over time and to ensure the effect of pain inhibition as it is maximal during application of the conditioning stimulus (Wolf & Hardy, 1941). However, it has been proposed that presentation of the test stimulus immediately after application of the conditioning stimulus provides a cleaner representation of pain modulation, which might be free of biases such as distraction (Yarnitsky *et al.*, 2015). Also, in this way, the event of possible delay of CPT effect could have been observed which is of added value as it is rather unknown how long it takes until the effect of CPT is maximal after the onset of immersion (Lewis *et al.*, 2012). Furthermore, postCPT measurements were performed after termination of CPT to observe the duration of CPT effect as it remains unknown and is known to be paradigm-dependent (Fujii *et al.*, 2006; Graven-Nielsen *et al.*, 1998; Graven-Nielsen & Mense, 2001; Tuveson *et al.*, 2006; Yarnitsky *et al.*, 2015). By proceeding the NDT-EP measurement as soon as possible after termination of CPT, observation of the exact duration of pain inhibition after termination of CPT was potentially enabled, which is not possible in traditional parallel and sequential CPM paradigms.

The generalizability of CPT effect in FBSS patients is limited by the small number of included patients. However, as part of the central aim and main objectives were to explore the general feasibility of the NDT-EP method combined with CPT in a clinical environment, there remains scope for improvement by including more chronic low back pain patients. Next to this, three out of six included FBSS patients were not considered as last-resort patients in which neuromodulation was considered. Supported by their relatively low CSI score compared to last-resort patients and shorter exposure to chronic pain, it is thought that these patients might be less centrally sensitized, possibly resulting in less affected descending pain modulatory mechanisms.

This study lacks the validation of successful CPM activation enabling pain inhibition through supraspinal inhibitory pathways. As exploration of the general feasibility of NDT-EP method combined with CPT in a clinical environment, was a major objective of this study, and to some extent the responsiveness of the NDT-EP method to perturbation, it was decided to disregard validated QST measures after application of CPT, due to the already comprehensive pain assessment battery. Therefore, a degree of caution is necessary before drawing any solid conclusions regarding activation of descending pain inhibitory pathways and the responsiveness of NDT-EP method to supraspinal pain inhibition.

5.4. RECOMMENDATIONS FOR FURTHER RESEARCH

Six chronic low back pain patients were enrolled in this study. Based on power calculations in previous studies, at least fifteen subjects should be included for data analysis in order to observe significant differences. Therefore, the first step subsequent investigations should be extending the number of FBSS patients. Next to this, the influence of medication intake on outcomes should be investigated as two patients used medication, i.e. tramadol, prior to the experiment which is known to have analgesic properties, possibly resulting in altered (effect of CPT on) detection probabilities and/or EP amplitudes (Chaparro *et al.*, 2013).

In the present study, the twenty independent components (ICs) responsible for the biggest variance in the signal were visually inspected for all epochs. Electro-oculogram (EOG) components generated by the eye and signal drifts, were removed from EEG data using ICA. Next to this, trials showing deviated variances compared to the general dataset were removed manually as well. As it is assumed that CPT generated a substantial perturbation resulting in increased variances during CPT compared to preCPT and postCPT epochs, it is thought that data cleaning mainly affected EEG data obtained during CPT. As a result, it is thought that artefacts affecting preCPT and postCPT epochs were minimally removed. In further research, it is recommended to subdivide each EEG dataset into three separate subsets enabling proper data cleaning for each CPT phase which could improve general data quality.

As results in the present study indicated, an altered effect of CPT on NDTs and EP amplitudes was observed across age groups. However, due to the small number of healthy subjects in each age group, proper statistical analysis without generation of computational errors could not be conducted. By enlarging the healthy subject group, exploration of the effect of age could be achieved and a more solid conclusion regarding a possible age-related CPT effect might be drawn. Research has shown that decreased sensitivity to the effects of a conditioning stimulus was observed with advancing age (van Wijk & Veldhuijzen, 2010). CPT was found to significantly increase thresholds, however, this effect was less so in older subjects. Also, decreased inhibition was found starting in middle-aged subjects (between 40 and 55 years) compared to lower aged subjects (Lariviere *et al.*, 2007). Therefore, it is recommended to focus on enlarging the lower age group to possibly observe pain modulation evoked by CPT.

Preliminary results of subanalyses of healthy subjects comprising gender analyses, revealed striking differences in EP amplitudes between men and women. These findings are supported by multiple studies in which gender differences have been reported in experimental pain studies (Popescu *et al.*, 2010; van Wijk & Veldhuijzen, 2010). Also, greater stability of CPM in women has been found in chronic pain patients (Martel *et al.*, 2013). However, other studies revealed no major gender differences in CPM (Razavi *et al.*, 2014). Based on preliminary findings, and as there is no consensus regarding the influence of gender on CPM efficacy, further research of the influence of gender on pain inhibition is clearly warranted.

6. CONCLUSION

This explorative study assessed the general feasibility of the NDT-EP method combined with CPT. Results demonstrate that both healthy subjects and FBSS patients seem to be able to properly execute the experiment and tolerate the intensity of the conditioning stimulus. Test-retest results in healthy subjects demonstrate good reliability of single pulse stimulation in detection probabilities, NDTs and EP amplitudes, whereas test-retest reliability of double pulse stimulation was relatively poor. A significant effect of CPT on detection probabilities and average group NDTs in both stimulus properties is found in healthy subjects, however, a moderate effect of CPT is observed in EP amplitudes. Also, detection probabilities and EP amplitudes in FBSS patients were not affected by CPT, whereas average group NDTs were moderately affected. Results of healthy subjects suggest efficient pain inhibition through descending pain inhibitory pathways evoked by CPT compared to FBSS patients, however the effects of psychological factors, such as expectancies and distraction, cannot be excluded. Additional research, including subject training and application of control CPT experiment, is warranted to investigate the influence of psychological factors on pain modulation.

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APPENDIX A

Deelnemers gezocht voor studie naar hersensignalen gerelateerd aan pijn

In het St. Antonius Ziekenhuis onderzoeken we een nieuwe manier om de verwerking van (pijn)prikkels te meten. We hopen dat deze metingen in de toekomst het stellen van diagnoses kan vergemakkelijken. Ook hopen we dat de metingen uiteindelijk kunnen bijdragen aan een beter begrip van het werkingsmechanisme van pijn, zodat nieuwe behandelingen kunnen worden ontwikkeld. We zoeken voor dit onderzoek deelnemers voor de volgende twee groepen:

Groep 1: Gezonde personen van 18 jaar of ouder.
Groep 2: Patiënten met de diagnose FBSS (met chronische pijn) van 18 jaar of ouder.

