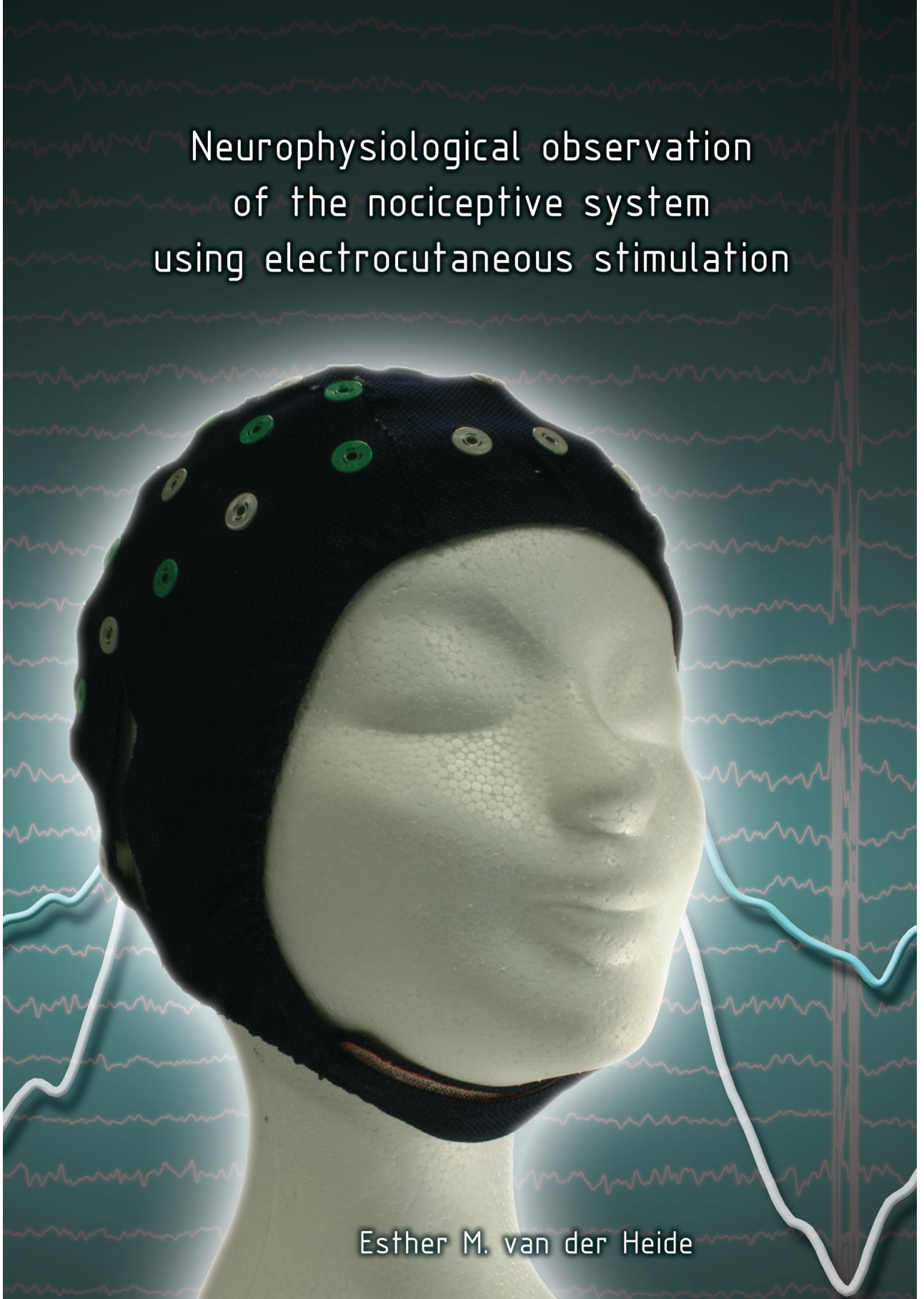
Neurophysiological observation of the nociceptive system using electrocutaneous stimulation



Esther M. van der Heide

NEUROPHYSIOLOGICAL OBSERVATION OF THE NOCICEPTIVE SYSTEM USING ELECTROCUTANEOUS STIMULATION

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Prof. dr. E. Marani
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Chapter 1

General introduction

1.1 History of pain research

Pain is an intriguing and important sensation which has been the subject of research for ages. Ancient civilizations related pain to evil, magic and demons. Pain relief was provided by sorcerers and priests. The theory of sensation was first introduced in Greek and Roman times. This theory describes that the brain and nervous system play a role in producing the perception of pain. The Greek physician Hippocrates made various observations related to pain sensation. He considered pain purely as a clue to disease [104]. Interestingly, in one of Hippocrates' aphorisms he states: 'If a patient is subject to two pains arising in different parts of the body simultaneously, the stronger blunts the other' [54]. This is still a generally agreed upon statement; the so called cold pressor test, discussed in section 1.3, uses this observation.

Leonardo da Vinci proposed that the brain was the central organ responsible for sensations such as pain. He developed the idea that the spinal cord transmits sensation to the brain [141].

In 1664, the French philosopher René Descartes described the concept of a pain pathway [96]. He describes how particles from fire near the foot set in motion the touched spot of the skin (figure 1.1), by this means pulling upon the delicate thread which is attached to the spot in the skin. The thread arises via the leg and back into the head (striking a bell). Pain is felt and the person responds to it. This theory led to the specificity theory which proposes that specific nerves transmit information of pain receptors to the pain center in the brain [96].

Figure 1.1: Descartes concept of the pain pathway. Particles of fire A set in motion spot B on the foot. The signal arises to the head F similar to pulling at one end of the thread [96].

The specificity theory was refuted by Melzack and Wall in 1965 [96]. They stated that no psychophysical evidence existed for the one-to-one relationship between pain perception and intensity of the stimulation [96]. As an example, the paradoxical stories from soldiers in the battlefield suggested that the specificity theory did not hold; soldiers did not feel any pain after extensive injuries [95]. It was suggested that the pain sensation of these soldiers was modulated by cognitive processes.

Melzack and Wall proposed the gate control theory which states that pain is modulated in the dorsal horn by an interaction between different nerve fibers (Aβ, Aδ and C-fibers). Psychological factors such as past experience and attention influence pain experience by acting on the gate control system [96]. Research in the past years debated the gate control theory and suggests that pain signals are also modulated at other levels [61].

The international association for the study of pain (IASP) defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in such damage" [57]. The complex character of pain is enclosed in this definition: physiological, psychological as well as social aspects influence the pain sensation. Pain can be felt without an injury or disease; loss of a beloved person or emotional suffering like exclusion can cause pain [40]. Pain is necessary to survive. People born with congenital insensitivity to pain with anhidrosis disease do not feel pain sensation and are not warned for injury, diseases or danger [25]. This can lead to permanent injuries.

Acute pain is directly related to damaged or diseased tissue. Usually acute pain lasts for a relatively limited time and remits with the course of the healing process. When pain continues beyond the normal course of the disease or healing time of an injury it is called chronic pain. Chronic pain can exist in the absence of tissue damage or a likely pathophysiological cause [57]. The transition from acute to chronic pain is called chronification.

Although a lot of clinical and fundamental research has been performed to the pain system still our knowledge about the various processes involved in the chronification of pain is limited. Adequate observation techniques are required to explore changes in the nociceptive system in pain patients. Chronic pain is related to neuroplastic changes at several levels of the nervous system [24; 41; 93]. Neuroplastic changes are not restricted to chronic pain states, the pain system can change rapidly in other conditions as well [136; 163]. In this thesis neurophysiological observation methods of the pain system are explored. In this introductory chapter several aspects of the neurophysiology of pain and observation techniques will be treated.

1.2 Neurophysiology

Explaining all details of the neurophysiology of pain is far beyond the scope of this thesis. In this section a short overview will be given of those aspects relevant for this thesis.

1.2.1 Peripheral nerve fibers and endings

Throughout the body different kinds of cellular receptors can be found in the skin. These receptors are contacted by the peripheral nerve endings of dorsal root ganglion neurons. There are different nerve fibers; such as Aβ-, Aδ- and C fibers, that are extensions of the dorsal root ganglions cells involved in pain.
Mechanoreceptors are receptors which mediate tactile, vibration and joint position sense. Different kinds of mechanoreceptors with a different morphology are activated by these different stimuli. The majority of mechanoreceptors are innervated by Aβ-fibers. The Aβ-fibers are relative thick myeliniated fibers with a conduction velocity of 30-70 m/s (diameter (Ø): 6-12 µm).
Nociceptors are the free nerve endings of Aδ- and C-fibers. Nociceptors respond to mechanical damage, temperature extremes or chemicals. Aδ-fibers are thin myelinated afferents (Ø: 1-6 µm) conducting the so called 'first pain sensation'. The conduction velocity varies from 4-36 m/s. C-fibers are unmyelinated (Ø: 0.2-1.5 µm) and conduct the so called 'second pain sensation'. Due to the lack of myelin the conduction velocity is low and varies between 0.4-2.0 m/s.
The distribution and local fiber density of the receptors differ throughout the body [22; 72; 106]. Although research is done on local fiber density and distribution in the skin in the fingertip and forearm still no exact values are available; data is limited and not consistent. However, nerve fibers are more densely arranged in the skin of the finger than in the forearm [147].

1.2.2 Dorsal horn

The ventral, lateral and dorsal horns are all located in the grey matter in the spinal cord. The grey matter is subdivided in a number of Rexed laminae (see figure 1.2). The laminae are named after the neuroanatomist who first described these laminae in the 1950s [124; 125]. The dorsal horn is situated posteriorly in the spinal cord comprising Rexed laminae I to VI. The lateral horn is found between ventral and dorsal horn (only in the thoracolumbar spinal cord levels), and includes Rexed lamina VII. Anteriorly in the spinal cord is the ventral horn located, containing Rexed laminae VIII to IX. The Rexed laminae are based on cytoarchitectonic changes in neuron distribution.
The afferent fibers terminate in different laminae of Rexed in the dorsal horn in the spinal cord. The Aδ-fibers terminate in laminae I and V, C-fibers in laminae I and II [147]. The Aβ-fibers project to laminae III-VI [147]. Different kinds of laminae neurons

exist, such as nociceptive specific (NS), wide dynamic range (WDR) and polymodal nociceptive neurons. NS neurons are a type of laminae I neurons responding only to noxious mechanical and heat stimuli. Mainly Aδ-fibers activate these NS neurons. Laminae V cells are mostly WDR neurons, receiving primarily input of Aβ- and Aδ-fibers.

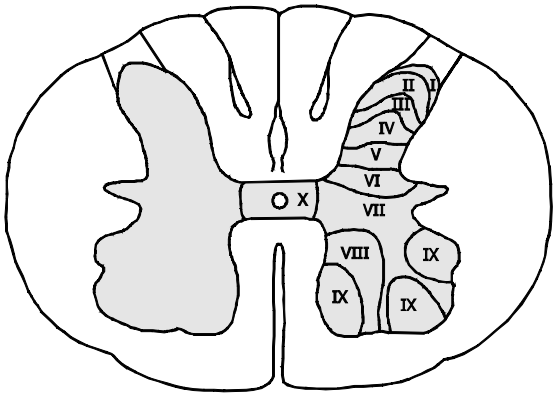

Figure 1.2: Illustration of the Rexed Laminae located in the grey matter of the spinal cord. The laminae are only shown in the right and middle part of the grey matter.

1.2.3 Spinal pathways

Several spinal pathways transmit information from the dorsal horn to the thalamus. A part of the tactile information is directly relayed via the ipsilateral cuneate tract in the dorsal column and synapses with the internal cuneate nucleus in the medulla. The axons of these neurons cross the midline and form the medial lemniscus the tract terminating in the ventroposterior lateral (VPL) nucleus in the thalamus (see figure 1.3). Transmission via the dorsal column- medial lemniscus (DCML) pathway is fast with a conduction velocity of 60 m/s. Branches of Aβ-fibers also synapse in the dorsal horn in, among other laminae, laminae V (WDR) neurons [92; 147].

Projections from dorsal horn neurons ascend mainly contralaterally in the anterolateral quadrant to the thalamus. The anterolateral system comprises several pathways such as the spinothalamic tract (STT), and the spinoreticular tract (SRT) [92]. The STT mediates sensations of pain, cold warmth and touch [147]. The STT is divided in an anterior and lateral part [26]. Axons of laminae V (WDR) neurons ascend via the anterior STT and terminate to the VPL nucleus in the thalamus. The STT and DCML project to closely related but different parts in the VPL. The lateral STT transmits signals from NS neurons (laminae I) to the ventromedial posterior (VMpo) and the ventroposterior inferior (VPI) nucleus in the thalamus. Besides the differences in projection targets in the thalamus, both STT pathways have different conduction velocities. The conduction velocity of

anterior STT is around 16.8 m/s faster than the lateral STT which is around 10 m/s [145]. Both the DCML and STT are somatotopically organised.

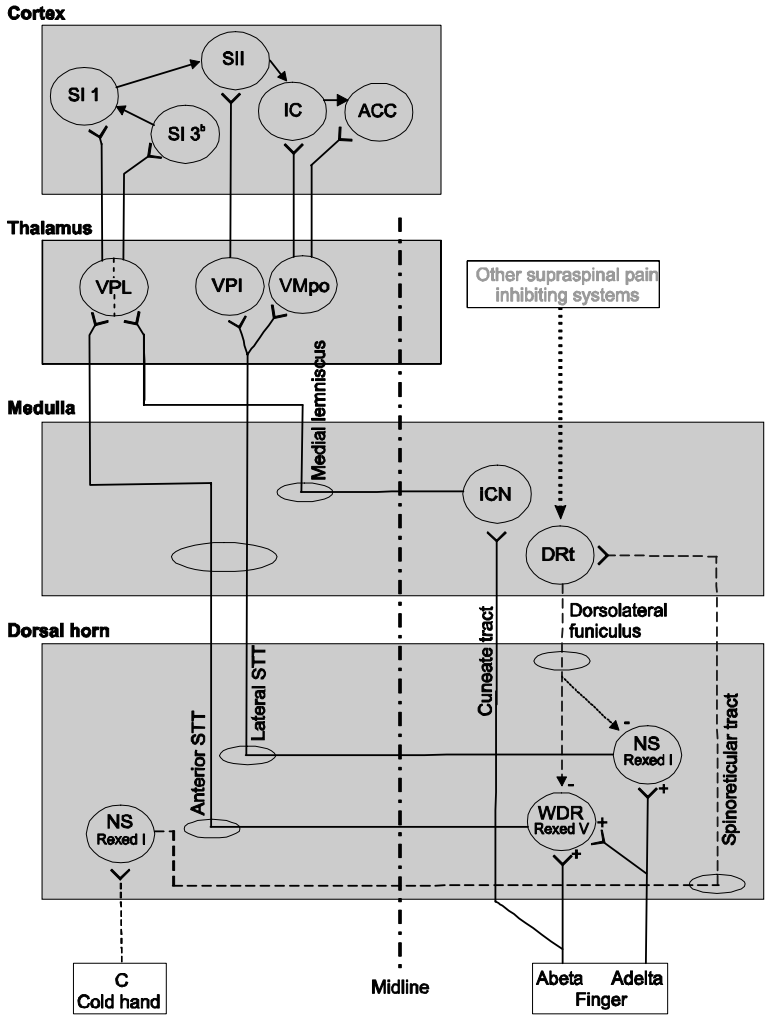

Figure 1.3: Simplified representation of anatomical connections, based on literature, relevant for pain processing. Dashed lines (--) represent the DNIC system. ACC: anterior cingulate cortex, DRt: dorsal reticular nucleus, IC: insular cortex, iCN: internal cuneate nucleus, NS: nociceptive specific neurons, Rexed I: lamina I in the dorsal horn, Rexed V: lamina V in the dorsal horn, SI 1: primary somatosensory cortex area 1, SI 3b: primary somatosensory cortex area 3b, SII: secondary somatosensory cortex, STT: spinothalamic tract, VPI: ventroposterior inferior nucleus, VPL: ventroposterior lateral nucleus, VMpo: ventromedial posterior nucleus, WDR: wide dynamic range neurons.

1.2.4 From thalamus to cortex

Various brain areas are involved in the processing of tactile and nociceptive activations [13; 113; 144]. The activated regions in the thalamus project to the primary somatosensory cortex (SI), the secondary somatosensory cortex (SII), the insular cortex (IC), the anterior cingulate cortex (ACC).

The SI is located in the postcentral gyrus. Based on cytoarchitecture the SI is divided in different area so called Brodmann's areas (areas 3a, 3b, 1 and 2). All areas are located in different parts of the postcentral gyrus; area 3a in the fundus of the central sulcus, area 3b in the rostral bank of the postcentral gyrus, area 1 in the crown of the postcentral gyrus and area 2 in the caudal bank of the postcentral gyrus (see figure 1.4).

The SI is primarily involved in the discrimination of the stimulus location and the stimulus intensity [144; 147]. Similar to the STT and DCML the SI is somatotopically organised [147].

Tactile and nociceptive activations are processed differently in these areas in the SI. Tactile information, relayed via the DCML, is firstly projected from the VPL in the thalamus to Brodmann's area 3b. Sequentially area 1 is activated [62; 117]. Nociceptive information, projected via the anterior STT, is processed in area 1 of the SI. The activated region in area 1 responds to both tactile and nociceptive stimulation; probably in the anterior fissural region [62; 117].

The SII is located in the upper bank of the sylvian fissure. The SII is involved in pain-related and tactile activations [55; 62; 140; 143]. Activations transmitted via the lateral STT activate the SII but also direct connections between SI and SII exist. The SII seems to encode the stimulus intensity to a certain extent [137; 140].

However, the representation of the intensity in SII differs to that of the SI [137; 140]. Similar to the SI, the SII also demonstrates a somatotopic organisation [130].

Activations in the VMpo nucleus in the thalamus are among other things projected to the IC. Furthermore, connections between SII and IC exist. The IC is part of the limbic system. The IC plays a role in the affective and cognitive aspects of pain [113].

The ACC receives afferents from the VMpo and the IC. The ACC, also part of the limbic system, is involved in several emotional and cognitive-evaluative aspects of pain [13; 147]. Different subareas of the ACC are involved in different aspects of pain. The ACC plays a role in attentional functions and the association of pain with unpleasantness [118; 120].

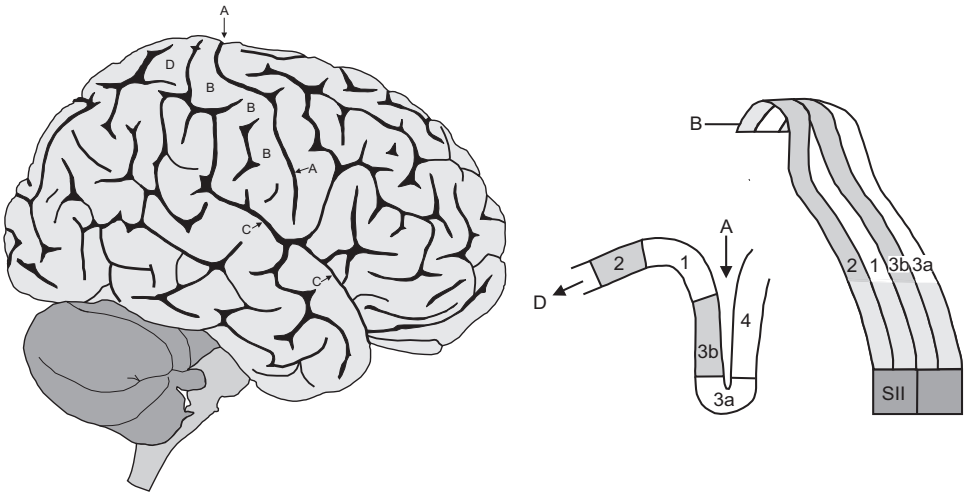

Figure 1.4: Lateral view of the brain (left) and illustration of the Brodmann areas in the primary somatosensory cortex (right). In the lateral view of the brain the central sulcus (A), postcentral gyrus (B), sylvian fissure (C) and parietal gyrus (D) are indicated. The SI is situated in the postcentral gyrus (B). Brodmann areas 3a, 3b, 1 and 2 comprise the SI.

1.2.5 Modulation

At different levels in the pain system noxious information can be modulated. The gate control theory [96] for example (see also section 1.1), describes the interaction in the dorsal horn between activated large (tactile) and small (nociceptive) afferents: variation in activated afferents will result in differences in perceived stimulus strength, i.e. differences in pain strength. Noxious activity can also be inhibited by a supraspinal mechanism, the so called diffuse noxious inhibitory control (DNIC) system.

The DNIC system was first discovered in rats by Le Bars [81; 82] and has been extensively studied since then. Activity of most convergent neurons (WDR) and some NS neurons [139] in the dorsal horn and trigeminal nucleus caudalis (responsible for facial pain [146]) is inhibited by heterotopic noxious stimulation. In contrast, activity from low threshold mechanoreceptive neurons is not decreased [82; 139]. Involvement of the caudal medulla, or more specifically the dorsal reticular nucleus (DRt) (figure 1.3) in the supraspinal loop of the DNIC was demonstrated in rats [11]. Ascending information of this supraspinal loop is transmitted via the spinoreticular pathway to the caudal medulla [162]. Inhibiting descending activity is mediated via the dorsolateral funiculus to the dorsal horn. By making lesions in rats it was demonstrated that several supraspinal structures such as periaqueductal gray (PAG), cuneiform nucleus, parabrachial area, locus coerulus/subcoerulus, rostral ventromedial medulla are not directly involved in the DNIC [8; 9]. Effects of the DNIC act by a final post-synaptic inhibitory mechanism [160; 161] involving hyperpolarization of the neuronal membrane.

Inhibition by the DNIC is reduced or completely blocked by systemic administering of low doses of morphine [12; 78]. The lifting effect of morphine was found in both rats and humans [78; 80]. It is suggested that though the PAG is not directly involved in the DNIC, it may be involved indirectly when opioid systems are activated [12].
The inhibiting effect of heterotopic noxious stimulation is also observed in humans. Several studies with healthy subjects have shown inhibition of responses due to a test stimulus as a result of noxious stimulation of a part of the body distal to this test stimulus [20; 149; 166]. A dysfunctional DNIC has been demonstrated for some chronic pain syndromes [75; 114] but is absent in others [63; 83; 84] .

1.3 Stimulation methods

In order to study the pain system several stimulation methods can be applied [1; 49]. The pain system can be activated by a phasic or tonic external stimulus. Phasic pain stimuli produce only very brief stimulation, whereas tonic pain stimuli produce a long stimulation.

1.3.1 Phasic stimulation

Among all kinds of stimulation methods, laser and electrical stimulation are mostly used. Laser stimulation permits selective stimulation of nociceptive fibers at different locations of the body [3; 13; 56; 110]. A disadvantage is the receptor fatigue and peripheral sensitisation. Besides, since nociceptors are activated by heat conduction, timing of activation is difficult [164].
Electrical stimulation used to be a common used method to evoke pain sensations. Electrical stimulation of the skin surpasses the receptors and activates the nerve fibers directly [13; 64]. A major advantage of electrical stimulation is good control of timing of neural activations. A shortcoming of electrical stimulation is the simultaneous activation of both tactile and nociceptive fibers. Due to this lack of selectivity electrical stimulation has become a less popular stimulation method. Several electrical stimulation methods are described in literature. The intracutaneous electrical stimulation was first introduced by Bromm [14] and was repeatedly used since then [17; 52; 100]. The stimulation electrode is attached to the fingertip in a small opening in the upper layer of the skin. This method produces a definite and well-localised pain sensation and similar reactions were obtained in repeated measurement sessions.
More recently the intra-epidermal needle electrodes were introduced by Inui [58]. This electrode was an improvement to earlier electrodes and most likely activates preferentially Aδ-fibers [58].
Despite the shortcoming concerning selective stimulation, electrical stimulation is still an interesting stimulation method especially due to its good control of timing. We

question if stimulation of both nerve fibers types by electrical stimulation does exclude activation of the nociceptive system.
The stimulus strength of both electrical and thermal (laser) stimuli can be varied in a spatially or temporally manner. In this thesis two methods of changing the stimulus strength of an electrical stimulus will be explored. Commonly, the stimulus current amplitude of an electrical stimulus is varied [17; 87; 133; 140]. Changing the electrical stimulus strength in a temporal fashion (pulse trains) is rarely reported [47]. In chapter 2 the so called single pulse (SP) and pulse train (PT) method will be introduced and further explained.

1.3.2 Tonic stimulation

The DNIC can be induced by diverse tonic noxious stimulation methods, such as ischemic tourniquet pressure [46], capsaicin [150] or cold pressor test (CPT) [115]. Ischemic tourniquet pressure produces pain by inflating a tourniquet for a couple of minutes. Capsaicin is better known from for example sambal, it is the active component of chilli peppers producing the burning sensation. Cold pressor pain is induced by immersing an extremity (e.g. hand or foot) in ice water for several minutes. The CPT is not only used to produce tonic pain but also as a cardiovascular test to predict hypertension [53; 97]. The painfulness of the CPT is a function of the temperature of the water [102].

1.4 Electroencephalography

To observe the neurophysiology of the central processing of noxious stimuli an objective measurement method is required. Cortical activations reflect the central processing of stimuli and can be measured by various methods including electroencephalography (EEG), magnetoencephalography (MEG), functional magnetic resonance imaging (fMRI), and positron emission tomography (PET). Compared to EEG and MEG, the latter two methods have a better spatial resolution. However, the temporal resolution of EEG and MEG is superior to fMRI and PET. In the research described in this thesis EEG was used. Early evoked activity was analysed, therefore a method with a high temporal resolution was preferred. Consequently, in this section some features of EEG will be treated.
In 1875 Richard Caton discovered the electric nature of the brain by measuring directly on the surface of the brain of rabbits and monkeys [135]. EEG was first measured in through the intact human scalp surface by the German psychiatrist Hans Berger in 1924 [71]. Berger noticed that rhythms in the EEG changed by consciousness and characterised wave patterns such as alpha and beta rhythm [135].
The EEG is the recording of time varying electrical signals generated by brain structures measured from electrodes on the human scalp. Most of the electric potential measured at

the scalp is generated in the cerebral cortex. The cerebral cortex consists of 10^{10} neurons which are strongly interconnected. The surface of a single neuron may be covered with 10^4 to 10^5 synapses [108]. The apical dendrites of the pyramidal neurons in the cortex receive a variety of synaptic input. Excitatory and inhibitory postsynaptic potentials (EPSP and IPSP respectively) are two types of synaptic inputs. EPSPs produce an active sink in the extracellular space near dendrites and a passive source near the soma. For IPSPs the situation is reversed compared to EPSPs. Each dipole represents a sink-source combination. Synchronised activity of a large group of neurons creates an electric field which can be measured at the scalp surface.

1.4.1 Somatosensory evoked potentials

An evoked potential (EP) is a direct response in the EEG to an external stimulus such as nociceptive, visual or auditory stimuli. The EP reflects the central processing of the stimulus. Typically EPs are stimulus locked averages of responses to repeated stimuli. By averaging the spontaneous EEG is removed. The nociceptive EP following electrical stimulation at the fingertip is composed of some characteristic components depending on the recording electrode. Commonly EPs recorded at the vertex electrode referenced to the earlobes (C_Z-A_1A_2) are used (for electrode position see figure 1.7A). At this electrode the largest signal is measured. An example of an EP measured at C_Z-A_1A_2 is shown in figure 1.5.

By definition the positive and negative axis are plotted reversed; the negative axis points up and the positive axis points down. The first component is a positive component around 100 ms, called the P100. The second component is the N150 (negative peak around 150ms). The peak-to-peak amplitude N150-P200 often correlates with subjective rating of pain intensity. The last peak, the P300, has been related to cognitive processes. All EP components reflect the activation of one or more brain regions.

Although vertex electrode data is mostly shown, it can be seen in figure 1.6 that the position of the maximum amplitude of the EP changes with latency. In this figure the EP activity measured at the central line of the scalp are shown following fingertip stimulation [151]. Early contralateral activity is probably better represented at an electrode contralateral to the side of stimulation. Thus since in this thesis stimulation is applied to the left fingertip also data recorded from the contralateral C_4 referenced to F_Z will be analysed.

