Pain perception and modulation in acute and chronic pain states

L.C.J. Oudejans

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CONTENTS

Chapter 1

Introduction

PERIPHERAL AND CENTRAL MECHANISM IN CHRONIC PAIN

The pain system has long been regarded as a simple connection between a peripheral sensor sending information, and the brain as the awareness center interpreting this information. This basic description of the perception of pain as proposed by Descartes in the 17th century, has evolved into an elaborate system involving much more than sensory information alone. We have come to understand that pain is a complicated experience integrating prior exposures, expectations, attention, mood, genetics, peripheral and central nervous system physiology as well as neurochemical and anatomical variation [1-3] (Figure 1).

During the previous century elaborate research on nerve fiber morphology, velocity of signal conduction and neuronal responses to thermal, mechanical and noxious stimuli in both animals and humans has unveiled some of the mysteries of how the peripheral and central nervous system are wired to generate and control pain [4-6]. The peripheral

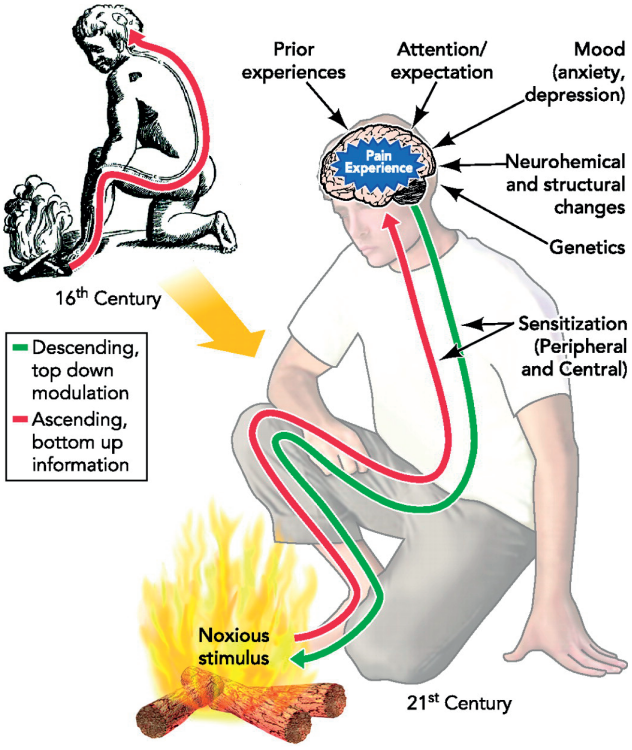

Figure 1. The view on pain perception in the 16th century has evolved into our current understanding of the complexity of pain perception in the 21st century. (With permission from: Tracey, I. & Mantyh, P. W. The cerebral signature for pain perception and its modulation. *Neuron* **55**, 377-391).

nervous system consists of first order neurons that have their cell bodies in the dorsal root ganglia and end at sensory receptors in the skin or in the visceral organs. Nociceptors are small unmyelinated (C) or thinly myelinated (Aδ) fibers that respond to thermal, mechanical or chemical stimuli [7-10]. Peripheral nerves first connect to the central nervous system in the dorsal horn of the spinal cord. Nociceptive signals that pass the dorsal root ganglion, synapse onto a second order neuron as soon as they arrive in the spinal cord and the second order neuron further conveys the pain signal to several brain regions involved in pain perception, such as the thalamus, the insula and the somatosensory cortex [3]. From the cortical regions, multiple descending pathways involving the periaqueductal grey and the nucleus raphe magnus send signals back to the spinal cord, where incoming pain signals are modulated. When this central modulation of pain is inhibitory it is known as descending inhibition [11,12]. To the contrary, a pain amplifying mechanism is central sensitization or facilitation of pain signaling: the amplification of incoming signals from primary nerves at synapses in the spinal cord or at supraspinal sites [13,14]. Sustained afferent nociceptive input can induce a long-term increase in excitability of second order neurons which may lead to hypersensitivity and hyperalgesia [15]. Both the increase in facilitation of pain and a disruption of descending inhibition are thought to play a major role in the chronification of pain.

Chronic pain is usually preceded by a focal lesion or trauma or may be a consequence of systemic diseases that disrupt peripheral small nerve fiber function and/or central modulation of nociception. When the lesion or disease causing the pain is affecting the somatosensory system, the disorder is classified as neuropathic and may be manifested as large and/or small fiber pathology [16,17]. In contrast to large fiber neuropathy, the exact mechanism of the degeneration of nerve fibers in small fiber neuropathy is still unknown, even when it is present as a complication of diseases such as diabetes and sarcoidosis [18-20]. However, decreased nerve fiber density in the skin or cornea and functional impairment can be clearly demonstrated in patients with small fiber neuropathy.

FIBROMYALGIA

Fibromyalgia is a disorder of unknown etiology mainly defined by widespread pain and fatigue, and was previously considered to be caused predominantly by central nervous system dysfunction. This idea is supported by the observation that patients with fibromyalgia often suffer from additional centrally mediated problems such as sleep disturbance, irritable bowel syndrome, depression and mild cognitive symptoms, *i.e.* forgetfulness and verbal memory problems. However, in 2013, two separate research groups [21,22] showed decreased intraepidermal nerve fiber density in skin biopsies, a capital sign of small fiber pathology, in cohorts of fibromyalgia patients. Ramirez and

colleagues [23] were the first to demonstrate the presence of small fiber pathology in patients with fibromyalgia by use of cornea confocal microscopy, a relatively new method to quantify and qualify small nerve fibers. These studies imply that besides central mechanisms, peripheral nerves are also involved in the generation of pain in fibromyalgia (this view is explored in **chapter 5**).

MEASURING PAIN PERCEPTION AND MODULATION

For the measurement of pain a number of instruments are available. A distinction needs to be made between measuring chronic pain and acute, or experimental pain. For the evaluation of chronic pain, its occurrence and intensity, quality and impact on daily life, several questionnaires exist such as the brief pain inventory [24], PainDetect [25], DN4Q [26], the RAND-36 [27] and the Neuropathic Pain Symptoms Inventory [28]. In most of these questionnaires, at least one of the questions concerns rating daily pain on a numerical rating scale, usually from 0 to 10, 0 representing no pain and 10 the worst pain imaginable (**chapter 2** explores the complexities of pain rating).

In most experimental studies, acute pain perception is evaluated by means of psychophysical tests: applying various pain stimuli, such as electrical, ischemic, heat, cold and pressure pain and recording individual's responses to these stimuli, also known as quantitative sensory testing (QST). Often, the lowest intensity of a stimulus that elicits a feeling of pain, *i.e.* the pain threshold, or the highest endurable pain, *i.e.* pain tolerance, is recorded. As it is known which kind of small nerve fibers (C or Aδ fibers) are responsible for conduction of signals from cold, warm and mechanical stimuli and because normative values are available, the class of dysfunctional nerve fibers can be identified by the modalities that show abnormal test results. Moreover, some tests can specifically identify peripheral or central sensitization [29,30].

In contrast to static tests, dynamic tests give an indication of the status of the endogenous pain modulatory system. Examples of such pain modulation tests are conditioned pain modulation (CPM) and offset analgesia (OA). CPM is performed by application of two noxious stimuli at two separate sites on the body, during which the second stimulus reduces the perception of pain evoked by the primary stimulus. This test represents endogenous modulation of pain based on spatial signal integration in the spinal cord [31]. OA, on the other hand, represents a temporal integration of signals. OA is the rapid onset and large reduction in pain perception after a small reduction in temperature during a noxious heat stimulus [32] (these two paradigms are explored in **chapter 3 and 4**).

Apart from psychophysical testing and questionnaires, two objective measurements can be used to assess the state of the small sensory nerve fibers specifically. Skin biopsies and cornea confocal microscopy allow the determination of nerve fiber density

and morphology of small nerve fibers. Skin biopsies are usually taken from the thigh or lower leg and the number of small nerve fibers per mm of epidermis, the intraepidermal nerve fiber density (IENFD), is measured. This technique is currently the gold standard to confirm the diagnosis of small fiber pathology [33]. Alternatively, a confocal microscopic technique called cornea confocal microscopy (CCM) can be used to visualize small nerve fibers that innervate the cornea. Cornea nerve fiber density, cornea nerve fiber length, and cornea branching density (*i.e.* the number of smaller nerves branching from main nerve fibers) can be determined by this technique allowing the assessment of small fiber pathology rapidly, repetitively and non-invasively [34].

With all these tests at our disposal, it is possible to apply these in characterization of chronic pain patients and combine the results to construct a somatosensory phenotype of an individual patient. Analysis of these phenotypes can be used to divide the heterogeneous groups of chronic pain patients, even within a disease entity, into more homogeneous cohorts. Treatment regimens may subsequently be based on the characteristics present in each cohort (phenotype analysis was performed for fibromyalgia patients in **chapter 5** and for diabetes mellitus type 2 and sarcoidosis patients in **chapter 6**).

THESIS OUTLINE

In **Chapter 2** the ability of chronic and acute pain patients and healthy volunteers to grade experimental painful stimuli on a number based scale is evaluated. Additionally it is described how opioids affect the ability to rate painful stimuli.

Chapter 3 compares the response of patients with fibromyalgia to several offset analgesia paradigms with the response of healthy volunteers, and describes the influence of impaired OA responses on the onset and offset of pain.

In **Chapter 4** a novel contact heat thermode device, the Q-sense CPM, is evaluated for its ability to induce a sufficiently large CPM effect. Moreover, several CPM paradigms are compared to explore which model generates the optimal CPM effect.

In **Chapter 5** CCM is performed to assess small nerve fiber morphology of patients with fibromyalgia. The results are used in combination with QST and questionnaires to construct phenotypes of patients.

Chapter 6 describes phenotypes of patients with diabetes mellitus type 2 and patients with sarcoidosis based on CCM and QST.

REFERENCES

1. Loeser, J. D. & Melzack, R. Pain: an overview. *Lancet* 353, 1607-1609, doi:10.1016/S0140-6736(99)01311-2 (1999).
2. Melzack, R. Pain: past, present and future. *Canadian journal of experimental psychology = Revue canadienne de psychologie experimentale* 47, 615-629 (1993).
3. Tracey, I. & Mantyh, P. W. The cerebral signature for pain perception and its modulation. *Neuron* 55, 377-391, doi:10.1016/j.neuron.2007.07.012 (2007).
4. Julius, D. & Basbaum, A. I. Molecular mechanisms of nociception. *Nature* 413, 203-210, doi: 10.1038/35093019 (2001).
5. Le Bars, D., Dickenson, A. H. & Besson, J. M. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain* 6, 283-304 (1979).
6. Melzack, R. & Wall, P. D. Pain mechanisms: a new theory. *Science* 150, 971-979 (1965).
7. Davis, K. D., Meyer, R. A. & Campbell, J. N. Chemosensitivity and sensitization of nociceptive afferents that innervate the hairy skin of monkey. *Journal of neurophysiology* 69, 1071-1081 (1993).
8. LaMotte, R. H. & Campbell, J. N. Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgments of thermal pain. *Journal of neurophysiology* 41, 509-528 (1978).
9. Sherrington, C. S. *The integrative action of the nervous system*. (Charles Scribner's Sons, 1906).
10. Woolf, C. J. & Costigan, M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. *Proceedings of the National Academy of Sciences of the United States of America* 96, 7723-7730 (1999).
11. Ossipov, M. H., Dussor, G. O. & Porreca, F. Central modulation of pain. *The Journal of clinical investigation* 120, 3779-3787, doi:10.1172/JCI43766 (2010).
12. Wall, P. D. The laminar organization of dorsal horn and effects of descending impulses. *The Journal of physiology* 188, 403-423 (1967).
13. Price, D. D., Hayes, R. L., Ruda, M. & Dubner, R. Spatial and temporal transformations of input to spinothalamic tract neurons and their relation to somatic sensations. *Journal of neurophysiology* 41, 933-947 (1978).
14. Price, D. D., Hu, J. W., Dubner, R. & Gracely, R. H. Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. *Pain* 3, 57-68 (1977).
15. Woolf, C. J. Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 152, S2-15, doi:10.1016/j.pain.2010.09.030 (2011).
16. Jensen, T. S. *et al.* A new definition of neuropathic pain. *Pain* 152, 2204-2205, doi:10.1016/j.pain.2011.06.017 (2011).
17. Merskey H.; Bogduk, N. *Classification of chronic pain*, 1994).
18. Hoitsma, E. *et al.* Small fibre neuropathy in sarcoidosis. *Lancet* 359, 2085-2086 (2002).
19. Jin, H. Y., Baek, H. S. & Park, T. S. Morphologic Changes in Autonomic Nerves in Diabetic Autonomic Neuropathy. *Diabetes & metabolism journal* 39, 461-467, doi:10.4093/dmj.2015.39.6.461 (2015).
20. Tavee, J. & Culver, D. Sarcoidosis and small-fiber neuropathy. *Current pain and headache reports* 15, 201-206, doi:10.1007/s11916-011-0180-8 (2011).
21. Oaklander, A. L., Herzog, Z. D., Downs, H. M. & Klein, M. M. Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. *Pain* 154, 2310-2316, doi:10.1016/j.pain.2013.06.001 (2013).
22. Uceyler, N. *et al.* Small fibre pathology in patients with fibromyalgia syndrome. *Brain : a journal of neurology* 136, 1857-1867, doi:10.1093/brain/awt053 (2013).

23. Ramirez, M. *et al.* Small fiber neuropathy in women with fibromyalgia. An in vivo assessment using corneal confocal bio-microscopy. *Seminars in arthritis and rheumatism* 45, 214-219, doi: 10.1016/j.semarthrit.2015.03.003 (2015).
24. Cleeland, C. S., Ladinsky, J. L., Serlin, R. C. & Nugyen, C. T. Multidimensional measurement of cancer pain: comparisons of US and Vietnamese patients. *Journal of pain and symptom management* 3, 23-27 (1988).
25. Freynhagen, R., Baron, R., Gockel, U. & Tolle, T. R. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Current medical research and opinion* 22, 1911-1920, doi:10.1185/030079906X132488 (2006).
26. Bouhassira, D. *et al.* Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain* 114, 29-36, doi: 10.1016/j.pain.2004.12.010 (2005).
27. Ware, J. E., Jr. & Sherbourne, C. D. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Medical care* 30, 473-483 (1992).
28. Bouhassira, D. *et al.* Development and validation of the Neuropathic Pain Symptom Inventory. *Pain* 108, 248-257, doi:10.1016/j.pain.2003.12.024 (2004).
29. Magerl, W. *et al.* Reference data for quantitative sensory testing (QST): refined stratification for age and a novel method for statistical comparison of group data. *Pain* 151, 598-605, doi:10.1016/j.pain.2010.07.026 (2010).
30. Rolke, R. *et al.* Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. *Pain* 123, 231-243, doi:10.1016/j.pain.2006.01.041 (2006).
31. Talbot, J. D., Duncan, G. H. & Bushnell, M. C. Effects of diffuse noxious inhibitory controls (DNICs) on the sensory-discriminative dimension of pain perception. *Pain* 36, 231-238 (1989).
32. Grill, J. D. & Coghill, R. C. Transient analgesia evoked by noxious stimulus offset. *Journal of neurophysiology* 87, 2205-2208, doi:10.1152/jn.00730.2001 (2002).
33. Lauria, G. *et al.* Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. *Journal of the peripheral nervous system: JPNS* 15, 202-207, doi:10.1111/j.1529-8027.2010.00271.x (2010).
34. Brines, M., Swartjes, M., Tannemaat M.R., Dunne, A., van Velzen, M., Proto, P., Hoitsma, E., Petropoulos, I., Chen, X., Niesters, M., Dahan, A., Malik R., Cerami, A. . Corneal nerve quantification predicts the severity of symptoms in sarcoidosis patients with painful neuropathy. *Technology* 1, 1-7, doi: 10.1142/S2339547813500039 (2013).

Chapter 2

Translation of random painful stimuli into numerical responses in fibromyalgia and perioperative patients

LCJ Oudejans, M van Velzen, E Olofsen, R Beun, A Dahan, M Niesters.

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INTRODUCTION

In contemporary medicine, number-based assessment tools are frequently used to evaluate the perception of pain in both acute and chronic pain patients and to determine the effect of pain management [1]. Most popular methods are the visual analogue scale (VAS) and numerical rating scale (NRS), as these are simple and equally sensitive methods that are considered superior to categorical pain scales (e.g., none, mild, moderate, and severe pain) or narrative reports of pain [1, 2]. For the NRS, patients are instructed to verbally rate their pain's quality (this can be any of many pain-related dimensions such as pain intensity or satisfaction with pain relief) on an 11-point scale ranging from 0 (no pain perceived) to 10 (worst intense pain imaginable or tolerable) [1]. Although simple in theory, the use of a numerical scale in the rating of pain requires the ability of the patient to translate a sensory stimulus into a relative number on an abstract pain scale. This is a rather complex task, and, additionally, rating pain up to "the worst pain imaginable" is a concept that requires an intuitive imagination and an adequate memory of previous pains endured. Nevertheless, various validation studies show that patients are able to use numerical pain-rating scales to adequately score their pain and quantify the effect of pain management [3-7]. Rating scores on a VAS or NRS are considered the gold standard of pain testing [6]. Still, the use of numerical scoring systems may be affected by changes in cognition induced by diseases such as chronic pain or by drugs that act at the central nervous system such as opioids. Wolrich *et al.* [8] made an important observation in this respect. They showed that the ability of number sensing, *i.e.*, the ability to name and mark a number, is negatively affected in chronic pain patients more than in acute pain patients, possibly because of functional changes in brain areas involved in understanding numbers and their proportions [8]. This may particularly affect the ability of chronic pain patients to score pain using VAS or NRS.

The primary aim of this study was to determine the ability of chronic and acute postoperative pain patients to adequately score their response to randomly applied noxious stimuli on the NRS relative to healthy sex-matched and age-matched controls and to assess the effect of treatment with opioids. After defining experimental pain threshold (NRS = 1) and pain tolerance (NRS = 10), 8 noxious stimuli, in intensity linearly distributed in between NRS 1 and 10, were applied in a randomized blinded fashion and the NRS was recorded. The data were then analyzed using a penalty score system based on the assumption that stimuli of higher intensity should be scored with a greater NRS.

The second aim of the study was to assess the linearity of the stimulus–NRS relationship. Some studies suggest that the numerical pain scales are linear, whereas others suggest a sigmoid relationship [9-11]. This is an important issue, as only a linear relationship will allow treatment of numerical scales as ratios or percentage change. We applied population-based mixed-effects models on the stimulus–response data to determine linearity of the relationship in healthy controls, chronic and acute pain patients.