Wat houdt het onderzoek in?
Als deelnemer komt u voor twee afspraken van ca. 90 minuten per bezoek naar het St. Antonius Ziekenhuis (Nieuwegein).

Vervolgens krijgt u op uw hand milde elektrische prikkels toegediend. Deze zult u licht voelen. Daarnaast wordt voor gedurende een korte tijd gevraagd uw voet in een bak met ijswater onder te dompelen. Tijdens het onderzoek meten we tegelijk uw gevoelsdrempels en de signalen in uw hersenen. Na uw afspraak kunt u gewoon naar huis.

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

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

ST ANTONIUS
een santeon ziekenhuis

UNIVERSITY OF TWENTE.

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APPENDIX B

Vragenlijst voor screening op mogelijke Corona infectie

Voorafgaand aan het onderzoek verzoeken wij u onderstaande vragenlijst in te vullen. Hiermee hopen wij het risico op een mogelijke besmetting te minimaliseren.

Wanneer u de vragenlijst invult, denk dan hoe u zich op dit moment voelt en of het om nieuwe klachten gaat, die u sinds kort heeft. Dus vergelijk uw huidige klachten met uw normale klachten of aandoeningen (zoals bijvoorbeeld hooikoorts).

- Heeft u op dit moment Corona (een bewezen COVID-19 infectie)?

  ☐ Ja ☐ Nee

- Heeft u afgelopen week contact gehad met iemand die een bewezen Corona (COVID-19) infectie heeft?

  ☐ Ja ☐ Nee

- Heeft u koorts (is uw temperatuur boven 38.0ºC)? (bij paracetamolgebruik is koorts een temperatuur boven 38.5ºC)

  ☐ Ja ☐ Nee

- Heeft u last van luchtwegklachten zoals: hoesten, benauwdheid, keelpijn, neusverkoudheid?

  ☐ Ja ☐ Nee

- Als u de komende dagen alsnog een van deze klachten krijgt of u twijfelt hierover, neem dan contact op met de onderzoeker.

  ☐ Ik heb het begrepen ☐ Ik heb het niet begrepen

Hartelijk dank voor het invullen van de vragenlijst.

Naam proefpersoon:

Handtekening proefpersoon: Datum: __/__/__

Naam onderzoeker:

Handtekening onderzoeker: Datum: __/__/__

APPENDIX C

KENMERK
TV

VERSIE
V2.0

ABR NUMMER
NL71927.100.19

ONDERWERP
Toestemmingsverklaring

TITEL ONDERZOEK
CPT-Studie

Ik heb de informatiebrief, kenmerk "PIF-CPTF (chronische pijnpatiënten)" versie 'V2.0' gelezen. Ook kon ik vragen stellen. Mijn vragen zijn voldoende beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.

Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.

Ik geef toestemming voor het opvragen van informatie bij mijn specialist die mij behandelt over de vastgestelde datum van diagnose en huidig gebruik van medicatie.

Ik geef toestemming voor het verzamelen en gebruiken van mijn gegevens voor de doeleinden in dit onderzoek.

Ik weet dat voor de controle van het onderzoek sommige mensen toegang tot al mijn gegevens kunnen krijgen. Die mensen staan vermeld in de informatiebrief. Ik geef toestemming voor die inzage door deze personen.

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

Ik wil meedoen aan dit onderzoek.

Naam proefpersoon:
Handtekening: Datum: __ / __ / __

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

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

Naam onderzoeker:
Handtekening: Datum: __ / __ / __

APPENDIX D

Meting documentatie

Studieparameters

Studienummer: _____ | **Locatie:** St. Antonius Nieuwegein ICU

Deelnemernummer: _____ | **Datum:** __ __ / __ __ / __ __ __ __
d d m m j j j j

Neurologisch onderzoek	
Stomp (L/R)	/
Scherp (L/R)	/

Bloeddruk	
Systolisch	mmHg
Diastolisch*	mmHg

* Bloeddrukband plaatsen op 20 mmHg onder diastolische bloeddruk

T = 35 °C	
t =	T =
0	
1	
2	

T = 1 °C	
t =	T =
0	
1	
2	
3	
4	
5	
6	
7	
SRP pre-CPT	/230
SRP post-CPT	/230

NRS pre-CPT	
NRS post-CPT	
NRS post-exp	
pQST (PPDT)	N
CPT (L of R)	
Menstruatie (ja of nee)	

Aantekeningen

APPENDIX E

Centrale Sensitisatie Vragenlijst (CSI)

Mayer TG, Neblett R, Cohen H, Howard KJ, Choi YH, Williams MJ, Perez Y, Gatchel RJ: The development and psychometric validation of the central sensitization inventory. Pain Pract 2012;12:276-285.
Nederlandse vertaling: van Wilgen P, Meeus M, Descheemaeker F, Cagnie B. 2013.

CENTRALE SENSITISATIE VRAGENLIJST: DEEL A

Geef aan in welke mate u de volgende klachten heeft. Omcirkel één van de antwoorden.