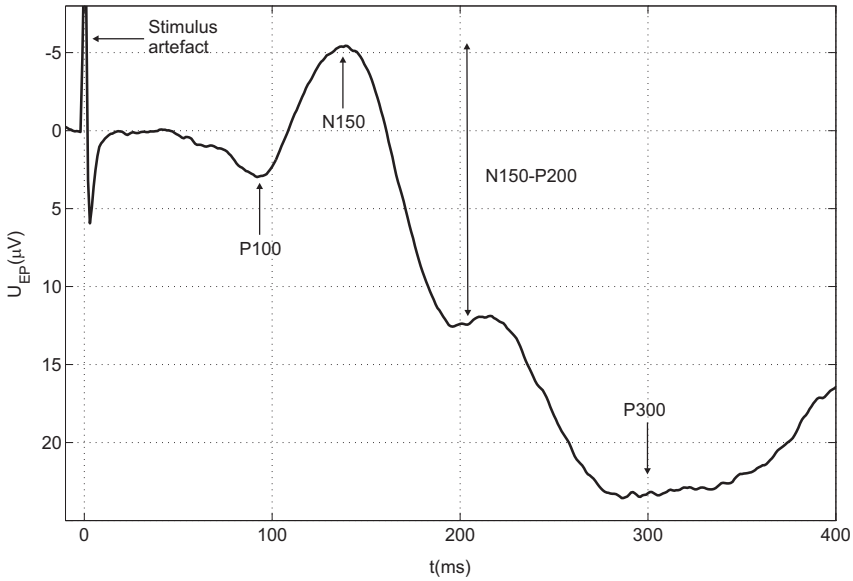

Figure 1.5: Illustration of a grand average EP (mean of 27 healthy subjects) following electrical fingertip stimulation. The EP is measured at C_Z referenced to A_1A_2. Typical components of the EP are indicated. Unpublished data of earlier research [151].

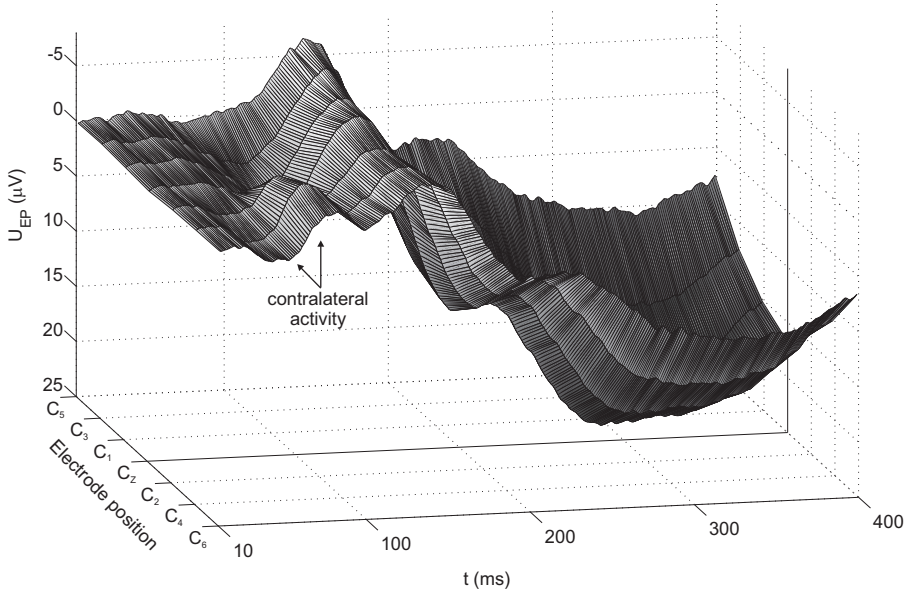

Figure 1.6: Grand average EPs (mean of 27 subjects) of 7 neighbouring electrodes at the central line of the scalp. Data is interpolated. EPs measured after electrical stimulation at the fingertip. Electrodes C2, C4 and C6 are situated contralateral to the stimulation site. Clear early (before 100 ms) lateralised activity at contralateral electrodes. Unpublished data of earlier research [151].

In figure 1.7A shows the electrode positions of 64 electrodes placed according to the international 10-5 system. The scalp distribution of evoked activity measured with the 64 channels EEG is illustrated in figure 1.7B. The scalp distribution at 88ms shows focal negative activity around electrode C_4 (contralateral to the side of stimulation). This is in accordance with activity around 90 ms in figure 1.6.

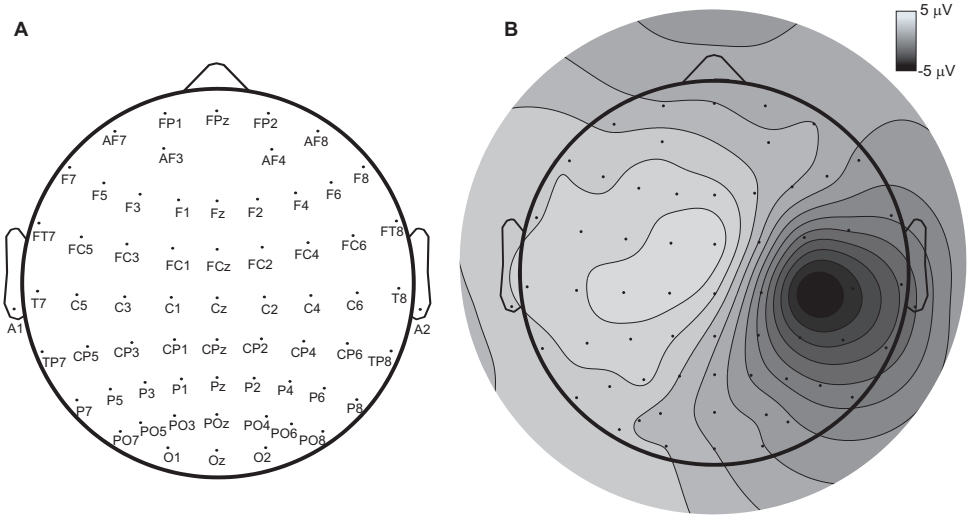

Figure 1.7: Electrode placement of 64 EEG electrodes according to the international 10-5 system (A). Figure B shows scalp potential distribution at 88ms of grand average EP (mean of 24 subjects) following electrical stimulation at the left fingertip.

1.4.2 Source modelling

The potential distribution measured at the scalp surface (such as Figure 1.7) results from one or more intracerebral (equivalent) dipole current sources (neural assemblies). The prediction of the location and strength of the sources of a measured potential distribution is the so-called inverse problem [74]. Several methods are developed to calculate the inverse solution of the scalp distribution based on models of the head and scalp [74]. The inverse problem has no unique solution, already described by Helmholtz in 1853. The inverse problem can fit different sets of sources on the same scalp distribution [98]. A priori constraints can help to decrease the number of solutions. The solutions can be limited to a maximum number of sources and an approximate location [74].

The dipole source localisation method is commonly used to calculate equivalent current dipoles in a volume conductor head model [28; 108]. Due to the non-uniqueness of the inverse problem a single dipole localisation is preferred [98]. The accuracy of the dipole fit can be evaluated by for example the goodness of fit procedure. The goodness of fit is

a measure of the percentage of variance in the potential distribution that can be explained by the potential distribution of the calculated dipole.
The accuracy of the source localisation depends on different measurement and modelling aspects. The head model used for this purpose is of great importance. The realistic head model consists of three compartments: the cortex, skull and scalp. Each has different conductivities. The boundary element method is used to provide a more accurate electrical model of the head [28; 45; 98]. The dimensions of the three compartments are related to the anatomy of the real head. Although a realistic head model gives more accurate source locations than other used models, such as the simplified spherical head model, still one must be cautious about localisation errors introduced. For example, the assumptions that are made about the conductivity, skull and scalp thickness [27; 28]. Besides these assumptions also contamination of the EEG signal by noise can result in localisation errors [157]. Deep cortical sources are more affected by noise than sources near the surface [165].

1.5 Goal of this thesis

To further explore the changes in the nociceptive system playing a role in chronification of pain adequate measurement techniques are required. The aim of this work is to explore the merits of electrocutaneous SP and PT stimulation as observation techniques of the nociceptive system. Using EEG the central processing of the SP and PT stimuli will be analysed, especially EPs measured at the vertex and contralateral electrode. Source localisation techniques can increase our knowledge about parts of the brain involved in the generation of the EPs. We are especially interested in which parts of the brain are involved in the generation of early contralateral activity. To analyse the relevance of both methods to explore changes in the central pain processing it is important to perform measurements with patients suffering from pain. Due to the clear cause of the pain complaints we choose to measure patients suffering from lumbosacral radiculopathy (LSR). Activation of by tonic stimulation methods such as the CPT can induce inhibition by the DNIC. We are interested if the CPT influences the central processing of the SP and PT stimuli in healthy subjects. A dysfunctional DNIC has been demonstrated in for chronic pain syndromes but is absent in others. Although it is unknown if the DNIC is dysfunctional in patients with LSR we are interested if we can measure differences concerning the effect of the DNIC between healthy subject and patients.

1.6 Outline of this thesis

First, both the SP and PT method will be introduced in chapter 2; the effect on subjective ratings and EPs is investigated and compared. In chapter 3, differences between the two methods are further analysed by using a heterotopic noxious stimulation (CPT). In this

chapter, the effect of CPT on the processing of SP and PT on EPs and subjective rating was evaluated. Next in chapter 4, a dipole source localisation technique was used to investigate which brain regions were involved in the generation of the early contralateral component around 90 ms. In the measurements of chapter 2 to 4 only healthy subjects were included. In chapter 5, the processing of SP and PT stimuli and the effect of CPT was studied in patients with lumbosacral radiculopathy. The results were compared with data of healthy subjects. Finally, in chapter 6 all results are considered in a general discussion.

Chapter 2

Single pulse and pulse train modulation of cutaneous electrical stimulation: a comparison of methods

Abstract - Changing the amplitude of single rectangular pulse stimuli (SP) has the disadvantage of recruiting tactile and nociceptive fibers in a changing, unknown proportion. Keeping the amplitude constant, but applying a varying number of pulses in a train is another way of stimulus variation, keeping the proportion constant. So, pulse trains (PT) with a variable number of pulses (NoP) but fixed amplitude might be more suitable to study nonperipheral aspects of processing of stimuli. In this study, we compared the effects of PT and SP stimulation on subjective Numeric Rating Scale (NRS) scores of perceived stimulus strength and evoked potentials (EP). A total of 41 healthy subjects were electrically stimulated at the left forearm or left middle fingertip using SP and PT stimuli. NRS scores and EPs were averaged from 105 randomized stimuli at 5 stimulus amplitudes or NoP for each subject. The relationships between stimulus amplitudes or NoP, EP components and NRS scores differed depending on the stimulation method and stimulus location. Although the repeatedly reported NRS-EP (N150-P200) correlation was reproduced for SP at the fingertip, no significant correlation was found for SP stimulation at the forearm. For PT this correlation was found for both stimulus locations. These findings demonstrate that SP and PT involve different ways of processing. The two methods result in different NRS scores and EP components. Furthermore, PT stimulation is less dependent on stimulus location

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2.1 Introduction

In both clinical and fundamental pain research, peripheral and central changes in neural functions are widely acknowledged to play a key role in chronifying pain [1; 24; 170]. However, observation of the underlying neurophysiological mechanisms remains difficult.

Perceived pain strength, for example reported by the Numeric Rating Scale (NRS), is frequently used for measuring the subjective pain experience. Yet, for understanding the mechanisms of pain the subjective pain experience is not sufficient and neurophysiological measures are required. Therefore, in several studies evoked potentials (EPs) are used to measure cortical activations that reflect central processing of noxious stimuli, applied using thermal energy (laser or contact heat) or electrical current (see for reviews : [13; 66; 142]) .

The measured peak-to-peak EP amplitudes appear to correlate with subjectively reported pain intensities: subjective ratings of identical stimuli are correlated to EP components and peak-to-peak EP amplitudes [56; 91]. Other studies have used different stimulation strengths e.g. in order to evaluate if generated EPs could be related to subjective ratings [19; 69] or to explore the differences in activation of cortical areas by changing stimulus amplitudes [140]. Naturally, well defined stimuli are essential for such studies with varying strength.

Laser stimulation permits selective stimulation of cutaneous nociceptive fibers at different locations on the body [13; 66; 68]. In some studies the stimulus strength is modulated by varying the power of a laser pulse [103; 109; 137]. In other studies, increasing the duration of laser stimuli resulted in a linear increase of subjective ratings and EP components and besides a relationship between peak-to-peak amplitudes and subjective ratings was reported [69]. On the other hand, Chen et al [18] showed that subjective ratings of contact heat are not only changed by increasing energy levels but also by increasing the area of stimulation. In spite of these merits of heat stimulation, a disadvantage of laser stimulation is receptor fatigue and peripheral sensitisation, both disturbing the transduction of stimulus power into neural activity [2].

Intracutaneous electrical stimulation (IES) [14] was also commonly used to evoke pain sensations. An advantage of electrical stimulation over heat stimulation is a good control of timing of neural activation. In most studies using different electrical stimulation strengths, the amplitude of a single pulse is varied (see e.g. [17; 133]). Often a linear increase of subjective ratings and modulated EP amplitudes or peak-to-peak amplitudes by a changing stimulus amplitude was reported [17; 19; 44]. Conversely, multiple pulses of equal amplitude are also perceived stronger and are applied even in combination with changing stimulus amplitudes [15; 35; 61]. Additionally, a train of increasing numbers of pulses resulted in increased subjective ratings, and tend to

saturate for higher levels [47]. However, since both nociceptive and tactile afferents are activated, electrical stimulation became less popular after introduction of laser stimulation. To improve electrical selective stimulation, an alternative method of electrical stimulation, using a pushpin-like needle electrode (epidermal stimulation, ES), has been introduced recently, which preferentially stimulates Aδ-fibers [59].

From the above it follows that changing the stimulus strength of both thermal and electrical stimulation results in modulation of the neural activity. This activity can be modulated in two different manners: spatially and temporally. By increasing the area of a thermal stimulus more receptors are activated resulting in a changing number of activated fibers. Increasing the stimulus amplitude of a single electrical pulse enlarges the area of recruitment resulting in a similar change in activation. However, due to unknown local distribution of tactile and nociceptive fibers, these fiber types are activated in variable and unknown proportion with changing electrical stimulus amplitude. This proportion is largely unknown in most skin areas. Interaction between activated fibers, e.g., in the dorsal horn (gate control theory) [96], may result in differences in perceived stimulus strength. Conversely, the activity can be changed by temporal modulation of the amount of neural activity in a relative constant proportion of fibers. Due to the coding mechanisms of the receptor, varying the thermal energy results in fibers firing more action potentials with a higher frequency [70]. It is widely acknowledged that electrical stimuli directly activate neural fibers instead of skin receptors [13; 64]. Hence, by increasing the number of pulses (NoP) in a train of pulses with fixed amplitude more action potentials are generated in a constant number of activated fibers (with an inter-pulse interval larger than the refractory period). Although with ES Aδ-fibers are stimulated more preferentially, an increase of stimulus amplitude would result in a changing distribution of activated fibers. Yet, by changing the NoP in a train this could be improved, leading to a well defined varying electrical stimulus strength.

Both spatial and temporal modulations of neural activity change subjective ratings and EPs. The question arises if both modulations are equivalent and cause similar effects. A linear relationship between subjective ratings and stimulus strength was shown for increasing stimulus amplitude [19] but in contrast a non-linear relationship was reported for change of number of electrical pulses in a train [47]. Besides, an EP component showed non-linear modulation for an increasing thermal pulse length [69] whereas increasing electrical stimulus amplitudes changed EP components linearly [19]. A systematic comparison between the two modulations has not been reported before.

Therefore, the objective of this study was to investigate differences in subjective ratings and EP components by spatial and temporal modulations using electrical stimuli. The neural activity will be modulated in two manners; by changing the stimulus amplitude of a single pulse (SP) or by changing the NoP in a pulse train (PT).

In the present study a similar electrode was used as in IES stimulation. The differential effect of both SP and PT on stimulus processing was evaluated using two response scores: subjective pain rating scale (NRS) scores and both contralateral and vertex EP component amplitudes.

2.2 Methods

2.2.1 Subjects

A total of 36 right-handed, healthy female subjects (age 22.51 ± 2.81) participated. All subjects gave written informed consent according to the Declaration of Helsinki. The study was approved by the ethical committee of the Academical Hospital Maastricht.

2.2.2 Electrical stimulation

The subjects were electrically stimulated at the left anterior lateral forearm or left middle fingertip. Stimulation at the fingertip corresponds to the IES method [14]. We expected that PT is less sensitive to the local fiber distribution compared to the SP method. Hence, stimuli were also applied at the forearm where the local fiber density is lower [22; 106] and the distribution different.
An electrode with a 1 mm diameter tip of gold in an insulating material was used. A small opening was drilled in the upper layer of the skin of the fingertip using a dental gimlet with the same diameter as the tip of the stimulation electrode [14]. If the sensation threshold (I_S) was higher than 1 mA the preparation was regarded insufficiently and tried again. As no thick horn layer is present at the forearm, no special preparation was required there. A rectangular surface electrode (a 4x9 cm Klinerva Blue Electrode) was placed with a distance of at least 10 cm at the upper part of the left forearm as an anode. The stimuli were generated by a battery-driven computer controlled current stimulator. The stimulus was a current bipolar rectangular pulse with a stimulus duration of 0.2 ms. Such a stimulus produces a clear pinprick sensation. The electrode was placed in a way that all subjects reported a mild pricking sensation at I_S.

2.2.3 Sensation and pain threshold

For each subject, the stimulus amplitudes corresponding to the subjective I_S and pain threshold (I_P) were determined before a protocol. Thresholds were obtained by the ascending method of limits by increasing the stimulus amplitude with steps of 0.1 mA starting at a level of zero. Mean I_S and I_P for both electrode locations are shown in table 2.1.

Table 2.1: Ranges and means (± SD) of I_S and I_P for the forearm (N = 26) and fingertip (N = 30). Means obtained of all subjects of the four groups.

	I_S (mA)		I_P (mA)	
Location	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Fingertip	0.1 – 1.0	0.46 ± 0.26	0.5 – 3.3	1.76 ± 0.72
Forearm	0.1 – 1.3	0.47 ± 0.26	0.7 – 4.0	2.06 ± 0.74

2.2.4 SP method

For SP, the stimulus amplitude of a single pulse was varied depending on the obtained I_S and I_P (see equations below).

- $I_{-50\%} = I_P - 0.50 \cdot (I_P - I_S)$ (2.1)
- $I_{-25\%} = I_P - 0.25 \cdot (I_P - I_S)$ (2.2)
- $I_{0\%} = I_P$ (2.3)
- $I_{+25\%} = I_P + 0.25 \cdot (I_P - I_S)$ (2.4)
- $I_{+50\%} = I_P + 0.50 \cdot (I_P - I_S)$ (2.5)

In anticipation of habituation effects [99], the minimum stimulus amplitude was set in between I_S and I_P. Decreasing the amplitude further below this minimum stimulus amplitude would probably result in large numbers of unperceived stimuli.

2.2.5 PT method

The fixed stimulation current for PT was chosen similar to the minimum stimulus amplitude $I_{-50\%}$ of SP (equation 2.1). Since we used an IES electrode, selective stimulation of nociceptive afferents (Aδ-fibers) alone is probably not possible. To activate Aδ-fibers as selective as possible we therefore chose the minimum stimulus amplitude of SP as stimulus amplitude of PT.

The NoP for PT varied from 1, 3, 5, 7, to 9 pulses. The inter-pulse interval (IPI) between two subsequent pulses in the pulse train was 5 ms. With 5 ms IPI, i.e., well outside the refractory period, fibers have enough time to regenerate. To make sure that stimulation by PT was tolerable, the five NoP were applied in increasing order before the protocol. Although the stimulus amplitude of the single pulse of PT stimulus was below the subjective pain threshold, subjects described stimulation by a train of five pulses as a clear pricking painful sensation.

2.2.6 EEG recordings

Electrical brain activity was continuously recorded using a 64-channel EEG Refa-72 system (ANT, the Netherlands). Ag/AgCl electrodes were placed according to the

international 10-5 system (Waveguard EEG cap). All scalp electrode impedances were less than 5kΩ. The ground electrode was placed at the forehead. An electrode was placed above and under the left eye for electrooculogram (EOG) recording. Furthermore, subjects were instructed to fix their eye on a point in front of them. Data recorded at C_Z referred to linked earlobes (A_1A_2) and data recorded at C_4 referred to F_Z were analysed. EEG was recorded at a sample frequency of 1 kHz. The signals were filtered offline at band-pass 0.3-120 Hz. Data from -10 up to -100 ms pre-stimulus was used for baseline correction. The time window of analysis was 100 ms pre-stimulus to 400 ms post-stimulus. EEG data was recorded using ASA software (ANT software BV, the Netherlands) and data analysis was performed in Matlab®.

2.2.7 Numeric rating scale

Subjects were asked to rate orally the perceived strength of each stimulus on an 11 point NRS. Zero corresponded to "no sensation" whereas 10 corresponded to "strongest imaginable pain". The first stimulus corresponded for SP with the pain threshold $I_{0\%}$ (equation 2.3) and for PT with a train of 5 pulses at $I_{-50\%}$ (equation 2.1). The subjects were instructed to rate the first stimulus with a six.

2.2.8 Procedure

Four experiments consisting of two protocols were performed. In table 2.2 the four experiments and sample sizes can be found. In each protocol, one combination of stimulus location and stimulation method was tested. The order of protocols was randomized in each experiment.

Table 2.2: Composition of 4 experiments (exp) each consisting of 2 protocols. A protocol is a combination of stimulus location and modulation method. The number of subjects of each protocol in an experiment is shown.

	SP	**PT**
Forearm	exp 1 (N = 6)	exp 1 (N = 6)
	exp 4 (N = 11)	exp 2 (N = 9)
Fingertip	exp 4 (N = 11)	exp 2 (N = 9)
	exp 3 (N = 10)	exp 3 (N = 8)

A protocol consisted of a total of 105 stimuli with 21 stimuli for each of the five stimulus amplitudes (SP) or five NoP in a pulse trains (PT). The stimuli were applied semi-randomly. The inter stimulus interval between two successive stimuli was varied randomly between 10 and 12 seconds.

2.2.9 Data analysis

Grand average EPs (C_Z-A_1A_2, C_4-F_z,) were obtained of each of the five stimulus amplitudes or NoP of all protocols. First, trials with an EOG artefact exceeding ±100μV in a time window of -10 to -100 ms pre stimulus and 60 to 400 ms post stimulus were rejected. Subsequently, the accepted data was visually inspected for missed EOG artefacts and muscular artefacts. At least 11 trials should be accepted for each of the five subject EPs obtained in a protocol. If one of the 5 subject EPs had fewer than 10 accepted trials, the subject was excluded from analysis of the concerning protocol.
Furthermore, mean NRS scores were obtained at all five stimulus amplitudes (SP) or at all five NoP (PT).
To allow pooling of the data (NRS scores and EPs) of subjects participating in identical protocols in different groups, the data were statistically tested for difference using a one-way Analysis of Variance (ANOVA). Furthermore, the one-way ANOVA was used to analyse the difference between the I_S and I_P of stimulation at the forearm and finger.
For each protocol both NRS scores and prominent EP component amplitudes were analysed against stimulus amplitudes or NoP, using one-way ANOVA. We analysed the following EP components, recorded at C_Z-A_1A_2: P90 at 90 ms, P300 at 290 ms and N150-P200 peak-to-peak EP amplitude with N150 at 140 ms and P200 at 190 ms. Furthermore we analysed EP components recorded at C_4-F_Z and C_3-F_Z: P50 at 50 ms, N90 at 90 ms. A linear regression analysis was performed to determine the correlation between NRS scores and EP components and N150-P200 peak-to-peak amplitude.
The effect of stimulus location was analysed by using recorded EPs at the minimum stimulus amplitude (equation 2.1) of experiment 2 and 4. In these experiments subjects were stimulated at both fingertip and forearm with SP or PT. Since the minimum stimulus amplitude was equal for both, the EPs (C_4-F_Z and C_3-F_Z) were statistically tested for the effect experiment using a one-way ANOVA. Subsequently, the data of the experiments was pooled and the effect of stimulus location on the early contralateral P50 and N90 was statistically tested with a repeated measured ANOVA.
All statistical tests were performed at a level of significance $p < 0.05$.

2.3 Results

2.3.1 NRS scores of SP

Mean NRS scores were obtained for each of the five stimulus amplitudes by SP. The mean NRS scores for stimulation at the fingertip and the forearm are shown in figure 2.1A. A linear relationship was found between stimulus amplitude and NRS score for both locations. The effects were significant (fingertip: $F(4,100) = 26.45$; $p < 0.0001$ and forearm: $F(4,80) = 26.76$; $p < 0.0001$).

2.3.2 NRS scores of PT

Mean NRS scores were obtained for each of five NoP. The scores are shown in figure 2.1B. The relationship between NRS scores and NoP was comparable for both stimulus locations. The effect was significant (fingertip: $F(4,80)=28.13$; $p < 0.0001$ and forearm: $F(4,70) = 13.44$; $p < 0.0001$).

2.3.3 Effect of stimulus location

Figure 2.2A shows the pooled grand average EPs (C_4-Fz) for stimulation at the fingertip and forearm. These grand averages were obtained by pooling the data of both SP and PT at the minimum stimulus amplitude.

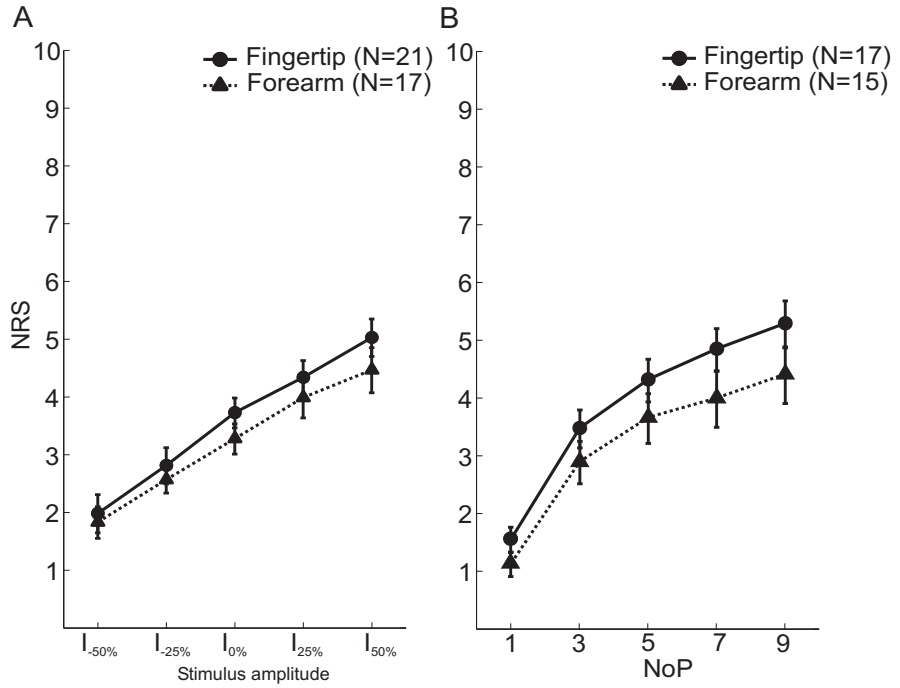

Figure 2.1: Mean NRS scores (±SEM) of all five stimulus amplitudes for SP (A) and all five NoP for PT (B) for stimulation at the forearm and fingertip. Each symbol represents the mean NRS score of all accepted sweeps of all included subjects at the stimulus amplitude or NoP under test.

Stimulation at the fingertip resulted in a clear positive peak around 50 ms (C_4-F_Z), significantly different from the potential for stimulation at the forearm ($F(1,19)=13.55$; $p<0.002$). Furthermore, the N90 (C_4-F_Z) was significant different for stimulus location ($F(1,19)=9.41$; $p<0.006$). Besides C_4-F_Z we also analysed pooled grand average EPs measured at C_3-F_Z (figure 2.2C). For potentials measured at the ipsilateral electrode C_3 versus F_Z no significant difference for stimulus location was found.