METHODS

The study was performed after approval was obtained from the LUMC Human Ethics Committee (Commissie Medische Ethiek, Leiden University Medical Center, 2300 RC Leiden, the Netherlands) from January 2013 to October 2014. The protocol was registered in the Netherlands Trial Register under number 3769. All patients gave oral and written informed consent before enrolment into the study.

Participants

Healthy volunteers without pain (controls, n = 37), chronic pain patients (n = 30), and American Society of Anesthesiologists (ASA) class I and 2 surgical patients (n = 62) participated in the study. Exclusion criteria were age <18 years, body mass index >35 kg/m^2, presence of a medical condition (such as systemic, cardiovascular, pulmonary, renal, liver, or infectious disease), pregnancy or lactation, and history of illicit drug or alcohol abuse. For controls and surgical patients, the presence of an acute or chronic pain syndrome or the use of pain relief medication (excluding acetaminophen) in the 6 months before the study were additional exclusion criteria. All chronic pain patients were diagnosed with fibromyalgia and included if they had an NRS pain score ≥5 for most of the day and met the 2010 American College of Rheumatology diagnostic criteria [12, 13]. These criteria included a widespread pain index (WPI) ≥7 (on a scale from 0 to 19) and a symptom severity (SyS) score ≥5 (on a scale from 0 to 12) or a WPI of 3 to 6 and a SyS score ≥9. The WPI defines the number of body areas in which a patient experienced pain during the last week; the SyS score indicates the level of other core symptoms of fibromyalgia such as fatigue, nonrefreshing sleep, and cognitive symptoms. Additionally, tender point examinations were performed according to the 1990 American College of Rheumatology diagnostic criteria [14]; however, these results were not considered for inclusion or exclusion. The presence of autonomic complaints such as diarrhea or obstipation, dizziness, and dry mouth/eyes was no reason for exclusion in the chronic pain patient group, as these are symptoms consistent with the fibromyalgia syndrome [12]. In chronic pain patients, the presence of pain syndromes other than fibromyalgia was a final exclusion criterion for entrance in the study.

Controls and chronic pain patients were instructed to refrain from taking any medication and consuming alcohol, caffeinated beverages or caffeinated foods on the days of the experiment. Preoperative preparation of the surgical patients was according to local protocol.

Nociceptive assays and pain scoring

Controls and chronic pain patients underwent 2 pain tests: nociceptive thermal and nociceptive electrical testing. Heat and electrical pain stimuli were alternated with a 3 to

5 minutes resting period maintained between tests. For logistic reasons of which time constraints were most important, surgical patients underwent electrical pain testing only.

Nociceptive thermal stimulation

Heat pain was induced by placing a 3 × 3 cm thermal probe (Pathway Neurosensory Analyzer; Medoc Ltd, Ramat Yishai, Israel) on the volar side of the right forearm of the subject. Temperatures increased by 6°C per second from a baseline temperature of 32°C to a preset target temperature that was maintained for 5 seconds. Subjects were instructed to score the highest pain sensation they felt during the stimulation. To overcome adaptation or sensitization, the stimulus zone was divided into 3 separate blocks, which were used sequentially [15]. Heat stimulations at the same skin site occurred at 25 to 30 minutes intervals.

Nociceptive electrical stimulation

Electrical pain was induced by placing 2 electrodes (surface area, 0.8 cm^2; space between the electrodes, 2 cm) on the tibial surface of the right leg. Electrical currents were applied using a locally designed and constructed computer interfaced current stimulator (CICS, Leiden University Medical Center, Leiden, the Netherlands) [15]. A preset constant current (a 5-second train of 200 μs pulses at a frequency of 10 Hz) was delivered to the skin, and subjects were instructed to score the highest pain sensation they felt during the stimulation.

Pain scoring

All participants were initially familiarized with the study design, pain tests, and scoring system. Pain intensity was scored using an 11-point NRS ranging from 0 (no pain) to 10 (worst pain imaginable). Only integers were allowed for scoring. The first part of the study was the accurate assessment of pain threshold (PTh, NRS = 1) and pain tolerance (PTol, NRS = 10). This was performed for heat and electrical pain. To define PTh, a subthreshold stimulus lasting 5 seconds was applied (39°C and 8 mA) and the NRS was scored. Next in steps of 0.5°C and 0.5 mA, the stimuli were increased in intensity. The lowest value causing an NRS of 1 was used as PTh. For pain tolerance, a similar approach was applied, with the lowest temperature and current causing an NRS of 10 as PTol set point. This procedure was repeated 2 to 3 times to be certain of a reliable estimation of PTh and PTol. The procedure was ended when the sequential estimates were within ±0.5°C and ±0.5 mA. The values of PTh and PTol were used to construct a linear distribution of 8 interpolated temperatures and currents. For example, if PTh was 11 mA and PTol 20 mA, the interpolated currents were 12, 13, 14, 15, 16, 17, 18, and 19 mA. The 8 temperatures and 8 currents were subsequently presented in randomized (using a

random number generator), blinded fashion to the participants, each with a duration of 5 seconds. Heat and electrical stimuli were alternated. If the PTol was not reached at the maximum temperature of 52°C, the highest pain score was used as upper limit and a linear distribution of 8 interpolated temperatures was made between the temperature of PTh and 52°C. All subjects were blinded to the sequence and intensity of the stimuli.

Study design

Stimulus–response data were obtained in all participants under baseline conditions (without the administration of any opioids) and in most participants during administration of opioid medication.

Controls and chronic pain patients

Twenty controls and all chronic pain patients received a continuous intravenous infusion of alfentanil on one occasion and no treatment (NoT) on the other. Sessions were randomized with at least 1 week between experiment days; time of testing was similar on both sessions. Alfentanil (Rapifen; Janssen-Cilag BV, Tilburg, the Netherlands) was administered using a target controlled infusion system (Orchestra Base Primea; Fresenius Kabi, Zeist, the Netherlands) programmed with the alfentanil pharmacokinetic set of Maitre *et al.* [16]. The participants were infused for 2 hours at a target concentration of 200 ng/mL. This concentration was chosen as it provides robust analgesia without causing serious side effects. Seventeen additional controls participated on one occasion and received no analgesic medication during testing. Testing was performed during the later part of the infusion (from t = 40 to t = 120 minutes) when stable alfentanil concentrations were assumed.

Surgical patients

All patients were tested before surgery on the day of operation. Premedication consisted of 1000 mg oral acetaminophen just before testing; no sedative or opioid premedication was allowed. Thirty minutes after arrival in the postanesthesia care unit, the patients were retested. Only patients with a Ramsay sedation score of 2 were tested. Spontaneous pain scores before the stimulus–response tests were noted. All patients received intravenous bolus morphine or methadone infusions for acute pain relief.

Data and statistical analyses

The deviation of stimulus–response relationship to an ideal relationship (Fig. 1A) was calculated by subtracting each pain score (j) from the previous score (j – 1),

$$d(j) = NRS(j) - NRS(j-1).$$

Next, the value of d was translated into a penalty score rather than into an error score. In both regression and prediction analyses, errors are taken into account by their squared

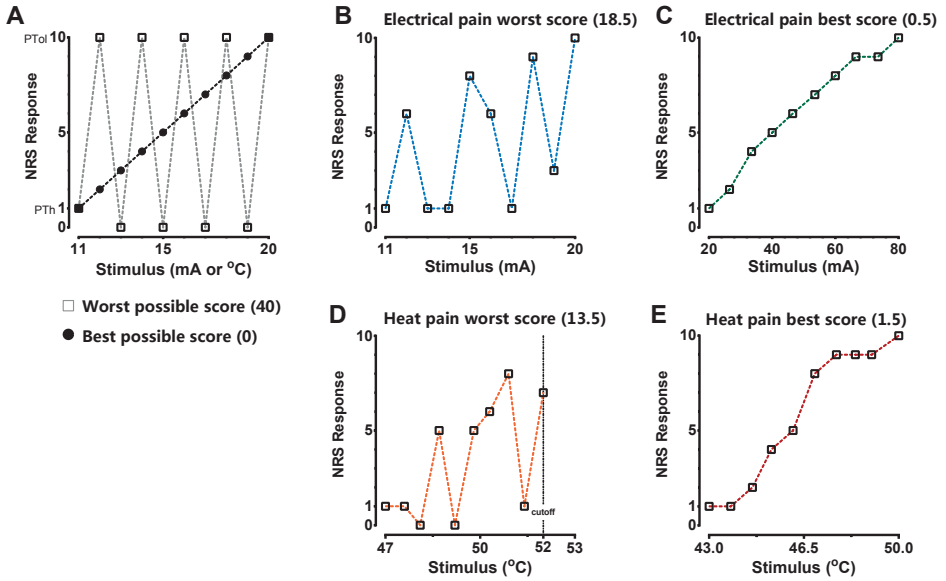

Figure 1. Pain stimulus–NRS response data. **(A)** Best and worst possible scores with respective summed penalties 0 and 40. **(B)** Worst observed score for the electrical pain test with a summed penalty score of 18.5 in a preoperative surgical patient. **(C)** Best observed score for the electrical pain test with a summed penalty score of 0.5 in a postoperative surgical patient. **(D)** Worst observed score for the heat pain test with a summed penalty score of 13.5 in a chronic pain patient during the administration of alfentanil. The patient did not reach pain tolerance at a temperature ≤52°C (cutoff). Consequently, the NRS value observed at 52°C (in this case an NRS of 7) was set as upper limit and a linear distribution of 8 interpolated temperatures was made between the pain threshold temperature and 52°C. **(E)** Best observed score for the heat pain test with a summed penalty score of 1.5 in a chronic pain patient under baseline conditions. NRS is numerical rating score.

values. However, this would also penalize scores going in the expected direction on an increase or decrease in stimulation. The penalty score awards points to a negative (unwanted) event and is an objective tool for the assessment of the general performance of a system in which specific performances are expected such as in our case increasing NRS values at increasing stimulus intensities. Deviations from the expected performance receive penalty scores, which are defined as follows:

(1) if d(j) > 0 (ie, a stimulus j with a higher intensity is perceived as more painful than the stimulus with the lower intensity j-1), no penalty was applied,

(2) if d(j) = 0 (ie, a stimulus j with a higher intensity is perceived as equally painful than the stimulus with the lower intensity j-1), a penalty of 0.5 points was applied,

(3) if d(j) < 0 (ie, a stimulus j with a higher intensity is perceived as less painful than the stimulus with the lower intensity j-1), a penalty of the observed change in score, ie, d(j) was applied.

The total penalty score is the sum of all separate penalty scores, *i.e.*, from j = 2 (the first measurement above PTh) to j = 10 (PTol). Theoretically the summed penalty scores range from 0 (a perfect ever increasing NRS) to 40 (a score that depicts the maximum penalty score), see also Figure 1. On the basis of a blinded visual check of the complete data set, we divided the summed penalty scores into 3 cohorts, representing "good," "mediocre," and "poor" stimulus–response relationships, with respective sum scores ≤3.5 (good), 4 to 7 (mediocre) and ≥7.5 (poor).

Separate summed penalty scores were obtained for each session (no treatment or baseline, opioid, preoperative, postoperative) and nociceptive assay (heat, electrical). The penalty scores were analyzed using IBM SPSS statistics version 20. All data analyses were by nonparametric tests. Fisher's exact tests were used to assess whether the distributions of subjects over the summed penalty score classes good, mediocre, and poor scores were different among groups and to determine whether the distributions of penalty score classes were influenced by opioid use. The presence of sex differences was assessed by the Mann–Whitney U exact test; age effects were evaluated by Spearman's ρ.

To assess the linearity of the stimulus–response relationship, 2 models were fitted to the data. (1) A linear function with parameters S1 and S10, which are the values of the stimulus (current or temperature) yielding an NRS of 1 and 10, respectively; (2) a sigmoidal function with parameters N5 and shape parameter γ, where N5 is the value of the stimulus yielding an NRS of 5. Opioid effect was assessed by a multiplicative factor Z where N5(opioid) = Z × N5(baseline). The fact that the NRSs are integers between 0 and 10 was addressed by assuming an underlying normally distributed variable. Nonlinear mixed-effects analysis by NONMEM (a statistical package for nonlinear mixed-effects modelling; ICON Development Solutions, Hanover, MD) [17] was performed, and the difference between the minimum values of the objective function using the linear and nonlinear models was inspected to test the linearity of the stimulus–NRS response relationship.

RESULTS

Subjects

Thirty-seven healthy controls, 30 chronic pain patients and 62 surgical patients participated in the study. The age range of participants was similar for healthy controls (n = 37, age range: 18-57 years) and chronic pain patients (n = 30, 19-58 years); acute pain patients were on average older (n = 61, 21-84 years). See Table 1 for relevant patient characteristics. Chronic pain patients had an average spontaneous pain score of 6.5 (95% confidence interval 5.9-7.1). One surgical patient used chronic opioids and was excluded. All others completed the preoperative tests. In 19 cases, no postoperative tests

were possible because of either residual sedation or inadequate pain control causing interference with testing. Of the remaining surgical patients, 18 received morphine for postoperative pain relief before psychophysical testing (mean dose: 0.15 ± 0.11 mg/kg, median dose: 0.12 mg/kg, range: 0.03-0.4 mg/kg) and 24 patients received methadone (mean: 0.11 ± 0.08 mg/kg, median: 0.08 mg/kg, range: 0.02-0.19 mg/kg). Psychophysical testing was performed 140 minutes (median) after arrival in the postanesthesia care unit (range: 86-311 minutes). At the moment of testing, the average postoperative pain score in the 42 tested patients was 4.0 (95% confidence interval 3.1-4.9). All participants completed the study without any unforeseen adverse effects.

Baseline heat pain thresholds and tolerances were in controls: 43.1 ± 2.0°C (mean ± SD) and 50.7 ± 1.5°C and in chronic pain patients: 42.2 ± 2.7°C and 48.6 ± 1.9°C. Electrical pain thresholds and tolerances were in controls: 11.5 ± 3.5 mA and 27.6 ± 9.2 mA, in chronic pain patients: 11.4 ± 6.2 mA and 25.1 ± 13.6 mA, and in preoperative surgical patients: 13.9 ± 6.0 mA and 36.8 ± 17.7 mA. Opioid treatment caused an increase in threshold and tolerance values; the effect was largest for the electrical pain test (Table 1).

Table 1. Participants' characteristics and pain threshold and tolerance values

	Healthy controls	**Chronic pain patients**	**Surgical patients**
Number of subjects	**37**	**30**	**61**
Sex (M/F)	17/20	2/28	26/35
Age (years)	32.8 ± 13.6	37.1 ± 11.2	54.7 ± 14.0
Age range (years)	18-57	19-58	21-84
Body mass index (kg/m^2)	23.7 ± 3.3	25.1 ± 5.1	25.4 ± 4.0
Spontaneous pain (NRS)	0	6.5 ± 1.6	4.0 ± 3.0*
Without opioid treatment			
Electrical pain threshold (mA)	11.5 ± 3.5	11.4 ± 6.2	13.9 ± 6.0
Electrical pain tolerance (mA)	27.6 ± 9.2	25.1 ± 13.6	36.8 ± 17.7
Heat pain threshold (°C)	43.1 ± 2.0	42.2 ± 2.7	-
Heat pain tolerance (°C)	50.7 ± 1.5[a]	48.6 ± 1.9[b]	-
During opioid treatment			
Number of subjects	**20**	**30**	**42**
Electrical pain threshold (mA)	16.3 ± 5.3	16.9 ± 9.1	18.1 ± 9.6
Electrical pain tolerance (mA)	35.6 ± 11.2	40.6 ± 31.0	53.5 ± 31.0
Heat pain threshold (°C)	44.2 ± 2.7	44.2 ± 2.9	-
Heat pain tolerance (°C)	51.2 ± 1.1[c]	49.6 ± 1.9[d]	-

All values are mean ± SD unless otherwise stated; * Obtained after surgery in 42 patients. Several patients did not reach heat pain tolerance values at the maximum temperature of 52°C, causing a reduced number of subjects from which heat pain tolerance data were calculated. **a**. n = 31/37; **b**. n = 25/30, **c**. n = 12/20, **d**. n = 22/30.

Penalty scores

Examples of stimulus–response data are given in Figure 1. Baseline (no treatment) penalty scores ranged from 1.5 to 9 (heat pain) and 1.0 to 8.5 (electrical pain) in healthy controls. Corresponding ranges for chronic pain patients were 1.5 to 8.0 (heat pain) and 1.5 to 13.0 (electrical pain). In surgical patients, the scores ranged from 1.0 to 18.5. Heat pain seemed to be more difficult to assess than electrical pain with more individuals with higher penalty scores for heat pain than for electrical pain scores (P = 0.03 in controls and P = 0.04 in chronic pain patients, Table 2). In none of the study populations, a significant age or sex effect on the penalty scores could be detected (data not shown).