1	Ik voel me niet uitgeslapen 's morgens als ik wakker word	Nooit Zelden Soms Vaak Altijd
2	Mijn spieren voelen stijf en pijnlijk	Nooit Zelden Soms Vaak Altijd
3	Ik heb angstaanvallen	Nooit Zelden Soms Vaak Altijd
4	Ik knars of klem met mijn tanden	Nooit Zelden Soms Vaak Altijd
5	Ik heb last van diarree en/of constipatie	Nooit Zelden Soms Vaak Altijd
6	Ik heb hulp nodig bij het uitvoeren van dagelijkse activiteiten	Nooit Zelden Soms Vaak Altijd
7	Ik ben gevoelig voor fel licht	Nooit Zelden Soms Vaak Altijd
8	Ik ben snel moe bij fysieke activiteiten	Nooit Zelden Soms Vaak Altijd
9	Ik heb pijn over mijn gehele lichaam	Nooit Zelden Soms Vaak Altijd
10	Ik heb last van hoofdpijn	Nooit Zelden Soms Vaak Altijd
11	Ik heb een ongemakkelijk gevoel in mijn blaas en/of een branderig gevoel bij het plassen	Nooit Zelden Soms Vaak Altijd
12	Ik slaap niet goed	Nooit Zelden Soms Vaak Altijd
13	Ik kan me moeilijk concentreren	Nooit Zelden Soms Vaak Altijd
14	Ik heb huidproblemen zoals droge huid, jeuk of huiduitslag	Nooit Zelden Soms Vaak Altijd
15	Stress verergert mijn lichamelijke klachten	Nooit Zelden Soms Vaak Altijd
16	Ik voel me neerslachtig of depressief	Nooit Zelden Soms Vaak Altijd
17	Ik heb weinig energie	Nooit Zelden Soms Vaak Altijd
18	Ik heb spierspanning in mijn nek en schouders	Nooit Zelden Soms Vaak Altijd
19	Ik heb pijn in mijn kaak	Nooit Zelden Soms Vaak Altijd
20	Bepaalde geuren, zoals parfums, maken me duizelig en misselijk	Nooit Zelden Soms Vaak Altijd
21	Ik moet vaak plassen	Nooit Zelden Soms Vaak Altijd

22	Mijn benen voelen ongemakkelijk en rusteloos wanneer ik 's avonds wil gaan slapen	Nooit Zelden Soms Vaak Altijd
23	Ik heb moeite om dingen te onthouden	Nooit Zelden Soms Vaak Altijd
24	Als kind heb ik traumatische gebeurtenissen meegemaakt	Nooit Zelden Soms Vaak Altijd
25	Ik heb pijn in mijn bekkenregio	Nooit Zelden Soms Vaak Altijd

CENTRALE SENSITISATIE VRAGENLIJST: DEEL B

Zijn er door een arts in het verleden bij u één van volgende aandoening gediagnosticeerd?
Vink het vakje rechts aan voor elke diagnose en schrijf het jaar van de diagnose indien van toepassing.

		nee	ja	Jaar diagnose
1	Restless legs syndrome (Rusteloze benen)			
2	Chronische vermoeidheidssyndroom			
3	Fibromyalgie			
4	Kaakklachten			
5	Migraine of spanningshoofdpijn			
6	Prikkelbare darm syndroom			
7	Overgevoeligheid voor chemische stoffen			
8	Nekletsel (inclusief whiplash)			
9	Angst- of paniekaanvallen			
10	Depressie			

APPENDIX F

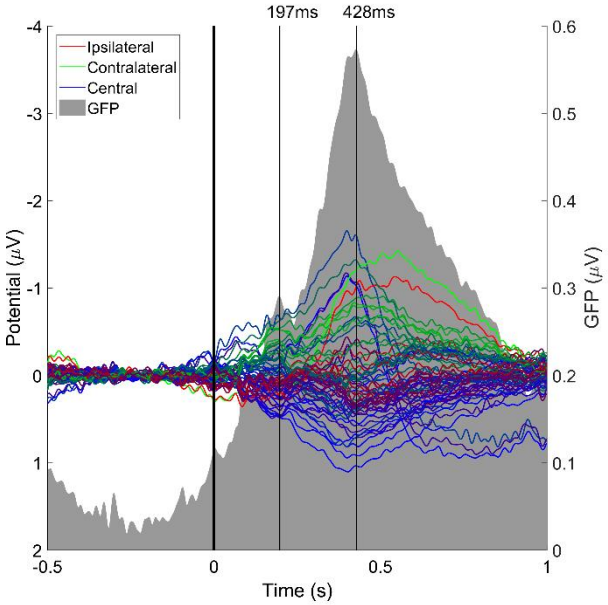

Figure 27: Butterfly plots of average EPs for test-retest analysis. The first solid vertical black line represents the time of intra-epidermal stimulation. Vertical black lines at 197 ms and 428 ms are based on local global field power (GFP) maxima and were used for statistical analysis of contralateral derivations (T7-F4 or T8-F3, depending on the side of electrical stimulation) and central derivation (CPz-A1A2), respectively.

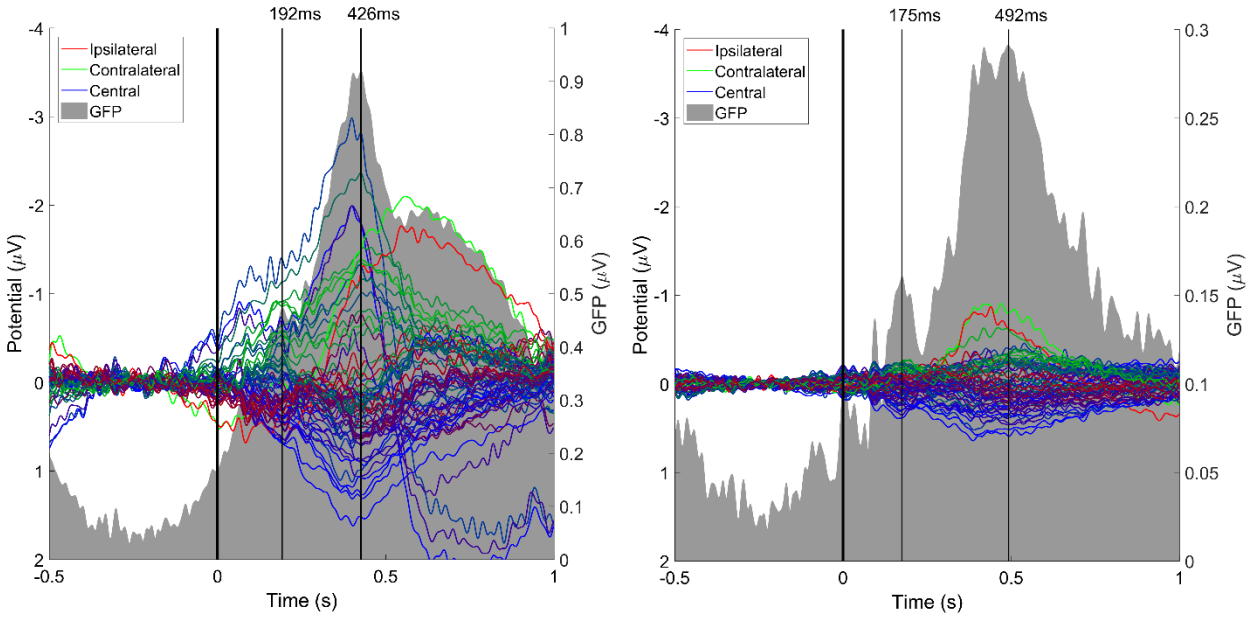

Figure 28: Butterfly plots of average EPs. Left panel shows butterfly plot of test measurements and right panel shows butterfly plot of retest measurements, both healthy subjects. The first solid vertical black line represents the time of intra-epidermal stimulation. Vertical black lines at 192 ms, 175 ms, 426 ms and 492 ms are based on local global field power (GFP) maxima and were used for statistical analysis of contralateral derivations (T7-F4 or T8-F3, depending on the side of electrical stimulation) and central derivation (CPz-A1A2).