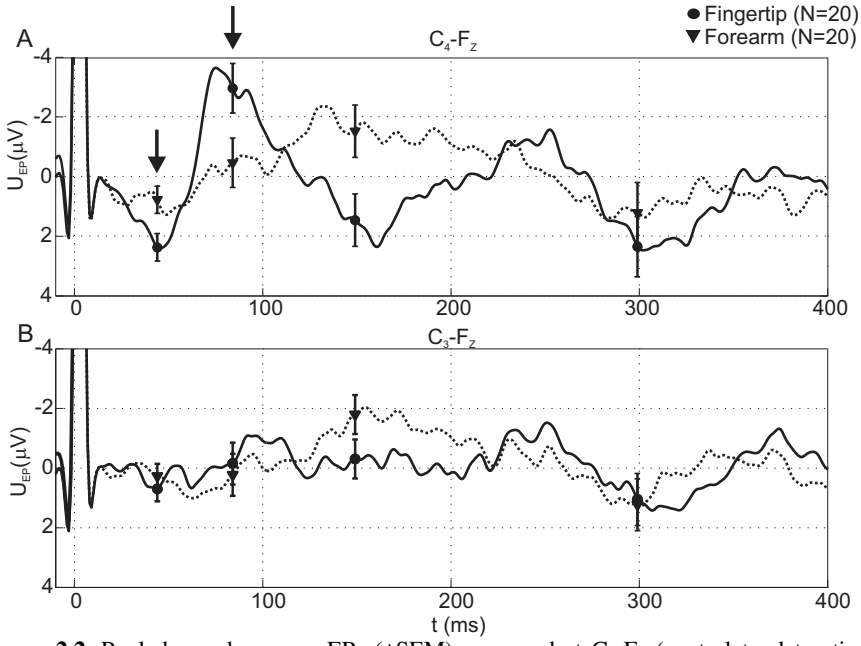

Figure 2.2: Pooled grand average EPs (±SEM) measured at C_4-F_Z (contralateral to stimulus location) (A) and at C_3-F_Z (ipsilateral to stimulus location) (B) for both stimulus locations. Data was pooled of experiment 2 and experiment 4 of both the SP and PT method for stimulation with a single pulse at minimum stimulus amplitude $I_{50\%}$. Significant effect ($p < 0.05$) indicated by an arrow.

2.3.4 EPs of SP

Grand average EPs (C_Z-A_1A_2) of five stimulus amplitudes for stimulation at the fingertip and forearm are shown in figures 2.3A and 2.3C respectively. For both stimulus locations, the relationship between the P300 EP component amplitude and stimulus amplitude (see figure 2.3E) was comparable to the relationship between NRS and stimulus amplitude (figure 2.1). Increasing stimulus amplitude resulted in increasing EP component amplitude. The effect of stimulus amplitudes on the P300 EP component was only significant for stimulation at the fingertip (fingertip: $F(4,100)=5.31$, $p<0.0001$ and forearm $F(4,80)=2.15$, $p=0.082$). Furthermore, stimulus amplitude had no effect on N150-P200 for both fingertip ($F(4,100)=1.25$, $p=0.30$) and forearm ($F(4.80)=0.16$, $p=0.96$).

2.3.5 EPs of PT

Figures 2.3B and 2.3D show grand average EPs (C_Z-A_1A_2) of all five NoP for stimulation at the fingertip and the forearm. A stimulation artefact can be distinguished during the first milliseconds of the EPs, lasting up to 45 ms for stimulation with 9 pulses.

Although the stimulus duration increases with the NoP, latency shifts of the EP components did not follow accordingly (significance not tested).
A significant modulation of the amplitudes the P300 EP component by the PT method was observed for both stimulus locations (fingertip: $F(4,80)=4.11$, $p<0.0044$ and forearm $F(4,70)=7.14$, $p<0.0001$). Figure 2.3F illustrates the relationship between P300 EP component amplitudes and the NoP in a pulse train. Again it was comparable with the relationship between NRS and the NoP. The effect of NoP on EP components was also significant for N150-P200 peak-to-peak EP amplitude for both fingertip ($F(4,80)=3.73$, $p=0.0078$) and forearm ($F(4.70)=2.69$,$p=0.038$).

2.3.6 EPs C_4-F_Z for stimulation at the fingertip

In figure 2.4 the grand average EPs recorded contralaterally at C_4-F_Z for both SP and PT are shown, for stimulation at the fingertip. For the PT method, a significant effect of increasing NoP in a pulse train on the EP amplitude appears for the N90 ($F(4,80)=3.60$, $p=0.009$)**.** For SP no significant effect on N90 was obtained ($F(4,100)=0.16$, $p=0.96$). For the SP method the effect of stimulus amplitude on only the P50 EP amplitude (C_4-F_Z) was significant ($F(4,100)=3.47$, $p=0.01$).

2.3.7 Correlation NRS-EP

For both SP and PT method and for both stimulus locations, the relationship between the measured NRS scores and the EP components (measured at C_Z-A_1A_2) was tested by a linear regression analysis. The P300 EP component and the N150-P200 peak-to-peak amplitude were tested. Figures 2.5A and 2.5B show the correlations. For both SP and PT at both stimulus locations a significant relationship between P300 EP amplitude and NRS can be seen. It is notable that for the N150-P200 peak-to-peak amplitude no relationship was found for SP at the forearm whereas this relationship was found for SP at the fingertip and PT at both stimulus locations.

2.4 Discussion

In the current study, a comparison between SP and PT stimulation methods was performed. Both methods, applied at fingertip as well as forearm, influence NRS scores and EP components in specific ways, as discussed below.

2.4.1 Effect of SP and PT on NRS scores

A linear relationship was found between SP stimulus amplitudes and NRS scores (figure 2.1A). Linearity was also reported by Chapman [17] for electrical SP stimuli at the fingertip and by Chen [19] for dental stimuli. Furthermore, linearity showed up for power modulated laser stimuli at the dorsum of the left hand [21; 69; 103; 109].

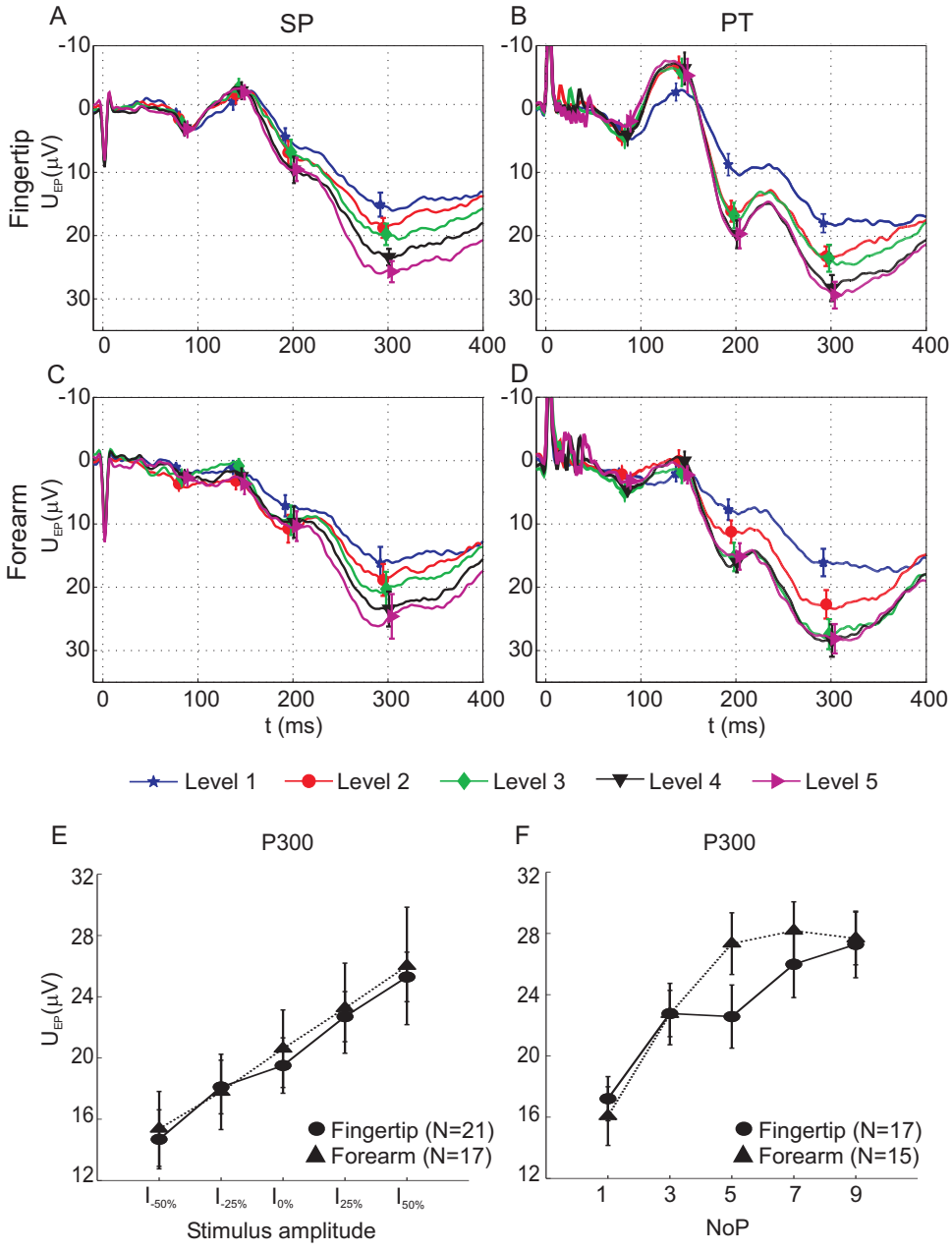

Figure 2.3: Grand average EPs (±SEM) measured at C_Z-A_1A_2 of five stimulus amplitudes for stimulation at the fingertip (A) and forearm (C) by SP. Grand average EPs (±SEM) measured at C_Z-A_1A_2 of five NoP for stimulation at the fingertip (B) and forearm (D) by PT. Amplitude (±SEM) of P300 EP component measured at C_Z-A_1A_2 for SP (E) and PT (F) for stimulation at the fingertip and forearm. The levels mentioned in the figure correspond to stimulus amplitudes (SP) or NoP (PT).

The PT method yielded a curved relationship (figure 2.1B). In literature, only Giffin [47] presents comparable results, for electrical PT stimuli at the forehead.

2.4.2 Relationship between NRS scores and EP components

The relationship between NRS scores and (peak-to-peak) EP amplitudes (figure 2.5) is reported for several stimulation methods at different locations at the body. The reports pertain not only to modulated stimuli [19; 67; 69] but also to stimulation with identical stimuli [6; 56].

P300

For all four protocols P300 and NRS showed a linear dependency. This suggests that the P300 can be used as a neurophysiological correlate of the subjective perceived stimulus strength. However, P300 not only reflects sensory processing but also cognitive processes like attention/distraction [5; 123; 172]. It should be noted that in our experiment attention to the stimulus is controlled by the task to rate each stimulus. Therefore, this cognitive component is similar for all protocols and all stimuli. Thus this cognitive component does not influence the P300.

N150-P200

In this study NRS scores varied almost linearly with N150-P200 peak-to-peak amplitudes, for PT at both stimulus locations. Furthermore, variation was also found for SP at the fingertip, but not for SP at the forearm. An explanation for the latter can be sought in differences in local fiber density and distribution, at fingertip and forearm. Modulation by SP changes the recruitment and proportion of the activated fiber types (tactile and nociceptive) in the skin depending on local fiber density and distribution. The local fiber density is larger at the fingertip than at the forearm [22; 72; 86; 106; 112].

2.4.3 Effect of SP and PT method on N150-P200 (C_Z-A_1A_2)

For both stimulus locations, the N150-P200 peak-to-peak amplitude (C_Z-A_1A_2) varied under the influence of PT stimuli, but not by SP stimuli (figure 2.3A-D). No significant change of EP amplitude by SP was found for stimulation at the fingertip, which is contrary to earlier studies using SP with IES at the fingertip [44; 100]. This might be attributed to differences in stimulation charge in (mA·s). The small stimulation charges in our study might not have resulted in significant effect of stimulus amplitude.

On the other hand a clear change in amplitude was found by PT stimulation at the fingertip and forearm. Notably, this change was obtained using pulse trains at minimum stimulus amplitude ($I_{-50\%}$).
The absence of N150-P200 variation with SP is remarkable. For SP a sufficient change in number and proportion of activated fibers should result in changing N150-P200 amplitudes. Possibly, the differences between the five stimulus SP amplitudes in the current study may not have been sufficient.

2.4.4 Effect of SP and PT method on P300 (C_Z-A_1A_2)

The P300 EP amplitude (C_Z-A_1A_2) varied along with SP variation (only fingertip stimulation) as well as with PT variation (at both stimulus locations). Although SP at the forearm did not show significant modulation a linear increase of EP amplitude with stimulus amplitude was found (figure 2.3E). Inui reported a P300 EP component, for both preferential Aδ stimulation and non-noxious electrical stimulation, using a larger electrode [59]. The P300 may also reflect cognitive processes (see section 2.4.2).

2.4.5 Contralateral EP components P50 and N90

The P50 and N90 EP components are clearly represented in EPs measured at the contralateral electrode C_4 versus F_Z. The EP amplitude of these two components was sensitive for SP or PT stimulation. The relationship between EP component amplitude and stimulus amplitude or NoP was similar to that between NRS and stimulus amplitude or NoP.

Effect of stimulus location
The effect of two stimulus locations on EPs was tested, at minimum stimulus amplitude $I_{-50\%}$. EPs measured at the contralateral electrode (C_4-F_Z) showed significant effects of different location for the P50 and N90 component (figure 2.2B). An explanation for this may be that differences in local fiber density, as present between fingertip and forearm, result in different distributions and number of stimulated afferents. Furthermore, both stimulus locations have a different cortical representation (somatotopic organisation) which may also lead to different EP shapes and amplitudes.

Effect of SP and PT method on P50
The early P50 EP component (C_4-F_Z) amplitude was significantly sensitive for SP at the fingertip. This was not observed for PT at the fingertip, although a clear peak is present (figure 2.4, due to stimulation artefacts modulation of P50 could only be tested for 1, 3 and 5 pulses (data not shown). Incoming Aβ information, which is fast in the periphery [70] and relayed to the fast dorsal column-medial lemniscus [32; 92; 168], is held responsible for P50.

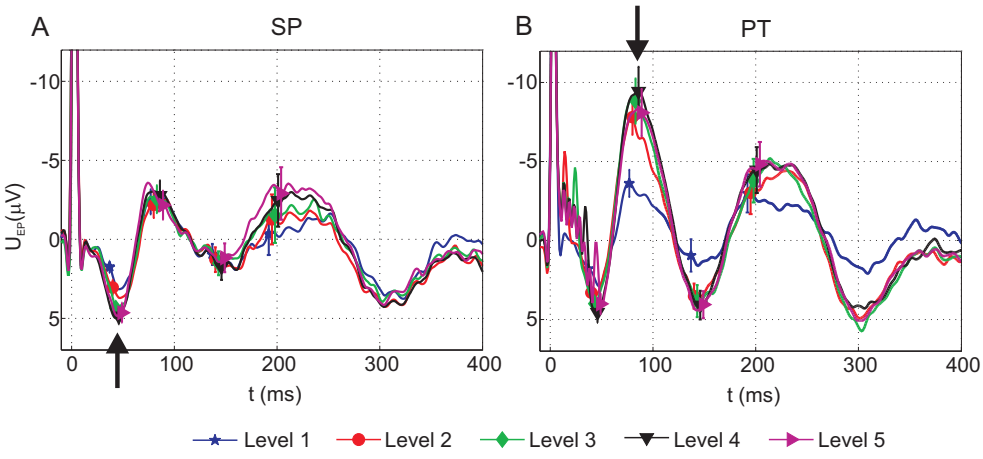

Figure 2.4: Grand average EP (± SEM) measured at the contralateral electrode (C_4-F_Z) of all five stimulus amplitudes by SP (A: N=21) and all five NoP by PT (B: N=17) for stimulation at the fingertip. Significant effect ($p < 0.05$) indicated by an arrow. The levels mentioned in the figure correspond to stimulus amplitudes (SP) or NoP (PT).

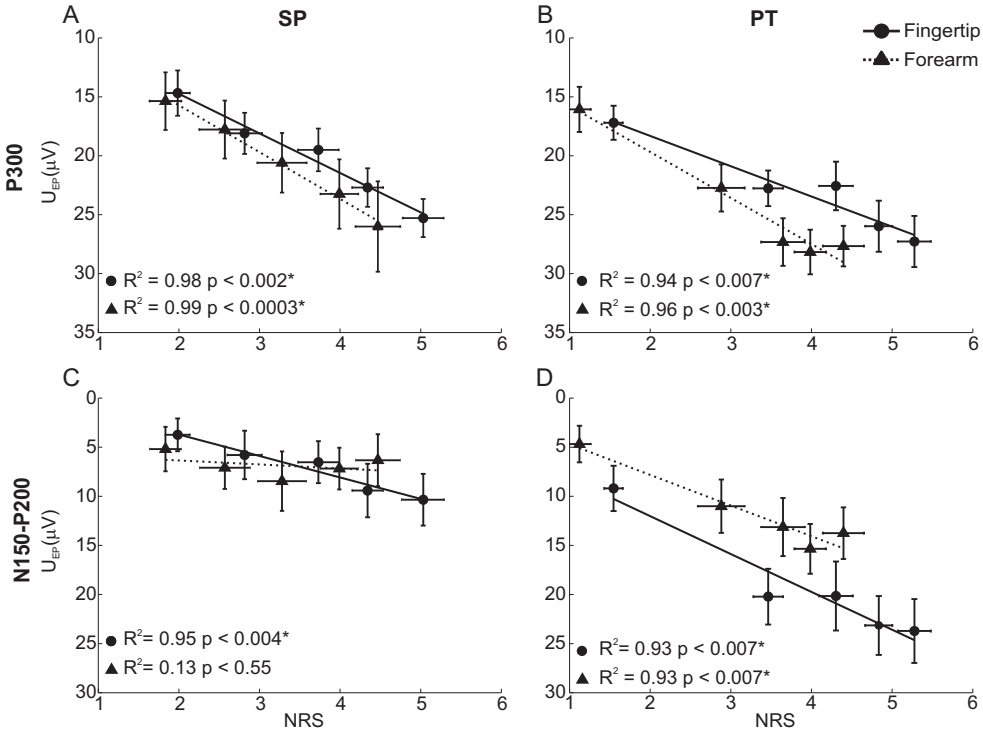

Figure 2.5: Linear regression analysis for correlation between NRS scores (±SEM) and amplitudes EP components (±SEM) measured at the vertex (C_Z-A_1A_2) for both SP (A: N=21, C: N=17) and PT (B: N=17, D: N=15) at the fingertip and forearm. Correlations are shown for both P300 amplitude (A, B) and peak-to-peak amplitude N150-P200 (C, D).

The P50 was also reported following mechanical pulses or vibration [51]. The changing P50 amplitude for SP at the fingertip is probably a result of an increasing number of activated Aβ-fibers, resulting in increased neural activity.
No significant change in P50 amplitude was found at the forearm, both for SP and PT (data not shown). The effect of stimulus location ascribed above may explain the differences in P50 potentials between fingertip and forearm.

Effect of SP and PT method on N90
Contrary to the P50, the amplitude of the N90 (C_4-F_Z) wave did not significantly change for SP at the fingertip. At the forearm, no significant change in N90 amplitude was found for both SP and PT (data not shown).
However, for PT at the fingertip the EP amplitude changed distinctly with NoP (figure 2.4). Furthermore, although the total stimulus duration increases with NoP, no latency shift was observed for the N90 latency with NoP.
In several studies, N90 potentials are observed after mechanical stimulation as well as non noxious electrical stimulation [51; 60; 62]. Hence, at least Aβ activity is likely to be involved in N90 generation. Using preferential Aδ activation with epidermal stimulation Inui reported SI activity starting around 93 ms [60]. Recently Wang [164] showed that laser stimulation evokes potentials peaking around 109-119 ms but with onset latencies of 88-105 ms. In their study a new analysis method was used taking into account latency jittering. The N90 in the current study could be associated with (interactive) processing of Aβ and/or Aδ activation.

2.4.6 Conclusions

The current results show that SP and PT stimulation act differently on EP components, at different stimulus sites. Some EP components varied only by one of both methods and some by both. SP changed the amplitude of P50, at the fingertip. For SP variation of NRS with N150-P200 was observed only for stimulation at the fingertip. The amplitude of the N90 EP component changed only under PT stimulation at the fingertip. PT results for both stimulus sites in similar relationships between NRS and N150-P200 peak-to-peak EP amplitudes. Stimulation at different locations of the body can be useful to research cortical reorganization in chronic pain patients [42].
The used stimulation electrode activates both Aβ and Aδ fibers. Nevertheless, at I_S subjects reported a pricking sensation indicating the activation of nociceptive fibers. So, although the stimulus amplitude of PT was chosen below the subjective pain threshold, yet Aδ-fibers were activated.
Increasing the stimulus amplitude in the SP method increases the number of activated fibers depending on local fiber density and distribution. The change in proportion of activated Aβ-fibers and Aδ-fibers by SP is unknown, resulting in an unknown change in neural activity. PT is a more controlled method, keeping the proportion of activated

nociceptive and tactile fibers constant and giving better temporal control of neural activity.

It was shown that the PT method results in comparable results as the SP method; they both change EP components and subjective ratings. Yet, the seemingly saturating modulation by PT and difference in modulation of the N90 is remarkable. The question arises if parts of the nociceptive system are involved in the N90. Further research is required to interpret the obtained differences in modulation by SP and PT in terms of neurophysiological mechanisms.

Chapter 3

Effect of cold pressor on electrocutaneous stimuli: N90 reflects spinothalamic activity

Abstract - Recently, we showed that single pulse (SP) and pulse train (PT) electrocutaneous stimuli at various strengths influence subjective ratings and evoked potentials (EP) components differently. Especially, the change of contralateral N90 by PT but not by SP was remarkable. The involvement of parts of the nociceptive system in this potential was questioned. The cold pressor test (CPT) as a noxious modulation stimulus might give further insight in the differences in processing. CPT evokes a diffuse noxious inhibitory control (DNIC) effect. Here, we analysed the effect of the CPT on the processing of SP and PT stimuli in healthy subjects using subjective pain ratings and EPs. Healthy subjects were electrically stimulated at the left middle fingertip during two protocols where the right hand was immersed in water of 0-1°C (CPT) or 32°C (control). Subjects had to withdraw and re-immerse their hand after subsequently 3 and 1 minute until the end of the protocol. Grand average EPs and numeric rating scale (NRS) scores were averaged from 105 stimuli of 5 stimulus amplitudes (SP) or number of pulses (PT). For both SP and PT, NRS scores and EP component amplitudes decreased by CPT. The different effects for SP and PT were not changed by CPT. Inhibition by CPT might be ascribed to activation of endogenous pain modulation by DNIC. For PT, the N90 was decreased by CPT but for not SP. The results suggested involvement of the spinothalamic tract in the N90 by PT. PT might be useful as a tool to further explore changes in the pain system.

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Submitted for publication

3.1 Introduction

Various observation techniques are used to study pain processing in man. This includes the use of evoked potentials (EPs) to measure cortical activations reflecting central processing of pain. Recently, we compared single pulse (SP) and pulse train (PT) electrocutaneous stimulation at the fingertip and forearm [152]. Both nociceptive (Aδ) and sensory (Aβ) fibers are activated by electrical stimuli. Changing the stimulus amplitude by SP results in a change in the proportion of nociceptive and sensory fibers activated, also depending on local fiber densities. Increasing the number of (fixed amplitude) pulses (NoP) in a train increases activity in a fixed proportion of fibers. Using the intracutaneous electrical stimulation (IES) method [14], we showed that both SP and PT influence subjective ratings as well as EP components, but differently. The vertex P300 was modulated by both methods, while the contralateral N90 was changed by PT only. Furthermore, the relationship between stimulus amplitude or NoP and subjective ratings or EP amplitudes was linear for SP whereas for PT it was not. In particular the difference in modulation of the contralateral N90 was remarkable. The N90 was more sensitive for temporal modulation of neural activity by PT. As electrocutaneous stimulation activates both tactile and nociceptive fibers, we questioned if parts of the nociceptive system are involved in this N90 potential.

Using an additional modulating stimulus might increase our knowledge about involvement of the nociceptive system in the N90 potential. Several studies in man have shown that responses due to a test stimulus can be inhibited by diverse noxious modulating stimuli applied at body areas remote to the test stimulus [111; 149; 166]. This phenomenon was first observed in rats by Le Bars [81] and is called diffuse noxious inhibitory control (DNIC). Activity of most convergent neurons (wide dynamic range; WDR) [16] and some nociceptive specific (NS) neurons [139] in the dorsal horn are inhibited by stimulation of nociceptive fibers in an area in the body distal from their excitatory receptive field [81]. Descending activity from the caudal medulla (dorsal reticular nucleus) mediated through the dorsalateral funiculis reduces the activity of spinal and trigeminal WDR and NS neurons [10; 159]. In man, various studies suggest the existence of a DNIC as well [37; 122]. Inhibition of late P300 EP component and N150-P200 peak-to-peak amplitude was assigned to activation of the DNIC. On the other hand, early EP components induced by a test stimulus were not inhibited by a modulating stimulus [20; 48]. This early response is mediated via tactile somatosensory pathway; the dorsal column-medial lemniscus. This indicates that the dorsal column-medial lemniscus pathway is not affected by DNIC.

In this study we used the cold pressor test (CPT) at the contralateral hand as the remote noxious stimulus to modulate the processing of SP and PT stimuli in healthy subjects. If parts of the nociceptive system are involved in the N90 potential by PT, an effect of the CPT on this peak amplitude could be expected. Furthermore, late EP components and NRS scores are presumed to decrease as well.

3.2 Methods

3.2.1 Subjects

Twelve male and thirteen female right-handed, healthy subjects (age 40.2 ± 13.8) participated in the study. All subjects gave written informed consent according to the Declaration of Helsinki. The study was approved by the ethical committee of the Medisch Spectrum Twente, Enschede.

3.2.2 Electrical stimulation

The subjects were electrically stimulated at the left middle fingertip. Stimulation at the fingertip corresponds to the IES method [14]. An electrode with a 1 mm diameter tip of gold in an insulating material was used. A small opening was drilled in the upper layer of the skin of the fingertip using a dental gimlet with the same diameter as the tip of the stimulation electrode [14]. If the sensation threshold (I_S) was higher than 1 mA the preparation was regarded insufficiently and tried again. A rectangular surface electrode (a 4x9 cm Klinerva Blue Electrode) was placed with a distance of at least 10 cm at the upper part of the left forearm as an anode. The stimuli were generated by a battery-driven computer controlled current stimulator. The stimulus was a current bipolar rectangular pulse with a stimulus duration of 0.2 ms.

For each subject, the stimulus amplitudes corresponding to the subjective sensation threshold (I_S=0.28 ± 0.20 mA) and pain threshold (I_P=1.36 ± 0.62 mA) were determined once before the first protocol. Thresholds were obtained by the ascending method of limits by increasing three times the stimulus amplitude with steps of 0.1 mA starting at a level of zero.

3.2.3 SP and PT method

The SP and PT method used in this study have been described in detail previously [153]. For SP, the stimulus current amplitude of a single pulse was varied in discrete steps depending on the obtained I_S and I_P according to:

$$I = I_P + q \cdot (I_P - I_S) \quad q = -0.5, -0.25, 0, 0.25, 0.5 \qquad (3.1)$$

The fixed stimulation current for PT was chosen similar to the minimum stimulus amplitude $I_{50\%}$ of SP (equation 1, q=-0.5). The NoP for PT varied from 1, 3, 5, 7, to 9 pulses. The inter pulse interval (IPI) between two subsequent pulses in the pulse train

was 5 ms. To make sure that stimulation by PT was tolerable, the five NoP were applied in increasing order before the protocol. Although the stimulus amplitude of the single pulse of PT stimulus was below the subjective pain threshold, subjects described stimulation by a train of five pulses as a clear pricking painful sensation.