Penalty score distributions observed under baseline conditions (ie, without opioid treatment) are given in Figure 2 and Table 2. Baseline scores differed significantly between healthy controls and chronic pain patients for heat pain tests with 27% (controls) *vs.* 55.1% (chronic pain patients) of scores >3.5 (X^2 Fisher's exact test: P = 0.03) but not for electrical pain tests (P = 0.46). Preoperative scores from electrical testing in surgical patients did not differ from the scores of healthy controls (P = 0.33) or chronic pain

Table 2. Mean penalty scores and distribution into cohorts good (≤ 3.5), mediocre (4-7) and poor (≥ 7.5)

	Healthy controls		**Chronic pain patients**		**Surgical patients**
	Heat pain	**Electrical pain**	**Heat pain**	**Electrical pain**	**Electrical pain**
	No Treatment	No Treatment	No Treatment	No Treatment	Preoperative
Median	3.0	2.5[a]	4.0[b]	3.0[c]	3.0
range	1.5 - 9.0	1.0 - 8.5	1.5 - 8.0	1.5 - 13.0	1.0 - 18.5
Good	73.0%	81.1%	44.8%	66.7%	70.5%
95% CI	55.9-86.2%	64.8-92.0%	26.4-64.3%	47.2-82.7%	57.4-81.5%
Mediocre	21.6%	16.2%	51.7%	26.7%	27.9%
95% CI	9.8-38.2%	6.2-32.0%	32.5-70.6%	12.3-45.9%	17.1-40.8%
Poor	5.4%	2.7%	3.4%	6.7%	1.6%
95% CI	0.7-18.2%	0.1-14.2%	0.1-17.8%	0.8-22.1%	0-8.8%
	Opioids	Opioids	Opioids	Opioids	Postoperative
Median	4.8[d]	2.3	5.5[e]	4.0	3.0
range	1.5 - 10.5	0.5 - 7.5	1.5 - 13.5	2.0 - 14.5	0.5 - 13.5
Good	40.0%	70.0%	32.1%	41.4%	69.0%
95% CI	19.1-63.9%	45.7-88.1%	15.9-52.4%	23.5-61.1%	52.9-82.4%
Mediocre	30.0%	25.0%	35.7%	44.8%	23.8%
95% CI	11.9-54.3%	8.7-49.1%	18.6-55.9%	26.4-64.3%	12.1-39.5%
Poor	30.0%	5.0%	32.1%	13.8%	7.1%
95% CI	11.9-54.3%	0.1-24.9%	15.9-52.4%	3.9-13.7%	1.5-19.5%

a. Electrical pain *versus* heat pain (within controls, no treatment) p = 0.03; **b**. Chronic pain patients *versus* controls (heat pain, no treatment) p = 0.028; **c**. Electrical pain *versus* heat pain (within chronic pain patients, no treatment) p = 0.04; **d**. Opioids *versus* no treatment (within controls, heat pain) p = 0.015; **e**. Opioids *versus* no treatment (within chronic pain patients, heat pain) p = 0.016.

patients (P = 0.44). To assess whether subjects were consistent in their scoring ability between heat and electrical pain testing, contingency tables were created (Table 3). Healthy controls performed best in the 2 nociceptive assays with 68% overlap in scoring between heat pain and electrical pain (with 60% of scores in cohort "good"). In contrast,

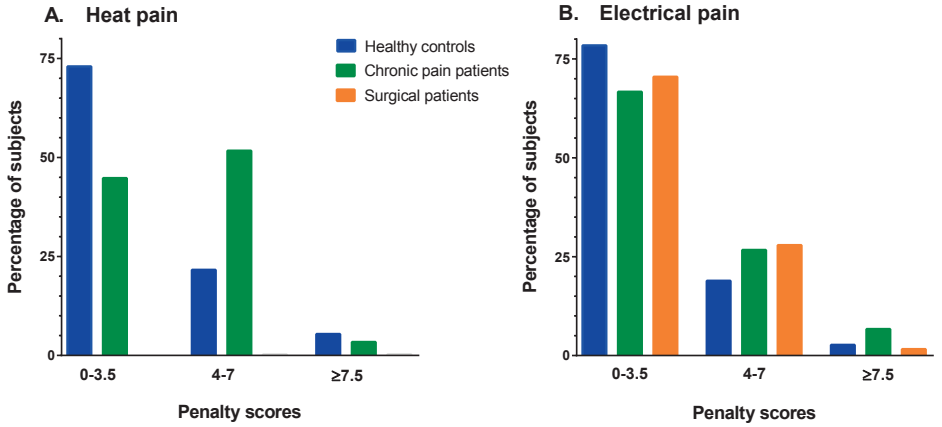

Figure 2. Penalty score distribution under baseline conditions (without opioid treatment) for heat pain **(A)** and electrical pain **(B)** tests in healthy controls (blue bars), chronic pain patients (green bars), and preoperative surgical patients (orange bars). The penalty scores are divided into 3 cohorts: "good" (scores 0-3.5), "mediocre" (4-7), and "poor" (≥7.5).

Table 3. Contingency table of good (≤ 3.5), mediocre (4-7) and poor (≥ 7.5) penalty scores for heat *versus* electrical pain in healthy controls and chronic pain patients, and for preoperative *versus* postoperative testing in surgical patients

Contingency			
Healthy controls		Penalty scores heat pain	
Penalty scores electrical pain	**≤ 3.5**	**4-7**	**≥ 7.5**
≤ 3.5	59.5%	13.5%	5.4%
4-7	10.8%	8.1%	-
≥ 7.5	2.7%	-	-
Chronic pain patients		Penalty scores heat pain	
Penalty scores electrical pain	**≤ 3.5**	**4-7**	**≥ 7.5**
≤ 3.5	26.7%	36.7%	3.3%
4-7	16.7%	6.7%	-
≥ 7.5	3.3%	6.7%	-
Surgical patients		Preoperative penalty scores	
Postoperative penalty scores	**≤ 3.5**	**4-7**	**≥ 7.5**
≤ 3.5	52.4%	14.3%	2.4%
4-7	19.0%	4.8%	-
≥ 7.5	2.4%	-	-

overlap in scoring was just 33% in chronic pain patients (with 27% of scores in cohort "good").

Opioid effect on stimulus–response relationship

Opioids negatively influenced heat pain scoring in both controls and chronic pain patients (Table 2 and Fig. 3) with a significant shift in distribution towards higher penalty scores in controls (P = 0.02) and chronic pain patients (P = 0.02). These effects were not observed for the electrical nociceptive assay in any of the study population (controls P = 0.77, chronic pain patients P = 0.13, postoperative patients P = 0.45; Table 2). There was good correspondence between preoperative and postoperative scores in surgical patients with an overlap of 57.2% (52.4% in cohort "good"; Table 3).

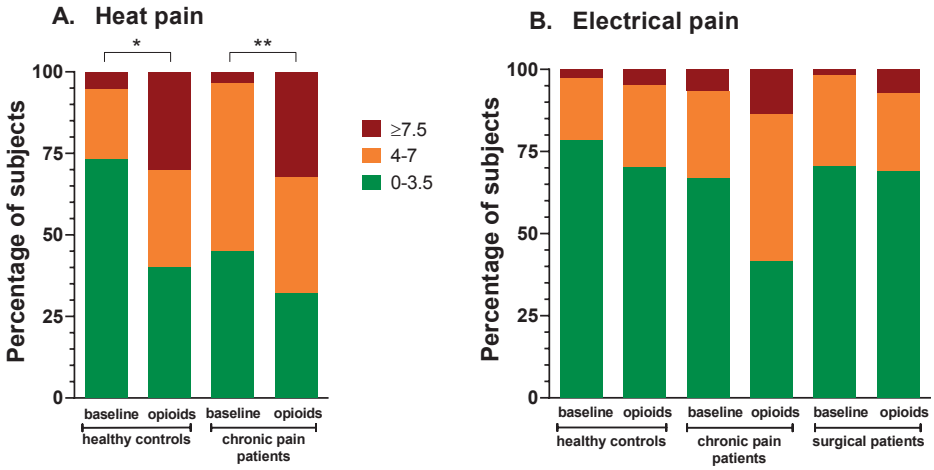

Figure 3. Effect of opioid treatment on penalty score distribution for heat pain **(A)** and electrical pain **(B)** models in healthy controls, chronic pain patients, and preoperative and postoperative surgical patients. The penalty scores are divided into 3 cohorts: "good" (scores 0-3.5), "mediocre" (4-7), and "poor" (≥7.5). *P = 0.015, **P = 0.016.

Nonlinearity of stimulus–response relationship

For both electrical and heat pain, the sigmoidal model of the stimulus–response data provided a significantly better fit compared with the linear model with a difference in the objective function value of more than 100 (P < 0.0001). Parameter estimates of the sigmoidal model are given in Table 4. In Figure 4, examples of data fits and population fits obtained under baseline conditions and during opioid treatment are shown. The population fits give a clear indication of the effect of opioid treatment on the stimulus–NRS relationship with a rightward shift of the curve that differed between nociceptive assays (5% rightward shift for heat pain *vs.* 46%-55% for electrical pain). In perioperative patients, postoperative curves were 28% shifted to the right relative to preoperative tests.

Table 4. Parameter estimates of sigmoid stimulus-NRS relationship

	Controls		Chronic pain patients		Surgical patients
	Electrical pain	**Heat pain**	**Electrical pain**	**Heat pain**	**Electrical pain**
N5[a]	16.6 ± 1.1 mA	46.3 ± 0.45°C	17.9 ± 1.6 mA	45.2 ± 0.5°C	22.0 ± 1.3 mA
γ	8.4 ± 0.7	33 ± 3.5	9.9 ± 1.1	53 ± 6.8	7.0 ± 0.7[b]
Z[c]	1.46	1.05	1.55	1.05	1.28

a. Stimulus at which an NRS of 5 is estimated; **b**. the postoperative value of parameter γ was significantly reduced by factor 0.84 ± 0.03; **c**. Z = opioid effect on N5

DISCUSSION

In this study, we determined the ability of 3 distinct pain populations (healthy controls without pain, fibromyalgia patients, and perioperative patients) to score experimental pain in a consistent fashion. Most important findings are that the presence of chronic pain and the administration of opioids negatively affect the scoring ability of thermal noxious stimuli and to a lesser extent of electrical stimuli. Moreover, the stimulus–NRS relationship was best described by a sigmoidal function irrespective of stimulus type, disease state, or opioid treatment.

We applied a series of random noxious stimuli to assess scoring capabilities of our participants during exposure to heat and electrical pain. Application of random stimuli has been used previously in pharmacological studies [18, 19] and studies on the validation of various pain-rating scales including VAS and NRS [20, 21]. For example, Ferreira-Valente *et al.* [20] used cold pressure tests at fixed temperatures (range: 1-7°C) and Herr *et al.* [21] used fixed heat stimuli (43-51°C) to assess the validity of pain-rating scales in young male *vs.* female [20] and old *vs.* young volunteers [21]. We did not apply fixed stimuli but used 8 randomized, blinded stimuli that in terms of intensity were linearly dispersed in between subject-specific pain threshold and tolerance values. We did not correlate the given NRS scores to the assumed values, as we were initially uninformed on the linearity of the NRS. We simply assumed that stimuli with greater intensity would produce higher numerical rating scores. Deviations from this assumption resulted in penalties that could range from 0.5 to 10 per stimulus with a maximum (summed) penalty score of 40 for the train of 8 stimuli (for a graphical explanation, Fig. 1). In the population analysis, we observed that the stimulus–response relationships were sigmoidal rather than linear (Fig. 4). Consequently, the minimum penalty score is most likely >0. Indeed, we observed minimum scores of 0.5 and 1.5 for electrical and heat pain, respectively (Figs. 1C and E). Consequently, the cohort "good" includes data with scores from 0.5 to 3.5 (electrical pain) and 1.5 to 3.5 (heat pain).

Controls without pain were well able to consistently score the random stimuli. Summed penalty scores ≤3.5 (cohort "good") were observed in 73% to 81% of subjects

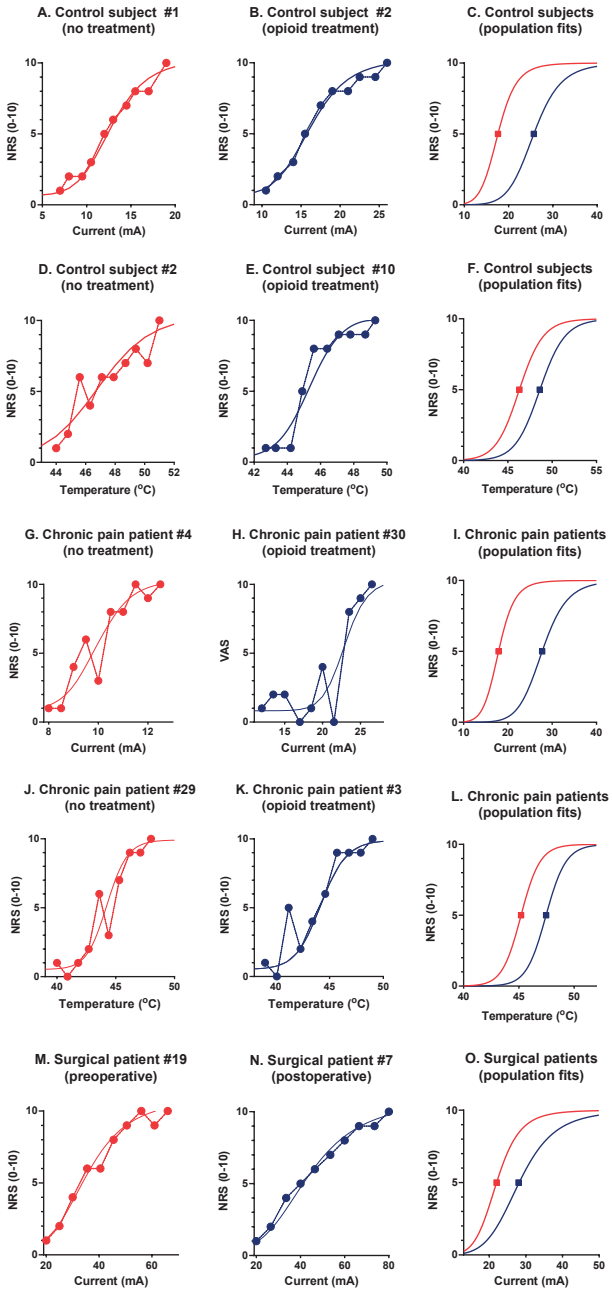

Figure 4. Examples of individual data fits and population fits for the electrical stimulus–NRS relationship in controls **(A-C)**, chronic pain patients **(G-I)**, and surgical patients **(M-O)** and heat stimulus–NRS relationship for controls **(D-F)** and chronic pain patients **(J-L)**. Closed circles are the measured data; the continuous lines are the data fits. Population fits (without and with opioid treatment) are given in panels **(C, F, I, L and O).** The closed squares denote the N5 values of the stimulus intensity at which the model predicts an NRS of 5. NRS is numerical rating score. Penalty scores are 1.0 (A), 1.0 (B), 3.5 (D), 2.5 (E), 3.5 (G), 6.5 (H), 1.5 (J), 0.5 (K), 1.5 (M), and 0.5 (N).

and scores ≥7.5 (cohort "poor") in just 3% to 5% of subjects. The infusion of alfentanil disturbed consistent scoring of heat pain causing a significant shift in cohort distribution with 40% of subjects in cohort "good" and 30% in cohort "poor." In contrast, opioid administration produced no such shift in distribution for the electrical pain model (70% in cohort "good," 5% in cohort "poor"). These data suggest that opioids negatively affect the ability to translate heat pain stimuli into numerical responses, whereas the translation of electrical stimuli is more resistant to the effects of opioids. Opioids produce sedation and consequently affect cognition and hence some deterioration of scoring was expected for both pain models [15]. Additionally, opioids may impact stimulus processing causing a more floating variable that becomes more difficult to score. The 2 pain models differ significantly in their mechanism by which they induce transcutaneous nociceptive stimuli. Electrical stimulation directly excites sensory and nonsensory nerves of the skin in an unnatural and synchronized fashion, bypassing the sensory nerve endings, whereas heat pain selectively activates Aδ and C-fibers at their nerve endings [22]. Consequently, central processing of these 2 distinct stimuli differs. Possibly, processing of the barrage of afferent input from cutaneous electrical stimulation into numerical ratings bypasses opioid-sensitive brain centers involved in this specific function. This hypothesis requires further study.

Fibromyalgia patients were less able to consistently score the random stimuli compared with age-matched and sex-matched controls. Summed penalty scores ≤3.5 (cohort "good") were observed in 45% to 67% of patients and scores 4 to 7 (cohort "mediocre") in 27% to 52% of patients. Although relative to controls, the score distributions were worse in both pain models; this reached the level of significance for heat pain but not for electrical pain (Table 2). Opioids further worsened the penalty score distribution (heat pain 32%, electrical pain 41% in cohort "good"). These are important observations and indicate that chronic pain patients lack the ability to score (experimental) pain in a consistent manner, an effect that is further worsened by opioid treatment. There is evidence that chronic pain induces structural and functional changes in the brain that correlate with impaired cognition [23, 24]. For example, Apkarian *et al.* [23] showed that chronic low back pain is associated with brain atrophy in the prefrontal cortex and thalamus. Wolrich *et al.* [8] observed impairment in number sensing in chronic pain patients that may be ascribed to functional changes in the prefrontal and parietal cortices. This, however, is just one part of the complex process of translation of an incoming sensory stimulus into a verbal statement. To properly score pain, patients need to construct an abstract pain scale in their minds and position the incoming painful stimuli on that virtual scale. This requires various cognitive functions such as imagination, retrieval of a numerical memory, ability to size incoming stimuli by comparing them to remembered stimuli, and number sensing. Evidently, even small impairments of cognition may affect any of these processes and hence may hinder the proper scoring of random nociceptive stimuli. Ad-

ditionally, our population of chronic pain patients may be affected by small (and large) fiber neuropathy in their skin [25, 26]. These pathophysiological changes may have caused alterations in the afferent input to the spinal cord and brain with consequently modifications in the central processing of the applied experimental stimuli. Moreover, in chronic pain, the continuous but also highly variable nociceptive input from their chronic pain may have further altered central processing with a lesser ability to score the delivered stimuli. These are important issues that merit further study.

Because attempts to test both models lead to logistic problems in the perioperative setting, we applied just one nociceptive modality (electrical pain) in our surgical patient population. As expected, baseline penalty score distribution in this ASA 1-2 patient population without chronic pain was similar to that observed in healthy controls with 71% of scores in the cohort "good." Postoperatively the distribution of scores remained unaffected by acute pain (average NRS 4.0) and opioid treatment. These data contrast the observations in chronic pain patients (41% in cohort good during opioid treatment, Table 2), an indication that pain per se does not affect pain scoring but that long-term neuroplastic changes in the central and possibly peripheral nervous system are responsible for the observed inadequacy of pain scoring in chronic pain. Our findings are in agreement with those of others [8, 27] and indicate that NRS is a valid tool to describe pain and pain relief from analgesic treatment in postoperative patients.

Linear and nonlinear functions were fitted to the data to assess whether the stimulus–response (NRS) relation was linear or sigmoidal. Irrespective of pain modality, study population or opioid treatment, a sigmoidal function provided significant better data fits. A nonlinear relation indicates that the NRS is best not used as relative or percentage change in magnitude of pain sensation because, for example, an increase in NRS from 0 to 1 does not represent a similar change in pain intensity as an increase from 4 to 5, unlike previously suggested [7]. Our analysis further shows the opioid effect on the stimulus–response curves (Fig. 4). The rightward shift was most pronounced for the electrical pain model with a shift ranging from 28% to 46%. In contrast, the heat pain curves shifted by 5% (parameter Z in Table 4). We may have underestimated the effect of the opioid on heat pain curves to some extent, as we did not discriminate between immediate and late pain sensations evoked by the brief heat stimulus. Late pain sensations are most sensitive to opioids [28]. The finding of a lesser opioid effect on heat pain together with the more robust scoring in healthy controls and chronic pain patients likely renders the electrical pain model the more attractive assay for experimental and phase 1 studies of analgesic compounds.