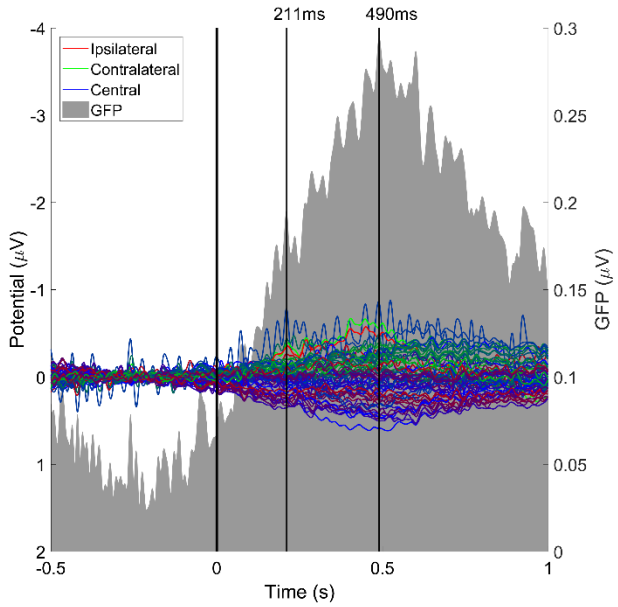

Figure 29: Butterfly plots of average EPs for FBSS patients' analysis. The first solid vertical black line represents the time of intra-epidermal stimulation. Vertical black lines at 211 ms, and 490 ms are based on local global field power (GFP) maxima and were used for statistical analysis of contralateral derivations (T7-F4 or T8-F3, depending on the side of electrical stimulation) and central derivation (CPz-A1A2), respectively.

APPENDIX G

1. preCPT comparison between test and retest of healthy subjects

Table A1: Regression parameter estimates of the fixed effects of the linear mixed model (LMM), corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P422. The model was fitted to test and retest results of healthy subjects. The test results were used as the reference level.

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-1.00	[-1.69 -0.31]	-2.86 (615)	0.004*
Amplitude SP	3.17	[0.29 6.06]	2.23 (38)	0.032*
Response	4.35	[1.76 6.93]	3.39 (43)	0.001*
Retest	-0.86	[-2.01 0.29]	-1.48 (185)	0.140
Trial number	-0.01	[-0.02 0.01]	-0.17 (5278)	0.865
Amplitude DP	-1.56	[-3.79 0.67]	-1.37 (3808)	0.170
Amplitude SP x retest	-4.26	[-8.59 0.08]	-1.96 (59)	0.054
Response x retest	-1.69	[-5.34 1.96]	-0.93 (43)	0.356
Response x trial number	-0.03	[-0.05 -0.01]	-2.88 (5397)	0.004*
Retest x trial number	-0.01	[-0.02 0.02]	-0.16 (4950)	0.877
Retest x amplitude DP	3.27	[-0.16 6.7]	1.87 (2864)	0.061
Response x retest x trial number	0.05	[0.02 0.08]	3.01 (5393)	0.003*

Table A2: Regression parameter estimates of the fixed effects of the linear mixed model (LMM), corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P197. The model was fitted to test and retest results of healthy subjects. The test results were used as the reference level.

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-0.01	[-0.57 0.55]	-0.03 (170)	0.977
Amplitude SP	-0.11	[-1.74 1.51]	-0.15 (10)	0.880
Response	-0.77	[-1.62 0.07]	-1.79 (5355)	0.073
Retest	0.49	[-0.30 1.28]	1.22 (271)	0.222
Trial number	-0.01	[-0.02 0.01]	-0.87 (4398)	0.382
Amplitude DP	2.28	[0.38 4.17]	2.36 (726)	0.019*
Amplitude SP x retest	0.04	[-2.33 2.40]	0.03 (14)	0.974
Response x retest	0.55	[-0.64 1.74]	0.91 (5362)	0.363
Response x trial number	0.01	[-0.02 0.02]	0.08 (5404)	0.940
Retest x trial number	0.01	[-0.00 0.03]	1.46 (4819)	0.145
Retest x amplitude DP	-1.62	[-4.38 1.13]	-1.16 (494)	0.247
Response x retest x trial number	-0.01	[-0.04 0.02]	-0.70 (5412)	0.482

2. PerCPT comparison between test and retest of healthy subjects

Table A3: Regression parameter estimates of the fixed effects of the linear mixed model (LMM), corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P428. The model was fitted to test and retest results of healthy subjects. The test results were used as the reference level.

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	0.12	[-1.06 1.30]	0.20 (48)	0.842
Amplitude SP	-1.70	[-3.23 -0.18]	-2.20 (174)	0.029*
Response	0.96	[-0.15 2.06]	1.70 (4053)	0.089
Retest	-1.48	[-3.15 0.19]	-1.78 (48)	0.082
Trial number	0.002	[-0.02 0.02]	0.22 (3927)	0.825
Amplitude DP	-2.58	[-4.50 -0.67]	-2.65 (437)	0.008*
Amplitude SP x retest	0.25	[-2.13 2.63]	0.21 (180)	0.835
Response x retest	1.01	[-0.66 2.68]	1.20 (88)	0.233
Response x trial number	0.01	[-0.02 0.05]	0.78 (4060)	0.437
Retest x trial number	-0.004	[-0.03 0.03]	-0.30 (3867)	0.768
Retest x amplitude DP	3.25	[0.48 6.03]	2.30 (420)	0.022*
Response x retest x trial number	-0.04	[-0.09 0.01]	-1.66 (4072)	0.097

Table A4: Regression parameter estimates of the fixed effects of the linear mixed model (LMM), corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P197. The model was fitted to test and retest results of healthy subjects. The test results were used as the reference level.