3.2.4 CPT and control protocol

A polystyrene squared vessel was filled with ice water 0-1°C (CPT) or 32±0.5°C (control). The right hand was immersed up to the wrist in the water. During CPT the subjects were stimulated to keep their hand in the water as long as possible with a maximum of three minutes. Subjects had to withdraw and re-immerse their hand after subsequently 3 and 1 minute until the end of the protocol (about 9.5 minutes). Time to hand withdrawal and re-immersion was recorded. Pain intensity and unpleasantness increases rapidly [169] and peaks in the first 20-45 seconds [134]. Therefore, electrical stimuli at the left fingertip were applied 30 seconds after hand immersion.

3.2.5 EEG recordings

Electrical brain activity was recorded using a 64-channel EEG system (A.N.T. Enschede, the Netherlands). Ag/AgCl electrodes were placed according to the international 10-5 system (Waveguard EEG cap). The ground electrode was placed at the top of the nose. All scalp electrode impedances were less than 5kΩ. An electrode was placed under the left eye for electrooculogram (EOG) recording. Furthermore, subjects were instructed to fix their eye on a point in front of them. The sample frequency was 1 kHz and filter settings were 0.3-120Hz. Data from -10 to 100 ms pre-stimulus was used for baseline correction. The time window of analysis was 100 ms pre-stimulus to 400 ms post-stimulus.

3.2.6 Numeric rating scale

Subjects rated the perceived strength of each electrocutaneous stimulus on an 11 point NRS scale (“no sensation” = 0, “strongest imaginable pain” = 10). The first electrical stimulus corresponded for SP with the pain threshold $I_{0\%}$ (equation 3.1, q=0) and for PT with a train of 5 pulses at $I_{50\%}$ (equation 3.1, q=-0.5). The subjects were instructed to rate the first stimulus with a six. Furthermore, after CPT subjects were asked to rate orally the perceived strength of the right hand during the measurement on a similar NRS scale.

3.2.7 Procedure

The experiment consisted of two blocks of three protocols; a block for both SP and PT. A block consisted of an identical stimulus (IS), CPT and a control protocol. The order of the SP and PT blocks and the order of CPT and control protocol were randomized.

During the IS protocol a total of 100 identical electrical stimuli were applied at the left middle fingertip. For SP the stimulus was a single pulse at pain threshold (equation 3.1, q=0) and for PT 5 pulses at minimum stimulus amplitude (equation 3.1, q=-0.5). Data of the IS measurement is not analysed in this paper.
During the CPT and control protocol a total of 105 randomized electrical stimuli were applied at the left middle fingertip with 21 stimuli for each of the five stimulus amplitudes (SP) or five NoP in a pulse train (PT). The inter stimulus interval between two successive stimuli was randomly varied between 4 and 6 seconds.
The inhibitory effect of the CPT can persist for 5-10 minutes after withdrawal of the hand [134]. Therefore, to be sure that there was no effect of CPT in a subsequent protocol we waited 15 minutes between the CPT and the control protocol and between the two blocks.

3.2.8 Data analysis

At least 11 sweeps were needed for each of the five subject-EPs obtained in a measurement. If one of the five subject-EPs had fewer than 10 accepted sweeps, the subject was excluded from analysis of the concerning measurement. Grand average EPs were calculated from C_Z versus A_1A_2 and C_4-F_Z recordings for each of the five stimulus amplitudes or NoP for the CPT and control protocol. Trials with an EOG artefact exceeding ±70 µV in the time windows of -10 to -100 ms pre-stimulus and 60 to 400 ms post-stimulus were rejected. Subsequently, accepted data was visually inspected for missed EOG artefact or muscular artefacts. Mean NRS scores were obtained at all fives stimulus amplitudes (SP) or at all five NoP (PT) for both CPT and control.
For both SP and PT we analysed NRS scores, EP component P300 at 290 ms and N150-P200 peak-to-peak amplitude (P200 at 200ms and N150 at 150ms) all recorded at C_Z-A_1A_2 and N90 at 88 ms recorded at C_4-F_Z. Besides these EP components for SP also the P50 at 52 ms measured at C_4-F_Z was analysed. Due to the stimulation artefact duration this component could not be analysed for PT.
For statistical analysis SPSS 15.0 (SPSS, Chicago, IL) was used. A repeated measures multivariate analysis of variance (MANOVA) was used to test for the two factors: stimulus method (stimulation amplitude or NoP) and condition (control or CPT). Significant effects were followed by a post-hoc repeated measures analysis of variance (ANOVA). To correct for sphericity assumption violation a Greenhouse-Geisser degrees of freedom adjustment was applied (p value indicated by p_{GG}). The effect of CPT for each stimulus amplitude or NoP was tested post-hoc by a paired-sample student's t-test. The null hypothesis was that NRS scores and EP component amplitudes measured during CPT were smaller than those measured during the control protocol. All statistical tests were performed at a level of significance of $p<0.05$.

3.3 Results

3.3.1 NRS scores

Figure 3.1A shows mean NRS scores for SP with the control and CPT protocol. A significant decrease of the NRS scores by CPT was observed ($F(1,23)=10.64$, $p=0.003$). The linear relationship between NRS scores and stimulus amplitudes was unchanged by CPT. NRS was significantly changed by stimulus amplitude ($F(1.3,29.8)=56.89$, $p_{GG}<0.0005$). Furthermore, each of the stimulus amplitudes significantly decreased by CPT (figure 3.1A).

In figure 3.1B mean NRS scores for PT can be seen. Except for 1 pulse, the NRS scores for CPT are lower than control scores ($F(1,23)=10.48$, $p=0.004$). Both relationships clearly show a curved effect. A significant effect for NoP was obtained ($F(1.7,29.0)=138.63$, $p_{GG}<0.0005$).

After removal of the hand subjects rated the perceived pain from CPT, during the measurement, of the right hand with NRS = 8.20±1.23.

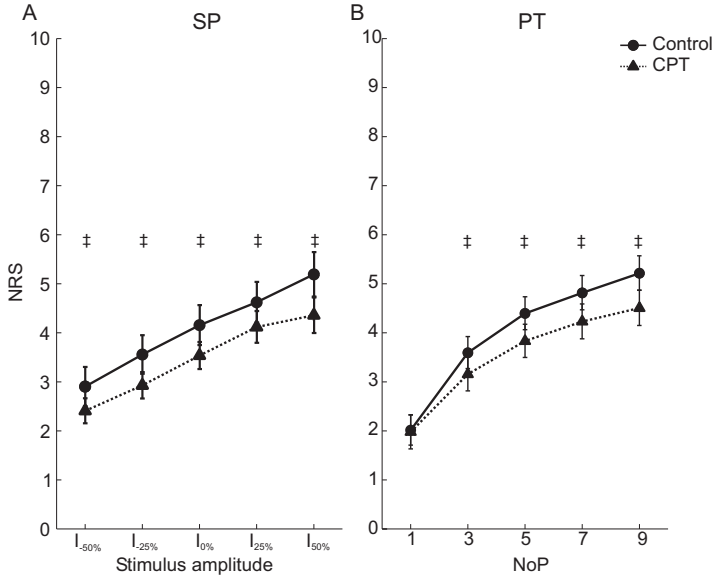

Figure 3.1: Mean NRS (± SEM) of all 5 stimulus amplitudes (A) and all five NoP (B) for CPT or control. Each symbol represents the mean NRS score of all included subjects at the stimulus amplitude or NoP under test. Significant post-hoc effect of CPT per level (NoP or stimulus amplitude) marked with ‡.

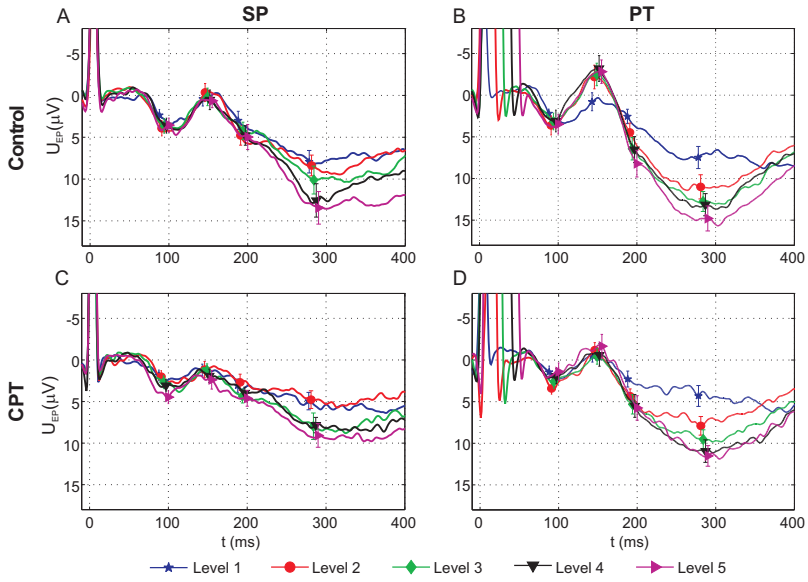

Figure 3.2: Grand average EPs (±SEM) recorded at C_Z-A_1A_2 of all five stimulus amplitudes for SP: control (A) or CPT (C). Grand average EPs (C_Z-A_1A_2) of five NoP for control (B) or CPT (D) protocol. A stimulation artefact can be seen in first milliseconds of the EPs. The five levels mentioned in the figure legend correspond to stimulus amplitude or NoP.

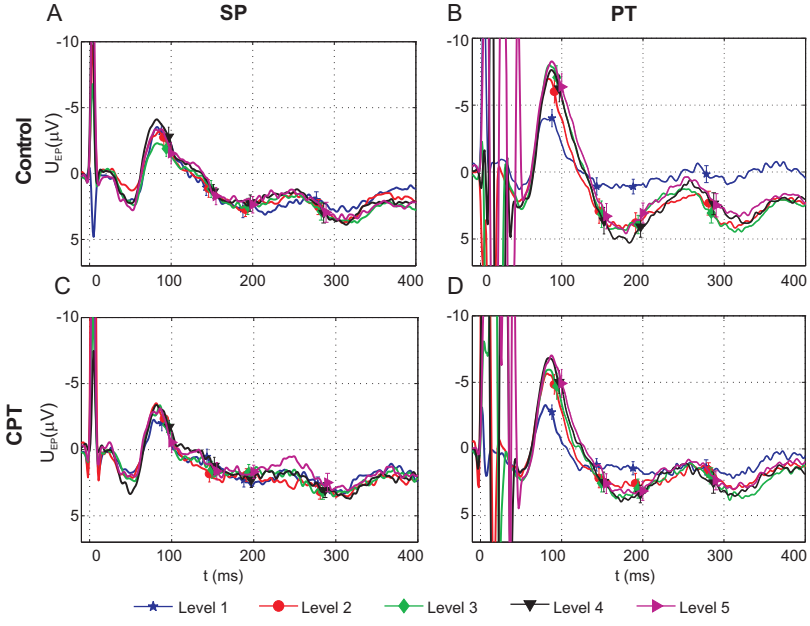

Figure 3.3: Grand average EPs (±SEM) recorded contralaterally at C_4-F_Z of all five stimulus amplitudes for SP: control (A) or CPT (C). Grand average EPs (C_4-F_z) of five NoP for control (B) or CPT (D) protocol. A stimulation artefact can be seen in first milliseconds of the EPs. The five levels mentioned in the figure legend correspond to stimulus amplitude or NoP.

3.3.2 Vertex EPs of SP

Figure 3.2A and 3.2B show grand average EPs (C_Z-A_1A_2) of the five stimulus amplitudes in combination with control or CPT protocol. The relationship between P300 EP component amplitude and stimulus amplitude is comparable with the relationship between NRS and stimulus amplitude ($F(2.4,55.0)=13.79$, $p_{GG}<0.0005$).
Figure 3.4D shows the P300 EP peak amplitude for all stimulus amplitudes for both control and CPT. A significant reduction of the P300 amplitude by CPT can be seen in this figure ($F(1,23)=22.87$, $p<0.0005$). Furthermore, N150-P200 peak-to-peak amplitude (figure 3.4C) showed no effect of stimulus amplitude ($F(2.7,62.3)=0.66$, $p_{GG}=0.56$) but was changed by CPT ($F(1,23)=9.43$, $p=0.005$).

3.3.3 Vertex EPs of PT

Grand average EPs (C_Z-A_1A_2) of five NoP with control or CPT are shown in figure 3.2B and 3.2D respectively. A stimulation artefact can be seen in the first milliseconds of the EPs, lasting up to 45 ms for 9 pulses.
Especially the P300 EP component shows a curved effect for NoP ($F(2.4,55.8)=21.76$, $p_{GG}<0.0005$), this effect is similar to the relationship between NRS and NoP. The effect is unchanged by CPT. The decrease of the P300 EP amplitude is shown in figure 3.4G ($F(1,23)=36.89$, $p<0.0005$). Besides the P300 also the peak-to-peak amplitude N150-P200 showed an effect by both NoP ($F(2.4,54.7)=16.74$, $p_{GG}<0.0005$) and CPT ($F(1,23)=15.00$, $p=0.001$). Similar to the NRS scores no significant effect of CPT was found for the minimum NoP (figure 3.4F).

3.3.4 Contralateral EPs of SP and PT

Grand average EPs recorded contralaterally to the stimulus location at C_4 referred to F_Z are shown in figure 3.3. For the PT method (Figure 3.3B and D), EPs show a clear peak around 90 ms for both control and CPT protocol. Increasing the NoP increases the EP amplitude in a similar manner as described for the P300 amplitude recorded at C_Z-A_1A_2 ($F(2.7,61.8)=31.40$, $p_{GG}<0.0005$).
Keeping the relationship between NoP and amplitude unchanged a reduction of EP N90 amplitude by CPT can be seen in figure 3.4E ($F(1,23)=10.18$, $p=0.004$). For SP no relationship between N90 EP amplitude and stimulus amplitude was found ($F(2.0,46.6)=1.10$, $p_{GG}=0.34$) nor a decrease of this amplitude by CPT ($F(1,23)=2.21$, $p=0.15$). For SP, for both control and CPT around 50 ms a positive peak was observed which was altered by stimulus amplitude ($F(4,92)=2.88$, $p=0.027$). CPT did not change the P50 amplitude (figure 3.4A, $F(1,23)=0.374$, $p<0.55$).
Due to the stimulus artefact this peak is less obvious for PT. Hence, for PT the P50 peak was not tested for effect of NoP or CPT.

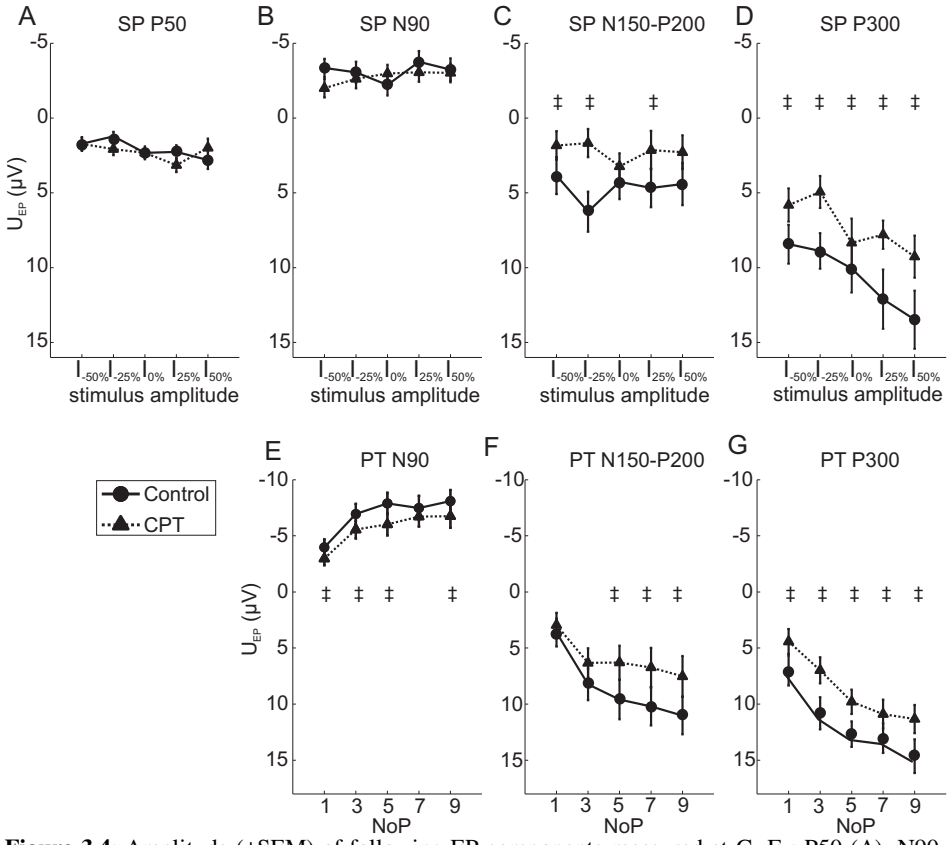

Figure 3.4: Amplitude (±SEM) of following EP components measured at C_4-F_Z: P50 (A), N90 (B,E) and EP components measured at C_Z-A_1A_2: P300 (C,F) and N150-P200 (D,G) for SP (A-D) or PT (E-G) for control and CPT protocol. Significant post-hoc effect of CPT per level (NoP or stimulus level) marked with ‡.

3.4 Discussion

We analysed the effect of a CPT on the processing of SP and PT stimuli. For both SP and PT NRS scores, P300 and N150-P200 amplitudes were decreased by CPT. However, the contralateral N90 was modulated by CPT for PT only. In our previous study we compared SP and PT stimulation and found that they were processed differently. We demonstrated that the P300 EP component was changed by both methods, the contralateral P50 was varied only by SP and the contralateral N90, and vertex N150-P200 amplitudes showed an effect of only PT. Furthermore, the effect of SP was linear and by PT not. These effects were reproduced in the current study.

In man, in several studies reduced NRS scores and reduced EP amplitudes by a modulating stimulus following a test stimulus are shown [65; 115; 166]. Inhibition of

activity in convergent neurons in the dorsal horn and trigeminal nucleus caudalis by stimulation of a remote area of the body was first observed in anesthetised rats by [81; 82]. Since then an increase of research on the DNIC was performed in both animals and humans. In [139] it was shown that in rats besides activity of most wide dynamic range (WDR) neurons also activity of some nociceptive specific neurons is decreased by noxious stimulation. None of the low threshold mechanoreceptive neurons showed reduced activity [82; 139]. Effects of the DNIC act by a final post-synaptic inhibitory mechanism [160; 161] involving hyperpolarization of the neuronal membrane.

In figure 3.5 a schematic illustration is shown with the connections and structures relevant for the current study including activations by electrocutaneous stimuli and the CPT. The dorsal tier of the dorsal reticular nucleus (DRt) in the caudal medulla is part of the supraspinally mediated DNIC system [11; 159]. Descending information from the DRt mediated via the dorsolateral funiculus inhibits activity from dorsal WDR and some nociceptive specific (NS) neurons (see figure 3.5).

For both SP and PT NRS scores were reduced (figure 3.1A and B). Inhibition of subjective ratings by CPT is in accordance with other studies: a decrease was obtained for electrical sural nerve stimuli, thermal stimuli [50; 134] and laser stimuli [166].

The linear effect by SP and the nonlinear effect by NoP were not changed by adding CPT. For both SP and PT, the difference between NRS scores of CPT and control was different for all stimulus amplitudes or NoP. Unequal inhibition effects by CPT were also reported for laser stimuli with changing energy [115]. Largest effect of CPT were found near pain threshold and no effect for stimuli below pain threshold [115]. On the contrary, laser modulating stimuli resulted in almost equal inhibitory effects regardless of stimulus amplitudes of electrical tooth stimulation [111]. Talbot et al. [134] also showed inhibition of both perceived non-noxious and noxious heat stimuli by CPT. For PT the NRS scores for the minimum NoP is similar for both control and CPT. The lack of inhibition for the minimum NoP is remarkable since this stimulation level is similar to the minimum level of SP for which the NRS still was inhibited.

The P50 was tested only for the SP method. Similar to earlier results [153] the P50 amplitude was significantly changed by stimulus amplitude. The CPT did not influence the P50; the amplitude was not decreased. The P50 is assigned to tactile information, mediated directly via the fast dorsal column-medial lemniscus (see figure 3.5). No effect of CPT is also reported for amplitudes of early EP responses triggered by electrical sural nerve stimulation [37; 48]. This lack of inhibition was demonstrated for two different time intervals; between 45-55 ms and between 69-85ms [37; 48]. Responses in both intervals were ascribed to innocuous-related activity [36; 38]. Besides CPT also ischemic pain did not change early EP components following electrical fingertip stimulation [20]. Hence, no effect of CPT on the P50 is in accordance with literature.

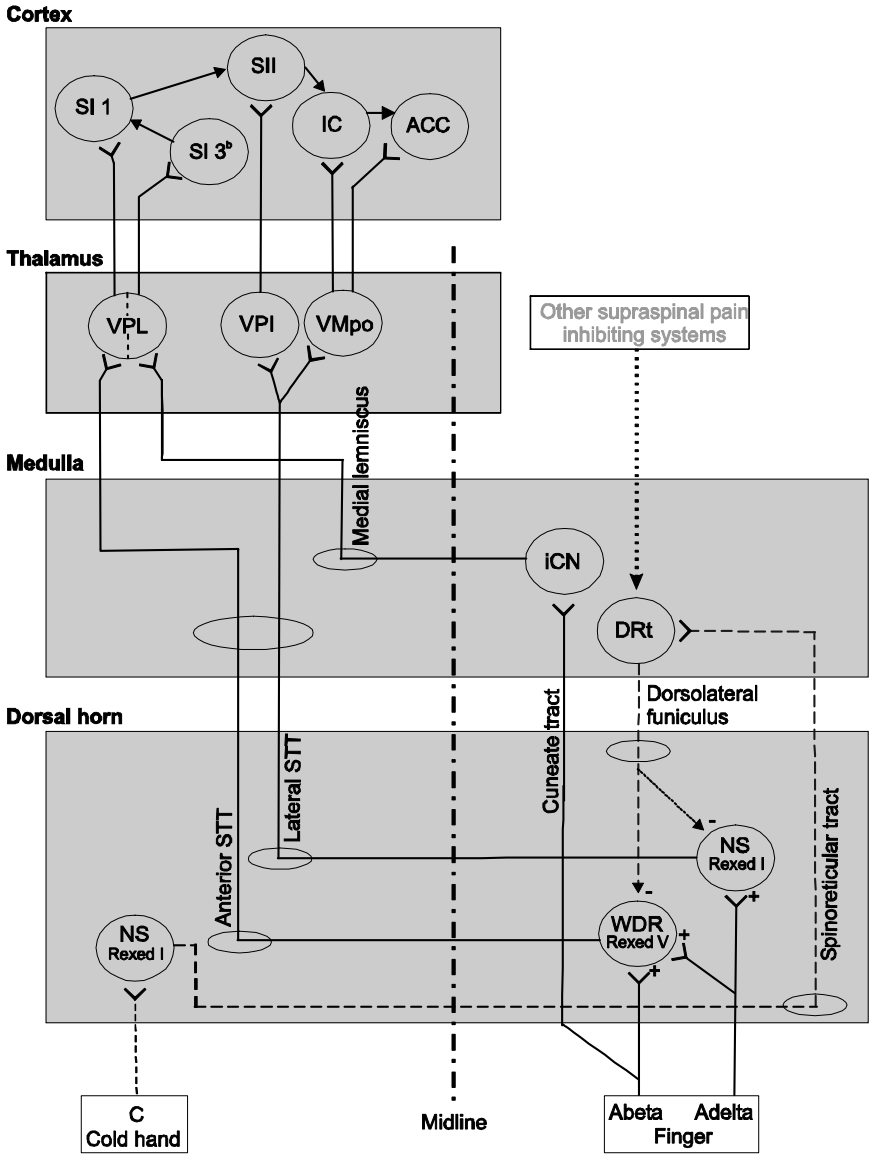

Figure 3.5: Simplified representation of anatomical connections, based on literature, relevant for pain processing. Dashed lines (--) represent the DNIC system. ACC: anterior cingulate cortex, DRt: dorsal reticular nucleus, IC: insular cortex, iCN: internal cuneate nucleus, NS: nociceptive specific neurons, Rexed I: lamina I in the dorsal horn, Rexed V: lamina V in the dorsal horn, SI 1: primary somatosensory cortex area 1, SI 3b: primary somatosensory cortex area 3b, SII: secondary somatosensory cortex, STT: spinothalamic tract, VPI: ventroposterior inferior nucleus, VPL: ventroposterior lateral nucleus, VMpo: ventromedial posterior nucleus, WDR: wide dynamic range neurons

For PT, the NoP modulated the N90 EP component for both control and CPT protocol. Interestingly, a significant inhibition of the N90 component by CPT was shown for PT (figure 3.3 B,D and 3.4E). This result might be assigned to activation of endogenous pain modulation by DNIC. Chen et al [20] showed inhibition by ischemia pain of P80-N150 peak-to-peak amplitude (vertex) following electrical fingertip stimulation with 5 pulses. It is not clear from this study if both components are decreased or mainly one of both components. Furthermore, the amplitude of the vertex N100 response (between 90-120ms) by sural nerve stimulation was reduced by CPT [48]. To the best of our knowledge no other studies described inhibition of a contralateral potential around 90 ms. The N90 might be attributed to activation of Aδ as well Aβ fibers. It has been shown that DNIC inhibits activity of most WDR and some NS neurons in the dorsal horn. No effect of DNIC was found for non-nociceptive cells in the dorsal horn [82; 139]. Yet, non-nociceptive sensory inputs may be affected by the DNIC via WDR neurons [10]. Information of the WDR and NS neurons is relayed through the spinothalamic tract (STT). The STT can be divided in two parts [26]; activity of the WDR neurons is relayed through the anterior STT (also called dorsal and ventral STT) and of the NS on the lateral STT (see figure 3.5). The conduction velocities are different and information is projected to different areas in the cortex [145]. The anterior STT projects via the thalamus to the primary somatosensory cortex (SI). Considering this and the obtained reduction of EP amplitude this potential might be explained by information of WDR neurons relayed through the anterior STT. Yet, the spinal pathway is short so the difference in conduction velocity probably does not result in distinct differences in peak latencies in the EP for both pathways.

Notable, for SP neither stimulus amplitude nor CPT had an effect at the N90 amplitude (figure 3.3A, C and 4B). Hence, N90 is influenced by temporal modulation (PT) of neural activity but not by spatial modulation (SP). The differences caused by different modulations of neural activity may indicate activation of two different mechanisms, possibly in the dorsal horn.

For PT we suggest that the N90 reflects activity relayed through the anterolateral system. But for SP it is unknown via which spinal pathway the information is mediated.

Several studies showed a decrease of the N150-P200 peak-to-peak amplitude by different modulation techniques [20; 46; 122; 143]. In our study we obtained attenuation of the N150-P200 by CPT for both SP and PT as well. The nonlinear effect of NoP was obtained for control as well as CPT. No significant effect of CPT was obtained for minimum NoP (figure 3.4F) that corresponds to the obtained NRS scores (figure 3.1B). For SP, the N150-P200 peak-to-peak amplitude was not altered by stimulus amplitude (figure 3.4C). For both SP and PT the P300 EP amplitude (C_Z-A_1A_2) was reduced by CPT and showed an effect of stimulus amplitude or NoP (Figure 3.2, 4D and G). Attenuation of the P300 laser evoked potential by CPT was described [3]. Late EP components can also be influenced by attention or distraction. Furthermore, Plaghki et

al. [115] obtained inhibition of EEG and subjective rating by both a CPT and mental task but in a dissimilar manner. In the present study, subjects were asked to focus their attention to the electrical stimuli by rating each of them during both control and CPT. Nevertheless, the modulating stimulus is a very strong stimulus and it might distract from the test stimulus. Consequently, cognitive processes may indeed have influenced both the P300 and the N150-P200. For PT, the larger relative effect (difference between CPT and control) for N150-P200 and P300 compared to N90 possibly reflects involvement of more processes than the DNIC only.