Although we applied a randomized design to prevent a learning effect and rotated the heat stimulus between 3 locations on the skin, it remains unclear to what extent the repeated stimulation of the skin has influenced the patients' pain perception. Both adaptation/habituation and sensitization may theoretically have occurred [29]. However,

we observed no signs of either manifestation during or after testing in any of our subjects. We applied acute experimental pain stimuli that are intrinsically different from spontaneous pain [30], and discriminating between intensity levels in real-life pain may be mediated by other factors than tested by us. Irrespective, we contend that our study allows assessment of the reliability of the NRS as scoring tool of acute pain.

In conclusion, consistency to grade experimental pain using an 11-point NRS is high in healthy controls without pain but deteriorates in chronic pain and during opioid administration to healthy volunteers and patients in chronic pain but not in patients with acute pain.

REFERENCES

1. Breivik H, Borchgrevink PC, Allen SM, Rosseland LA, Romundstad L, Hals EKB, Kvarstein G, Stubhaug A: Assessment of pain. Brit J Anaesth 2008, 101:17-24.
2. Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA: Studies with Pain Rating-Scales. Ann Rheum Dis 1978, 37:378-81.
3. Bijur PE, Silver W, Gallagher EJ: Reliability of the visual analog scale for measurement of acute pain. Acad Emerg Med 2001, 8:1153-7.
4. Chanques G, Viel E, Constantin JM, Jung B, de Lattre S, Carr J, Cisse M, Lefrant JY, Jaber S: The measurement of pain in intensive care unit: Comparison of 5 self-report intensity scales. Pain 2010, 151:711-21.
5. Cushman D, McCormick Z, Casey E, Plastaras CT: Discrepancies in Describing Pain: Is There Agreement Between Numeric Rating Scale Scores and Pain Reduction Percentage Reported by Patients with Musculoskeletal Pain After Corticosteroid Injection? Pain Med 2015, 16:870-6.
6. Dworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP, Kerns RD, Stucki G, Allen RR, Bellamy N, Carr DB, Chandler J, Cowan P, Dionne R, Galer BS, Hertz S, Jadad AR, Kramer LD, Manning DC, Martin S, McCormick CG, McDermott MP, McGrath P, Quessy S, Rappaport BA, Robbins W, Robinson JP, Rothman M, Royal MA, Simon L, Stauffer JW, Stein W, Tollett J, Wernicke J, Witter J: Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain 2005, 113:9-19.
7. Price DD, McGrath PA, Rafii A, Buckingham B: The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 1983, 17:45-56.
8. Wolrich J, Poots AJ, Kuehler BM, Rice AS, Rahman A, Bantel C: Is number sense impaired in chronic pain patients? Br J Anaesth 2014, 113:1024-31.
9. Kersten P, White PJ, Tennant A: Is the Pain Visual Analogue Scale Linear and Responsive to Change? An Exploration Using Rasch Analysis. Plos One 2014, 9.
10. Myles PS, Troedel S, Boquest M, Reeves M: The pain visual analog scale: Is it linear or nonlinear? Anesth Analg 1999, 89:1517-20.
11. Wallenstein SL, Heidrich G, 3rd, Kaiko R, Houde RW: Clinical evaluation of mild analgesics: the measurement of clinical pain. Br J Clin Pharmacol 1980, 10 Suppl 2:319S-27S.
12. Niesters M DA: Fibromyalgia. Encyclopedia of the neurological sciences. Edited by Aminoff MJ DR. Oxford: Academic Press, 2014. pp. 288-92.
13. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis care & research 2010, 62:600-10.
14. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, *et al.*: The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis and rheumatism 1990, 33:160-72.
15. Olofsen E, Romberg R, Bijl H, Mooren R, Engbers F, Kest B, Dahan A: Alfentanil and placebo analgesia: no sex differences detected in models of experimental pain. Anesthesiology 2005, 103:130-9.
16. Maitre PO, Vozeh S, Heykants J, Thomson DA, Stanski DR: Population Pharmacokinetics of Alfentanil - the Average Dose-Plasma Concentration Relationship and Interindividual Variability in Patients. Anesthesiology 1987, 66:3-12.
17. Bauer R: NONMEM user's guide: introduction to NONMEM 7.3.0. . Hanover, MD: ICON Development Solutions, 2014.

18. Angst MS, Drover DR, Lotsch J, Ramaswamy B, Naidu S, Wada DR, Stanski DR: Pharmacodynamics of orally administered sustained- release hydromorphone in humans. Anesthesiology 2001,94: 63-73.
19. Lotsch J, Kobal G, Stockmann A, Brune K, Geisslinger G: Lack of analgesic activity of morphine-6-glucuronide after short-term intravenous administration in healthy volunteers. Anesthesiology 1997, 87:1348-58.
20. Ferreira-Valente MA, Pais-Ribeiro JL, Jensen MP: Validity of four pain intensity rating scales. Pain 2011, 152:2399-404.
21. Herr KA, Spratt K, Mobily PR, Richardson G: Pain intensity assessment in older adults - Use of experimental pain to compare psychometric properties and usability of selected pain scales with younger adults. Clin J Pain 2004, 20:207-19.
22. Olesen AE, Andresen T, Staahl C, Drewes AM: Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. Pharmacological reviews 2012, 64:722-79.
23. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR: Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. The Journal of neuroscience : the official journal of the Society for Neuroscience 2004, 24:10410-5.
24. Seminowicz DA, Wideman TH, Naso L, Hatami-Khoroushahi Z, Fallatah S, Ware MA, Jarzem P, Bushnell MC, Shir Y, Ouellet JA, Stone LS: Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function. The Journal of neuroscience : the official journal of the Society for Neuroscience 2011, 31:7540-50.
25. Oudejans L, He X, Niesters M, Dahan A, Brines M, van Velzen M: Cornea nerve fiber quantification and construction of phenotypes in patients with fibromyalgia. Scientific reports 2016, 6:23573.
26. Uceyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C: Small fibre pathology in patients with fibromyalgia syndrome. Brain : a journal of neurology 2013, 136:1857-67.
27. DeLoach LJ, Higgins MS, Caplan AB, Stiff JL: The visual analog scale in the immediate postoperative period: Intrasubject variability and correlation with a numeric scale. Anesth Analg 1998, 86: 102-6.
28. Cooper BY, Vierck CJ, Yeomans DC: Selective Reduction of 2nd Pain Sensations by Systemic Morphine in Humans. Pain 1986, 24:93-116.
29. Jepma M, Jones M, Wager TD: The Dynamics of Pain: Evidence for Simultaneous Site-Specific Habituation and Site-Nonspecific Sensitization in Thermal Pain. J Pain 2014, 15:734-46.
30. Beecher HK: The measurement of pain; prototype for the quantitative study of subjective responses. Pharmacological reviews 1957, 9:59-209.

Chapter 3

The influence of offset analgesia on the onset and offset of pain in patients with fibromyalgia

LCJ Oudejans, JM Smit, M van Velzen, A Dahan, M Niesters.

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INTRODUCTION

Fibromyalgia is a chronic pain syndrome characterized by widespread pain, often accompanied by fatigue, sleep disturbances, cognitive dysfunction, and episodes of clinical depression [1,2]. The etiology of fibromyalgia has not been completely clarified. Currently, the most accepted hypothesis is that fibromyalgia is a central pain syndrome in which the central nervous system is the origin of the pain state or is involved in the pathological amplification of nociceptive input. Evidence supporting this hypothesis includes the observation of increased neuronal activation in brain regions involved in pain processing during nonnoxious stimulation and the presence of a dysfunctional endogenous pain modulatory system [1-6].

The endogenous pain modulation system is an important modulator of pain perception. It consists of inhibitory and facilitatory descending pathways originating in the brain and projecting to the dorsal horn of the spinal cord, where they inhibit or enhance the passage of nociceptive input to central sites [7]. An imbalance between facilitatory and inhibitory properties of the descending pathways has been associated with chronic central pain states, including fibromyalgia, as measured by a decrease in diffuse noxious inhibitory control (currently known as conditioned pain modulation) [4,5,8,9]. A relatively new experimental paradigm that is used to evaluate the function of the endogenous pain modulation system is offset analgesia (OA). Offset analgesia is characterized by the perception of profound analgesia upon a small decrease in temperature during noxious thermal stimulation, which is more pronounced than would be expected from the rate of the temperature decrease [10,11].

In this study, the presence of OA in patients with fibromyalgia was investigated. We hypothesized that patients with fibromyalgia would have a decreased OA response compared with control subjects. Furthermore, the effect of several variations to the OA paradigm was evaluated to understand the role of OA (or its lack) in the development of pain in control subjects and patients with fibromyalgia. To assess whether the magnitude of OA could be enhanced, repetition of the OA paradigm and additional downward 1°C temperature steps (downward steps test) were applied. To assess whether OA affects onset of pain, we applied OA steps at increasing temperatures (upward OA steps test).

METHODS

Subjects

Sixty-eight individuals participated in 1 or more pain tests, 34 patients with fibromyalgia and 34 age-matched and sex-matched healthy control subjects (Table 1 for the number of subjects who participated in each of the tests). Recruitment began after approval of

the protocol by the local medical ethics committee (Commissie Medische Ethiek LUMC, Leiden, the Netherlands), and the study was registered in the Netherlands trial register under number NTR4023 (www.trialregister.nl). All participants gave written informed consent and underwent a physical examination before enrollment in the study. Patients with fibromyalgia were included if they had a pain score ≥5 (on a scale of 0-10) for most of the day and met the 2010 American College of Rheumatology diagnostic criteria [12]. These criteria included a widespread pain index (WPI; 0-18 points) that defined the number of body areas in which a patient experienced pain during the last week, and a symptom severity score (SyS-score; 0-12 points), which indicated the level of other core symptoms of fibromyalgia such as fatigue, unrefreshing sleep and cognitive symptoms. Inclusion criteria were a WPI ≥7 with a SyS-score ≥5 or a WPI of 3 to 6 with a SyS-score ≥9. Exclusion criteria for control subjects and patients included age <18 or >75 years, an inability to give written informed consent, a medical disease such as cardiac, liver, renal, or vascular disease that could influence pain perception according to the investigator, and a history of psychiatric disease, pregnancy, and obesity (body mass index >35). Patients were allowed to continue the use of their pain medication as long as the dose used was constant for the 8 weeks before the study day.

Table 1. Subject characteristics and number of subjects per test

	Control subjects	**Patients with fibromyalgia**	***P***
Sex (M/F)	8/26	8/26	
Age (y)	36.5 [23.8 – 47.3]	38.0 [24.8 – 46.3]	0.753
Height (cm)	175.7 ± 20.0	171.5 ± 6.4	0.151
Weight (kg)	68.8 ± 21.6	77.0 ± 18.0	0.150
BMI (kg/cm^2)	22.1 ± 2.9	26.1 ± 5.0	0.016
NRS	0	6.7 ± 1.2	
Disease duration (y)		14.0 ± 12.8	
WPI		13.9 ± 2.9	
SyS-score		8.1 ± 2.3	
Number of subjects in each test			
Test 1: 1-step OA	34	34	
Test 2: repeated OA	12	28	
Test 3: downward step test	12	12	
Test 4: constant stimulus test	12	28	
Test 5: upward OA step test	12	12	
Test 6: Ramp test	12	12	

Values are presented as numbers, means ± SD or medians (interquartile range). BMI, body mass index; NRS, numerical rating scale, with 0 indicating "no pain" and 10 indicating "worst pain imaginable"; OA, offset analgesia; SyS-score, symptom severity score; WPI, widespread pain index.

Pain measurements

Noxious thermal stimulation was applied on the skin of the volar side of the non-dominant arm using the 3 × 3 cm thermal probe of the Pathway Neurosensory Analyzer (Medoc Ltd, Ramat Yishai, Israel). The system was tested and calibrated according to the specifications of the manufacturer using a surface thermometer (K-Thermocouple thermometer, Hanna Instruments, Woonsocket, RI). To prevent sensitization or adaptation, the thermode was sequentially moved among 3 different locations on the skin of the forearm [13]. There were at least 15 minutes between the various heat tests. During the induction of pain, the visual analog score (VAS) was measured using a slider on a computerized potentiometer that ranged from 0 mm (no pain) to 100 mm (worst pain imaginable). This allowed for the continuous quantification of the intensity of the noxious stimulus.

Study design

At the start of the study session, the temperature that induced a VAS score of 50 mm was determined. To that end, a series of heat stimuli were applied in the range of 42°C to 49°C for 10 seconds. The temperature that evoked a VAS of 50 mm was used during the remainder of the study and defined as the "test" temperature. Next, 6 different heat pain tests were performed in random order; each pain test was performed 3 times and all tests were performed on 1 day.

Tests 1, 2, and 3 were designed to characterize OA (test 1) and evaluate whether repeated OA steps (test 2) or OA followed by downward steps (test 3) could enhance the magnitude of the OA response. Test 4, the constant stimulus test, served as a control test and enabled measurement of response adaptation in patients with fibromyalgia. In contrast to tests 1 to 3, tests 5 (repeated OA at increasing temperatures) and 6 (ramp test) were designed to assess whether OA affects the onset of a pain response.

Pain test 1: one-step offset analgesia

A regular 1-step OA test was induced using the 3-temperature paradigm as previously described [14,15]. In short, the temperature of the heat probe was increased by 1.5°C/s from a baseline temperature of 32°C to the individual's test temperature and kept constant for 5 seconds. Next, the temperature was raised by 1°C for 5 seconds after which it returned back to the individual test temperature (*i.e.*, a decrease by 1°C). This temperature was kept constant for 20 seconds followed by a quick return at 6°C/s toward the baseline temperature (Fig. 1A). We define the 1°C increase which is kept constant for 5 seconds and subsequent 1°C decrease as the OA paradigm.

Pain test 2: repeated offset analgesia

During the repeated OA test, the OA paradigm (1°C increase followed after 5 seconds by a 1°C decrease) was repeated 4 times with an interval of 10 seconds between the 1°C temperature variations (Fig. 1B).

Pain test 3: downward steps test

This is one OA test followed by a stepwise 1°C temperature decreases at 5-second intervals until the baseline temperature was reached (Fig. 1C).

Pain test 4: constant stimulus test

For this test, constant heat stimulation was applied. The heat probe was ramped with 1.5°C/s to the individual test temperature and kept constant for 80 seconds. Next, the temperature returned to baseline (temperature decrease rate = 6°C/s; Fig. 1D).

Pain test 5: upward offset analgesia steps test

This is a repeated OA test at increasing temperatures. During this test, the heat probe temperature was increased in steps of 2°C followed after 3 seconds by a 1°C temperature decrease. After another 3 seconds, the sequence was repeated. The sequence was initiated at 32°C (first step from 32 to 34°C followed by a decrease to 33°C) and continued until the VAS reached a value of 80 mm. At this point, the test was terminated and the temperature quickly returned (at 6°C/s) to 32°C (Fig. 2A). The safe fail temperature was set at 51°C.

Pain test 6: ramp test

A ramp heat stimulus was induced by increasing the temperature of the thermode by 0.5°C/s from baseline (32°C) until the VAS reached a score of 80 mm. At that point, the stimulus was ended and the temperature returned at a rate of 6°C/s to the baseline temperature (Fig. 2B). The safe fail temperature was 51°C.

Data and statistical analyses

Sample size calculation based on data from Ref. [15] indicated that 12 controls and 12 patients with fibromyalgia would be sufficient to detect a significant difference in OA response. However, the groups were expanded based on the availability of subjects.

Tests 1, 2, and 3

Offset analgesia responses were quantified as previously described [14,15]. In short, for each OA paradigm, the decrease in VAS from the peak VAS value to the VAS nadir was measured (ΔVAS) within a 10-second time frame after the 1°C decrease in temperature.

This value was next corrected for the value of the peak VAS: (ΔVAS/[peak VAS] × 100%) and defined as ΔVAS corrected or ΔVASc.

Test 4

To quantify the adaptation response, area-under-the-curve values were calculated for each test.

Tests 5 and 6

The temperature at which the VAS reached 80 mm was determined and compared between populations and between tests.

Statistical significance between controls and patients was tested using an unpaired 2-tailed Student *t* test for normal distributed data and a Mann–Whitney *U* test for non-normal distributed data. Statistical significance between different tests within a population was tested using a paired 2-tailed student *t* test. The OA responses in test 2 were analyzed by repeated measures analysis of variance with a Dunnett's test for comparisons *vs.* the first OA response. The analyses were performed using GraphPad Prism version 6.0 for Mac (GraphPad Software Inc, La Jolla, CA).

Data are presented as mean ± SD or 95% confidence interval unless otherwise stated; *P* values <0.05 are considered significant.

RESULTS

Subject characteristics are shown in Table 1. Patients and control subjects were comparable in age and sex distribution. A 15% higher body mass index was observed for the patients with fibromyalgia. All patients fitted the diagnostic criteria for fibromyalgia according to the 2010 criteria as set by the American College of Rheumatology [12]. The following pain medication was continued during the study: acetaminophen, NSAIDs, tramadol, amitriptyline, paroxetine, venlafaxine, sertraline, and duloxetine. The average individual test temperature for control subjects was 46.9 ± 2.3°C (mean ± SD) *vs.* 44.5 ± 2.3°C for patients with fibromyalgia ($P = 0.004$). No difference was observed in the corresponding VAS scores, which were 51.5 ± 14.5 mm *vs.* 51.4 ± 9.2 mm, respectively ($P = 0.97$). This indicates that a pain score of 50/100 mm was obtained at a 2.4°C lower temperature in patients with fibromyalgia compared with control subjects, an indication of hyperalgesia in the fibromyalgia population.

Pain test 1: one-step offset analgesia

Thirty-four control subjects and 34 patients with fibromyalgia participated in test 1. Compared with the control group, patients with fibromyalgia had a 32.0% reduced OA

response: ΔVASc scores = 97.8 ± 4.7% (control subjects) *vs.* 65.3 ± 26.2% (patients with fibromyalgia, $P < 0.001$; Fig. 1A). No differences in peak VAS scores were present: 60.1 ± 17.9 mm (control subjects) *vs.* 64.1 ± 15.7 mm (patients with fibromyalgia, $P = 0.30$).