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-0.87	[-1.65 -0.09]	-2.18 (702)	0.029*
Amplitude SP	0.75	[-0.50 1.99]	1.17 (771)	0.241
Response	0.88	[-0.38 2.15]	1.37 (4077)	0.171
Retest	1.50	[0.39 2.61]	2.64 (1317)	0.008*
Trial number	-0.03	[-0.05 -0.01]	-2.42 (4071)	0.015*
Amplitude DP	3.13	[0.77 5.49]	3.18 (7)	0.017*
Amplitude SP x retest	-0.65	[-2.57 1.28]	-0.66 (429)	0.510
Response x retest	-0.92	[-2.70 0.86]	-1.01 (4077)	0.311
Response x trial number	0.02	[-0.01 0.06]	1.28 (4077)	0.200
Retest x trial number	0.03	[-0.01 0.06]	1.69 (4051)	0.092
Retest x amplitude DP	-2.77	[-6.03 0.50]	-1.99 (7)	0.085
Response x retest x trial number	-0.02	[-0.08 0.03]	-0.84 (4077)	0.401

3. PostCPT comparison between test and retest of healthy subjects

Table A5: Regression parameter estimates of the fixed effects of the linear mixed model (LMM), corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P428. The model was fitted to test and retest results of healthy subjects. The test results were used as the reference level.

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-0.04	[-1.48 1.40]	-0.06 (39)	0.955
Amplitude SP	-1.18	[-2.69 0.33]	-1.64 (19)	0.118
Response	1.20	[0.33 2.07]	2.71 (6464)	0.007*
Retest	-1.50	[-3.53 0.54]	-1.49 (39)	0.144
Trial number	0.01	[-0.00 0.02]	1.69 (101)	0.093
Amplitude DP	-0.66	[-1.96 0.63]	-1.00 (1052)	0.316
Amplitude SP x retest	2.21	[-0.10 4.52]	1.97 (27)	0.060
Response x retest	0.77	[-0.93 2.47]	0.92 (39)	0.365
Response x trial number	0.01	[-0.01 0.02]	0.23 (6197)	0.821
Retest x trial number	-0.01	[-0.02 0.01]	-1.00 (100)	0.320
Retest x amplitude DP	2.21	[0.14 4.28]	2.10 (898)	0.036*
Response x retest x trial number	-0.01	[-0.03 0.02]	-0.54 (6111)	0.590

Table A6: Regression parameter estimates of the fixed effects of the linear mixed model (LMM), corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P192. The model was fitted to test and retest results of healthy subjects. The test results were used as the reference level.

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-1.20	-1.75	-4.27	<0.001*
Amplitude SP	1.81	1.00	4.38	<0.001*
Response	1.79	0.93	4.08	<0.001*
Retest	1.75	0.97	4.37	<0.001*
Trial number	-0.01	-0.01	-0.49	0.624
Amplitude DP	-0.05	-1.09	-0.10	0.921
Amplitude SP x retest	-1.59	-2.78	-2.62	0.009*
Response x retest	-1.96	-3.15	-3.21	0.001*
Response x trial number	0.016	-0.00	1.79	0.074
Retest x trial number	0.00	-0.01	0.13	0.893
Retest x amplitude DP	-0.05	-1.60	-0.06	0.951
Response x retest x trial number	-0.01	-0.03	-1.00	0.318

4. Effect of CPT on EP amplitudes

4.1. Test measurements

*Table A7: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test measurements of healthy subjects. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P426. PreCPT test results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	Parameter estimate	95% confidence interval	Effect χ^2 (df)	P-value
(Intercept)	-0.74	[-3.69 2.21]	-0.49 (421)	0.624
Amplitude SP	2.46	[0.19 4.74]	2.13 (428)	0.034
Response	-0.84	[-5.33 3.65]	-0.37 (1797)	0.714
Trial number	0.00	[-0.02 0.02]	0.32 (774)	0.750
Condition 1	0.12	[-2.85 3.09]	0.08 (921)	0.938
Condition 2	-0.54	[-3.89 2.80]	-0.32 (655)	0.750
Amplitude DP	-1.82	[-4.80 1.16]	-1.20 (1737)	0.232
Response x trial number	-0.03	[-0.06 -0.01]	-2.35 (1961)	0.019*
Amplitude SP x condition 1	-3.25	[-5.75 -0.75]	-2.57 (183)	0.011*
Amplitude SP x condition 2	-3.32	[-5.91 -0.73]	-2.53 (193)	0.012*
Response x condition 1	1.58	[-3.04 6.20]	0.67 (8117)	0.502
Response x condition 2	1.23	[-3.87 6.33]	0.47 (8084)	0.636
Trial number x condition 1	0.00	[-0.04 0.03]	-0.15 (103)	0.878
Trial number x condition 2	0.00	[-0.02 0.03]	0.26 (349)	0.798
Condition 1 x amplitude DP	0.33	[-3.13 3.80]	0.19 (639)	0.850
Condition 2 x amplitude DP	1.33	[-1.92 4.57]	0.80 (1146)	0.422
Response x trial number x condition 1	0.04	[-0.01 0.09]	1.75 (5605)	0.080
Response x trial number x condition 2	0.04	[0.01 0.07]	2.30 (7979)	0.022*

*Table A8: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test measurements of healthy subjects. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P192. PreCPT test results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	Parameter estimate	95% confidence interval	Effect χ^2 (df)	*P*-value
(Intercept)	-0.07	[-3.08 2.94]	-0.05 (5322)	0.961
Amplitude SP	-0.40	[-2.26 1.47]	-0.42 (225)	0.676
Response	-0.74	[-5.53 4.05]	-0.30 (8173)	0.762
Trial number	-0.01	[-0.02 0.01]	-0.52 (6752)	0.605
Condition 1	-0.98	[-4.18 2.21]	-0.60 (7445)	0.547
Condition 2	-0.32	[-3.95 3.31]	-0.17 (3545)	0.864
Amplitude DP	2.21	[-0.65 5.06]	1.52 (1429)	0.129
Response x trial number	0.00	[-0.03 0.03]	0.02 (8148)	0.987
Amplitude SP x condition 1	1.07	[-1.27 3.40]	0.90 (1859)	0.370
Amplitude SP x condition 2	1.72	[-0.49 3.92]	1.53 (558)	0.126