In conclusion, we showed that the CPT as a modulating stimulus affects SP and PT responses differently. Both subjective ratings were changed by CPT. For PT, all EP components under test were inhibited by CPT. Additionally, all components changed by NoP for both control and CPT. For SP, only the contralateral response was not inhibited with CPT. Besides only the P50 and P300 showed an effect by stimulus amplitude. So it seems that temporal modulation of neural activity is more sensitive to CPT than spatial modulation.

Inhibition by CPT of the contralateral N90 can be assigned to activation of endogenous pain modulation by DNIC. Pain patients can show impairment of the DNIC and abnormal endogenous modulation [76; 167]. We suggest an activation of a part of the nociceptive system (anterolateral system) by PT, reflected by the N90. The PT method seems a promising technique to further explore changes in the pain system as these are presumed to occur in patients suffering from chronic pain.

Chapter 4

Primary somatosensory cortex is involved in N90 activity following single pulse and pulse train electrocutaneous stimulation

Abstract - The strength of an electrocutaneous stimulus can be varied by changing the amplitude of a single pulse (SP) or changing the number of (fixed amplitude) pulses (PT). Earlier we showed that dissimilarities between SP and PT processing are in particular reflected in the early contralateral N90 evoked potential component (C_4-F_Z). Involvement of the nociceptive system in the N90 was suggested. In this study we analyse which brain regions are involved in the generation of N90 activity using SP and PT. Healthy subjects were electrically stimulated at the left middle fingertip with one pulse at the pain threshold (SP) or with 5 pulses with a stimulus amplitude in between sensation and pain threshold (PT). We used electroencephalography to record brain activity. Dipole coordinates and orientation were analysed. For both SP and PT a majority of the dipoles were located in the postcentral gyrus which is the location of the primary somatosensory cortex (SI). PT resulted in a less dispersed group of dipoles of individual subjects. The PT dipoles were on average located significantly more frontally and oriented more in the anterior direction. We showed that differences in the N90 component using SP and PT are not the result of activation of different brain regions. Mean dipoles were located in the postcentral gyrus but at different positions. We suggest involvement of the anterior spinothalamic tract in the generation of the N90 by PT, which could be important for clinical observation of changes in the nociceptive system in pain patients.

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Submitted for publication

4.1 Introduction

Different brain regions are involved in the processing of nociceptive and tactile stimuli [1]. Knowledge about involved cortical areas can help to identify central processes involved in two different stimulation protocols.

Recently, we studied the effect of spatial and temporal modulated electrocutaneous stimuli on evoked potential (EPs) components and subjective ratings [153]. Intracutaneous electrical stimuli (IES) [14] can be modulated by increasing the amplitude of a single pulse (SP) or varying the number of (fixed amplitude) pulses (NoP) in a train (PT). Both tactile and nociceptive nerve fibers are activated by electrical stimuli. Consequently, increasing the amplitude by SP results in a change in the proportion of activated fibers depending on the local fiber densities. In contrast, by varying the NoP more action potentials are generation in unchanged proportions of nociceptive and tactile of fibers.

We showed that both SP and PT influenced EP components and subjective ratings, but differently. Not all EP components under test were affected by both SP and PT. Especially the modulation of the contralateral N90 (C_4-F_Z) by PT only was remarkable. Moreover, using the cold pressor test (CPT) as an additional stimulus (painful heterotopic stimulation) resulted in decreased N90 amplitudes by PT only [154]. The inhibition by the CPT may be ascribed to activation of descending noxious inhibitory control (DNIC). Dissimilarities between SP and PT processing were mainly reflected in the contralateral N90 EP component. In particular the early moment in the EP makes the N90 interesting. We hypothesised reflectance of the nociceptive system in the N90 by PT. The N90 might be a good indicator of the nociceptive system and involved mechanisms such as the DNIC.

So far, we analysed only EPs measured at the vertex or contralateral (C_4-F_Z) electrode. We questioned if different brain activations are involved in the N90 by SP and PT. Source localisation techniques can be used to further explore cortical areas involved in the generation of the N90 potential.

Previous studies to cortical processing of nociceptive and tactile stimuli have shown that several cortical areas are involved (for review see: [1]). Activity up to 100 ms is predominantly ascribed to activation of the contralateral primary somatosensory cortex (SI). Brodmann's area 1 of the SI is activated following activation of Aδ (nociceptive) fibers projected via the spinothalamic tract [117; 148]. While tactile stimuli projected via the dorsal column-medial lemniscus are first processed in area 3b and sequentially in area 1 [62; 117]. Besides SI activations also activations in the parasylvian region are reported in the first 100 ms after stimulation. Both the secondary somatosensory cortex (SII) and the insular cortex (IC) are located in this area. Early SII and IC involvement (before 100 ms) is merely obtained for stimuli activating mainly tactile fibers [62; 116].

Several studies investigated encoding of stimulus strength in different brain regions. Nociceptive stimulation such as laser stimuli resulted in encoding of varying stimulus energy (temporal modulation) by both contralateral SI and bilateral SII but differently [7; 137]. Furthermore laser energy is encoded in the parasylvian region too. Median nerve stimulation with changing electrical stimulus amplitudes (spatial modulation) also resulted in different activations of SI and SII [87; 140].
Thus several contralateral brain regions can be activated in the first 100 ms and all these regions modulate the stimulus strength. Therefore the objective of this work is to investigate which parts of the brain are involved in the generation of the N90 potential by electrocutaneous SP and PT stimulation. Dipoles are fitted on the N90 using electroencephalography (EEG) recordings and moving dipole source localisation technique. We used only one stimulus strength: the pain threshold for SP and for PT 5 pulses with a stimulus amplitude in between sensation and pain threshold. These stimulus strengths were chosen such that maximum difference can be analysed.

4.2 Method

4.2.1 Subjects

Twelve male and twelve female right-handed, healthy subjects (age 39.8 ± 14.0) participated in the study. All subjects gave written informed consent according to the Declaration of Helsinki. The study was approved by the ethical committee of the Medisch Spectrum Twente, Enschede.

4.2.2 Electrical stimulation

The subjects were electrically stimulated at the left middle fingertip. Stimulation at the fingertip corresponds to the IES method [14]. An electrode with a 1 mm diameter tip of gold in an insulating material was used. A small opening was manually drilled in the upper layer of the skin of the fingertip using a dental gimlet with the same diameter as the tip of the stimulation electrode [14]. If the sensation threshold was higher than 1 mA the preparation was regarded as insufficient and tried again. A rectangular surface electrode (a 4x9cm Klinerva Blue Electrode) was placed with a distance of at least 10 cm at the upper part of the left forearm as an anode. The stimulus was a current bipolar rectangular pulse with a stimulus duration of 0.2 ms. The stimuli were generated by a battery-driven computer controlled current stimulator.
For each subject, the stimulus amplitudes corresponding to the subjective sensation threshold (I_S=0.29 ± 0.20 mA) and pain threshold (I_P=1.40 ± 0.63 mA) were determined once before the first protocol. Thresholds were obtained by the ascending method of limits by increasing three times the stimulus amplitude with steps of 0.1 mA starting at a level of zero.

For both the SP and PT method one of the five stimulus levels described in [153] was used. For SP the stimulus was a single pulse at the pain threshold. For PT, the stimulus consisted of 5 pulses with a stimulus amplitude in between the sensation and pain threshold. The inter-pulse interval between subsequent pulses was 5 ms.

4.2.3 Procedure

The experiment consisted of two blocks of three protocols; a block for both SP and PT. A block consisted of an identical stimulus (IS), a cold pressor test (CPT) and a control protocol. Both blocks started with the IS protocol. The order of the SP and PT blocks and the order of CPT and control protocol were randomized. The analysis of the CPT and control protocol is discussed in [155].
During the IS protocol a total of 100 identical SP or PT electrical stimuli were applied at the left middle fingertip. The inter stimulus interval between two successive stimuli was randomly varied between 4 and 6 seconds.
Subjects rated each of the stimuli on an 11 point numeric rating scale (NRS) starting with a six. The rating task was used to minimize the effect of attention. Subjects were unaware of the fact that all stimuli were identical. They were told that the perceived intensity of the stimuli differed slightly during the experiment. The NRS data was not used for analysis.

4.2.4 EEG recordings

Electrical brain activity was recorded using a 64-channel EEG system (A.N.T. Enschede, the Netherlands). Ag/AgCl electrodes were placed according to the international 10-5 system (Waveguard EEG cap). The ground electrode was placed

at the top of the nose. All scalp electrode impedances were less than 5kΩ. An electrode was placed under the left eye for electrooculogram (EOG) recording. The sample frequency was 1 kHz and filter settings were 0.3-120Hz. Data from 15 to 100 ms pre-stimulus were used for baseline correction.

4.2.5 EEG data analysis

Grand average EPs were calculated from all EEG channels for both SP and PT. Trials with an EOG or EMG artefact exceeding 100μV were rejected. The automatic artefact detection was verified by visual inspection. To illustrate the difference between the obtained EPs for SP and PT, grand average EPs were calculated from C_Z versus A_1A_2 and C_4-F_Z recordings.
For dipole source localisation the latency of interest was obtained by using both the global field power (GFP) [85] and the EP measured at C_4. To make sure that the right latency was chosen both methods were used. Firstly, in a time window of 65-100 ms the

latency of the local maximum in the GFP was determined. Secondly, the latency of the negative peak of the EP measured at C_4 was obtained in the same time window (figure 4.2 A-D). The latency of interest was chosen between the most optimal GFP latency and C4 latency. This latency was obtained for each subject and for the grand average EP. For the majority of subjects the latencies of GFP and C4 differed 3 ms at most. For dipole source localisation an event of 5 ms duration was defined around the obtained latency.
Source modelling was performed using ASA 4.5 (A.N.T., Enschede, The Netherlands). A realistically shaped with three shell boundary element head model was used with conductivities 0.33, 0.0042 and 0.33 S/m for brain, skull and scalp respectively. The Levenberg-Marquardt minimisation algorithm was applied in the source localisation procedure. A moving dipole model was used on each event around the latency of interest. Both a single dipole fit and a dipole localisation with two dipoles were performed.
Only dipoles explaining at least 85% of the signal variance (goodness of fit (GOF)) were used for further analysis. The dipole with the largest GOF in the particular time window was used for further analysis. The obtained dipoles were superimposed onto standard MRI slices (provided by ASA 4.5 software).
ASA uses the nasion-ear coordinate frame. The origin is located midway between left and right pre-auricular points with x-axis through the nasion and y-axis towards the left ear. The z-axis is perpendicular to x- and y-axis and points upwards.

4.2.6 Statistical analysis

To evaluate the possible difference between SP and PT we analysed location, orientation and latency of the dipoles. For each method a group of dipoles with a GOF>85% was obtained. The location of the dipoles in a group can be rather scattered. For between groups analysis a homogeneous group of dipoles is desired. Therefore the robust distance (RD) was used as outlier detection method [129]. The RD is a more robust extension of the Mahalanobis distance and is more sensitive to recognize more than one outlier. The RD is based on robust estimators of multivariate location and scatter. Robust estimates are obtained by the minimum covariance determinant (MCD) estimator [127]. Under the normal assumption, the outliers are observations having a RD larger than the cut-off value $\sqrt{\chi^2_{p,0.975}}$. The RD was calculated with the robust statistics software package LIBRA [158] using an implementation of the FAST-MCD algorithm [128].
Furthermore the student's t-test was used to analyse the differences in location, latency and orientation of obtained clusters of SP and PT dipoles. The test was performed at a level of significance of $p<0.05$.

4.3 Results

4.3.1 Grand average EPs

In figure 4.1 the grand average EPs recorded at the vertex (C_Z-A_1A_2 see figure 4.1A) and contralaterally (C_4-F_Z, figure 4.1B) to the stimulus location are shown for both SP and PT. A stimulus artefact can be seen in the EPs, lasting up to 25 milliseconds for PT.
The morphology of both the vertex (A) and contralateral (B) EPs is similar for SP and PT. The contralateral N90 component is clearly visible (figure 4.1B) for both SP and PT. The EP amplitude of the N90 component by PT is significantly larger than the N90 amplitude by SP. Furthermore, also the vertex EP peak amplitude at 150 ms by PT was significantly larger.

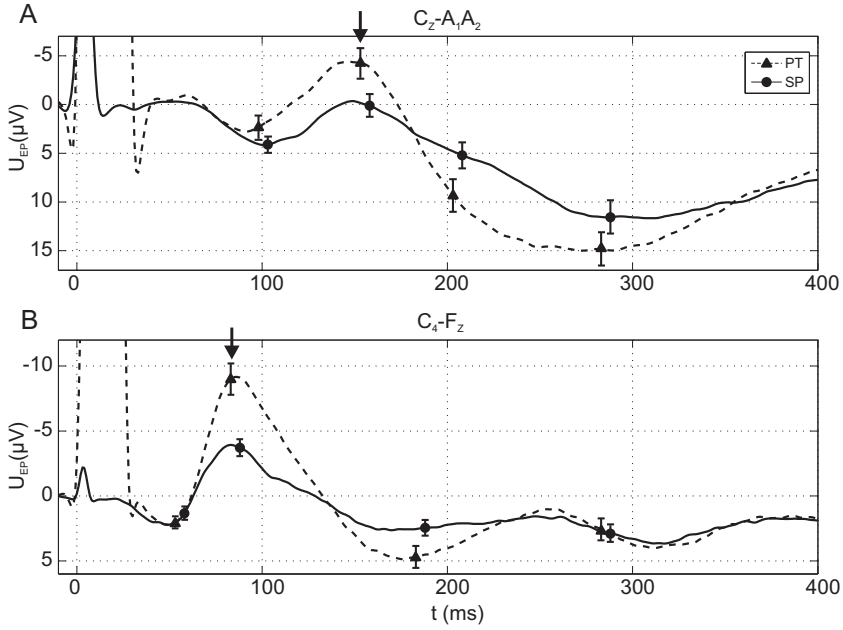

Figure 4.1: Grand average EPs (±SEM) of both SP and PT recorded at the C_Z-A_1A_2 (vertex, A) and C_4-F_Z (contralateral, B). A stimulation artefact can be seen in the first milliseconds of the EP. Significant difference ($p<0.05$) indicated by an arrow.

4.3.2 Grand average scalp distribution

GFP, grand average EPs (C_4) and grand average scalp distributions of the N90 can be seen in figure 4.2. For this case the latency of interest is determined by grand average GFP across 61 electrodes (A,B) and peak latency of the N90 in the EP recorded at C_4 (C,D). For SP the determined latency of interest was 81 ms and for PT 84 ms. For both SP and PT the grand average scalp distribution are obtained at the latency of interest (E,F,G and H).

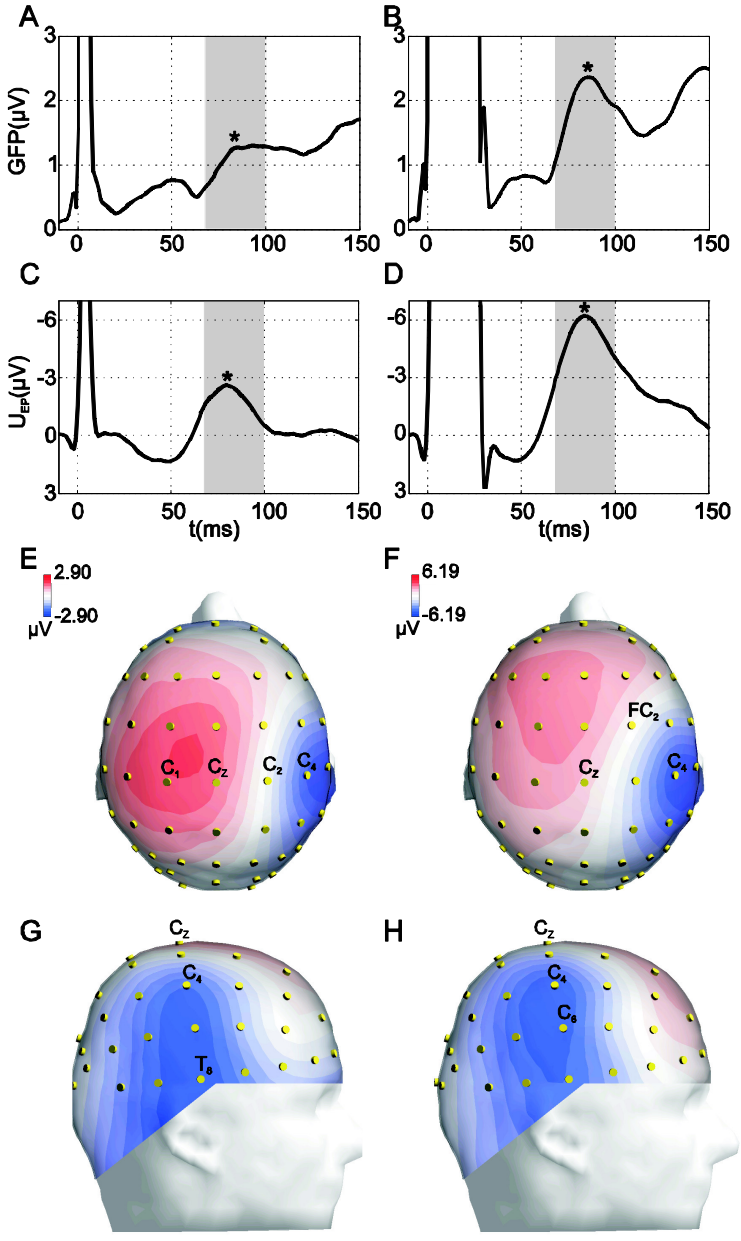

Figure 4.2: Here we show the method to obtain the latency of interest and the corresponding scalp distributions. GFP across 61 electrodes for the grand average EPs of both SP (C) and PT (B) and the grand average EPs recorded at C_4 with average reference (SP:C and PT:D). In a time window of 65-100 ms (grey area) the latency of interest of the N90 component is chosen between the most optimal GFP peak latency and C4 peak latency (illustrated with a star). For SP scalp the distributions at 81 ms are shown (E,G) and for PT the scalp topography at 84ms (F,H).

Both scalp distributions show negative activity contralateral to the stimulus (G and H). For SP the maximum negative activity starts just below the C_4 electrode up to T_8. There is a change in potential from negative to positive around C_2 with a focused maximum positive activity starting in front of C_1. The negative component for PT is focused around C_4 and C_6 electrodes. The potential transition from negative to positive activity can be seen around FC_2. Compared to SP the maximum positive activity of PT is located more frontally and is less focused

4.3.3 Dipole localisation SP method

Dipole source analysis was performed on the N90. The analysis with two dipoles did not result in improved GOF and/or dipole localisations. Therefore, only the results of the single dipole localisations are presented. In figure 4.3A-C the dipoles with a GOF>85% for SP are presented. The obtained dipoles were projected at a standardised MRI model. According to the GOF criterion, dipoles were obtained from 17 subjects.

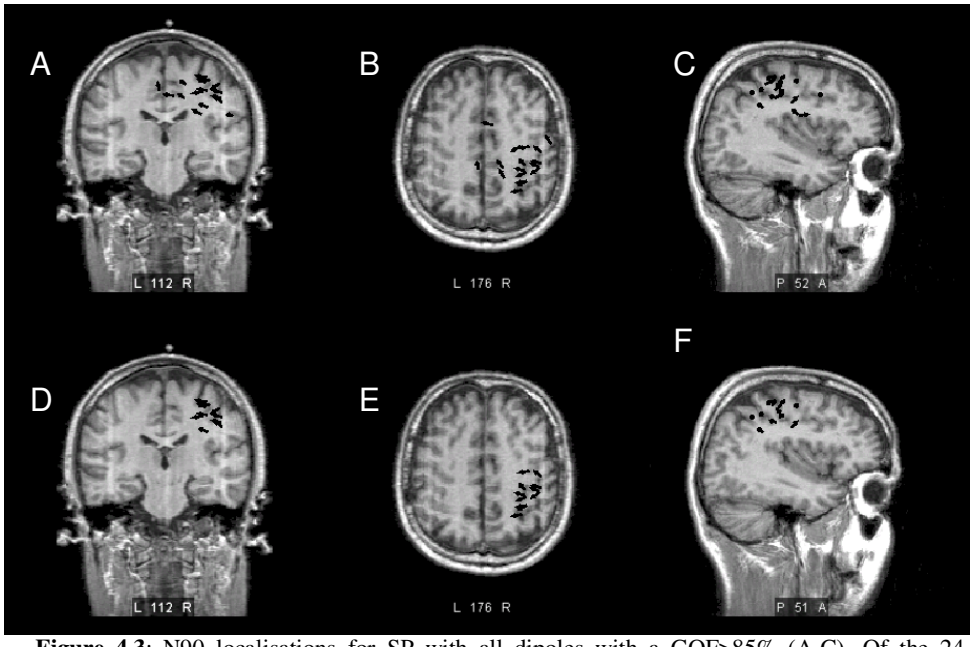

Figure 4.3: N90 localisations for SP with all dipoles with a GOF>85% (A-C). Of the 24 subjects 17 subjects had a localisation with GOF>85%. Dipoles are projected onto coronal (A,D), axial (B,E) and sagittal (C,F) views of a standard MRI model. After outlier detection using the RD 11 dipoles remained (D-F). Please note that all dipoles were projected at the same MRI slices.

Most of the dipoles (except one) are located in the right hemisphere. It can be seen that a group of dipoles is clustered around the postcentral gyrus. In the axial (B) and sagittal

view (C) two dipoles are located more caudally and ventrally relative to the cluster of dipoles. One of them is located near supra lentiform and the other more laterally near the precentral gyrus. Furthermore, four dipoles are located near the midsagittal plane (see coronal and axial view). In figure 4.3D-F the remaining 11 dipoles after outlier detection are shown. It can be seen that the 6 dipoles described above are removed. The remaining dipoles are located in the postcentral gyrus.

4.3.4 Dipole localisation PT method

For PT the dipoles fulfilling the GOF criterion are shown in figure 4.4A-C. Similar to SP only the dipoles obtained after single dipole localisation are shown. From the 24 included subjects, 16 subjects had a dipole with a GOF exceeding 85%. In the coronal (A) and axial (B) view two clusters of dipoles can be distinguished. One located around the postcentral gyrus and the other more caudally and ventrally compared to other cluster.

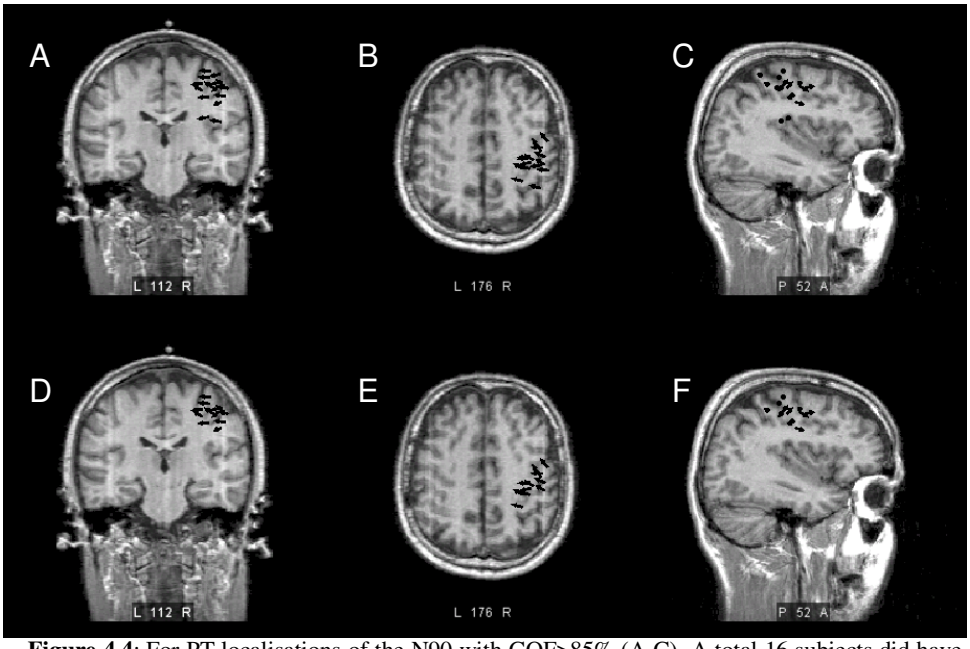

Figure 4.4: For PT localisations of the N90 with GOF>85% (A-C). A total 16 subjects did have a localisation with GOF>85%. Dipoles are projected onto coronal (A,D), axial (B,E) and sagittal (C,F) views of a standard MRI model. Outliers of the major group of dipoles were obtained using the RD. The remaining 12 subjects are shown in D-F. Please note that dipoles were projected at the same MRI slices.

The latter group consists of only two dipoles which are located near the insular cortex. In the axial view (B) it can be seen that two dipoles in the large cluster are located more dorsally. Using the RD the main group of dipoles is obtained by removing remote

dipoles. The remaining group of dipoles consists of 12 dipoles (figure 4.4D-F). These dipoles are located in the postcentral gyrus. Furthermore, the cluster of dipoles is oriented caudally (sagittal view) and frontally (axial view) indicating that the cluster is following the course of the postcentral gyrus. Compared to SP, the PT dipoles are on average located more superficially and frontally (see axial (E) and sagittal views (F)). A total of 8 subjects had dipoles in both SP and PT clusters.

4.3.5 Mean dipole location, latency and orientation

For SP and PT, mean dipole coordinates, orientation and latencies from the remaining clusters of dipoles were calculated. The determined two dipoles are represented in figure 4.5 (see for mean coordinates table 4.1). The mean x-coordinate was significantly different ($t(1,21)=-2.57$, $p=0.018$).

Table 4.1: Mean coordinates (±SD) and latency (±SD) of N90 dipoles of both SP and PT after RD outlier detection. Total number of subjects is 11 for SP and 12 for PT. Significant difference indicated by a star.

	x (mm) *	y (mm)	z (mm)	latency (ms)
SP	-23.24±11.78	-35.27±6.46	44.57±7.92	80.18±8.75
PT	-11.54±10.08	-38.71±7.66	46.89±7.43	81.08±6.04

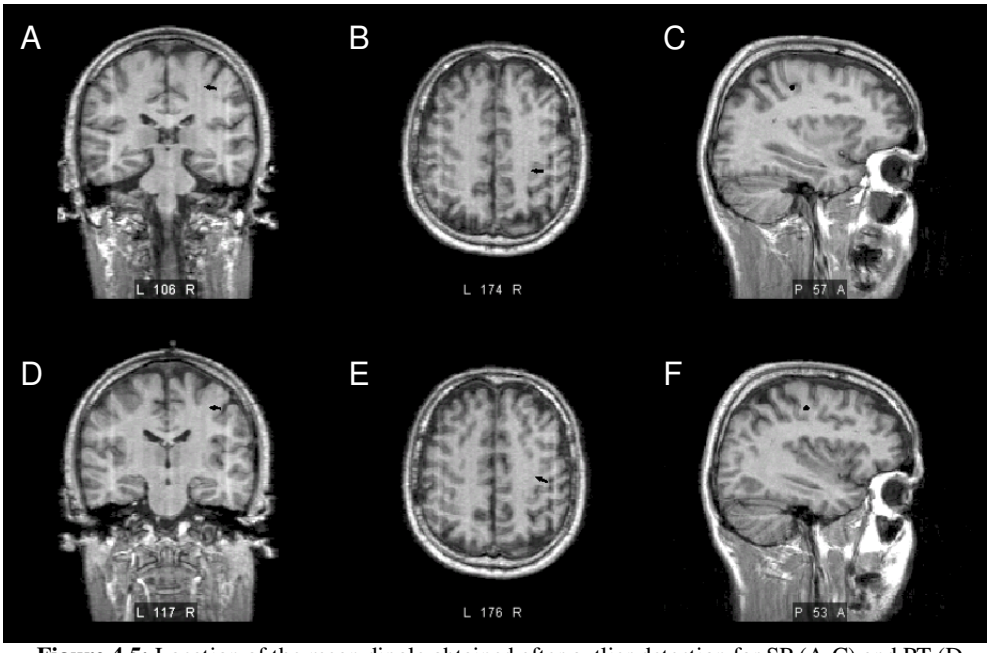

Figure 4.5: Location of the mean dipole obtained after outlier detection for SP (A-C) and PT (D-F). The mean coordinates are projected onto coronal (A,D), axial (B,E) and sagittal (C,F) views of a standard MRI model. The dipoles differed significantly in anterior-posterior direction.