Pain test 2: repeated offset analgesia

Twelve control subjects and 28 patients with fibromyalgia participated in test 2. In both populations, the second to fourth peak VAS scores decreased significantly from the first one (main effect $P < 0.001$; Fig. 1B). Consecutive peak VAS scores for fibromyalgia patients were 61.7 ± 12.4 mm, 45.6 ± 12.8 mm ($P < 0.001$), 45.7 ± 14.5 mm ($P < 0.001$), and 44.4 ± 13.5 mm ($P < 0.001$). For the controls, these scores were 63.1 ± 18.5 mm, 48.1 ± 15.3 mm ($P < 0.001$), 47.0 ± 22.2 mm ($P < 0.001$), and 32.9 ± 33.8 mm ($P < 0.001$). No differences in peak VAS scores were observed between control subjects and patients with fibromyalgia (main effect $P = 0.16$). In control subjects, the consecutive ΔVASc scores were of similar magnitude (main effect $P = 0.58$): 95.3 ± 5.9%, 94.9 ± 7.8%, 94.6 ± 10.9%, and 98.0 ± 6.8%. Also in patients with fibromyalgia, consecutive ΔVASc scores

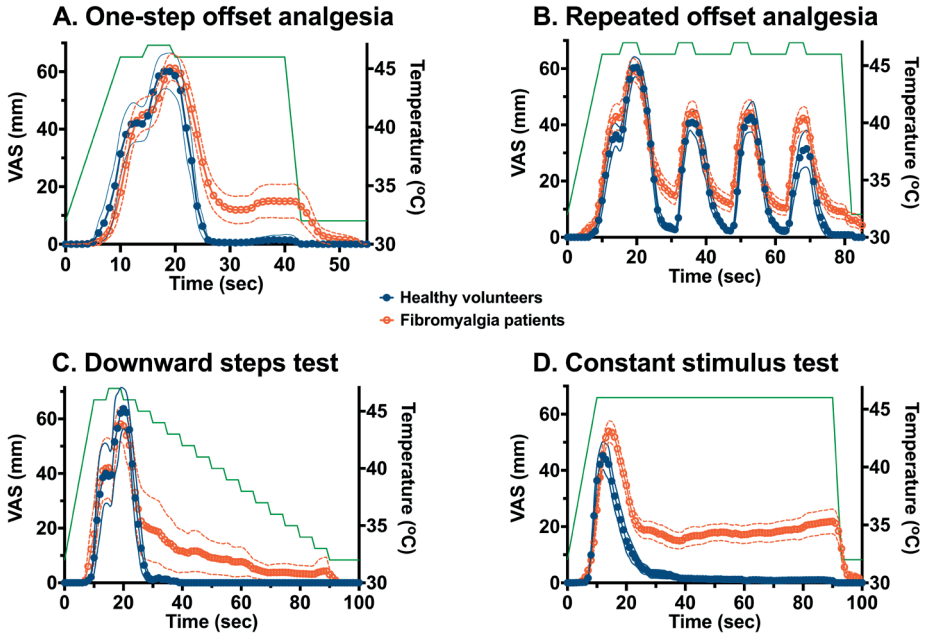

Figure 1. Visual analog scores in response to **(A)** the 1-step OA paradigm in 34 controls and 34 patients with fibromyalgia, **(B)** the repeated OA paradigm in 12 controls and 28 patients with fibromyalgia, **(C)** the downward steps test in 12 controls and 12 patients with fibromyalgia, and **(D)** the constant stimulus test in 12 control subjects and 28 patients with fibromyalgia. The continuous green lines reflect examples of the temperature paradigms applied. Blue symbols are the data from control subjects; orange symbols are the data from patients with fibromyalgia. The data are mean (circles) ± 95% CI (thin continuous or broken lines). CI, confidence interval; OA, offset analgesia; VAS, visual analog score.

were similar: 75.0 ± 23.4%, 77.7 ± 25.5%, 78.9 ± 26.5%, and 79.8 ± 27.0% (main effect P = 0.46). The OA responses in patients with fibromyalgia were reduced compared with the control subjects by about 21.3% (P = 0.03).

Pain test 3: downward steps test

Twelve control subjects and 12 patients with fibromyalgia participated in test 3. No differences were observed in the peak VAS scores between populations: 64.4 ± 10.5 mm (control subjects) *vs.* 60.8 ± 8.8 mm (patients with fibromyalgia, P = 0.38). The first 1°C temperature step-down resulted in an OA response that was larger in control subjects than in patients with fibromyalgia: ΔVASc = 97.5 ± 3.7% (control subjects) *vs.* 66.9 ± 33.7% (patients with fibromyalgia, P = 0.006; Fig. 1C). As OA was not or incompletely engaged in patients with fibromyalgia, we hypothesized that another 1°C temperature decrease (after the initial OA paradigm) might complete (or at least in part) the OA response. However, after the initial OA response, no additional OA was produced in patients with fibromyalgia (Fig. 1C).

Pain test 4: constant stimulus test

Twelve control subjects and 28 patients with fibromyalgia performed this test. No difference was observed in peak VAS scores between populations (control subjects: 51.5 ± 11.6 mm *vs.* patients with fibromyalgia: 59.1 ± 15.4 mm, P = 0.15; Fig. 1D). Patients with fibromyalgia displayed a significant decreased adaptation response compared with the controls during the constant heat stimulation test. Average area-under-the-curve scores were 538 ± 99 mm·s for the control subjects *vs.* 1875 ± 220 mm·s for the patients with fibromyalgia (P < 0.001).

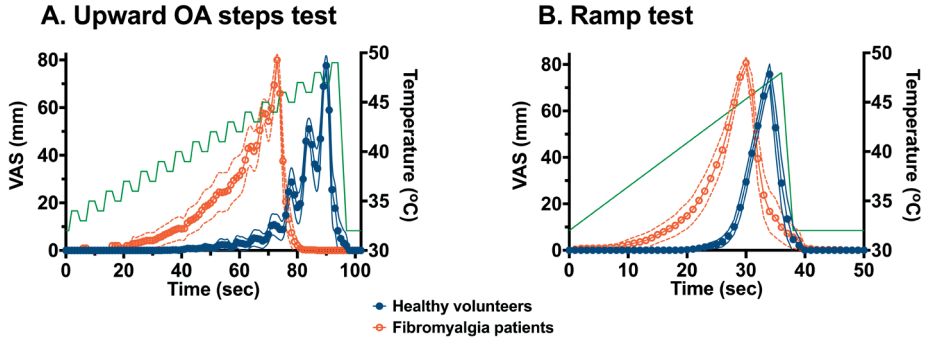

Figure 2. Visual analog scores in response to **(A)** the upward OA steps test (cutoff is a VAS of 80 mm) in 12 control subjects and 12 patients with fibromyalgia, and **(B)** the ramp test (cutoff is a VAS of 80 mm) in 12 control subjects and 12 patients with fibromyalgia. The continuous green lines reflect examples of the temperature paradigms applied. Blue symbols are the data from control subjects; orange symbols are the data from patients with fibromyalgia. The data are mean (circles) ± 95% CI (thin continuous or broken lines). CI, confidence interval; OA, offset analgesia; VAS, visual analog score.

Pain test 5: upward OA steps test

Twelve control subjects and 12 patients with fibromyalgia participated in test 5. The average temperature reached at the cutoff (VAS 80 mm) was 48.8 ± 0.7°C in control subjects *vs.* 46.5 ± 1.4°C in patients with fibromyalgia ($P < 0.001$) with corresponding VAS scores within 3% of the cutoff VAS (controls: 77.9 ± 7.1 mm and patients with fibromyalgia: 81.9 ± 1.4 mm, $P = 0.24$). None of the subjects reached the safe fail temperature of 51°C before they reached the cutoff VAS. Offset analgesia was observed with every decrease in temperature in the control subjects but not in the patients with fibromyalgia (Fig. 2A).

Pain test 6: ramp test

Twelve control subjects and 12 patients with fibromyalgia participated in this test. In this ramp test, the average temperature that was reached at the cutoff (VAS 80 mm) was 48.1 ± 5.8°C in control subjects *vs.* 46.2 ± 1.4°C in patients with fibromyalgia ($P < 0.001$). None of the subjects reached the safe fail temperature of 51°C before they reached the cutoff VAS. Cutoff VAS scores were within 3% of target (controls: 76.8 ± 7.5 mm and patients with fibromyalgia, 82.2 ± 4.1 mm, $P = 0.08$). This indicates that the same pain intensity was reached at a 1.9°C lower temperature in patients with fibromyalgia (Fig. 2B).

Pain test 5 vs. pain test 6

A significant higher temperature was reached at the cutoff VAS value during the upward OA step test (test 5) compared with the ramp test (test 6) in the control subjects (48.8 ± 0.7°C [test 5] *vs.* 48.1 ± 5.8°C [test 6]; $P < 0.001$) but not in the patients with fibromyalgia (46.5 ± 1.4°C [test 5] *vs.* 46.2 ± 1.4°C [test 6]; $P = 0.24$). Cut-off VAS-scores were similar though: control subjects 77.9 ± 7.1 mm (test 5) versus 76.8 ± 7.5 mm (test 6; $p = 0.56$); fibromyalgia patients: 81.9 ± 1.4 mm (test 5) versus 82.2 ± 4.2 mm (test 6; $p = 0.76$). This indicates that effective OA engagement (test 5) results in a difference in onset of pain (ie, more pain is tolerated) compared with a condition in which no OA was generated (test 6) in the control subjects. In contrast, in patients with fibromyalgia, reduced OA responses (in test 5) lead to the absence of a difference in the temperature at VAS 80 mm between the 2 tests, hence there was no difference in the onset of pain.

DISCUSSION

In this study, the presence of OA was investigated in patients who experienced chronic pain diagnosed with fibromyalgia and compared with sex-matched and age-matched healthy controls. We observed that compared with control subjects, patients with fibromyalgia show significantly reduced OA and adaptation responses, with an inability to enhance or restore the decreased OA responses by repeating the OA paradigm or

initiating multiple consecutive 1°C temperature decreases. Additionally, we showed that patients with reduced engagement of OA experience their first perception of pain and pain tolerance at lower stimulus intensities compared with controls with more effective OA activation (Fig. 2).

Offset analgesia

Grill and Coghill [10] first described OA in 2002 as a phenomenon that engages temporal filtering in pain processing. The mechanism is activated when a small decrease (1°C-2°C) in temperature during noxious stimulation evokes a disproportionately large reduction in pain perception. Offset analgesia is generally considered a part of the central pain modulation system as activation of the mechanism coincides with activation of brain regions involved in descending pain inhibition [16,17]. Offset analgesia is different from the spatial contrast enhancement mechanism conditioned pain modulation (CPM; formerly known as diffuse noxious inhibitory control), which is likewise used to evaluate descending inhibition of pain. In CPM, central inhibition of a focal stimulus is induced by the administration of a noxious stimulus at a remote area (ie, spatial filtering) [9,14,18]. Recently, Nahman-Averbuch *et al.* [18] showed in a functional magnetic resonance imaging study that temporal and spatial filtering of nociceptive information engage different inhibitory processes in the central nervous system. Within the same individual, OA and CPM activated distinctive brain regions, and magnitudes of OA and CPM were not correlated, indicative of 2 separate forms of endogenous inhibition of pain.

The site at which OA originates remains currently unknown. Functional imaging studies showed that OA activation coincides with activation of brain regions involved in descending pain inhibition [16-18]. A peripheral origin of OA is supported by evidence from neurophysiological research in monkeys where discharge of heat-sensitive nerve fibers innervating the skin was nearly completely suppressed during a 1°C cooling pulse [19]. Furthermore, central acting drugs (opioids, opioid antagonists, and NMDA receptor antagonists) are unable to modify OA responses [9,14,15,20]. This latter observation stands in sharp contrast to CPM, which is readily affected by central acting drugs such as ketamine and tapentadol [8,9,14]. These data indicate that OA may be initiated by dynamic responses in primary afferents or spinal processes. Whether the reduced OA responses observed in our cohort of patients with fibromyalgia are related to small fiber pathology remains unknown. However, since there is a marked resemblance between the responses observed in patients with painful peripheral neuropathy and fibromyalgia, it may well be that the abnormal OA responses are related to a similar underlying pathophysiological mechanism of peripheral origin (see also below).

We previously measured OA responses in a large population of volunteers without pain in an age range of 6 to 88 years [15]. Irrespective of age and sex, the observed OA responses were all of similar magnitude with ΔVASc values ranging between 92% and

99%. In contrast, a recent study by Naugle *et al.* [21] observed a (small) reduction in OA responses in an elderly population (60 years and above). Since in our study, the age range of both study populations was 24 to 47 years, we do not expect that age was a confounding factor.

Fibromyalgia and reduced pain inhibition

Just 2 studies evaluated OA responses in patients who experienced chronic pain [9,15]. Both studies involved patients diagnosed with painful peripheral neuropathy and reduced OA responses were observed comparable with the observation made in this study in patients with fibromyalgia. In painful peripheral neuropathy a ΔVASc cutoff of 88% discriminated between health and disease with 90% sensitivity and 91% specificity [15]. According to this criterion, the population patients with fibromyalgia in this study, with an average ΔVASc score of 65%, demonstrate a nonhealthy OA response. This is the first observation of a reduced temporal pain inhibition in fibromyalgia. Since previous findings show that patients with fibromyalgia also lack the ability to induce CPM (and hence have a reduced spatial pain inhibition), fibromyalgia seems characterized by a general inability to activate descending inhibitory pain mechanisms. There is an ongoing debate on the causative mechanism of fibromyalgia. Although a central cause underlying the pathophysiological mechanism of fibromyalgia has long been considered as most important [2], recent evidence suggests the involvement of peripheral factors. For example, several studies confirmed the presence of small fiber pathology using quantitative sensory testing, confocal cornea microscopy, and skin biopsies [22-24]. Furthermore, Serra *et al.* [25] showed abnormal C-fiber nociceptor activity with signs of hyperexcitability similar to observations in small fiber neuropathy.

Patients with fibromyalgia are known to be more responsive (hyperexcitable) to several sensory stimuli, such as heat, cold, pressure, and electrical pain stimulation [26]. Compared with healthy controls, a hyperalgesic response was observed for both moderate (VAS 50 mm) and intense (VAS 80 mm) heat pain stimuli in patients with fibromyalgia (probe temperature in patients with fibromyalgia ~2.5°C lower at VAS 50 and 80 mm). To the best of our knowledge, fibromyalgia is the first pain syndrome in which observations are made that hyperexcitability to painful stimuli coincides with reduced endogenous pain inhibition of both spatial (CPM) and temporal (OA) nature. Whether the hyperexcitability is a direct cause of altered pain inhibition or a secondary phenomenon remains unknown. Irrespective, we argue that multiple neurophysiological mechanisms underlie fibromyalgia-related pain. Whether these are peripheral factors, central factors or both needs further research. Our data suggest a role for the loss of proper OA engagement in the hyperexcitability to heat pain.

Variations to the offset analgesia paradigm

As OA in patients with fibromyalgia was reduced or incomplete, we hypothesized that repeating the OA paradigm or initiating additional 1°C temperature decreases (after the initial OA test) would improve or complete the OA response. However, both variations to the OA paradigm (test 2 and 3) did not improve the magnitude of the OA response in patients. In test 3 (downward step test, Fig. 1C), we observed no additional OA in patients with fibromyalgia. Instead, the reduction in pain scores showed an almost linear relation to the decline in temperature (Fig. 1C). This may be disease specific but could additionally be related to the test paradigm. Possibly, OA can only be initiated when the temperature decline that initiates the offset response is preceded by a heat stimulus intensity increase [27].

Of interest is that OA was able to influence the onset of pain. During the upward OA step test (test 5, Fig. 2A), an OA response was observed with every step decrease in temperature in controls and of lesser magnitude in patients with fibromyalgia. Owing to the presence of these repeated and larger OA responses, control subjects were able to tolerate higher temperatures at a VAS score of 80 mm compared with the ramp test (test 6) where no OA was generated. Owing to the inability to properly engage OA, this was not observed in patients with fibromyalgia who tolerated similar temperatures in tests 5 and 6. These data suggest that OA is an important phenomenon that may influence the onset of pain perception.

Adaptation and habituation

We observed reduced adaptation responses in patients with fibromyalgia (Fig. 1D). The underlying mechanism of heat stimulus adaptation is not completely clarified. Neurophysiological studies suggest that moderate temperatures initiate an adapting discharge pattern in heat nociceptors and in a subgroup of C-fibers [28,29]. This would indicate that small fibers are involved in the process of adaptation. Offset analgesia is considered to be different from pain adaptation due to the larger and more rapid reduction of pain perception during OA. To compare the offset response initiated by the 1-step OA paradigm (test 1) with the constant stimulation test (test 4), the time interval between peak VAS and VAS nadir was calculated in control subjects. The time interval for the OA response was 4.8 ± 3.5 seconds *vs.* 16.8 ± 20.3 seconds for the adaptation response. This indicates that the offset response initiated by the OA paradigm was indeed a much faster response than the slow pain adaptation response observed during continuous heat stimulation and implies that OA and pain adaptation have a different underlying physiological mechanism. Irrespective of their mechanisms, both systems are dysfunctional in fibromyalgia. Peak pain responses during the repeated OA paradigm similarly decreased over time for both control subjects and patients (Fig. 1B), which suggest that the process of pain habituation was not affected in patients with fibromyalgia. Pain

habituation differs from pain adaptation; pain habituation is characterized by sensory fatigue on repeated stimulation and pain adaptation is a time-related reduction in pain perception in response to continuous heat stimulation [29,30].

In conclusion, we showed that patients with fibromyalgia have reduced OA and adaptation responses that influenced both the onset and offset of pain.