Response x condition 1	2.07	[-2.99 7.12]	0.80 (8159)	0.423
Response x condition 2	0.70	[-4.87 6.28]	0.25 (8163)	0.805
Trial number x condition 1	-0.02	[-0.06 0.01]	-1.40 (8035)	0.162
Trial number x condition 2	0.00	[-0.02 0.02]	0.03 (7441)	0.974
Condition 1 x amplitude DP	1.02	[-2.98 5.03]	0.54 (18)	0.598
Condition 2 x amplitude DP	-2.18	[-5.35 0.98]	-1.35 (3878)	0.176
Response x trial number x condition 1	0.02	[-0.03 0.08]	0.87 (8172)	0.387
Response x trial number x condition 2	0.01	[-0.02 0.05]	0.70 (8155)	0.486

4.2. Retest measurements

*Table A9: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to retest measurements of healthy subjects. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P492. PreCPT retest results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-1.87	[-3.68 -0.06]	-2.04 (288)	0.043*
Amplitude SP	0.40	[-0.97 1.76]	0.57 (961)	0.569
Response	7.05	[4.54 9.56]	5.50 (1177)	<0.001*
Trial number	-0.01	[-0.02 0.00]	-1.18 (7641)	0.239
Condition 1	0.63	[-1.05 2.31]	0.73 (8024)	0.464
Condition 2	0.40	[-1.46 2.26]	0.42 (7079)	0.673
Amplitude DP	0.94	[-0.91 2.79]	1.01 (81)	0.317
Response x trial number	0.03	[0.02 0.05]	3.95 (8216)	<0.001*
Amplitude SP x condition 1	0.15	[-1.29 1.59]	0.21 (1244)	0.835
Amplitude SP x condition 2	0.54	[-0.86 1.95]	0.77 (76)	0.442
Response x condition 1	-5.81	[-8.35 -3.26]	-4.47 (8221)	<0.001*
Response x condition 2	-4.99	[-7.75 -2.22]	-3.53 (8230)	<0.001*
Trial number x condition 1	0.00	[-0.02 0.02]	0.06 (8029)	0.951
Trial number x condition 2	0.01	[0.00 0.03]	2.00 (7873)	0.046*
Condition 1 x amplitude DP	1.46	[-0.68 3.61]	1.40 (26)	0.173
Condition 2 x amplitude DP	0.48	[-1.55 2.50]	0.48 (32)	0.636
Response x trial number x condition 1	-0.05	[-0.08 -0.03]	-3.78 (8225)	<0.001*
Response x trial number x condition 2	-0.04	[-0.05 -0.02]	-3.87 (8229)	<0.001*

*Table A10: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to retest measurements of healthy subjects. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P175. PreCPT retest results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**

(Intercept)	0.58	[-0.52 1.67]	1.04 (5071)	0.301
Amplitude SP	-0.07	[-0.91 0.78]	-0.16 (165)	0.876
Response	0.05	[-1.62 1.73]	0.06 (3030)	0.949
Trial number	0.00	[0.00 0.01]	0.81 (7199)	0.416
Condition 1	-0.51	[-1.68 0.65]	-0.86 (2464)	0.388
Condition 2	-0.58	[-1.86 0.70]	-0.89 (8170)	0.373
Amplitude DP	-0.26	[-1.35 0.82]	-0.48 (141)	0.631
Response x trial number	0.00	[-0.01 0.01]	0.41 (8229)	0.685
Amplitude SP x condition 1	0.30	[-0.70 1.30]	0.59 (254)	0.554
Amplitude SP x condition 2	0.54	[-0.35 1.42]	1.19 (1649)	0.234
Response x condition 1	0.08	[-1.68 1.83]	0.09 (8248)	0.930
Response x condition 2	-0.14	[-2.06 1.78]	-0.14 (3635)	0.889
Trial number x condition 1	-0.01	[-0.02 0.01]	-1.12 (7334)	0.264
Trial number x condition 2	0.00	[-0.01 0.01]	-0.57 (7432)	0.566
Condition 1 x amplitude DP	0.61	[-0.68 1.90]	0.92 (1159)	0.357
Condition 2 x amplitude DP	0.50	[-0.69 1.70]	0.82 (711)	0.410
Response x trial number x condition 1	0.02	[0.00 0.04]	1.80 (8213)	0.073
Response x trial number x condition 2	0.00	[-0.02 0.01]	-0.70 (8246)	0.485

4.3. FBSS measurements

*Table A11: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to FBSS measurements. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P490. PreCPT FBSS results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	0.28	[-1.82 2.38]	0.27 (57)	0.790
Amplitude SP	0.94	[-1.20 3.07]	0.96 (11)	0.356
Response	0.28	[-2.63 3.18]	0.19 (464)	0.851
Trial number	0.00	[-0.01 0.02]	0.53 (2076)	0.596
Condition 1	-0.92	[-2.87 1.04]	-0.92 (2242)	0.357
Condition 2	-0.51	[-2.99 1.97]	-0.41 (80)	0.684
Amplitude DP	-0.56	[-2.15 1.03]	-0.69 (735)	0.491
Response x trial number	0.00	[-0.02 0.02]	-0.09 (2233)	0.930
Amplitude SP x condition 1	0.03	[-1.85 1.91]	0.03 (461)	0.975
Amplitude SP x condition 2	-0.21	[-2.58 2.16]	-0.18 (34)	0.857
Response x condition 1	1.00	[-2.19 4.18]	0.62 (152)	0.539
Response x condition 2	0.41	[-3.18 4.01]	0.23 (2163)	0.821
Trial number x condition 1	0.00	[-0.02 0.03]	0.24 (2243)	0.807
Trial number x condition 2	-0.01	[-0.02 0.01]	-0.67 (1240)	0.506
Condition 1 x amplitude DP	0.76	[-1.43 2.96]	0.68 (1327)	0.495

Condition 2 x amplitude DP	2.07	[-3.24 7.38]	1.16 (3)	0.321
Response x trial number x condition 1	0.00	[-0.04 0.03]	-0.18 (2228)	0.857
Response x trial number x condition 2	0.00	[-0.02 0.03]	0.11 (2101)	0.914