This difference can also be distinguished in the axial and sagittal MRI views with a more frontally located PT dipole. Comparison of the two clusters of dipoles also showed an on average more frontal localisation for PT. For the y and z coordinates only small differences were obtained which were not significantly different.
The orientation of the dipoles is schematically illustrated in figure 4.6. Both dipoles are oriented ipsilaterally but the PT dipole points more in the anterior direction and less rostral. The difference in anterior-posterior direction (x-direction) is significant ($t(1,21)=-2.36$, $p=0.028$). Also in the coronal views in figure 4.5 (B,E) the difference in orientation can be observed.
The obtained mean latencies of SP and PT did not differ significantly (see table 1).

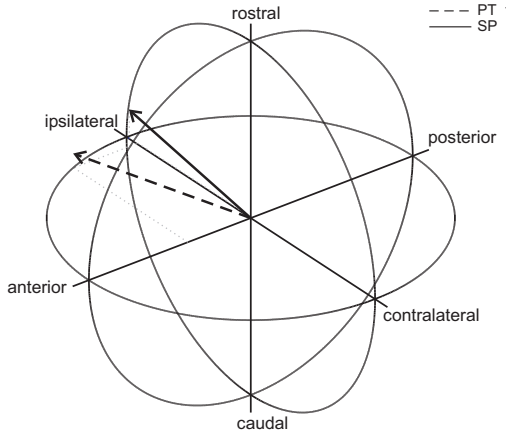

Figure 4.6: Schematic representation of the mean relative orientation of clusters of SP (solid line) and PT (dashed line) after outlier rejection. Both start in the middle of a sphere illustrating the relative orientation of the dipoles. Location of the dipoles is not taken into account in this figure. The orientation differed significantly in the anterior-posterior direction.

4.4 Discussion

We investigated which brain regions are involved in the generation of the contralateral N90 potential following SP and PT stimulation. Earlier we showed that the contralateral N90 EP component is changed by PT stimuli only [153]. Furthermore, the CPT (painful heterotopic stimulation) did reduce the N90 amplitude for PT only. Involvement of the nociceptive system in this early component was suggested. The morphology of the grand average EPs recorded at
C_Z-A_1A_2 and C_4-F_Z (Figure 4.1A and B) obtained in the current study corresponds to previous measured EPs [153]. For source analysis single moving dipole source localisations were used for both SP and PT, using individual EPs. For both methods the majority of dipoles were located in the postcentral gyrus. Nevertheless, compared to SP

the mean dipole of PT is located more anterior and additionally the mean orientation of PT points more anterior.
For dipole source localisations we used a standard realistic head model and a standard MR image of the head. Differences in conductivities, dimensions and shape between the standard head model and the actual head of individual subjects will lead to less accurate dipole solutions [28; 98]. The localisation accuracy by using a standard realistic head model is influenced by several factors with localisation errors in the order of 1 cm. For instance, variation in conductivity can produce an error of maximal 4 mm [28] and dissimilarities in skull and scalp thickness cause an error of much less than 1 cm [27]. Using a standard MR image instead of individual will also imply less accurate anatomical projections.
Nevertheless, for SP 17, and for PT 16 out of 24 subjects had a localisation of the N90 activity with a GOF exceeding 85%. For SP, all dipoles (except one) were scattered in the hemisphere contralateral to the stimulus (3A-C). Compared to SP, the PT dipoles were less dispersed in the right hemisphere and two clear groups of dipoles could be distinguished (figure 4.4A-C). Besides the use of a standard head model and MRI also the signal-to-noise ratio (SNR) of the recordings can influence the dipole source localisation [28; 157]. Since the N90 amplitude was significantly larger for PT the SNR of SP could be lower, which may explain the more scattered dipole localisations for SP.
After outlier detection mean coordinates, latencies and orientation were calculated for both clusters of remaining SP and PT dipoles. Just like the individual dipoles, mean dipoles were located in the postcentral gyrus (figure 4.5). Compared to SP, the dipole following PT stimulation is located significantly more frontally (figure 4.5B and 4.5E), and the PT dipole is oriented significantly more anteriorly (figure 4.6). This difference in orientation of the dipoles can also be seen in the scalp distributions (figure 4.2 E, F). The obtained dissimilarities in location and orientation between SP and PT dipoles indicate a different position at the postcentral gyrus. This gyrus meanders oblique in the posterior-anterior, dorso-ventral direction from the medial longitudinal fissure to the lateral sulcus. The PT dipole is probably located more anteriorly at the postcentral gyrus. In the mediolateral and dorso-ventral direction small (non significant) differences were obtained; the PT dipole is located more laterally and dorsally. Thus, albeit differences in effect on the N90 by SP and PT, both SP and PT dipoles were located in the postcentral gyrus but at different positions.
The postcentral gyrus is the location of the primary somatosensory cortex (SI). Activation of the contralateral SI following tactile and nociceptive stimulation is reported in several studies (for review see: [1; 113]). Tactile and nociceptive information are processed differently within the SI. Tactile stimuli activate two sources sequentially while only one source is activated by nociceptive stimuli [62; 117]. Tactile information, relayed via the dorsal column–medial lemniscus, activates firstly Brodmann's area 3b of the SI. This area occupies the rostral bank of the postcentral gyrus. Sequential responses

are generated in area 1 in the crown of the postcentral gyrus [62; 117]. Nociceptive stimulation is transmitted via the spinothalamic tract (STT) to area 1 in the SI. It was shown that location and orientation of the SI dipole in area 1 is similar for tactile and nociceptive information [62; 117]. Both probably originate from the anterior fissural parts of area 1 (central sulcus) [62; 117].

The stimulation electrode used in the current study activates both tactile and nociceptive fibers [153]. Therefore both area 3b and 1 are probably activated following SP and PT stimulation. Nevertheless considering the peak latency area 1 is more likely to be involved in generation of the N90 potential. Area 1 activity around 90 ms was also reported following preferentially Aδ-fibers stimulation by epidermal stimulation [62]. Both dipoles were oriented slightly in rostral direction. These dipoles cannot originate from the crown part of area 1 but the fissural part of area 1 could be involved.

In previous work we suggested involvement of the spinothalamic tract in the N90 by PT. The CPT (heterotopic pain stimulation) inhibited N90 amplitudes following PT stimulation [154]. This reduction may be ascribed to activation of the DNIC [81], Activity of both spinal wide dynamic range neurons (WDR) and some nociceptive specific (NS) neurons is inhibited by noxious heterotopic stimulation [16; 139]. WDR and NS neuron information is relayed through two different parts of the STT to the thalamus; the anterior and lateral STT respectively. The anterior STT projects via the thalamus to the SI and the lateral STT projects to the SII.

Earlier it was not possible to differentiate with certainty which of the two tracts was involved in the N90. However, the demonstrated involvement of the postcentral gyrus, which is the location of SI, in the generation of the N90 implicates that information is in all probability transmitted through the anterior STT.

In sum we have explored which brain regions are involved in the generation of the N90 potential using moving dipole source localisation. We showed that the neuronal networks in the postcentral gyrus are involved, most probably transmitted through the anterior STT. This is important for further research to changes in the nociceptive system in pain patient.

Chapter 5

Evoked potentials from single pulse and pulse train electrocutaneous stimulation in patients with lumbosacral radiculopathy

Abstract - Different observation techniques can be used to measure changes in the pain system. Earlier we showed in healthy subjects that changing the stimulus amplitude of a single electrical pulse (SP) or the number of pulses (NoP) in a train (PT) varies evoked potentials (EPs) and subjective ratings differently. Additional stimulation with a cold pressor test (CPT) reduced NRS scores and EP amplitudes. Inhibition by CPT was ascribed to activation of the diffuse noxious inhibitory control (DNIC). Here we studied the processing of SP and PT stimuli and the effect of CPT in patients with lumbosacral radiculopathy (LSR) and analysed possible differences with healthy subjects.

Patients with LSR and healthy subjects were electrically stimulated at the left middle fingertip during two protocols where the right hand was immersed in water of 0-1°C (CPT) or 32°C (control). Subjects had to withdraw and re-immerse their hand after subsequently 3 and 1 minute until the end of the protocol. Grand average EPs and numeric rating scale (NRS) scores were averaged from 105 stimuli of 5 stimulus amplitudes (SP) or NoP (PT).

In both groups similar EP components and NRS scores were influenced by SP and PT stimuli. But, EP amplitudes were larger or smaller in patients. Except for decreased NRS scores and P300 using SP stimuli, the effect of CPT lacked in patients. Ratios between grand average EP amplitudes of CPT and control protocol showed similarities between both groups.

Albeit equal modulations by SP and PT methods, the dissimilar EP amplitudes suggest that these methods can be used to measure changes in central pain processing in patients. The lack of inhibition by CPT seems to imply a dysfunctional DNIC, but for at least some components the equivalent EP ratios of both groups appear to argue with that. The possibilities of the two methods as a tool to analyse deficient DNIC should be further explored with a larger group of patients and patients with a proven dysfunctional DNIC.

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Submitted for publication

5.1 Introduction

At present our knowledge of various processes involved in chronification of pain is limited. Adequate observation techniques are required to explore changes in the nociceptive system of pain patients. Evoked potentials (EPs) can be used to measure cortical activations reflecting central processing of noxious stimuli. Somatosensory evoked potentials and laser evoked potentials are used in neurophysiological studies to explore changes in the pain system in several pain syndromes. For example, fibromyalgia was studied with both methods; compared to healthy subjects lower thresholds and higher amplitudes of EP components were revealed [34; 89]. Migraine, chronic low back pain and tension headache are examples of other pain syndromes examined using EPs [31; 43].

Previously, we investigated the effect of two different stimulation methods on EPs and subjective pain experience. In these methods the stimulus strength of intracutaneous electrical stimuli [14] was modulated by changing the stimulus amplitude of a single pulse (SP) or by varying the number of (fixed amplitude) pulses in a train (PT). Since both tactile and nociceptive fibers are activated increasing the stimulus amplitude by SP results in a change in the proportion of activated fibers, depending on the local fiber densities. Changing the stimulus strength by PT results in more action potentials in a similar proportion of fibers.

Both SP and PT influenced EP components and subjective ratings, however not all EP components were changed by both methods. The early contralateral N90 was changed by PT only. Furthermore, the cold pressor test (CPT) as an additional stimulus (heterotopic noxious conditioning stimulation) inhibited the amplitude of several EP components [155]. However, only the N90 component by PT was decreased by CPT. Inhibition by CPT is ascribed to the activation of the diffuse noxious inhibitory control (DNIC). Dissimilarities between SP and PT processing were in particular reflected in the early contralateral N90 EP component. Source localisation revealed that for both SP and PT the N90 activity was generated in the postcentral gyrus [156]. Taken together, we suggested that most likely the anterior spinothalamic tract is involved in the generation of N90 by at least PT. The N90 therefore might be a good indicator for the nociceptive system and involved mechanisms such as the DNIC.

The DNIC is a phenomenon which describes inhibition of activity of most wide dynamic range (WDR) neurons and some nociceptive specific (NS) neurons in the dorsal horn by heterotopic noxious conditioning stimulation [81; 139]. Descending information from the dorsal reticular nucleus reduces activity of spinal and trigeminal WDR and some NS neurons [10]. The DNIC was first described for animals [81] but several studies have shown that the DNIC can also be induced in healthy subjects [150; 166]. Dysfunction of the endogenous pain inhibition is suggested for patients with different chronic pain syndromes such as fibromyalgia [75; 77] or chronic tension-type headache [114].

However, a deficit of the DNIC is not inferred in chronic low back patients [63], patients with long-term trapezius myalgia [83] and rheumatoid arthritis [84].
Up to now the SP and PT stimulation method and the effect of CPT were only explored in healthy subjects. Measurement with patients suffering from pain can help to further analyse the potential of the SP and PT method as observation methods of changes in the nociceptive system.
Lumbosacral radiculopathy (LSR) is frequently diagnosed in clinical practice. About 10-40% of the patients with acute radiculopathy develop a chronic pain syndrome [132]. Lumbosacral radicular pain is caused by compression (figure 5.1), inflammation and/or injury to a spinal nerve root in the low back.
To analyse the relevance of stimulation methods to explore changes in the central processing of noxious stimuli, it is important to include a group of patients with a known cause of the pain complaints. In patients with radiculopathy the pathophysiology, the primary cause of the pain, is clear. It is unknown if the DNIC is dysfunctional in patients suffering from lumbosacral radicular pain.

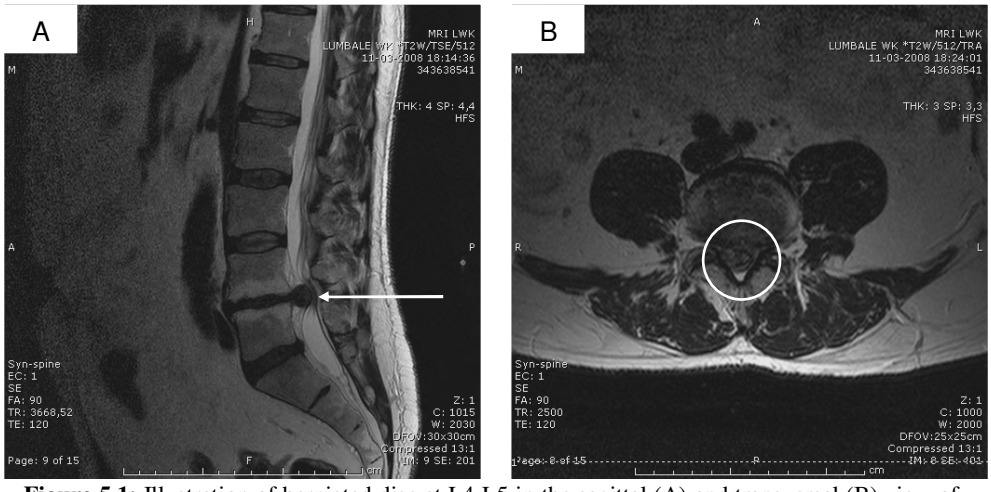

Figure 5.1: Illustration of herniated disc at L4-L5 in the sagittal (A) and transversal (B) view of MR image.

It is assumed that remote from the herniated disc the peripheral nerve system is unchanged. Thus, stimulation remote to the herniated disc might be useful to analyse changes in the central processing of noxious stimuli. These considerations have motivated us to use this group to explore our observation methods. Furthermore this group of patients was relatively easy accessible.

Here we study firstly the processing of the SP and PT stimuli and the effect of the CPT in patients with LSR. Secondly, we analyse possible differences in processing between these patients and healthy subjects. We hypothesise that a possible deficit in the endogenous pain inhibition could be reflected in a lack of inhibition of EP components by CPT.

5.2 Methods

5.2.1 Subjects

Seven male and two female patients were included in the study (age 44.4±10.3) all suffering from LSR. The subjects reported no chronic or other acute pain. All subjects were diagnosed for LSR by a neurologist on clinical grounds. Details of the clinical data are presented in table 5.1

Table 5.1: Demographic and clinical data of patients with LSR. Level of herniated disk, lateralisation of the radicular pain, and the VAS of the radicular pain (mean VAS of 4 measurements during experiment) are shown. Also, kind of analgesic and the moment of last intake of analgesics before the start of the experiment are presented. Analgesics are abbreviated as follows: P-paracetamol, D-diclofenac, O-oxycodone, and T- tramadol.

Subject	Sex	Age	Left/Right	Level of hernia	VAS hernia	Analgesia	Last intake (hours)
1	M	35	L	L5-S1	52.7	None	
2	M	52	L	L4-L5	41.5	O	-9h
3	M	56	R	L5-S1	47.8	O	-48
4	F	33	L	L5-S1	0	D	-24
5	M	37	R	L5-S1	52.5	T	-12
6	M	53	L&R	L4-L5	6.8	D	-8
7	F	57	L	L3-L4	9.0	O and P	-1
8	M	45	R	L4-L5	0	D and P	-48
9	M	32	R	L5-S1	58.0	None	

Seven of the nine subjects were treated with analgesics. The moment of last intake varied from 1 hour to 48 hours before the start of the experiment (see table 5.1).

As a control, twelve male and twelve female healthy subjects (age 40.2 ± 13.8) participated in the same experiment. None of the subjects used psychotropic medication. All subjects gave written informed consent according to the Declaration of Helsinki. The study was approved by the ethical committee of the Medisch Spectrum Twente, Enschede.

5.2.2 Electrical stimulation

The subjects were electrically stimulated at the left middle fingertip. This corresponds to the intracutaneous electrical stimulation method [14]. An electrode with a 1 mm diameter tip of gold in an insulating material was used. A small opening was drilled in the upper layer of the skin of the fingertip using a dental gimlet with the same diameter as the tip of the stimulation electrode [14]. If the sensation threshold was higher than 1 mA the preparation was regarded insufficiently and tried again. A rectangular surface electrode (a 4x9 cm Klinerva Blue Electrode) was placed with a distance of at least 10 cm at the upper part of the left forearm as an anode. The stimuli were generated by a battery-driven computer controlled current stimulator. The stimulus was a bipolar rectangular current pulse with a stimulus duration of 0.2 ms.

For each subject, the stimulus amplitudes corresponding to the subjective sensation threshold (I_S) and pain threshold (I_P) were determined before each measurement block. Thresholds were obtained by the ascending method of limits using amplitude steps of 0.1 mA starting from zero. This threshold search was repeated three times.

5.2.3 SP and PT method

The SP and PT method used in this study have been described in detail previously [153]. For SP, the stimulus current amplitude of a single pulse was varied in discrete steps depending on the obtained I_S and I_P according to:

$$I = I_P + q \cdot (I_P - I_S) \quad q = -0.5, -0.25, 0, 0.25, 0.5 \qquad (5.1)$$

The fixed stimulation current for PT was chosen similar to the minimum stimulus amplitude $I_{-50\%}$ of SP (equation 5.1, q=-0.5). The NoP for PT varied from 1, 3, 5, 7, to 9. The inter pulse interval (IPI) between two subsequent pulses in the pulse train was 5 ms. To make sure that stimulation by PT was tolerable, the five NoP were applied in increasing order before the protocol. Although the stimulus amplitude of a single pulse of PT stimulus was below the subjective pain threshold, subjects described stimulation by a train of five pulses as a clear pricking painful sensation.

5.2.4 CPT and control protocol

A polystyrene squared vessel was filled with ice water 0-1°C (CPT) or 32±0.5°C (control). The right hand was immersed up to the wrist in the water. During CPT the subjects were stimulated to keep their hand in the water as long as possible with a maximum of three minutes. Subjects had to withdraw and re-immerse their hand after subsequently 3 and 1 minute until the end of the protocol (about 9.5 minutes). Time to hand withdrawal and re-immersion was recorded. Pain intensity and unpleasantness increases rapidly [169] and peaks in the first 20-45 seconds [134]. Therefore, electrical stimuli at the left fingertip were applied 30 seconds after hand immersion.

5.2.5 EEG recordings

Electrical brain activity was recorded using a 64-channel EEG system (A.N.T. Enschede, the Netherlands). Ag/AgCl electrodes were placed according to the international 10-5 system (Waveguard EEG cap). The ground electrode was placed at the top of the nose. All scalp electrode impedances were less than 5 kΩ. An electrode was placed under the left eye for electrooculogram (EOG) recording. Furthermore, subjects were instructed to fix their eye on a point in front of them. The sample frequency was 1 kHz and filter settings were 0.3-120 Hz. Data from -10 to 100 ms pre-stimulus was used for baseline correction. The time window of analysis was 100 ms pre-stimulus to 400 ms post-stimulus.

5.2.6 Subjective ratings

Subjects rated the perceived strength of each electrocutaneous stimulus on an 11 point NRS scale ("no sensation" = 0, "strongest imaginable pain" = 10). The first electrical stimulus corresponded for SP with the pain threshold $I_{0\%}$ (equation 5.1, q=0) and for PT with a train of 5 pulses at $I_{50\%}$ (equation 5.1, q=-0.5). The subjects were instructed to rate the first stimulus with a six. Furthermore, after CPT subjects were asked to rate orally the perceived strength of the right hand during the measurement on a similar NRS scale.
Before each CPT and control measurement the patients were asked to rate their radicular pain on a visual analog scale (VAS) of 10 cm ('no pain" = 0 cm, "strongest imaginable pain" = 10cm).

5.2.7 Procedure

The experiment consisted of two blocks of three protocols; a block for SP and PT each. A block consisted of an identical stimulus (IS), CPT and a control protocol. The order of the SP and PT blocks and the order of CPT and control protocol were randomized.
During the IS protocol a total of 100 identical electrical stimuli were applied at the left middle fingertip. For SP the stimulus was a single pulse at pain threshold (equation 5.1, q = 0) and for PT 5 pulses at minimum stimulus amplitude (equation 5.1, q = -0.5). Data of the IS measurement is not analysed in this paper.
During the CPT and control protocol a total of 105 randomized electrical stimuli were applied at the left middle fingertip with 21 stimuli for each of the five stimulus amplitudes (SP) or five NoP in a pulse train (PT). The inter stimulus interval between two successive stimuli was randomly varied between 4 and 6 seconds.
The inhibitory effect of the CPT can persist for 5-10 minutes after withdrawal of the hand [134]. Therefore, to be sure that there was no effect of CPT in a subsequent protocol we waited 15 minutes between the CPT and the control protocol and between the two blocks.

5.2.8 Data analysis

At least 11 sweeps were needed for each of the five subject-EPs obtained in a measurement. If one of the five subject-EPs had fewer than 10 accepted sweeps, the subject was excluded from analysis of the concerning measurement. Grand average EPs were calculated from C_Z versus A_1A_2 and C_4-F_Z recordings for each of the five stimulus amplitudes or NoP for the CPT and control protocol. Trials with an EOG artefact exceeding ±70 µV in the time windows of -10 to -100 ms pre-stimulus and 60 to 400 ms post-stimulus were rejected. Subsequently, non rejected data was accepted after visual inspection for missed EOG artefact or muscular artefacts. Mean NRS scores were obtained at all five stimulus amplitudes (SP) or at all five NoP (PT) for both CPT and control. All statistical tests were performed at a level of significance of $p<0.05$.

Analysis of patient data

For both SP and PT we analysed NRS scores, EP component P300 at 300ms and N150-P200 peak-to-peak amplitude (P200 at 200 ms (SP) and 210 ms (PT) and N150 at 150 ms) all recorded at C_Z-A_1A_2 and N90 at 86 ms recorded at C_4-F_Z. Besides these EP components for SP also the P50 at 52 ms measured at C_4-F_Z was analysed. Due to the stimulation duration of the artefact this component could not be analysed for PT.

For statistical analysis SPSS 15.0 (SPSS, Chicago, IL) was used. A repeated measures analysis of variance (ANOVA) was used to test for the two factors: stimulus method (stimulation amplitude or NoP) and condition (control or CPT). To correct for sphericity assumption violation a Greenhouse-Geisser degrees of freedom adjustment was applied (p value indicated by p_{GG}). The effect of CPT for each stimulus amplitude or NoP was tested post-hoc by a paired-sample student's t-test.

Comparison of patient data with data of healthy subjects

The NRS scores, amplitudes of EP components, and stimulus currents of healthy subjects were compared with data of patients with LSR.

A one way ANOVA was used to test the difference in stimulus currents.

We analysed the differences in EP peak amplitude of the components of interest described earlier. The minimum stimulus strength is similar for both SP and PT. To maximise the difference between SP and PT stimulation only the four highest levels were used for the analysis of EP amplitudes. Thus for each component the mean EP amplitude of the four highest stimulus strengths are obtained. Mean NRS scores were calculated likewise. A one way ANOVA was used to test for the difference in mean EP amplitudes and NRS scores between both groups.

Of all grand average EP components the ratio between CPT amplitude and control amplitude was calculated. If this ratio is smaller than 1 the EP amplitude has decreased due to CPT. The mean ratio for all five stimulus strengths was calculated and compared. The ratio between NRS scores for CPT and control protocol was calculated similarly.

5.3 Results

Patients

5.3.1 NRS scores

Mean NRS scores for SP with control and CPT protocol are shown in figure 5.2A. For both control and CPT increasing stimulus amplitudes changed the NRS scores linearly. NRS scores were significantly varied by the stimulus amplitude ($F(4,32)=85.12$, $p<0.0005$). Except for the minimum stimulus amplitude, the NRS scores for CPT are lower than control scores ($F(1,8)=7.09$, $p<0.029$).

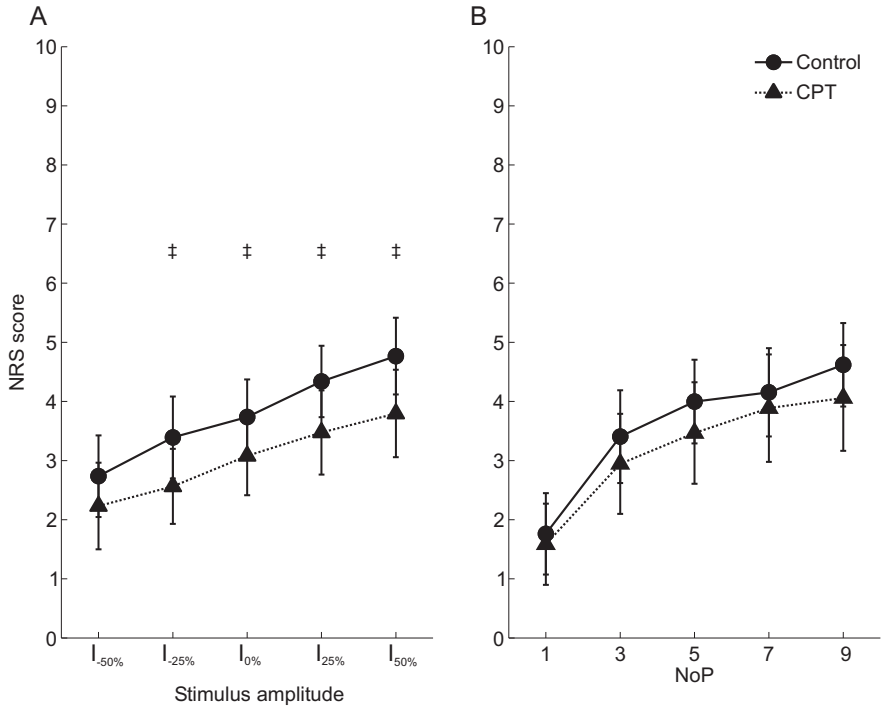

Figure 5.2: Mean NRS scores (± SEM) of all 5 stimulus amplitudes and NoP for both SP (A) and PT (B) with control and CPT protocol. Each symbol represents the mean NRS score of all included subjects at the stimulus amplitude or NoP under test. Significant post-hoc effect of CPT per stimulus amplitude marked with ‡.

The relationship between NRS and NoP was non-linear for both control and CPT (figure 5.2B). The effect of NoP was significant ($F(1.15,8.08)=22.70$, $p_{GG}<0.001$). NRS scores did not significantly decrease by the CPT ($F(1,8)=4.63$, $p=0.069$).
After removal of the hand out of the ice water subjects rated the perceived pain, during the measurement, with NRS = 7.13 ± 1.29.

Furthermore, prior to each control or CPT protocol subjects were asked to rate the perceived radicular pain. The VAS rating did not change significantly during the experiment ($F(3,29)=0.02$, $p=0.99$), the grand average VAS rating was 29.80 ± 25.08.

5.3.2 Vertex EPs of SP

Vertex grand average EPs were obtained for each of the five stimulus amplitudes for both control and CPT (figure 5.3A and C). The P300 EP amplitude significantly varied with stimulus amplitude ($F(4,32)=15,20$. $p<0.0005$). Furthermore, the P300 amplitude was significantly decreased by CPT ($F(1,8)=5.79$, $p=0.043$). Figure 5.5D shows the P300 peak amplitudes for all stimulus amplitudes for both control and CPT. The N150-P200 peak-to-peak amplitude (figure 5.5C) was not influenced by stimulus amplitude ($F(1.67,13.35)=1.14$, $p_{GG}=0.39$) nor by CPT ($F(1,8)=4.43$, $p=0.068$).