REFERENCES

1. Clauw DJ: Fibromyalgia: a clinical review. Jama 2014, 311:1547-55.
2. Schmidt-Wilcke T, Clauw DJ: Fibromyalgia: from pathophysiology to therapy. Nature reviews Rheumatology 2011, 7:518-27.
3. Jensen KB, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Giesecke T, Mainguy Y, Gracely R, Ingvar M: Evidence of dysfunctional pain inhibition in Fibromyalgia reflected in rACC during provoked pain. Pain 2009, 144:95-100.
4. Julien N, Goffaux P, Arsenault P, Marchand S: Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. Pain 2005, 114:295-302.
5. Kosek E, Hansson P: Modulatory influence on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects. Pain 1997, 70:41-51.
6. Lautenbacher S, Rollman GB: Possible deficiencies of pain modulation in fibromyalgia. The Clinical journal of pain 1997, 13:189-96.
7. Ossipov MH, Dussor GO, Porreca F: Central modulation of pain. The Journal of clinical investigation 2010, 120:3779-87.
8. Niesters M, Aarts L, Sarton E, Dahan A: Influence of ketamine and morphine on descending pain modulation in chronic pain patients: a randomized placebo-controlled cross-over proof-of-concept study. British journal of anaesthesia 2013, 110:1010-6.
9. Niesters M, Proto PL, Aarts L, Sarton EY, Drewes AM, Dahan A: Tapentadol potentiates descending pain inhibition in chronic pain patients with diabetic polyneuropathy. British journal of anaesthesia 2014, 113:148-56.
10. Grill JD, Coghill RC: Transient analgesia evoked by noxious stimulus offset. Journal of neurophysiology 2002, 87:2205-8.
11. Yelle MD, Rogers JM, Coghill RC: Offset analgesia: a temporal contrast mechanism for nociceptive information. Pain 2008, 134:174-86.
12. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis care & research 2010, 62:600-10.
13. Olofsen E, Romberg R, Bijl H, Mooren R, Engbers F, Kest B, Dahan A: Alfentanil and placebo analgesia: no sex differences detected in models of experimental pain. Anesthesiology 2005, 103:130-9.
14. Niesters M, Dahan A, Swartjes M, Noppers I, Fillingim RB, Aarts L, Sarton EY: Effect of ketamine on endogenous pain modulation in healthy volunteers. Pain 2011, 152:656-63.
15. Niesters M, Hoitsma E, Sarton E, Aarts L, Dahan A: Offset analgesia in neuropathic pain patients and effect of treatment with morphine and ketamine. Anesthesiology 2011, 115:1063-71.
16. Derbyshire SW, Osborn J: Offset analgesia is mediated by activation in the region of the periaqueductal grey and rostral ventromedial medulla. NeuroImage 2009, 47:1002-6.
17. Yelle MD, Oshiro Y, Kraft RA, Coghill RC: Temporal filtering of nociceptive information by dynamic activation of endogenous pain modulatory systems. The Journal of neuroscience : the official journal of the Society for Neuroscience 2009, 29:10264-71.
18. Nahman-Averbuch H, Martucci KT, Granovsky Y, Weissman-Fogel I, Yarnitsky D, Coghill RC: Distinct brain mechanisms support spatial *vs.* temporal filtering of nociceptive information. Pain 2014, 155:2491-501.

19. Darian-Smith I, Johnson KO, LaMotte C, Shigenaga Y, Kenins P, Champness P: Warm fibers innervating palmar and digital skin of the monkey: responses to thermal stimuli. Journal of neurophysiology 1979, 42:1297-315.
20. Martucci KT, Eisenach JC, Tong C, Coghill RC: Opioid-independent mechanisms supporting offset analgesia and temporal sharpening of nociceptive information. Pain 2012, 153:1232-43.
21. Naugle KM, Cruz-Almeida Y, Fillingim RB, Riley JL, 3rd: Offset analgesia is reduced in older adults. Pain 2013, 154:2381-7.
22. Giannoccaro MP, Donadio V, Incensi A, Avoni P, Liguori R: Small nerve fiber involvement in patients referred for fibromyalgia. Muscle & nerve 2014, 49:757-9.
23. Kosmidis ML, Koutsogeorgopoulou L, Alexopoulos H, Mamali I, Vlachoyiannopoulos PG, Voulgarelis M, Moutsopoulos HM, Tzioufas AG, Dalakas MC: Reduction of Intraepidermal Nerve Fiber Density (IENFD) in the skin biopsies of patients with fibromyalgia: a controlled study. Journal of the neurological sciences 2014, 347:143-7.
24. Uceyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C: Small fibre pathology in patients with fibromyalgia syndrome. Brain : a journal of neurology 2013, 136:1857-67.
25. Serra J, Collado A, Sola R, Antonelli F, Torres X, Salgueiro M, Quiles C, Bostock H: Hyperexcitable C nociceptors in fibromyalgia. Annals of neurology 2014, 75:196-208.
26. Desmeules JA, Cedraschi C, Rapiti E, Baumgartner E, Finckh A, Cohen P, Dayer P, Vischer TL: Neurophysiologic evidence for a central sensitization in patients with fibromyalgia. Arthritis and rheumatism 2003, 48:1420-9.
27. Morch CD, Frahm KS, Coghill RC, Arendt-Nielsen L, Andersen OK: Distinct temporal filtering mechanisms are engaged during dynamic increases and decreases of noxious stimulus intensity. Pain 2015, 156:1906-12.
28. Meyer RA, Campbell JN: Evidence for two distinct classes of unmyelinated nociceptive afferents in monkey. Brain research 1981, 224:149-52.
29. Treede RD, Meyer RA, Campbell JN: Myelinated mechanically insensitive afferents from monkey hairy skin: heat-response properties. Journal of neurophysiology 1998, 80:1082-93.
30. Hashmi JA, Davis KD: Effects of temperature on heat pain adaptation and habituation in men and women. Pain 2010, 151:737-43.

Chapter 4

Evaluation of a novel contact heat device (Q-sense CPM) for conditioned pain modulation testing in healthy volunteers

LCJ Oudejans, M van Velzen, A Dahan, M Niesters.

Submitted.

INTRODUCTION

Conditioned pain modulation (CPM) is an experimental design to assess endogenous modulation of pain. In classic CPM paradigms, two nociceptive stimuli are applied on two separate locations. One stimulus is referred to as the test stimulus, and the other as the conditioning stimulus. In an individual with intact endogenous pain modulatory pathways, the application of the conditioning stimulus will reduce pain perception of the test stimulus. This mechanism was first described in animal studies demonstrating that dorsal horn neurons are strongly inhibited by a feedback mechanism between spinal and brainstem neurons induced by extra-segmental noxious stimulation, a phenomenon called diffuse noxious inhibitory control or DNIC [1]. CPM differs from DNIC because it is influenced by higher brain centers such as the orbitofrontal cortex and amygdala [2]. The inability to engage descending pain inhibitory pathways is thought to play an important role in the development of chronic pain following tissue damage, for example after surgery. Moreover, the ability to induce efficient endogenous pain inhibition is impaired in patients with a variety of chronic pain conditions, such as fibromyalgia, irritable bowel syndrome, osteoarthritis and painful diabetic neuropathy [3,4]. Recent CPM studies indicate that deficits of the endogenous pain modulatory system may predict the development of persistent pain following surgery [5] and the efficacy of pain medication [4]. These data suggest an important role for CPM testing in the clinical setting.

The large variation in CPM paradigms that are used in experimental studies [6] complicates comparison of patient populations and study outcomes. Differences in methodology include the type of testing stimulus (heat, electrical, pressure pain), the type of conditioning stimulus (hot water, cold water, ischemia, capsaicin), the position of the conditioning stimulus and the duration of the stimuli [6]. Recently a novel sensory testing device has been specifically developed for induction and testing of CPM, the Q-sense CPM. It holds two contact heat thermodes that can be operated separately or simultaneously to apply the test and/or conditioning stimulus. The Q-sense CPM device is a lightweight mobile system that includes software that can be easily adjusted to tailor various CPM paradigms. The aim of this study was to compare the conditioning stimulus of the Q-sense CPM device with the more conventional cold water immersion on the ability to induce CPM. Additionally, we varied the location of the conditioning stimulus and the duration of the test stimulus to assess effects on the magnitude of CPM.

METHODS

Subjects

Twenty-four healthy volunteers (9 men/13 women) were recruited via local advertisement to participate in the study. Inclusion criteria were: age between 18 and 35 years, ability to give written informed consent and absence of a significant medical condition. Exclusion criteria were: a history of psychiatric disease or drug abuse, pregnancy or lactation and obesity (BMI > 35). All participants gave oral and written informed consent before enrolment in the study. All study procedures were performed according to Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. The protocol was approved by the local medical ethics committee (Commissie Medische Ethiek LUMC, Leiden, The Netherlands) prior to start of the study is registered in The Netherlands Trial Register (NTR) under number TC5414 (www.trialregister.nl).

Study design

The magnitude of the CPM effect was compared between the conditioning stimulus of the Q-sense CPM device - which consists of two 30 × 30 mm air-cooled heat probes – and a conventional cold water immersion test. In the first method one contact heat probe of the Q-sense CPM device was used as the test stimulus, and the second contact heat probe was used to induce CPM (CPMQ method). In the second method the contact heat probe of the Q-sense device was used as the test stimulus and immersion of the foot and ankle in cold water was used as conditioning stimulus (CPMW method). The heat probes are applied on the skin and can deliver a constant temperature in the range of 20 to 49°C using a ramp (0.1-2°C/s) and hold strategy. In our paradigms, the increase from a baseline temperature of 32°C was 1.5°C/s and applied until the target temperature was reached. For both methods, test stimuli were applied on the volar side of the non-dominant forearm. To induce CPMQ the conditioning stimulus was applied on medial side of the calf, contralateral or ipsilateral to the site of the test stimulus (Figure 1). To induce CPMW the ipsi- or contralateral foot and ankle were immersed in cold water. To measure the pain response, the visual analogue score (VAS) was measured electronically using a slide potentiometer (length 100 mm) that was moved from left (0 mm or no pain) to right (100 mm or most intense pain imaginable) using the dominant hand. Subjects were asked to constantly rate the pain intensity of the test stimulus during the entire test and were unaware of the purpose of the conditioning stimulus.

In the first 12 subjects, we used a 30-s constant test stimulus. In a second set of 12 subjects, we used a test stimulus that consisted of 3 × 10-s stimuli separated by 20-s of baseline temperature. For each method, the test stimulus was applied first and after a 3 min pause, the conditioning stimulus was combined with the test stimulus, with the conditioning stimulus starting 20-s before the test stimulus (Figure 2).

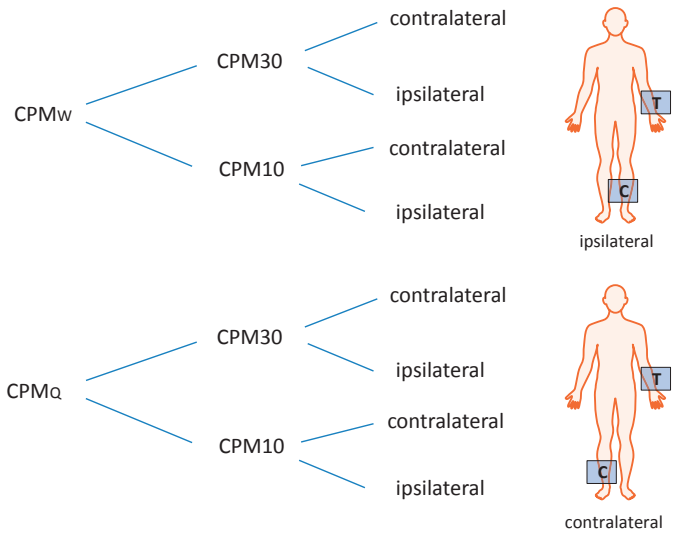

Figure 1. CPM testing protocols. CPM was tested with cold water immersion of the foot and ankle (CPMW) or the conditioning probe of the Q-sense CPM device (CPMQ) placed on the lower leg as the conditioning stimulus. A CPM 30-second (CPM30) test and a repeated CPM 10-second (CPM10) test were performed with the conditioning stimulus on both the contralateral and ipsilateral leg. T = teststimulus; C = conditioning stimulus.

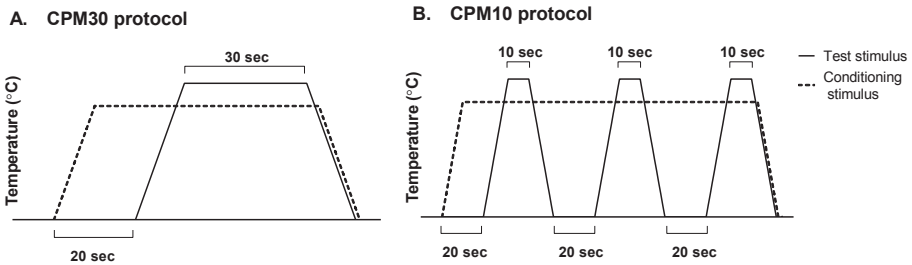

Figure 2. CPM paradigms. **(A)** The classic CPM test (CPM30) consisted of a single 30-second heat stimulus. **(B)** The repeated CPM test (CPM10) consisted of three 10-second heat stimuli with 20 seconds baseline warmth (32°C) in between. The conditioning stimulus was started 20 seconds before the test stimulus in both paradigms.

CPM30. The 30-s test stimulus CPMW and CPMQ tests (CPMW30 and CPMQ30) were tested 3 times at both locations of the conditioning stimulus (*i.e.* ipsi- and contralateral to the test stimulus). The order of the CPM tests was random. In between CPM tests there was at least a 15-minute pause.

CPM10. The 3 × 10-s test stimulus CPMW and CPMQ tests (CPMW10 and CPMQ10) were tested once at both locations of the conditioning stimulus (*i.e.* ipsi- and contralateral to the test stimulus). Tests were performed in random order with at least a 15-minute pause between tests.

Determination of stimuli intensities

All participants were initially familiarized with the pain tests and scoring system. Next, the temperature of the test stimulus was determined; we searched for a temperature that evoked a peak pain intensity of 50-60 mm. To that end, an initial stimulus of 41°C was applied for 10 seconds. If a peak VAS of < 50 mm was reached, subsequent stimuli were applied increasing the temperature by 0.5°C. This was repeated until a temperature causing a peak VAS of 50-60 mm was reached. This temperature was repeated two more times in order to be certain of a consistent determination of the test temperature. The same method was used to define the temperature of the conditioning stimulus (CPMQ method); we searched for a temperature that evoked a peak pain intensity of 30-40 mm. For the CPMW method the temperature of the water bath was determined by immersion of the foot and ankle for 1 min in water of different temperatures ranging from 6-14°C. The temperature that evoked a peak pain intensity of 30-40 mm was used in the CPMW experiments.

Data and Statistical analysis

Peak eVAS scores were determined for test-stimulus only (TS) and for test- and conditioning stimuli simultaneously (TCS). The values of the three CPMQ30 and CPMW30 tests or the three peaks per CPMQ10 and CPMW10 test obtained at a single location were averaged to get a single average value per subject. This resulted in the following scores for baseline and CPM tests: CPMQ30 contralateral; CPMQ30 ipsilateral; CPMW30 contralateral; CPMW30 ipsilateral; and CPMQ10 contralateral; CPMQ10 ipsilateral; CPMW10 contralateral; CPMW10 ipsilateral (see Fig. 1). Differences in Peak eVAS scores between TS and TCS were determined using Wilcoxon signed ranks tests. The magnitude of CPM was calculated by subtracting the peak eVAS value of TCS from peak eVAS values of TS. The resulting value, ΔPeak, was divided by TS and multiplied by 100 to get CPM% = (ΔPeak/TS)*100.

The CPM% values of the three CPMQ30 and CPMW30 tests or the three peaks per CPMQ10 and CPMW10 test obtained at a single location were averaged to get an average value per subject. Paired-t-tests or Wilcoxon signed ranks tests were used to assess whether (1) CPMQ and CPMW tests produced significant CPM responses, (2) magnitudes of CPMQ and CPMW tests differed, (3) magnitudes of CPM obtained at different conditioning stimulus locations differed and (4) magnitudes of CPM10 and CPM30 tests differed.

Statistical testing was performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA) and SPSS statistics 23 (IBM SPSS Statistics, Armonk, NY). P-values <0.05 were considered significant. Data are presented as average ± SEM, unless stated otherwise.

RESULTS

All 24 subjects completed the study according to protocol. Subject characteristics and the temperatures of the test and conditioning tests are summarized in Table 1. There were no differences in patient characteristics or baseline values (temperature of the test and conditioning CPMW and CPMQ stimuli) between subjects participating in the CPM30 and CPM10 experiments.

Table 1. Subject characteristics and baseline measurements

	CPM30	CPM10
Number of patients (n)	12	12
Men/women (n)	6/6	3/9
Age (years)	25.1 (18-34)	22.6 (19-31)
Body mass index (kg/m^2)	23 (18.3-26.7)	21.6 (17.1-25.9)
Test temperature (°C)	46.6 (42-48.9)*	46.5 (43-48.9)*
CPMQ Conditioning temperature (°C)	45.5 (41-48)	45.4 (41.5-46.9)*
CPMW Conditioning temperature (°C)	9.9 (6-14)	10.4 (5-14)

*The safety limit (maximum temperature) of the Q-sense CPM device for 30 seconds is 48.9°C. This temperature was reached in 1 (CPM30) and 2 (CPM10) cases. The safety limit of the Q-sense device for > 1 minute is 46.9°C. This temperature was reached in 4 (CPM10) cases. All values are mean (range) or numbers (n).

Peak eVAS scores and CPM effect. A representative example of a subject with a significant CPM effect in CPMW tests is shown in Figure 3. Peak eVAS scores for TS and TCS for both CPM30 and CPM10 are given in Table 2. Peak eVAS scores were significantly different between TS and TCS for CPMW experiments, but not for CPMQ experiments: CPMQ30 contralateral p = 0.20; CPMQ30 ipsilateral p = 0.86; CPMW30 contralateral p = 0.01; CPMW30 ipsilateral p =0.00; CPMQ10 contralateral p = 0.34; CPMQ10 ipsilateral p =

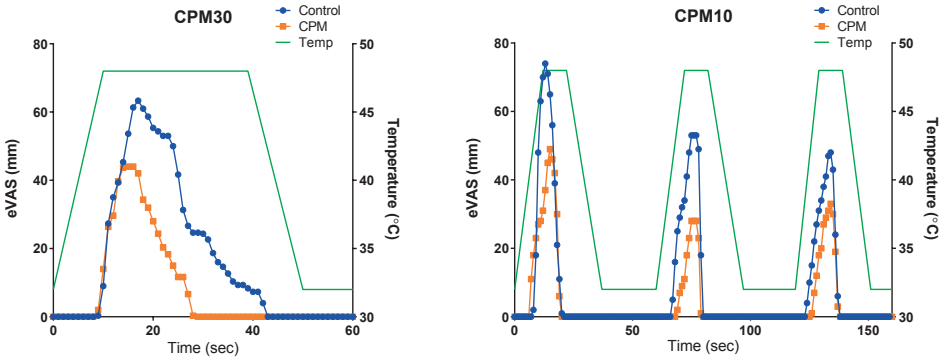

Figure 3. Representative examples of the time course of the CPM30 and CPM10 paradigms when a significant CPM effect was achieved. CPM = conditioned pain modulation.