*Table A12: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to FBSS measurements. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P218. PreCPT FBSS results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	0.26	[-0.75 1.28]	0.51 (1656)	0.608
Amplitude SP	0.41	[-0.33 1.16]	1.10 (117)	0.276
Response	-0.57	[-2.13 0.99]	-0.72 (2265)	0.472
Trial number	0.00	[-0.01 0.01]	-0.24 (2177)	0.809
Condition 1	-0.04	[-1.12 1.03]	-0.08 (2266)	0.935
Condition 2	-0.39	[-1.62 0.84]	-0.62 (2259)	0.534
Amplitude DP	0.57	[-0.24 1.39]	1.38 (1306)	0.169
Response x trial number	0.00	[-0.01 0.01]	-0.03 (2266)	0.975
Amplitude SP x condition 1	-0.20	[-1.16 0.76]	-0.40 (2264)	0.688
Amplitude SP x condition 2	-0.21	[-1.14 0.73]	-0.43 (987)	0.665
Response x condition 1	0.25	[-1.41 1.90]	0.29 (2265)	0.770
Response x condition 2	1.16	[-0.80 3.11]	1.16 (2203)	0.247
Trial number x condition 1	0.01	[-0.01 0.02]	0.80 (2266)	0.422
Trial number x condition 2	0.00	[-0.01 0.01]	0.42 (2202)	0.676
Condition 1 x amplitude DP	-0.64	[-1.82 0.53]	-1.07 (2267)	0.283
Condition 2 x amplitude DP	-0.47	[-1.46 0.52]	-0.93 (2194)	0.350
Response x trial number x condition 1	0.00	[-0.02 0.02]	-0.08 (2266)	0.938
Response x trial number x condition 2	-0.01	[-0.02 0.01]	-0.79 (2213)	0.431

4.4. Age analysis

4.4.1. Lower age

*Table A13: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test results of healthy subjects aged between 18 and 35 years. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P428. PreCPT results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-1.29	[-7.70 5.13]	-0.39 (726)	0.694
Amplitude SP	-4.37	[-17.18 8.43]	-0.69 (40)	0.494

Response	-1.78	[-11.46 7.90]	-0.36 (279)	0.718
Trial number	0.02	[-0.03 0.06]	0.73 (344)	0.468
Condition 1	-0.26	[-7.03 6.51]	-0.07 (2395)	0.941
Condition 2	-0.60	[-8.12 6.93]	-0.16 (246)	0.876
Amplitude DP	-5.24	[-20.91 10.43]	-0.66 (176)	0.510
Response x trial number	-0.06	[-0.12 0.00]	-1.96 (386)	0.051
Amplitude SP x condition 1	-5.78	[-24.28 12.72]	-0.62 (199)	0.538
Amplitude SP x condition 2	8.40	[-9.34 26.14]	0.94 (121)	0.350
Response x condition 1	4.95	[-4.82 14.73]	0.99 (2455)	0.321
Response x condition 2	2.38	[-8.28 13.04]	0.44 (2449)	0.662
Trial number x condition 1	-0.01	[-0.09 0.06]	-0.36 (54)	0.723
Trial number x condition 2	-0.01	[-0.06 0.05]	-0.28 (181)	0.783
Condition 1 x amplitude DP	-16.77	[-41.96 8.42]	-1.31 (728)	0.192
Condition 2 x amplitude DP	13.71	[-9.51 36.94]	1.16 (497)	0.247
Response x trial number x condition 1	0.08	[-0.02 0.18]	1.55 (2451)	0.121
Response x trial number x condition 2	0.07	[0.00 0.14]	1.98 (2455)	0.048*

*Table A14: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test results of healthy subjects aged between 18 and 35 years. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P193. PreCPT results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-1.87	[-7.98 4.23]	-0.60 (2446)	0.548
Amplitude SP	2.89	[-7.13 12.92]	0.57 (938)	0.571
Response	-0.26	[-9.24 8.72]	-0.06 (929)	0.955
Trial number	-0.02	[-0.06 0.02]	-1.02 (2453)	0.306
Condition 1	0.21	[-6.32 6.75]	0.06 (2430)	0.949
Condition 2	0.97	[-6.48 8.41]	0.26 (503)	0.798
Amplitude DP	9.98	[-3.87 23.83]	1.41 (2004)	0.158
Response x trial number	0.00	[-0.05 0.06]	0.12 (2446)	0.902
Amplitude SP x condition 1	3.62	[-15.09 22.33]	0.41 (15)	0.685
Amplitude SP x condition 2	-1.55	[-16.79 13.70]	-0.20 (724)	0.842
Response x condition 1	1.79	[-7.68 11.25]	0.37 (2450)	0.711
Response x condition 2	0.89	[-9.54 11.32]	0.17 (715)	0.867
Trial number x condition 1	0.00	[-0.07 0.07]	-0.02 (2438)	0.986
Trial number x condition 2	0.02	[-0.03 0.06]	0.67 (2460)	0.506
Condition 1 x amplitude DP	-7.58	[-31.46 16.29]	-0.62 (1931)	0.533
Condition 2 x amplitude DP	-19.54	[-40.82 1.75]	-1.80 (1249)	0.072
Response x trial number x condition 1	0.02	[-0.09 0.14]	0.41 (33)	0.687

Response x trial number x condition 2	0.01	[-0.05 0.08]	0.37 (2447)	0.708

4.4.2. Middle age

*Table A15: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test results of healthy subjects aged between 36 and 55 years. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P426. PreCPT results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	0.42	[-4.25 5.09]	0.18 (1966)	0.859
Amplitude SP	4.39	[0.65 8.14]	2.35 (59)	0.022*
Response	-0.71	[-8.01 6.59]	-0.19 (1331)	0.849
Trial number	0.00	[-0.04 0.04]	-0.09 (78)	0.932
Condition 1	-1.78	[-6.69 3.14]	-0.71 (2723)	0.479
Condition 2	-0.34	[-6.93 6.26]	-0.10 (39)	0.918
Amplitude DP	0.95	[-4.34 6.23]	0.35 (1349)	0.725
Response x trial number	-0.03	[-0.07 0.02]	-1.07 (930)	0.284
Amplitude SP x condition 1	-5.98	[-10.12 -1.84]	-2.84 (775)	0.005*
Amplitude SP x condition 2	-2.40	[-7.56 2.76]	-0.92 (97)	0.359
Response x condition 1	1.45	[-6.23 9.13]	0.37 (2850)	0.711
Response x condition 2	-0.52	[-9.12 8.07]	-0.12 (377)	0.905
Trial number x condition 1	-0.01	[-0.08 0.05]	-0.45 (24)	0.657
Trial number x condition 2	0.01	[-0.04 0.05]	0.24 (65)	0.809
Condition 1 x amplitude DP	-3.67	[-9.94 2.60]	-1.15 (1203)	0.251
Condition 2 x amplitude DP	5.92	[-9.38 21.21]	1.16 (3)	0.322
Response x trial number x condition 1	0.05	[-0.03 0.13]	1.22 (2418)	0.224
Response x trial number x condition 2	0.03	[-0.03 0.08]	0.96 (2692)	0.336