5.3.3 Vertex EPs of PT

Figure 5.3B and D show grand averages EPs (C_Z-A_1A_2) of five NoP with control or CPT. A stimulation artefact can be seen in the first milliseconds of the EPs, lasting up to 45 ms for 9 pulses.

Both P300 EP amplitude and N150-P200 peak-to-peak amplitude were varied significantly by NoP (respectively $F(4,28)=8.04$, $p<0.0005$ and $F(4,28)=8.02$, $p<0.0005$). An increasing NoP changes the N150-P200 in a similar non linear manner as the NRS scores (figure 5.5F).

The CPT did not significantly inhibit the P300 amplitude ($F(1,7)=2.33$,$p=0.171$) and the N150-P200 peak-to-peak amplitude ($F(1,7)=4.18$,$p=0.080$)

5.3.4 Contralateral EPs of SP and PT

Grand average EPs measured contralaterally to the stimulus location at C_4 referred to F_Z are shown in figure 5.4. The effect of stimulus strength and CPT on the P50 was only tested for SP (figure 5.5A). The stimulus amplitude significantly changed the P50 amplitude ($F(4,32)=4.99$, $p=0.003$), but the EP amplitude was not varied by CPT ($F(1,8)=0.39$, $p=0.55$). Furthermore, for SP no relationship between stimulus amplitude and N90 was obtained ($F(4,32)=2.01$, $p=0.116$), nor a reduction of this amplitude by CPT ($F(1,8)=2.10$, $p=0.19$, see figure 5.5B).

EPs of PT, with control and CPT, also show a clear N90 peak (figure 5.4B and D). This peak is significantly modulated by NoP ($F(4,28)=27.33$, $p<0.0005$) in a comparable non-linear fashion (figure 5.5E) as described for NRS scores and N150-P200 peak-to-peak amplitude. No effect of CPT on the N90 amplitude by PT was found ($F(1,7)=3.12$,$p=0.121$).

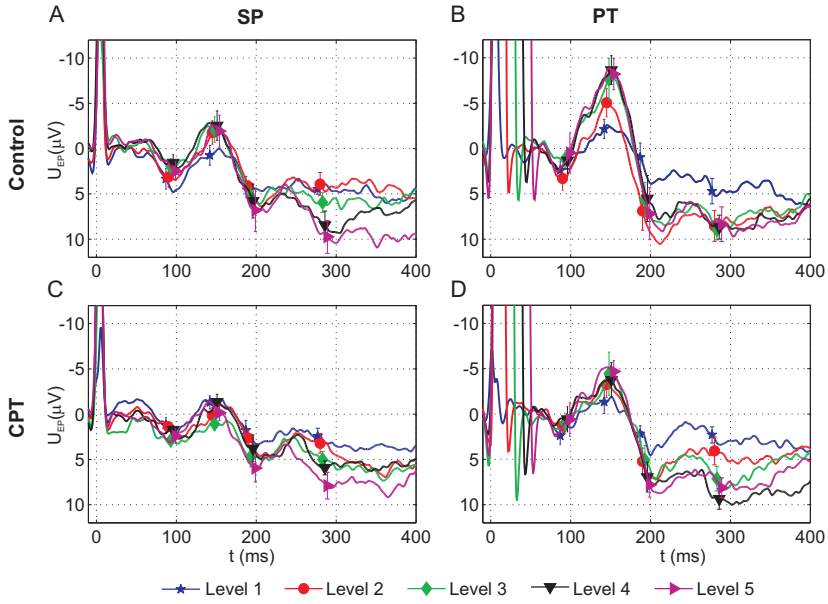

Figure 5.3: Grand average EPs (± SEM) measured at C_Z-A_1A_2 of each of the five stimulus amplitudes for SP with control (A) or CPT (C) protocol. Grand average EPs of PT with control (B) or CPT (D). A stimulation artefact can be seen in the first ms of the EPs up to 45 ms for 9 pulses. The five levels mentioned in the figure legend correspond to stimulus amplitude or NoP.

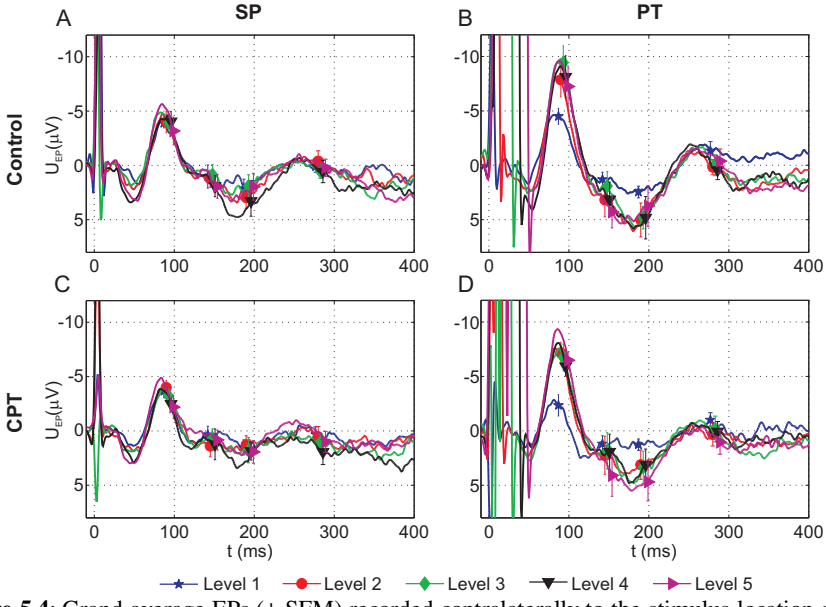

Figure 5.4: Grand average EPs (± SEM) recorded contralaterally to the stimulus location at C_4-F_Z of all five stimulus amplitudes for SP: control (A) or CPT (C). Grand average EPs (C_4-F_Z) of five NoP for control (B) or CPT (D). A stimulation artefact can be seen in the first ms of the EPs up to 45 ms for 9 pulses. The five levels mentioned in the figure legend correspond to stimulus amplitude or NoP.

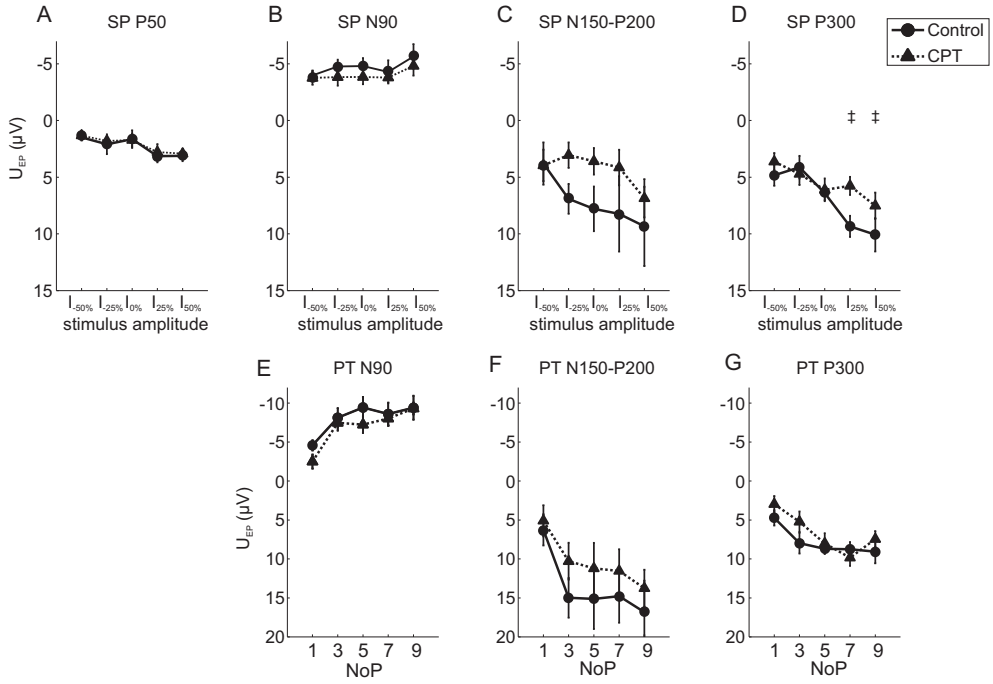

Figure 5.5: Amplitude (±SEM) of following EP components measured at C_4-F_Z: P50 (A), N90 (B,E) and EP components measured at C_Z-A_1A_2: P300 (C,F) and N150-P200 (D,G) for SP (A-D) or PT (E-G) for control and CPT protocol. Significant post-hoc effects of CPT at the P300 by SP are marked per stimulus amplitude with ‡.

Patients and healthy subjects

5.3.5 Pain and sensation thresholds

For two patients with LSR the I_S and I_P were adjusted before the second measurement block. This resulted in a I_S of: 0.35±0.21 mA (SP) and 0.34±0.23 mA (PT) and in a I_P: for 1.63±0.90 mA (SP) and 1.48±0.91 mA (PT). These sensation and pain thresholds were not significantly different. I_S (0.28 ± 0.20 mA) and I_P (1.36 ± 0.62 mA) of the healthy subjects were slightly smaller but not significantly different of the patient thresholds.

5.3.6 EP peak amplitudes and NRS scores

The grand average EP amplitudes of the components of interest (see data analysis) of the healthy subjects were compared with those of the patients. Mean amplitudes were obtained for all (except the minimum strength) stimulus strengths. Figure 5.6A (SP) and 5D (PT) illustrate EP grand average amplitudes of the N90 (control protocol) for each stimulus amplitude or NoP. EP amplitudes in the grey area are used to calculate a mean

EP for both subject groups. This mean can be found in 5B and 5E in the grey area. For SP (B,C) and PT(E,F) with both control (B,E) and CPT(C,F) the mean grand average EPs are obtained likewise.

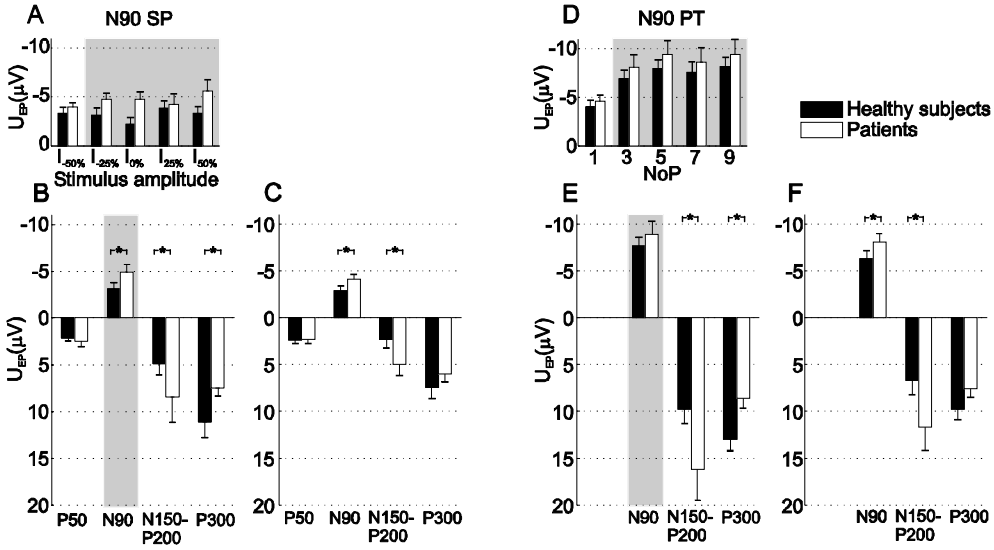

Figure 5.6: The N90 EP amplitudes (±SEM) of all five stimulus amplitudes (SP) or NoP (PT) with the control protocol (A and D) of both healthy subjects (black) and patients with LSR (white). From the amplitudes in the grey area (all except the minimum strength) a mean amplitude is calculated. The resulting means are shown in the grey areas in B and E. Of each component of interest mean amplitudes are similarly calculated of SP with control (B) or CPT (C) and PT with the control (E) or CPT (F) protocol. Significant differences between healthy subjects and patients are marked with a star.

Statistically significant differences are marked with a star. Of all four measurements, the N150-P200 peak-to-peak amplitudes of the patients were significantly larger. Conversely, the P300 amplitudes of SP and PT with control were significantly smaller for patients. The difference in P300 amplitudes of SP and PT with CPT was not significant.

The contralateral P50 amplitude was similar for both groups. But, the contralateral N90 of patients is also significantly larger for SP with control and SP and PT with CPT. Albeit a larger N90 EP amplitude of patients for PT with control, no significant difference was obtained.

Although the means of the grand average NRS scores of patients were lower than those of healthy subjects, no significant difference was obtained (data not shown).

5.3.7 Ratio EP amplitude and NRS between CPT and control protocol

For both SP and PT, ratios between EP amplitude of CPT and control protocol of all stimulus strengths of the components of interest were calculated. In figure 5.7 the median, maximum and minimum EP ratios are depicted. The grey area marks the ratios between 0 and 1.

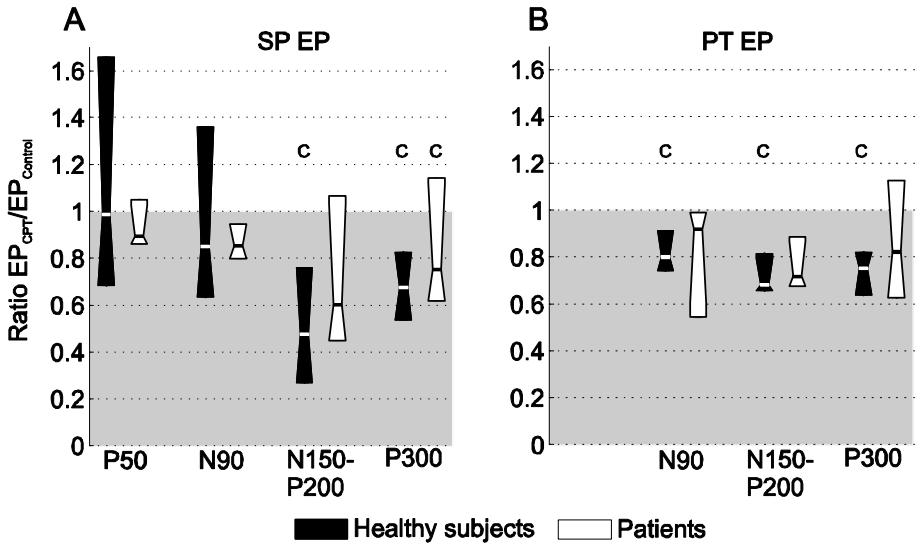

Figure 5.7: The ratio between EP amplitude with CPT and EP amplitude with control protocol for SP (A) and PT (B). For both subject groups ratios of all five stimulus strengths of each component of interest are calculated. The median, maximum and minimum are plotted in the boxplots. The letter C is used to mark components which were significantly changed by CPT (results of repeated measures ANOVA reported earlier).

Although the ratio of P50 of SP of healthy subjects was about 1, all median ratios for both groups were below 1. For SP, the maximum ratios of P50 and N90 of healthy subjects were above 1, as well as N150-P200 and P300 of patients with LSR. For the patient PT group, only the maximum ratio of the P300 exceeded 1.
In figure 5.8 the ratios of NRS scores are shown (SP:A and PT:B). All ratios were smaller than 1. EP components and NRS scores of each group which were significantly influenced by CPT were marked with a C (results of repeated measures ANOVA reported above (patients) and for healthy subjects in [155]).

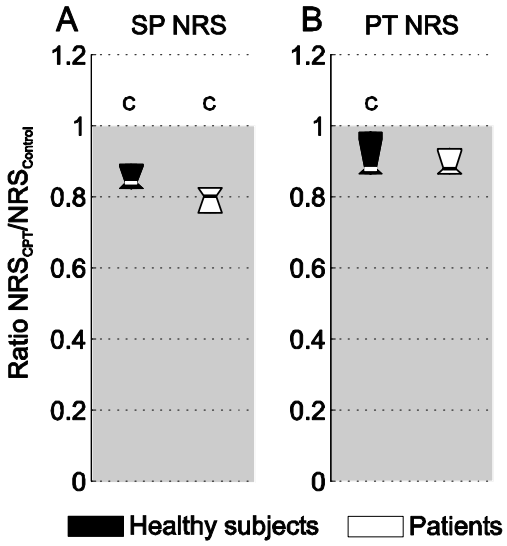

Figure 5.8: The ratio between NRS scores with CPT and NRS scores with control protocol. For both subject groups ratios of all five stimulus strengths are calculated. The median, maximum and minimum are plotted in the boxplots. The letter C is used to mark NRS scores which were significantly changed by CPT (results of repeated measures ANOVA reported earlier).

5.4 Discussion

In patients with LSR, NRS scores and P50 and P300 amplitudes were significantly changed by stimulus amplitude (SP). For PT, the NRS scores, as well as the N90 and P300 amplitude and N150-P200 peak-to-peak amplitude varied with NoP (figure 5.5). The influence of SP and PT in patients was similar to earlier results in healthy subjects [155]. Hence, radiculopathy did not influence the effect of stimulus amplitude or NoP.

5.4.1 EP peak amplitudes of patients with LSR and healthy subjects

Of several components under test the EP amplitudes of patients were significantly different from those of the healthy subjects (figure 5.6). With respect to the site of stimulation it was hypothesised that no differences in the peripheral sensory system exist. Patients with LSR and healthy subjects exhibited similar sensation and pain thresholds. The unequal EP amplitudes can therefore not be ascribed to dissimilar thresholds.

Electrical stimulation at the painful thumb (homotopic) in patients with cervical radiculopathy resulted in larger EP component amplitudes than stimulation at their non painful thumb or in healthy subjects [138]. These differences were ascribed to changes in neural activity at multiple levels of somatosensory system such as at the spinal level. However, in the current study the unaffected dermatome was stimulated. Hence, in

contrast to Tinazzi [138] in our study stimulation of a non-painful part of the body resulted in dissimilar EP amplitudes.
Apart from PT with control, for all measurements the early contralateral N90 component of patients was significantly greater. In fibromyalgia syndrome patients, compared to healthy subjects, temporal intracutaneous electrical stimulation (2.5Hz) at the arm [34] and laser stimulation [89] at the hand resulted in significant larger amplitudes of early and middle components (N80 and N170 respectively). Besides enhanced amplitudes pain thresholds were lowered [89]. Enhanced sensory processing in fibromyalgia pain subjects was suggested. The early contralateral N90 component in this study is also mainly ascribed to the somatosensory processing. For both SP and PT, the N90 activity was generated in the postcentral gyrus [156]. The significant larger amplitude in patients might also be ascribed to changes in the central processing of noxious stimuli. It seems that SP is more sensitive for these changes.
For all measurements the N150-P200 peak-to-peak amplitude of patients was significantly larger. Interestingly, reverse results were found for the P300 of both SP and PT with the control protocol; in patients the P300 amplitude was smaller. The difference in P300 amplitude by SP and PT with CPT was not significant. This might be ascribed to a difference in amount of decrease of the amplitude by CPT.
Late laser EP components (P390) were also enhanced in fibromyalgia subjects [88]. In contrast amplitudes in migraine patients subjects were similar to healthy subjects [31]. Late EP components can be influenced by several cognitive processes. Cognitive and affective processes can be different in pain patients. For example, migraine patients showed a lack of habituation and laser EP amplitudes were not inhibited by modulation of attention [29; 31]. Also chronic back pain patients lacked habituation to the stimulus [43]. In healthy subjects it is shown that the P300 reflects cognitive processes like attention/distraction [5; 172]. If also patients with LSR lacked inhibition by modulation of attention as in migraine patients also greater P300 amplitudes would be expected. In this view the smaller P300 amplitudes are striking. Analgesics can decrease EP peaks, this might have influenced the P300. However, it is notable that the N150-P200 is much larger in patients.
Although smaller, the subjective ratings of patients were not significantly different from those of patients. The reverse results of P300 probably indicate that different processes are involved in this component and N150-P200 peak-to-peak amplitude.

5.4.2 CPT in patients

CPT did only decrease NRS scores and P300 amplitude by SP. For PT, none of the tested variables were significantly inhibited. This lack of inhibition in patients is contrast with earlier results in healthy subjects [155]. (In healthy subjects for SP, NRS scores and P300 and also the N150-P200 peak-to-peak amplitude were inhibited. For PT, NRS scores and the amplitudes of all EP components under test decreased by CPT.)

In healthy subjects, Inhibition by CPT is ascribed to activation of the DNIC. The lack of inhibition in patients is possibly caused by a dysfunctional DNIC. Several studies with patients with chronic pain syndromes such as fibromyalgia have shown a deficient pain modulation by heterotopic noxious conditioning stimulation. Most of these studies focused on effects on thresholds such as the pressure pain threshold (fibromyalgia [75]), and the electrical detection or pain threshold (chronic tension headache [114]). Nevertheless, capsaicin decreased the vertex peak-to-peak EP amplitude following laser stimulation in healthy subjects but not in patients with migraine without aura [30].
Interesting, in patients with LSR, for PT the NRS scores and none of the tested EP components were significantly inhibited by CPT. In contrast, in healthy subjects these variables were inhibited. These current results might indicate a deficient functioning of the DNIC in patients with LSR.

5.4.3 CPT ratios in patients with LSR and healthy subjects

All median ratios between grand average EP amplitude with CPT and control protocol were below 1 (figure 5.8). This includes the components of which statistical analysis failed to reveal a significant effect of CPT. In healthy subjects and patients the P50 and N90 by SP did not reveal a significant effect by CPT. Although the medians are in between 0.9 and 1 it can be seen that both have a maximum clearly above 1. Although in patients with LSR the maximum ratio of these components was less large, medians were comparable.
The ratios of N150-P200 (SP), N90 (PT) and the P300 (SP and PT) are less comparable in both groups; median ratios of patients are larger. Still all these median ratios are below 1, and of the N90 the maximum ratio did not exceed 1.The median ratio of the N90 by PT was above 0.9 like the median for the SP P50 and N90 in healthy subjects which were not significantly affected by CPT. However the ratios of N90 by PT showed a large dispersion and are not univocal. Considering the DNIC in patients with LSR the results of the N90 are not conclusive. Nevertheless, the ratios might support the lack of inhibition described earlier.
For PT, the ratios of all components under test are less dispersed than SP ratios. This indicates that for PT reduction of amplitudes by CPT is equivalent for all stimulus strength.

5.4.4 Methodological remarks

The majority of patients were treated with different kinds of analgesics, such as opioids (tramadol and oxicodone), non-steroidal anti-inflammatory drugs (NSAID; diclofenac) and paracetamol. The last moment of intake before the experiment varied from 1 hour to 48 hours. Hence, influences of analgesia in at least a part of the subjects cannot be ruled

out. Also the mechanism of action of the analgesics may continue after the plasma and liquor half-life time.
Several studies have shown that analgesics can affect stimulation thresholds, EP components and the effect of the DNIC [131]. It is shown that besides opiods, NSAID and also paracetamol decreases the EP amplitude and pain thresholds following laser and intracutaneous electrical skin stimulation [4; 90; 131].
The effect of morphine (opioid) on the DNIC was tested in rats and humans. In rats and humans systemic (low dose) morphine reduced or completely blocked the DNIC [12; 79; 80; 126]. Besides, the magnitude of DNIC was significantly smaller in chronic pain patients treated with opioids than in non-opioid treated patients [121]. It is suggested that opioid receptors in the periaqueductal grey (PAG) are indirectly involved in the reduction of DNIC by morphine [12]. Consequently, possible effects of analgesia should be taken into account.
Although no significant effect of CPT was obtained for the majority of the tested EP components care should be taken with the interpretation of these results. In figure 5.5 it can be seen that for SP and PT, the N150-P200 peak-to-peak amplitudes with CPT are smaller than those of the control protocol. This holds also for other components but the difference is much smaller. Furthermore, considering the comparable median ratios between grand average EP amplitude with CPT and control protocol and the dispersion in ratios the lack of inhibition of especially the N150-P200 and NRS is notable. A lack of statistical power should be considered as an explanation of the lack of the inhibition of at least N150-P200 and the NRS scores by PT. Also, to the best of our knowledge a dysfunctional DNIC was not earlier reported in humans with radiculopathy.

5.4.5 Conclusion

To conclude, it was shown that although similar EP components under test were modulated by SP and PT in patients and in healthy subjects, EP amplitudes differed. These differences in EP amplitudes might indicate changes in central somatosensory processing and dissimilar cognitive processes in patients. However, the exact origins of these variations are still unclear. Nevertheless, we have shown that, by stimulation at the non-painful part of the body, with both methods it is possible to measure central changes in the pain system in patients suffering from LSR.
To evaluate the effect of the CPT in patients with LSR a larger group of subjects is required. The ratios suggest that with a larger group probably more tested variables will be significantly changed by CPT and may provide additional evidence for a functional DNIC. Consequently, further research to the sensitivity of SP and PT to measure an altered DNIC is required. We propose also the use of a group of patients with a proven deficient DNIC.

Chapter 6

General discussion

The aim of this study was to explore the merits of electrocutaneous SP and PT stimulation methods as observation techniques of the nociceptive system. Both techniques were applied in healthy subjects (chapter 2-4) and patients with LSR (chapter 5). Furthermore, the results of this thesis shed some light on the link between the neurophysiology of nociception and the recorded evoked potential following electrocutaneous stimulation.

6.1 Neurophysiological explanation of EPs

Activity induced by SP and PT stimuli is transmitted via several pathways to the cortex. The measured evoked potentials reflect not only cortical activations, but the entire central processing of SP and PT stimuli. In this subsection we couple EP peak activity to pathways.

In our measurements the P50 appears as the first contralateral EP component following SP stimulation at the fingertip (chapter 2). Although a clear P50 peak was also observed following PT stimulation at the fingertip, it was polluted with the stimulus artefact. As discussed in chapter 2, the P50 most likely results from activation of Aβ-fibers transmitted via the dorsal column-medial lemniscus (DCML, orange pathway in figure 6.1) to area 3b in the SI. The lack of effect of CPT on the peak amplitude, as seen in chapter 3, supports this view.

The N90 was the next contralaterally recorded EP component. This component was significantly altered by NoP (see e.g. chapter 2) and inhibited by the CPT (chapter 3). Inhibition by CPT is ascribed to activation of the supraspinal mediated DNIC system. Descending activity of the dorsal reticular formation acts on WDR and some NS neurons [81; 82; 139]. Information of the WDR neurons (Rexed laminae V) is relayed through the anterior STT and NS (Rexed lamina I) on the lateral STT. To further unravel the involved pathway source localisation was used (chapter 4). It was shown that the N90 following both SP and PT stimuli was generated in the postcentral gyrus. SI is located in this brain region. Thus taken together, the results of chapter 3 and 4 suggest that the activity transmitted via the anterior STT is reflected in the N90 (green pathway in figure 6.1). This would also imply that area 1 of SI is activated.

The N150-P200 peak-to-peak amplitude and the P300 component were recorded at the vertex electrode. For both SP and PT these components were influenced by the CPT. Most likely the lateral STT (blue pathway in figure 6.1) contributes to the generation of these EP peaks. These peaks are generated in several cortical areas such as the SII, IC and ACC. Other pathways and brain regions, not depicted in figure 6.1, may also be involved in these late components.