Table 2. Peak eVAS scores (mm) and percentage CPM effect (CPM%) for the 30-second (CPM30) and 10-second (CPM10) pain modulation tests

	TS	TCS	CPM%
CPM30 (n=12)			
CPMw contralateral	54 (6)	45 (8)*	17 (10)
CPMQ contralateral	57 (8)	50 (8)	10 (14)
CPMw ipsilateral	52 (6)	43 (6)*	19 (8)
CPMQ ipsilateral	51 (8)	55 (8)	-4 (12)
	TS	**TCS**	**CPM%**
CPM10 (n=12)			
CPMw contralateral	50 (8)	37 (10)*	25 (18)
CPMQ contralateral	54 (12)	50 (14)	7 (18)
CPMw ipsilateral	49 (10)	43 (12)*	14 (18)
CPMQ ipsilateral	49 (12)	47 (12)	-0.2 (12)

All values are mean (95% confidence interval). * $p < 0.05$ test stimulus (TS) *versus* test and conditioning stimulus (TCS). TS = peak eVAS score of the test stimulus without conditioning stimulus; TCS = peak eVAS score of the test stimulus with simultaneous presence of the conditioning stimulus.

0.48; CPMw10 contralateral $p = 0.04$; CPMw10 ipsilateral $p = 0.045$ (Figure 4). Because only the CPMw experiments demonstrated a significant CPM effect, further evaluations will only be discussed for CPMw results.

CPM30. The CPMw30 induced significant CPM effects (CPM%) of 17 ± 5% when the conditioning stimulus was applied on the contralateral leg and 19 ± 4% when the conditioning stimulus was applied on the ipsilateral leg. The magnitudes of CPM effects are summarized in Table 2 and Figure 5. Comparison of the contralateral and ipsilateral tests revealed no significant difference in CPM effect in the CPMw30 test ($p = 0.626$; Figure 5).

CPM10. A significant CPM response (CPM%) was induced by the CPMw10 test when the conditioning stimulus was applied on both the contralateral leg (25 ± 9%) and the ipsilateral leg (14 ± 6%; Table 2, Figure 4). No significant difference in CPM effect was shown between contralateral and ipsilateral conditioning: CPMw $p = 0.313$ (Figure 5).

CPM30 *vs.* CPM10. None of the tests showed a significant difference in CPM effect between the CPM30 and the CPM10 tests: CPMw30 contralateral $p = 0.733$; CPMw10 ipsilateral $p = 0.261$ (Figure 5).

DISCUSSION

The main aim of this study was to evaluate the ability of a device that holds two contact heat probes, to generate significant CPM responses. To that end, we used the Q-sense CPM device, which was recently introduced and is specifically developed to study CPM.

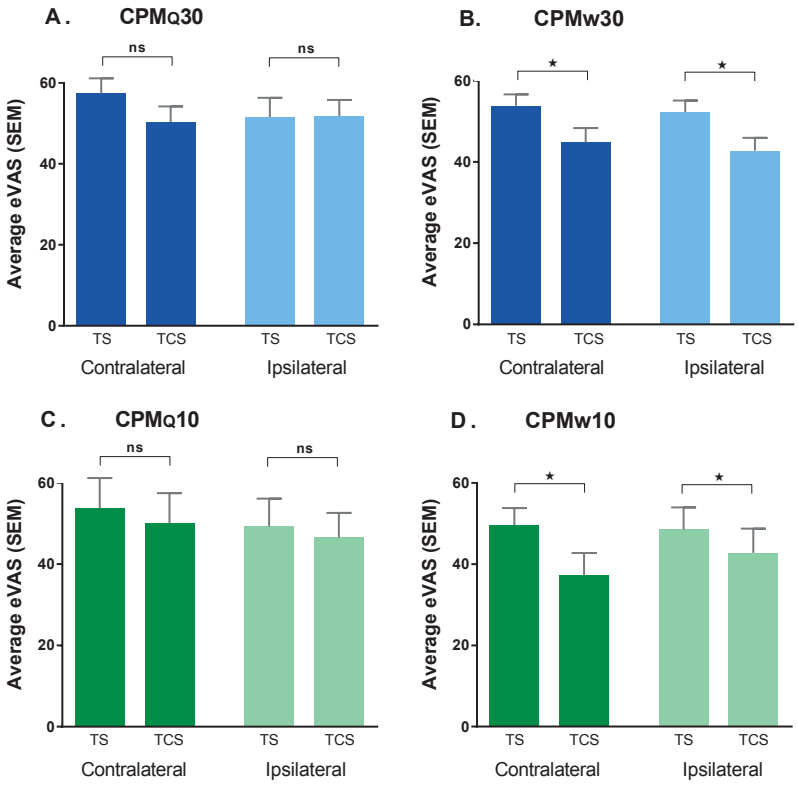

Figure 4. Average electronic visual analog scores (eVAS) for the test stimulus (TS) and the test- and conditioning stimulus (TCS) for contralateral and ipsilateral positioning of the conditioning probe. CPM30 paradigms using the Q-sense CPM device (CPMQ30) and cold water (CPMW30) as conditioning stimulus **(A and B).** CPM10 paradigms using the Q-sense CPM device (CPMQ10) and cold water (CPMw10) as conditioning stimulus **(C and D)**. Data are reported as average ± SEM. *$p < 0.05$; ns = not significant.

We employed the device under multiple conditions in which variations in test stimulus (30 seconds and 10 seconds) and conditioning stimulus (cold water and contact heat pain; ipsi- and contralateral sites to the test stimulus) were studied. The rationale behind the Q-sense CPM device is that it will enable the performance of standardized CPM tests in clinical settings. A device suitable for clinical use should meet a number of requirements of which the most important is a stable and sufficiently large induction of CPM in healthy volunteers.

We here show that using two contact heat probes did not produce significant CPM responses regardless of positioning of the conditioning probe or applied paradigm. In contrast, significant CPM responses were produced using cold water as conditioning stimulus with both contralateral and ipsilateral conditioning positions and in both 30 seconds and 10 seconds paradigms.

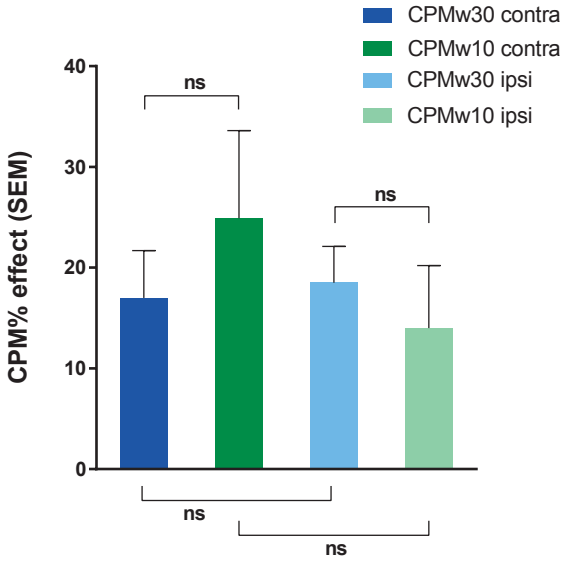

Figure 5. CPM effects (% CPM) of the CPM30 and the CPM10 paradigm using cold water (CPMw) as conditioning stimulus. The CPM effect is expressed as the relative difference between the peak eVAS in response to a test stimulus *with* and in response to a test stimulus *without* a conditioning stimulus. For example, a CPM effect of 17% indicates a reduction in pain score of 17% relative to the test stimulus without conditioning. For the CPM10 trials, the % CPM is the relative difference between 'the average of 3 peaks with conditioning stimulus' and 'the average of 3 peaks without conditioning stimulus'. Data are reported as average ± SEM. ns = not significant.

CPM measurements gained popularity over the last decade and are used to study endogenous pain modulation in healthy volunteers and patients with chronic pain. Two studies from Yarnitsky *et al.* [4,5] suggest that CPM testing has the potential to identify patients at risk of developing chronic pain, to evaluate efficacy of analgesics and possibly to make decision on choice of medication. These studies suggest that CPM testing may be used in chronic pain patient profiling (*i.e.*, phenotyping) and as such may be an important instrument in the decision tree. Still, pain testing is complex and influenced by many factors such as age, sex, psychosocial factors, drug intake (*e.g.* caffeine) and the (time of) day of testing [6]. CPM is a pain test that suffers from these same influences. Furthermore, additional problems from the large variation in techniques make interpretation and comparison of the CPM responses often difficult if not impossible.

We observed that the Q-sense CPM device produced inconsistent CPM responses over all three variations with low responses in all tests. This indicates that using cold water as conditioning stimulus is superior to a contact heat thermode to induce CPM. Possible explanations could be that the surface area of the conditioning contact heat probe is too small to induce a proper CPM response [7], that a heat stimulus induces a less effective CPM response than a cold stimulus, or that immersion of a limb in water induces a larger CPM effect because it is a much more pronounced and prominent stimulus compared to the 30 × 30 mm surface contact heat stimulus. Additionally, the conditioning heat stimulus may not induce constant pain during testing. Heat pain intensity is known to decrease over time in contrast to cold pain intensity induced by water which increases during the first couple of minutes of immersion. Indeed, some subjects indicated that

the constant heat stimulus adapted to near zero VAS scores over the 30 s exposure. We (and others) use the cold water conditioning test in our studies [8-12] and our current results show that consistent results are obtained using this paradigm under different experimental conditions. Hot water immersion, limb ischemia and chemical pain are other examples of conditioning stimuli. Recently, Lewis *et al.* [13] compared cold water and ischemic limb conditioning stimuli and concluded that the cold-water test was superior to the ischemic limb test.

We observed consistent CPM responses at ipsilateral and contralateral sites using the cold water as conditioning stimulus. Early publications on DNIC in animals reported analgesia caused by counter stimulation at a heterotopic anatomical site. Accordingly, most CPM protocols in humans have been performed by using a heterotopic, mostly contralateral location of the conditioning stimulus relative to the test stimulus [3]. Findings from studies that did use an ipsilateral positioning of the conditioning stimulus were unequivocal: one study did find pain reduction after ipsilateral conditioning stimulation [14], where others did not [15,16]. We argue that our observation of a significant CPM response at the ipsilateral site is due to the fact that cold water is a strong effective conditioning stimulus. Still, in line with the animal data and especially when a weaker conditioning stimulus is used (such as contact heat) we recommend the use of the contralateral site.

A number of limitations pertain to the current study. We did not study whether lower leg *versus* arm as heterotopic site for conditioning is preferable because subjects were asked to score their pain response continuously by an electronically recorded VAS. Using the contralateral arm for conditioning is applied in some studies and further investigations are needed to assess whether this approach enables generation of CPM responses. The difference between the test temperatures and the conditioning temperatures was small, while the temperatures were set to evoke different pain intensity ratings of eVAS 50-60 (test stimulus) and 30-40 (conditioning stimulus). This is probably caused by differential responses to heat stimuli in the upper and lower extremity. In our population, a heat stimulus of a particular temperature applied to the lower leg evoked a lower pain intensity score than the same temperature applied to the arm. Hence, there was only a minor temperature difference between test- and conditioning stimuli.

To be convenient to use (especially in clinical practice) any CPM paradigm should not take too much time. Determination of the temperatures for the test and conditioning stimuli is a time consuming procedure. Further studies should assess whether fixed and non-individualized temperatures are equally able to induce reliable CPM responses. It is our experience, however, that the consistency and magnitude of CPM responses is less when individual temperatures are not defined prior to CPM testing. Additionally, we recommend performing multiple CPM tests to take into account the within-subject and day-to-day variability [13]. As indicated above, the contact heat conditioning stimulus may not have been the constant mild painful stimulus that is required to induce CPM.

We decided not to increase the pre-set VAS of the conditioning stimulus above 30-40 mm as there is consensus that mild to moderate stimuli are sufficient to induce CPM responses [17] based on several studies that indicated there seems to be a ceiling effect in CPM efficiency [18-21]. Finally, we applied the conditioning stimuli 20-s prior to the start of the test stimulus. Granovsky *et al.* [7] recently assessed the possibility of starting the conditioning stimulus *after* the start of the test stimulus. Although the investigators concluded that this paradigm properly induces CPM, we do not believe that this approach would have improved CPM responses using the CPMQ methods.

In conclusion, we observed that using two contact heat probes to induce a noxious heat test stimulus applied on the arm and a heat conditioning stimulus applied on the leg, did not generate significant CPM responses. Consistent CPM responses were obtained under all three test conditions when cold water was used as conditioning stimulus. For the future application of CPM to evaluate endogenous pain modulation in experimental settings, we recommend the use of cold water as conditioning stimulus at the contralateral site of the test stimulus. The contact heat probe of the Q-sense CPM device can be used as the test stimulus. The use of the two contact thermal probes of the Q-sense CPM device to induce CPM in the clinical setting might be feasible if the surface area of the conditioning probe would be enlarged and if the contact probe would be able to deliver a noxious cold stimulus. We additionally suggest using individualized temperatures irrespective of CPM method.

REFERENCES

1. Le Bars D, Dickenson AH, Besson JM: Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. Pain 1979, 6:283-304.
2. Moont R, Crispel Y, Lev R, Pud D, Yarnitsky D: Temporal changes in cortical activation during conditioned pain modulation (CPM), a LORETA study. Pain 2011, 152:1469-77.
3. van Wijk G, Veldhuijzen DS: Perspective on diffuse noxious inhibitory controls as a model of endogenous pain modulation in clinical pain syndromes. The journal of pain : official journal of the American Pain Society 2010, 11:408-19.
4. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y: Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 2012, 153:1193-8.
5. Yarnitsky D, Crispel Y, Eisenberg E, Granovsky Y, Ben-Nun A, Sprecher E, Best LA, Granot M: Prediction of chronic post-operative pain: pre-operative DNIC testing identifies patients at risk. Pain 2008, 138:22-8.
6. Lewis GN, Rice DA, McNair PJ: Conditioned pain modulation in populations with chronic pain: a systematic review and meta-analysis. The journal of pain : official journal of the American Pain Society 2012, 13:936-44.
7. Granovsky Y, Miller-Barmak A, Goldstein O, Sprecher E, Yarnitsky D: CPM Test-Retest Reliability: "Standard" *vs.* "Single Test-Stimulus" Protocols. Pain medicine 2015.
8. King CD, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley JL, 3rd: Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. Pain 2009, 143:172-8.
9. Niesters M, Aarts L, Sarton E, Dahan A: Influence of ketamine and morphine on descending pain modulation in chronic pain patients: a randomized placebo-controlled cross-over proof-of-concept study. British journal of anaesthesia 2013, 110:1010-6.
10. Niesters M, Proto PL, Aarts L, Sarton EY, Drewes AM, Dahan A: Tapentadol potentiates descending pain inhibition in chronic pain patients with diabetic polyneuropathy. British journal of anaesthesia 2014, 113:148-56.
11. Olesen SS, Brock C, Krarup AL, Funch-Jensen P, Arendt-Nielsen L, Wilder-Smith OH, Drewes AM: Descending inhibitory pain modulation is impaired in patients with chronic pancreatitis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association 2010, 8:724-30.
12. Pud D, Granovsky Y, Yarnitsky D: The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. Pain 2009, 144:16-9.
13. Lewis GN, Heales L, Rice DA, Rome K, McNair PJ: Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. Pain research & management : the journal of the Canadian Pain Society = journal de la societe canadienne pour le traitement de la douleur 2012, 17:98-102.
14. Pud D, Sprecher E, Yarnitsky D: Homotopic and heterotopic effects of endogenous analgesia in healthy volunteers. Neuroscience letters 2005, 380:209-13.
15. Graven-Nielsen T, Babenko V, Svensson P, Arendt-Nielsen L: Experimentally induced muscle pain induces hypoalgesia in heterotopic deep tissues, but not in homotopic deep tissues. Brain research 1998, 787:203-10.
16. Svensson P, Hashikawa CH, Casey KL: Site- and modality-specific modulation of experimental muscle pain in humans. Brain research 1999, 851:32-8.

17. Yarnitsky D, Bouhassira D, Drewes AM, Fillingim RB, Granot M, Hansson P, Landau R, Marchand S, Matre D, Nilsen KB, Stubhaug A, Treede RD, Wilder-Smith OH: Recommendations on practice of conditioned pain modulation (CPM) testing. European journal of pain 2015, 19:805-6.
18. Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, Yarnitsky D: Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? Pain 2008, 136: 142-9.
19. Nir RR, Granovsky Y, Yarnitsky D, Sprecher E, Granot M: A psychophysical study of endogenous analgesia: the role of the conditioning pain in the induction and magnitude of conditioned pain modulation. European journal of pain 2011, 15:491-7.
20. Nir RR, Yarnitsky D, Honigman L, Granot M: Cognitive manipulation targeted at decreasing the conditioning pain perception reduces the efficacy of conditioned pain modulation. Pain 2012, 153:170-6.
21. Willer JC, Roby A, Le Bars D: Psychophysical and electrophysiological approaches to the pain-relieving effects of heterotopic nociceptive stimuli. Brain : a journal of neurology 1984, 107 (Pt 4): 1095-112.

Chapter 5

Cornea nerve fiber quantification and construction of phenotypes in patients with fibromyalgia

LCJ Oudejans, X He, M Niesters, A Dahan, M Brines, M van Velzen.

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INTRODUCTION

Fibromyalgia is characterized by chronic widespread pain accompanied by a range of symptoms including headache, fatigue, cognitive dysfunction, depression and sleep disturbances [1,2]. Diagnosis is based on symptoms that persist for at least three months and that are not explained by any other disease process. Since there is no clear and well-described pathophysiological substrate, fibromyalgia has long been considered a pain state that originates at central sites, *i.e.*, within the central nervous system. Evidence for this hypothesis comes from observations of increased neuronal activity during non-noxious stimulation in brain regions involved in pain processing, and indications of dysfunctional endogenous pain modulatory systems [2-4]. However, recent evidence suggests involvement of the peripheral nervous system in some patients with fibromyalgia. Proof of small fiber involvement comes from studies using skin biopsies, cornea confocal microscopy (CCM) and quantitative sensory testing (QST) [5-10]. For example, Ramirez *et al.* [9] observed in a small cohort of patients with fibromyalgia a 20% reduction of cornea nerve fiber density compared with control subjects. Additionally, there is proof of abnormal C-fiber nociceptor activity with hyperexcitability in patients with fibromyalgia, very similar to observations in patients with established small fiber neuropathy (SFN) [11]. At this point we would like to mention that with Clauw [12] and Üçeyler and Sommer [13] we make a distinction between SFN and small-fiber pathology. As stated by these authors, SFN is reserved for a subgroup of neuropathies in which impairment of small nerve fibers (causing changes in nerve density and autonomic functions) leads to superficial burning pain and abnormal sensations affecting predominantly the feet and hands of the patient. In fibromyalgia we use the term small fiber pathology or small fiber damage as these patients predominantly report deep pain in muscles and tendons and it remains currently unknown what the role is of the smallfiber pathology in the cause of symptoms of fibromyalgia [12,13].