*Table A16: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test results of healthy subjects aged between 36 and 55 years. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P193. PreCPT results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-0.60	[-5.32 4.13]	-0.25 (2122)	0.804
Amplitude SP	0.97	[-3.27 5.20]	0.48 (19)	0.638
Response	-0.20	[-7.50 7.10]	-0.05 (2412)	0.957
Trial number	-0.01	[-0.05 0.02]	-0.89 (2544)	0.376
Condition 1	0.07	[-4.83 4.97]	0.03 (2828)	0.978
Condition 2	2.12	[-3.44 7.67]	0.75 (2180)	0.455
Amplitude DP	3.34	[-1.55 8.23]	1.34 (1950)	0.181

Response x trial number	0.01	[-0.04 0.05]	0.35 (2870)	0.729
Amplitude SP x condition 1	0.83	[-2.86 4.52]	0.44 (2416)	0.661
Amplitude SP x condition 2	1.54	[-2.49 5.56]	0.77 (54)	0.447
Response x condition 1	2.70	[-5.06 10.46]	0.68 (2878)	0.496
Response x condition 2	-0.68	[-9.14 7.79]	-0.16 (2877)	0.876
Trial number x condition 1	0.01	[-0.04 0.07]	0.38 (2793)	0.706
Trial number x condition 2	0.00	[-0.03 0.04]	0.20 (2715)	0.839
Condition 1 x amplitude DP	-0.92	[-6.84 5.00]	-0.30 (2650)	0.761
Condition 2 x amplitude DP	-3.50	[-8.90 1.89]	-1.27 (1822)	0.203
Response x trial number x condition 1	0.00	[-0.09 0.08]	-0.07 (2880)	0.944
Response x trial number x condition 2	0.01	[-0.05 0.06]	0.18 (2876)	0.859

4.4.3. Higher age

*Table A17: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test results of healthy subjects aged between 56 and 75 years. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P426. PreCPT results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	-1.64	[-6.33 3.04]	-0.71 (42)	0.483
Amplitude SP	1.83	[-1.09 4.74]	1.24 (163)	0.218
Response	0.26	[-6.38 6.91]	0.08 (732)	0.938
Trial number	0.00	[-0.03 0.02]	-0.18 (2546)	0.854
Condition 1	1.86	[-2.35 6.07]	0.87 (272)	0.385
Condition 2	-0.27	[-5.11 4.57]	-0.11 (298)	0.914
Amplitude DP	-2.52	[-5.78 0.75]	-1.51 (533)	0.131
Response x trial number	-0.02	[-0.06 0.02]	-0.99 (997)	0.323
Amplitude SP x condition 1	-1.67	[-5.03 1.68]	-1.00 (49)	0.322
Amplitude SP x condition 2	-3.01	[-6.71 0.69]	-1.75 (14)	0.103
Response x condition 1	-0.25	[-7.16 6.65]	-0.07 (2771)	0.942
Response x condition 2	1.74	[-6.04 9.53]	0.44 (2752)	0.661
Trial number x condition 1	0.01	[-0.03 0.06]	0.49 (1540)	0.623
Trial number x condition 2	0.01	[-0.02 0.04]	0.70 (212)	0.485
Condition 1 x amplitude DP	1.92	[-1.94 5.79]	0.99 (116)	0.326
Condition 2 x amplitude DP	1.92	[-1.60 5.44]	1.08 (136)	0.282
Response x trial number x condition 1	0.00	[-0.07 0.08]	0.09 (2748)	0.928
Response x trial number x condition 2	0.02	[-0.03 0.07]	0.72 (2527)	0.474

*Table A18: Regression parameter estimates of the fixed effects of the linear mixed model (LMM) fitted to test results of healthy subjects aged between 56 and 75 years. Corresponding confidence intervals and the type III Wald statistics of the main effects evaluated at P192. PreCPT results were used as reference level (condition 0). Condition 1 and condition 2 represent per- and postCPT effects, respectively. Significant differences are indicated with *. Significance level: p = 0.05.*

Parameter	**Parameter estimate**	**95% confidence interval**	**Effect χ^2 (df)**	***P*-value**
(Intercept)	0.80	[-4.36 5.97]	0.30 (1824)	0.761
Amplitude SP	0.17	[-2.84 3.18]	0.11 (92)	0.910
Response	0.17	[-8.52 8.87]	0.04 (1567)	0.969
Trial number	0.00	[-0.03 0.04]	0.22 (2546)	0.828
Condition 1	-1.37	[-6.87 4.12]	-0.49 (2586)	0.624
Condition 2	-2.03	[-8.31 4.24]	-0.64 (2626)	0.525
Amplitude DP	0.97	[-2.89 4.83]	0.49 (539)	0.621
Response x trial number	0.00	[-0.05 0.06]	0.14 (2777)	0.890
Amplitude SP x condition 1	-0.10	[-4.20 4.00]	-0.05 (238)	0.962
Amplitude SP x condition 2	1.99	[-1.65 5.62]	1.07 (379)	0.283
Response x condition 1	-0.72	[-10.00 8.55]	-0.15 (2770)	0.878
Response x condition 2	-0.13	[-10.77 10.52]	-0.02 (523)	0.981
Trial number x condition 1	-0.06	[-0.12 0.00]	-1.88 (2671)	0.061
Trial number x condition 2	0.00	[-0.04 0.04]	-0.09 (2351)	0.928
Condition 1 x amplitude DP	2.71	[-4.34 9.76]	0.91 (7)	0.392
Condition 2 x amplitude DP	-0.66	[-4.95 3.64]	-0.30 (2269)	0.764
Response x trial number x condition 1	0.01	[-0.10 0.11]	0.17 (2770)	0.863
Response x trial number x condition 2	0.01	[-0.06 0.08]	0.26 (2461)	0.794