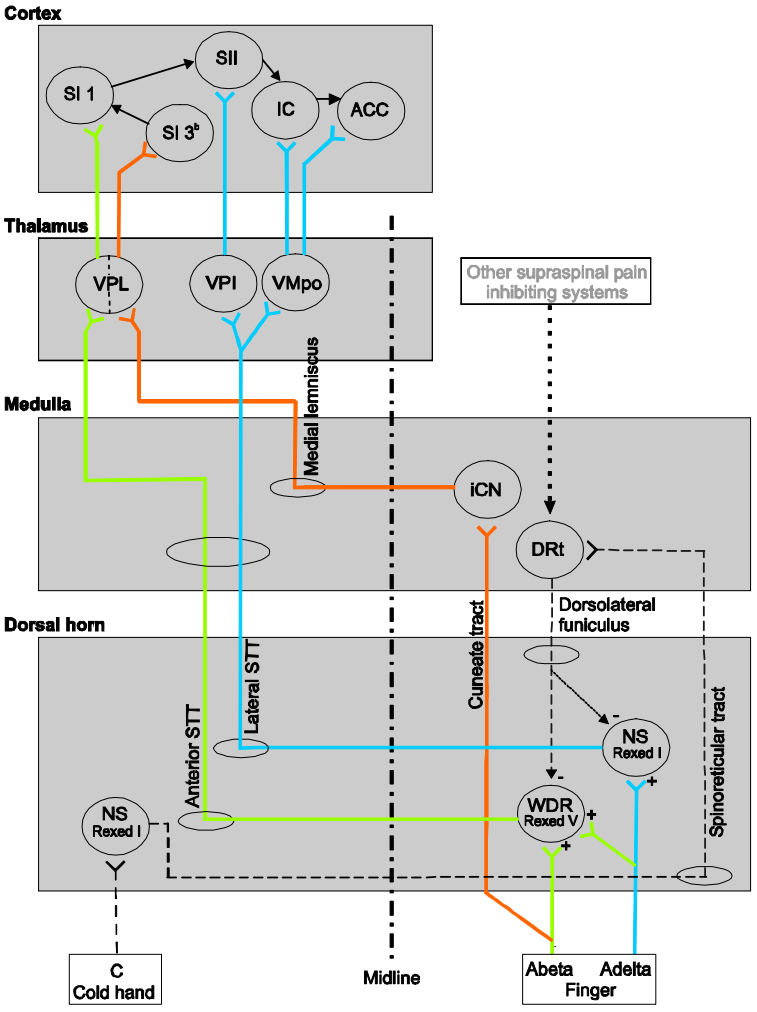

Figure 6.1: Simplified representation of anatomical connections, based on literature, relevant for pain processing. Orange represent pathways involved in contralateral P50. Green represent pathways involved in contralateral N90. Blue lines are pathways involved in late vertex potentials. Dashed black lines represent the DNIC system. ACC: anterior cingulate cortex, DRt: dorsal reticular nucleus, IC: insular cortex, iCN: internal cuneate nucleus, NS: nociceptive specific neurons, Rexed I: lamina I in the dorsal horn, Rexed V: lamina V in the dorsal horn, SI 1: primary somatosensory cortex area 1, SI 3b: primary somatosensory cortex area 3b, SII: secondary somatosensory cortex, STT: spinothalamic tract, VPI: ventroposterior inferior nucleus, VPL: ventroposterior lateral nucleus, VMpo: ventromedial posterior nucleus, WDR: wide dynamic range neurons

6.2 DNIC or attention?

A strong noxious stimulus can activate the DNIC system [81] (chapter 1 and 3). For both SP and PT, the vertex N150-P200 and P300 were inhibited by the CPT (chapter 3). Interestingly, only the contralateral N90 component by PT was decreased significantly by CPT. Reduced amplitudes of vertex EP components by a heterotopic noxious stimulation correspond to the results of earlier studies [3; 20; 46]. To the best of our knowledge, inhibition by CPT of the early contralateral N90 was not reported earlier.

Cognitive processes such as attention can also influence the amplitude of late potentials [6; 171]. Comparison between the effect of a CPT and distraction by a mental task showed that both affected late laser evoked potentials, but differently [115].

The effects of attention on early potentials are less obvious. A calculation task did not influence early components following electrical stimulation [171]. Conversely, other studies reported that attention indeed affected early potentials [33; 101]. These discrepancies might be ascribed to differences in the task protocols [171]. To the best of our knowledge for early EP peaks a comparison between CPT and distraction is not described in literature.

In all experiments in this thesis the attention to the test stimulus is controlled by the task to rate each electrical stimulus. Still, the CPT is a very strong stimulus and it might interfere with the task to rate the test stimulus. Possibly the inhibition reflects involvement of more processes than the DNIC only.

To analyse the effect of cognitive processes such as attention on the early and late EP components following SP and PT stimulation we propose to perform new experiments with distracting tasks such as arithmetic tasks.

6.3 Effect stimulus strength

Changing the current amplitude of a single pulse is more often used to change the stimulus strength than changing the NoP [17; 133]. A major disadvantage of the SP method is the unknown change in the proportion of activated tactile and nociceptive fibers. For PT stimulation this disadvantage is absent; a similar proportion of tactile and nociceptive fibers is activated repeatedly.

Especially the early contralateral EP components are interesting since these components reflect somatosensory processing of the stimulus and are less influenced by cognitive processes. For the P50, the effect of changing stimulus strengths was only tested for SP. The P50 amplitude significantly varies with the stimulus amplitude. Due to the stimulus artefact the effect of NoP could not be analysed. Although the P50 was not tested for PT, a clear peak was present and thus an effect of NoP cannot be ruled out. Interestingly, the contralateral N90 significantly varied with NoP only, and was not influenced by stimulus amplitude.

Both P50 and N90 are generated in the SI but in different areas (see section 6.1). There are different stages from periphery to the cortex where the stimulus strength might be encoded. Encoding of the stimulus strength in the SI is reported in some studies [87; 105; 137; 140]. Both thermal as well as electrical stimuli were used and of both methods the strength was encoded in the SI cortex. The results of studies on humans were in agreement with recordings in monkeys [23; 73]. The stimulus strength can also be encoded at earlier levels in the involved pathways.

Information from WDR neurons (Rexed lamina V) is relayed via the anterior STT. Both tactile and nociceptive fibers project to the WDR neurons. The question arises if and how the modulation by PT is influenced by the total number and the proportion of activated fibers. We propose the use of an electrode stimulating nociceptive cutaneous afferents more preferentially (for example the epidermal stimulation of Inui [58]) to analyse if the effect of NoP is similar for different diameter nerve types.

In this thesis stimulation parameters were similar in all experiments. In a follow-up study the effect different stimulus parameters might be explored to further our knowledge about both methods and especially the PT method. Examples are the inter-pulse interval and the used NoP. The inter-pulse interval of 5 ms was chosen such that it was well outside the total refractory period. However, it is unknown how the inter-pulse interval (IPI) influences the effect of the NoP. We wonder if the relationship between NoP and EP amplitude or NRS scores changes if the IPI is increased. This should be further analysed in a follow-up study.

In this study we stimulated with 1, 3, 5, 7 or 9 pulses. The difference between EP amplitudes and NRS scores of 5 and 9 pulses was much smaller than the difference between 1 and 3 pulses. To the best of our knowledge only one study reported the effect of changing the NoP from 1 to 7 pulses [47]. In their study subjective ratings from the single to double pulses and from double to triple pulses were significantly increased. Furthermore, the blink reflex was only significantly increased from single to double pulses. We wonder whether the EP amplitude and NRS scores following PT skin stimulation with 2 pulses fits in the curve between 1 and 3 pulses. Is the effect between single and double pulses comparable to the results of Giffin [47]? To analyse this, we suggest to use another range of pulses for example 1 to 5 pulses.

6.4 Stimulus location

An important feature of a somatosensory stimulation method is the possible applicability to different anatomical locations [13]. This would enable us to analyse changes in the pain system in patients by stimulating affected and unaffected sites of the body. In chapter 2 we compared stimulation at the fingertip and forearm. The local fiber density and proportion in fingertip and forearm differs; local fiber density in the fingertip is larger [22; 106; 107].

For the late vertex potentials, the effect of NoP on EP amplitudes and NRS scores was similar for stimulation at the fingertip and forearm (chapter 2). Also the relationships between subjective ratings and EP components were comparable for PT. For SP, stimulation at forearm revealed neither an effect of stimulus amplitude on the P300 amplitude nor a relationship between subjective ratings and N150-P200 peak-to-peak amplitude. Stimulation at the fingertip or forearm with PT resulted in more equivalent outcomes for late potentials.

As mentioned before, early evoked potentials are especially interesting since they reflect somatosensory processing. For stimulation with the minimum stimulus strength ($I_{-50\%}$) early contralateral P50 and N90 activity was less pronounced for forearm stimulation (figure 1.2 in chapter 1). The sensation and pain thresholds for fingertip and forearm stimulation were not significantly different, resulting in equivalent stimulus currents for both sites. The smaller contralateral EP amplitudes might be ascribed to the difference in fiber density which might influence the EPs; less activated fibres results in less neural activity. In addition, both stimulus locations have a different cortical representation due to the somatotopic organisation; the area representing the fingertip in the SI is larger represented than the forearm. This may also lead to differences in the EP shapes and amplitudes.

The early N90 activity is also present for stimulation at the forearm (figure A.1 in Appendix A), although not significantly changed by NoP like with fingertip stimulation. Still, the amplitude was increased for higher NoP, resulting in a clear N90 peak (figure A.1 and A.2 in Appendix A). Increasing the stimulus amplitude (SP) did not result in a clear N90 peak. Thus, it is most likely that the PT method is the preferred method for stimulation at different anatomical locations. Probably the PT method is less sensitive for differences in density and proportion of nerve fibers. However, in this thesis only one study was performed with two stimulation locations. The effect of PT on the N90 for different stimulus locations should be further analysed to ascertain that PT gives similar results for early EP components.

6.5 Observation of the nociceptive system?

The use of electrical stimulation became less popular since both nociceptive and tactile nerve fibers are activated. The activity in anterior STT contributes to the generations of the N90 (see section 6.1). The supplying WDR neurons (Rexed lamina V) respond to both noxious and non-noxious stimulation. Earlier, the anterior STT was associated with crude touch, pressure and movement sensation, and the lateral STT with pain and temperature sensation [26]. Nowadays the involvement of the anterior STT in nociception is disputed [26; 147]. Studies on humans using laser stimulation revealed that a least two distinct pathways are involved in the transmission of the noxious stimuli [145]. It is suggested that the sensory aspects of pain are encoded by the WDR neurons [94]. Although both tactile and nociceptive fibers are activated by our electrocutaneous

stimuli our results strongly suggest that properties of the nociceptive system are observed. Using the epidermal stimulation of Inui also revealed activation of the SI around 88 ms [58]. This suggests activation of the nociceptive pathway. We recommend the use of a preferential electrode in a follow-up study with at least the PT method.

6.6 Clinical relevance

The ultimate goal of fundamental research is, of course, to make it useful for clinical practice. In the clinical practice robust, reliable and easy to use measurement tools of the nociceptive system are desired for both diagnosis and further research to pain syndromes.

For instance, to diagnose radiculopathy the use of laser evoked potentials, stimulating the affected site, was proposed as an reliable and sensitive method to objectively record pathological dorsal root function in early monosegmental radiculopathy [119]. On the other hand the use of somatosensory evoked potentials, following sural nerve stimulation at the painful site, as a diagnostic tool for radiculopathy was questioned [39]. Besides a diagnostic tool, stimulation methods also enable us to increase our knowledge about pain syndromes and the transition from acute pain to chronic pain.

The SP and PT method were tested in patients with LSR. The subjects were stimulated on a non-painful part of the body. The effect of stimulus amplitude or NoP was similar in patients with LSR and healthy subjects. So, radiculopathy did not influence the effect of changing stimulus strength. Although the effects of SP and PT were similar for healthy subjects and patients with LSR, different results in patients with other pain syndromes or stimulation at an affected site of the body may be found.

Despite the lack of difference in intensity coding the EP amplitudes were different in healthy subjects and patients with LSR (chapter 5, figure 5.6). Thus central changes in the pain system in patients with LSR can be measured while stimulating an unaffected part of the body. It seemed that the SP method was more sensitive for this change resulting in enlarged N90 amplitudes also in the control protocol. For the late vertex potentials differences in cognitive activations, such as distraction, or habituation [29; 43] between patients and healthy subjects may also play a role. The aforementioned suggestion to implement a distraction task in the protocol might also apply here. Nevertheless, though it is not exactly obvious which change in the pain system is measured here, the stimulation methods can be valuable in clinical research.

A dysfunctional DNIC is reported for some pain syndromes but not for others [63; 75; 84; 114]. In contrast to healthy subjects where for PT all EP components under test were decreased by CPT, none of these components were decreased by CPT. For SP, compared to healthy subjects, in patients only the N150-P200 peak-to-peak amplitude was not inhibited by CPT (chapter 5). Especially the lack of inhibition of the contralateral N90 by the PT method could be interesting. However the group of included patients was small and some patients were under influence of analgesics. Therefore care should be

taken with the conclusions concerning the effect of the CPT. We recommend to perform further research with a group of patients with a determined dysfunctional DNIC system such as fibromyalgia. In this manner the strengths of both methods concerning measurement of a dysfunctional DNIC system can be evaluated more precisely.

Patients can also help to increase our knowledge about the pathways which are now unravelled by experimental results in combination with knowledge from literature. Stimulation of patients with disorders such as the lateral medullary syndrome (Wallenberg syndrome) might be an option. These patients can show a deficit in the pain and temperature sensation due to a dysfunctional lateral STT. The involvement of the lateral STT in the generation of vertex potentials may be analysed using this group of patients. This might give more insight in the effect of the lateral STT and cortico-cortical processes on vertex potentials.

How can these methods be implemented in further research? The measurement protocol used in this thesis is rather lengthy (about 10 minutes per stimulation method) which is not preferable in clinical practice. A slimmed version of the protocol is desired. The PT method favours the possibility of stimulation at different locations resulting in similar results. However, the SP method seems to be more sensitive for changes in the pain system resulting in increased EP amplitudes. With current knowledge it is preferable to combine both methods and use the 5 pulses of PT and the stimulation at the pain threshold of SP. The minimum level $I_{50\%}$ can be added if also the different effects of changing amplitude or NoP are to be analysed. To analyse for example neuroplasticity in chronic patients PT with 5 pulses is recommended. It was shown in chapter 4 that PT with 5 pulses results in less dispersed source localisation.

Taken together, to further analyse changes in the nociceptive system different stimulation protocols can be formulated based on the SP and PT method depending on the research question.

Appendix A

In this appendix contralateral recorded EP data of the experiments described in chapter 2 are shown. The data were not included in the article presented in chapter 2. However, the data were mentioned in the general discussion. In figure A.1 grand average EPs (C_4-F_Z) for both SP and PT at the forearm were shown (for fingertip stimulation see figure 2.4). None of the components (P50, N90) were significantly varied with stimulus amplitude or NoP. For PT a clear N90 peak is visible (figure A.1B) which is not present for SP (figure A.1A). In figure A.2 the amplitudes of the contralateral N90 are depicted for both SP and PT for stimulation at the fingertip (A) and forearm (B). The N90 amplitude varied significantly for PT.

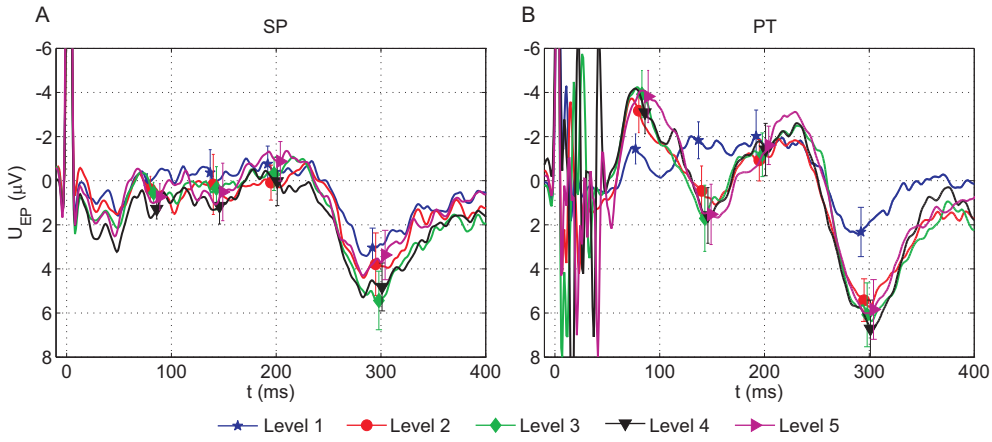

Figure A.1: Grand average EP (± SEM) recorded at the contralateral electrode (C_4-F_Z) of all five stimulus amplitudes by SP (A: N=17) and all five NoP by PT (B: N=15) for stimulation at the forearm.

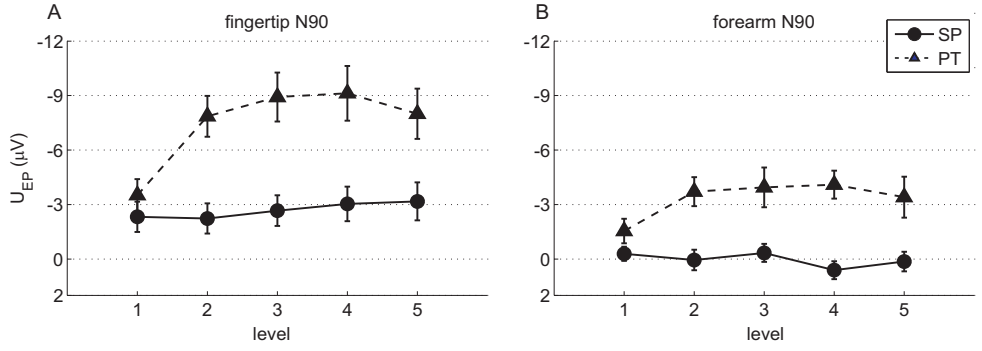

Figure A.2: Amplitude (± SEM) of contralateral N90 EP component for stimulation at the fingertip (A) and forearm (B) with SP and PT stimuli. The levels mentioned in the figure correspond to the stimulus amplitude (SP) or NoP (PT).

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List of acronyms

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
CPT	Cold pressor test
D	Diclofenac
DCML	Dorsal column-medial lemniscus
DNIC	Diffuse noxious inhibitory control
DRt	Dorsal reticular nucleus
EEG	Electroencephalography
EOG	Electrooculogram
EP	Evoked potential
EPSP	Excitatory postsynaptic potential
ES	Epidermal stimulation
Exp	Experiment
fMRI	Functional magnetic resonance imaging
GOF	Goodness of fit
IASP	International association for the study of pain
IC	Insular cortex
iCN	Internal cuneate nucleus
IES	Intracutaneous electrical stimulation
I_P	Pain threshold
IPI	Inter pulse interval
IPSP	Inhibitory postsynaptic potential
IS	Identical stimulus
I_S	Sensation threshold
LSR	Lumbosacral radiculopathy
MANOVA	Multivariate analysis of variance
MCD	Minimum covariance determinant
MEG	Magnetoencephalography

MR	Magnetic resonance
NoP	Number of pulses
NRS	Numeric rating scale
NS	Nociceptive specific
NSAID	Non-steroidal anti-inflammatory drugs
O	Oxycodone
P	Paracetamol
PAG	Periaquaductal grey
PET	Positron emission tomography
PT	Pulse train
RD	Robust distance
SD	Standard deviation
SEM	Standard error of mean
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SNR	Signal-to-noise ratio
SP	Single pulse
SRT	Spinoreticular tract
STT	Spinothalamic tract
T	Tramadol
VAS	Visual analogue scale
VMpo	Ventromedial posterior nucleus
VPI	Ventroposterior inferior nucleus
VPL	Ventroposterior lateral nucleus
WDR	Wide dynamic range

Summary

Pain has been subjected to research for ages. Nevertheless still our knowledge about the various processes involved in the chronification of pain is limited. Adequate observation techniques are required to explore changes in the nociceptive system in pain patients. In this thesis neurophysiological observation methods of pain system are explored. The focus of this thesis is to explore the merits of electrocutaneous single pulse and pulse train stimulation as observation techniques of the nociceptive system. To measure the central processing of these stimuli electroencephalography (EEG) was used. Especially evoked potentials measured at the vertex and contralateral electrodes were analysed.

The stimulus strength of an electrical stimulus can be varied in two different manners: spatially and temporally. Increasing the stimulus amplitude of a single pulse (SP) stimulus enlarges the area of recruitment. Due to the unknown local fiber density of tactile and nociceptive nerve fibers the proportion of activated fibers changes in an unknown manner. Changing the stimulus strength in a temporal fashion implies increasing the number of (fixed amplitude) pulses in a pulse train (PT). By increasing the number of pulses (NoP) more action potentials are generated in an unchanged proportion of tactile and nociceptive fibers.

In chapter 2 the effect of SP and PT stimulation on subjective ratings and EPs was compared. Electrical stimuli were applied to the fingertip and forearm. It is shown that both SP and PT influence EPs and NRS scores; however not all EP components were changed by both methods. Interestingly, for stimulation at the fingertip the contralateral N90 peak was changed by PT only. Although the N90 was present for stimulation at the forearm (appendix A), no effect of PT on was obtained. We concluded that different ways of processing are involved in both SP and PT method. PT is less dependent on stimulus location, which can be of interest for further research to changes in the nociceptive system of pain patients.

The obtained differences between both methods are further explored in chapter 3 using heterotopic noxious conditioning stimulation. We analyse the effect of the cold pressor test (CPT) as an additional stimulus on the processing of SP and PT stimuli. Subjects were electrically stimulated at the left middle fingertip during two protocols where the right hand was immersed in water of 0-1°C (CPT) or 32°C (control). The effect of SP and PT was not influenced by CPT. For both SP and PT the amplitude of several EP components were inhibited by CPT. Notably the amplitude of the early contralateral N90 was inhibited by CPT for PT but not for SP. Inhibition by the CPT was ascribed to activation of endogenous pain modulation by diffuse noxious inhibitory control (DNIC).

We suggest involvement of the spinothalamic tract in the generation of the N90 by at least PT.

To further unravel the pathway involved in the N90 in chapter 4 we analyse which brain areas are involved in the N90 following SP and PT stimuli. Dipole source coordinates and orientations of dipoles of both methods were compared. For both methods the majority of subject dipoles were located in the postcentral gyrus which is the location of the primary somatosensory cortex. For PT the subject dipoles were located less dispersed. We concluded that differences in N90 activity following SP and PT were not the result of activation of different brain regions. Taken together the results of chapter 3 and chapter 4 we suggest that the anterior spinothalamic tract is involved in the generation of the N90 by PT.

In the last chapter the experiments described in chapter 3 were performed with patients suffering from lumbosacral radiculopathy (LSR). We studied the processing of SP and PT stimuli and the effect of CPT in patients with LSR and analysed possible differences with healthy subjects. Similar EP components were modulated by SP and PT in both patients and healthy subjects. On the other hand EP amplitudes were larger or smaller in patients. This result suggests that the method can be used to measure changes in the central pain processing in patients. Also for the CPT differences were revealed between patients and healthy subjects. This might imply a dysfunctional DNIC. However the use of both methods as a tool to measure a dysfunctional DNIC should be further explored.

In this thesis we showed that both the SP and PT methods influence EP components and subjective ratings differently. The results shed some light on the link between neurophysiology of nociception and the recorded EPs. To further analyse changes in the nociceptive system in pain patients different measurement protocols can be formulated based on the SP and PT methods depending on the research question.

Samenvatting

Er wordt al eeuwenlang onderzoek gedaan naar pijn. Desondanks is onze kennis over de verschillende processen die een rol spelen in de chronificatie van pijn beperkt. Voor verder onderzoek naar de veranderingen in het nociceptieve systeem in pijnpatiënten zijn geschikte meettechnieken gewenst. Het doel van dit proefschrift is om meer inzicht te krijgen in de geschiktheid van electrocutane enkele-puls en pulstrein stimulatie als observatie techniek van het nociceptieve systeem. Voor het meten van de centrale verwerking van deze stimuli is er gebruik gemaakt van electro-encephalografie. Vooral opgewekte potentialen (EP's) gemeten op de vertex of contralaterale elektrode zullen worden geanalyseerd.

De stimulatiesterkte van een elektrische stimulus kan op twee verschillende manieren worden gevariëerd: spatiëel of temporeel. Door de amplitude van een enkele puls (SP) stimulus te verhogen worden er meer zenuwvezels in de huid geactiveerd. Omdat de lokale vezelverdeling van tast en nociceptieve zenuwvezels onbekend is zal de verhouding van geactiveerde zenuwvezels in een onbekende manier veranderen. Het temporeel veranderen van de stimulatie sterkte houdt in dat het aantal pulsen in een trein wordt verhoogd (met een vaste amplitude). Door de toename van het aantal pulsen (NoP) worden er meer actie potentialen gegenereerd in dezelfde tast en nociceptieve zenuwvezels.

In hoofdstuk 2 is een vergelijking gemaakt tussen het effect van SP and PT stimulatie op de gerapporteerde pijnervaring en EP's. Zowel het vingertopje van de linker middelvinger als de onderarm is gestimuleerd. Het blijkt dat zowel SP als PT stimuli de gerapporteerde pijnervaring en EP pieken amplitudes beïnvloeden. Echter niet alle EP pieken werden veranderd door beide methoden. Interessant is dat voor de N90 piekamplitude voor stimulatie op de vingertop alleen een effect van het veranderen van het NoP is gevonden. Hoewel voor onderarm PT stimulatie duidelijk een piek te zien is, wordt deze niet significant veranderd door PT (appendix A). We concluderen dat de SP en PT stimuli op verschillende manieren worden verwerkt. De PT methode is minder gevoelig voor plaats van stimulatie, dit kan interessant zijn voor verder onderzoek naar verandering in het nociceptieve systeem in pijnpatiënten.

Door gebruik te maken van een conditionerende stimulus zijn in hoofdstuk 3 de gevonden verschillen tussen beide methoden verder onderzocht. Er is gekeken naar het effect van de zogenoemde cold pressor test (CPT) op de verwerking van SP en PT

stimuli. In twee protocollen werden de proefpersonen gestimuleerd op de top van de linker middelvinger terwijl de proefpersoon de rechterhand in water van 0-1°C (CPT) of 32°C (controle) hield. Het eerder gemeten verschil tussen SP en PT stimulatie wordt niet beïnvloed door de CPT. Voor zowel SP als PT wordt de amplitude van EP componenten verminderd door de CPT. Opmerkelijk is dat een effect van de CPT (verlaging van de amplitude) op de N90 piek alleen is gevonden voor PT stimulatie. Het effect van de CPT wordt toegeschreven aan een pijnonderdrukkend system genoemd diffuse noxious inhibitory control (DNIC). De resultaten wijzen erop dat de spinothalamische baan betrokken is bij het ontstaan van de N90 door PT.

Om meer inzicht te krijgen in de het ontstaan van de N90 is er in hoofdstuk 4 onderzoek gedaan naar welke delen van de hersenen betrokken zijn in deze piek. Voor SP en PT zijn zowel de locatie als de oriëntatie van de dipolen vergeleken. Het bleek dat voor beide methoden het overgrote deel van de groep proefpersoon dipolen in de postcentrale gyrus waren gelokaliseerd. Dit is de locatie van de primaire somatosensorische cortex. Opvallend was er voor PT minder spreiding in de groep dipolen. We concluderen dat de gevonden verschillen in effect van SP en PT stimuli op de N90 niet toe te schrijven zijn aan de activiteit van verschillende delen van de hersenen. De gezamenlijke resultaten van hoofdstuk 3 en hoofdstuk 4 wijzen erop dat het voorste gedeelte de spinothalamische baan betrokken is bij de N90.

In het laatste hoofdstuk zijn dezelfde metingen uitgevoerd als in hoofdstuk 3, alleen nu met lumbosacrale radiculaire pijnpatiënten. De verwerking van SP en PT stimuli en het effect van CPT in deze patiëntgroep is onderzocht. De resultaten zijn vergeleken met die van gezonde proefpersonen. In patiënten was het effect van SP en PT stimuli gelijk aan gezonde proefpersonen. Echter, in patiënten waren de EP piekamplitudes groter of kleiner. Deze resultaten suggereren dat de beide methoden zouden kunnen worden gebruikt in onderzoek naar veranderingen in centrale pijnverwerking in patiënten. Ook voor de CPT werden verschillen gevonden tussen gezonde proefpersonen en patiënten. Dit kan wijzen op een verstoorde DNIC. Desondanks is er meer onderzoek nodig naar de bruikbaarheid van beide methoden voor het meten van de verstoorde DNIC.

In dit proefschrift hebben we laten zien dat SP en PT stimulatie de gerapporteerde pijnervaring en EP pieken op een verschillende wijze beïnvloeden. De resultaten hebben meer inzicht gegeven in de relatie tussen de neurofysiologie van nociceptie en de gemeten EP pieken. Afhankelijk van de vraagstelling, kunnen in toekomstig onderzoek naar de verandering in het nociceptieve systeem in pijnpatiënten verschillende meetprotocollen worden geformuleerd gebaseerd op de SP als PT methoden.

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