The diagnosis of small fiber pathology is usually based on assessment of neuropathic symptoms, quantitative sensory testing, electromyography and/or skin biopsies, with skin biopsies considered the gold standard for diagnosis. In addition to the invasive nature, intra-observer and intra-patient variability contributes to difficulties when using skin biopsies to diagnose peripheral neuropathy [14]. Cornea confocal microscopy is a relatively novel technique that has been developed to quantify small nerve fibers in the cornea [15,16]. CCM examines the densely innervated cornea as a surrogate for the small nerve fiber state, and can serve as a quantitative and qualitative measure of small fiber morphology in a reproducible, non-invasive manner. In most studies, nerve fiber counts in the cornea are generally correlated with skin biopsies, and correlate well with clinical symptoms of small fiber neuropathy especially in patients with patchy neuropathy [16-18].

To our knowledge, a comprehensive study in fibromyalgia patients including corneal nerve quantification, sensory testing, and questionnaires, is lacking. Combinatory test-

ing aids in the construction of patient phenotypes, identifying possible differences in disease mechanisms that could steer clinical decision-making. The main aim of the current study was to quantify the heterogeneity of the fibromyalgia patient population and assess whether multiple subgroups with distinct phenotypes may be detected based on the morphological state of small fibers, standardized quantitative sensory testing and neuropathic pain questionnaires (PainDetect and small fiber neuropathy screening list, SFNSL). We hypothesized that small fiber pathology, as detected by CCM, is present in a subset of patients with fibromyalgia and that abnormalities in cornea small fiber morphology overlap with abnormalities in QST and questionnaires.

METHODS

The protocol was approved by the Ethics Committee of the Leiden University Medical Center (Leiden, the Netherlands), and all study procedures were conducted according to GCP guidelines and adhered to the tenets of the Declaration of Helsinki. The study is registered in the Netherlands Trial Register (NTR3769).

Patients

Forty patients with fibromyalgia were recruited for the study. Inclusion criteria were: age between 18 and 75 years, fibromyalgia diagnosed by a rheumatologist according to the 1990 or 2010 American College of Rheumatologists criteria [19,20], and willing and able to provide informed consent. Exclusion criteria were: inability to read and understand written text in Dutch, a diagnosis diabetes mellitus, glucose intolerance, sarcoidosis or other diseases that are associated with small-fiber neuropathy, presence of a chronic pain condition other than fibromyalgia, prior eye surgery, use of contact lenses, and pregnancy/lactation. All study participants provided oral and written informed consent prior to study procedures. Fibromyalgia was re-assessed by a trained investigator using the 1990 and 2010 ACR criteria: nine bilateral pressure tender points were tested (18 in total) and the widespread pain index and symptom severity scale score were recorded.

Cornea Confocal Microscopy

Bilateral CCM was performed on 39 patients, using the Rostock Cornea Module with the Heidelberg Retina Tomograph III (Heidelberg, Germany). Images were acquired and quantified as follows: After topical anesthesia of both eyes, the microscope was placed at the surface of the cornea apex. Confocal images were acquired with a field of view of 400×400 µm and automatically quantified using ACCmetrics software (provided by the faculty of Medical and Human Sciences of the University of Manchester, United Kingdom). Cornea nerve fiber length (CNFL), cornea nerve fiber density (CNFD), and cornea

nerve branching density (CNBD) were quantified after manual selection (in a blinded fashion, by author MvV) of 5 to 10 representative, high-quality images per eye. Taken the good correlation between semi-automated (CCmetrics) and automated (ACCmetrics) corneal nerve fiber quantification [21,22], the data were compared to semi-automated acquired reference values from Tavakoli *et al.* [23].

PainDetect questionnaire and Small Fiber Neuropathy Screening List

To assess neuropathic pain involvement in daily pain, patients filled out the PainDetect questionnaire, a screening tool to detect neuropathic pain symptoms. This questionnaire assesses pain perception over the last 4 weeks and the current pain score, and patients are asked to localize and qualify their pain (burning, prickling, attacks, etc.). A score of 19 or higher indicates that a neuropathic pain component is likely. The validated Dutch version of the PainDetect was used. To screen for small fiber involvement, patients filled out the small fiber neuropathy screening list (SFNSL) which assesses complaints consistent with small fiber involvement such as indigestion, dry eyes, allodynia, tingling sensations, chest pain and others. The Dutch version validated for sarcoidosis patients was used [24]. A score of 37 or higher indicates that the presence of small fiber involvement is highly likely.

Quantitative Sensory Testing

QST was performed on the face (buccal surface), hand and foot (both dorsal surface) according to the protocol of the German Research Network on Neuropathic Pain using 13 tests per anatomic location [25]. In short, we tested the following modalities: cold and warm detection and pain thresholds (CDT, WDT, CPT HPT), thermal sensory limen (TSL), paradoxical heat sensation (PHS), mechanical detection and pain thresholds (MDT, MPT), mechanical pain sensitivity (MPS), dynamic mechanical allodynia (ALL), wind-up ratio (WUR), vibration detection threshold (VDT) and pressure pain threshold (PPT). Thermal tests were performed with the Pathway ATS device (Medoc, Ramat Yishai, Israel); mechanical detection and pain thresholds were obtained by using 0.2–588.4 mN von Frey filaments (Touch-Test®, Bioseb, France) and the PinPrick Stimulator set (8–512 mN; MRC-systems, Germany); dynamic mechanical allodynia was examined using brush and cotton-top strokes; pin prick for wind-up testing was performed with the PinPrick Stimulator set (8–512 mN; MRC-systems, Germany); vibration detection threshold was tested with a vibrating tuning fork (Martin Rydell Seiffer, Selles Medical, UK); and finally pressure pain threshold was tested with a handheld pressure algometer (Wagner Instruments, Greenwich, CT). For mechanical pain sensitivity, mechanical allodynia and wind-up, patients were asked to report a pain score based on a numerical rating scale (NRS) ranging from 0, no pain to 10, worst pain imaginable. Pain rating was adapted from a 0–100 point scale to a 0–10 point scale, to facilitate scoring in our Dutch population that is used to using a 10-point scoring system in general. QST results are expressed

as transformed *z*-scores, according to published reference values [26], where values less than −1.96*z (loss of function) or greater than 1.96*z (gain of function) are considered abnormal. Dynamic mechanical allodynia and paradoxical heat sensations were scored dichotomously.

Statistical analysis

Statistical testing was performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA) and SPSS statistics 20 (IBM SPSS Statistics, Armonk, NY). For correlation analysis Pearson's correlation coefficient or Spearman's ρ were used. Subgroups of patients (decreased *vs*. normal CNFL) were compared with the Fisher's exact test for normal *vs.* abnormal results of: the PainDetect and the SFNSL questionnaire; and all QST parameters. Other comparisons between subgroups were evaluated with the Mann Whitney U test. P values < 0.05 were considered significant. Data are presented as average ±95% confidence interval or (range), unless otherwise indicated.

RESULTS

Patient demographics and patient-reported symptoms

Patient characteristics of 39 patients that completed the study are given in Table 1. Data from one patient were not included in the analysis due to difficulty in obtaining reliable sensory assessments (QST). Of the remaining 39 patients, 3 were male. Fibromyalgia symptoms were present for 15 years (mean, range 2–37 years). The average number of positive tender points was 14 (range 4–18); 5 patients (13%) had less than 11 tender points. The average widespread pain index was 14 (range 6–18) and the average symptom severity score was 8 (range 4–12). With these ratings, the diagnosis of fibromyalgia was re-confirmed in all patients.

Cornea Confocal Microscopy

Figure 1 shows examples of confocal microscope photos of the cornea nerve plexus. Figure 1B shows the cornea plexus of a 21 years-old female fibromyalgia patient with a clear decrease in cornea nerve fiber density (CNFD), cornea nerve branching density (CNBD) and cornea nerve fiber length (CNFL) as compared with age- and sex-matched controls. In comparison, the cornea of a healthy 19 years-old female with normal cornea nerve fiber state is given in Fig. 1A (this photo is derived from a cohort of healthy individuals in our database). Additional images illustrate the corneas of a 57 years-old female patient with a normal cornea nerve fiber state (C), a 58 years-old female with an abnormal state (reduced CNFL) as compared with age- and sex-matched controls (D) [23], and a 53 years-

Table 1. Patient characteristics

Number of patients (n)	**39**
Females (%)	36 (92)
Age, years, mean (range)	39.2 (19-58)
Body mass index, kg/m^2, mean (range)	25.8 (19.6-38)
Years with fibromyalgia symptoms, mean (range)	15 (2-37)
Years with fibromyalgia diagnosis, mean (range)	6 (1-20)
Number of tender points, mean (range)*	14 (4-18)
Widespread pain index (WPI), mean (range)#	14 (6-18)
Symptom severity scale score (SyS), mean (range)#	8 (4-12)
PainDetect questionnaire score, mean (range)$	19 (8-30)
SFNSL total score, mean (range)§	32 (11-64)
SFNSL pain subscore	18 (7-32)
SFNSL autonomic dysfunction subscore	14 (3-33)

* A score of 11 points is indicative of fibromyalgia. # A combination of WPI ≥ 7 and SyS ≥ 5 or WPI 3-6 and SyS ≥ 9 is indicative of fibromyalgia. $ A score of 19 or higher is indicative of a neuropathic component of pain. § A score of ≥ 37 indicates that the presence of small fiber neuropathy is highly likely. SFNSL: small fiber neuropathy screening list.

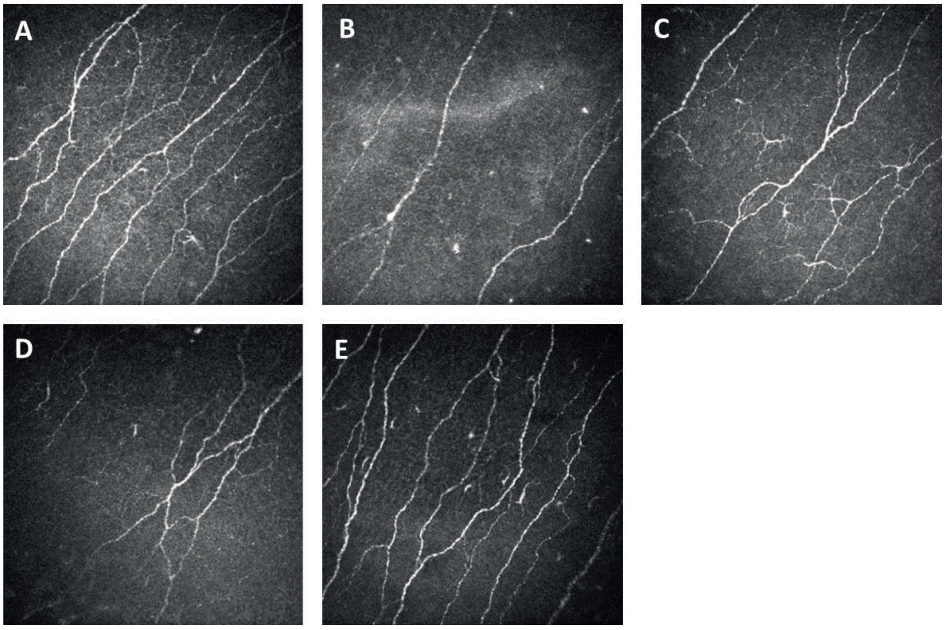


Figure 1. Representative cornea confocal images of fibromyalgia patients compared to healthy volunteers. Confocal microscope images from the cornea nerve plexus. **(A)** 19 years-old healthy female with normal cornea nerve fiber state. **(B)** 21 years-old female patient with significantly decreased cornea nerve fiber state. **(C)** 57 years-old female patient with normal cornea nerve fiber state. **(D)** 58 years-old female patient with significantly decreased cornea nerve fiber state. **(E)** 53 years-old male with normal nerve fiber state. Images were acquired with a field of view of 400 × 400 µm.

old male with a normal cornea nerve fiber density (E). These data demonstrate the effect of disease but also age and sex on the cornea nerve plexus.

Cornea nerve quantification of the left and right eye in each patient was similar (data not shown). Data were therefore averaged per patient. Average values (95% confidence intervals) for CNFD, CNFL and CNBD are given in Table 2. Compared with recently published reference values [23], abnormalities in cornea nerve fiber morphology were observed in 10–44% of patients. Forty-four percent of patients had CNFL values below the 5th percentile of their age and sex reference group. As reference values are given per age group, individual scores are presented per age categories in Fig. 2. Similarly, CNFD and CNBD were below the 5th percentile of controls in 10% and 28%, respectively. Ab-

Table 2. Quantification of cornea nerve fibers in fibromyalgia patients

Cornea confocal microscopy parameter	Average (95% CI)	Range (min-max)	Significantly decreased, n (%)*
Cornea nerve fiber density (n/mm^2)	23.3 (21.3-25.3)	10.6 – 36.9	4 (10)
Cornea nerve branching density (n/mm^2)	30.5 (26.7-34.3)	4.0 – 69.0	11 (28)
Cornea nerve fiber length (mm/mm^2)	13.7 (12.7-14.7)	6.0 – 20.5	17 (44)

CI: confidence interval.* Relative to reference values [23].

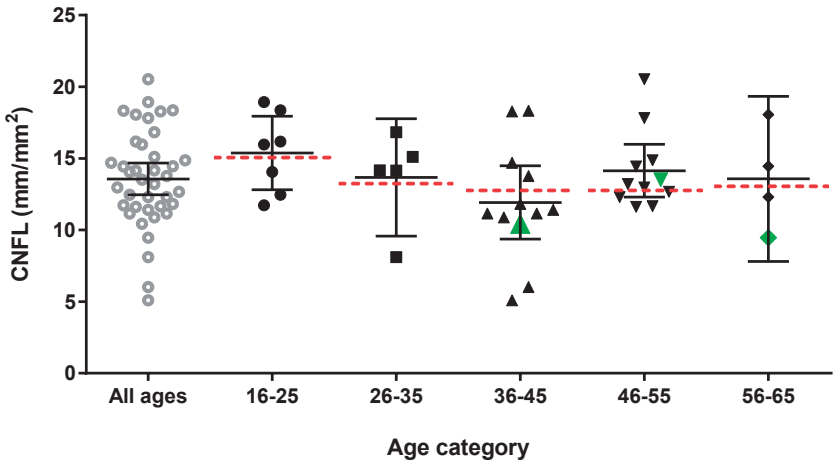


Figure 2. Cornea nerve fiber length (CNFL) individual data (with average and 95% confidence interval) compared to female reference values per age category [23]. The red dotted lines indicate the 5th percentile normative cutoff values for decreased CNFL. The green data points mark male fibromyalgia patients; only the CNFL of the male in age category 46–55 was not significantly decreased compared with the male reference value (not shown) in the corresponding age category.

normalities in cornea morphology were correlated. For example, CNFL values correlated with CNBD (Pearson's r = 0.81) and CNFD (Pearson's r = 0.90) ($p < 0.01$).

PainDetect questionnaire and Small Fiber Neuropathy Screening List

The average PainDetect score was 19 (range 8–30) points (Table 1). Twenty-two patients (56%) had a score above the neuropathy cutoff of 18 points (out of the possible maximum of 38 points). The SFNSL average score was 32 (range 11–64) points (out of the possible maximum of 84; Table 1). Fifteen patients (38%) scored ≥37 where the presence of small fiber neuropathy becomes highly likely.

Table 3. Quantitative sensory testing: number and percentage of fibromyalgia patients with abnormal values on at least one of three test locations: the facial buccal surface and the dorsal surface of hand and foot

QST parameter	Average Z-score (95% CI)	Range Z-score (min-max)	Loss of function, n (%)	Gain of function n (%)
Cold detection threshold	-0.86 (-1.23/-0.48)	-4.69/0.96	15 (38)	-
Warm detection threshold	-0.64(-0.94/-0.34)	-3.91/1.38	8 (21)	-
Thermal sensory limen	-0.08 (-0.33/0.17)	-2.60/1.79	2 (5)	-
Paradoxical heat sensations	-	-	9 (23)	-
Cold pain threshold	0.50 (0.05/0.95)	-2.28/1.83	1(3)	1(3)
Heat pain threshold	0.19 (-0.17/0.55)	-3.66/2.24	2 (5)	2 (5)
Mechanical detection threshold	-0.72 (-1.26/-0.18)	-10.61/1.64	9 (23)	-
Mechanical pain threshold	0.08 (-0.36/0.51)	-2.65/3.12	5 (13)	8 (21)
Mechanical pain sensitivity	-0.01 (-0.39/0.38)	-2.24/2.11	2 (5)	1 (3)
Vibration detection threshold	-1.17 (-1.58/-0.77)	-6.68/1.15	26 (67)	-
Pressure pain threshold	2.68 (2.18/3.18)	-0.98/6.49	-	27 (69)
Temporal summation (wind-up)	-0.08 (-0.10/0.74)	-1.48/3.91	-	10 (26)
Dynamic mechanical allodynia	-	-	-	5 (13)

Percentage of patients with decreased detection thresholds and increased pain thresholds expressed in z-scores compared with age- and sex-matched reference values [26] were calculated from 39 patients. For paradoxical heat sensations and dynamic mechanical allodynia, z-transformations do not yield realistic comparable numbers. Instead, the prevalence of these variables was recorded in a dichotomous fashion.

Table 4. Prevalence of paradoxical heat sensations and dynamic mechanical allodynia on face, hand and foot in fibromyalgia patients

	Paradoxical heat sensations n (%)	Dynamic mechanical allodynia n (%)
face	0 (0)	3 (8)
hand	1 (3)	3 (8)
foot	8 (21)	5 (13)

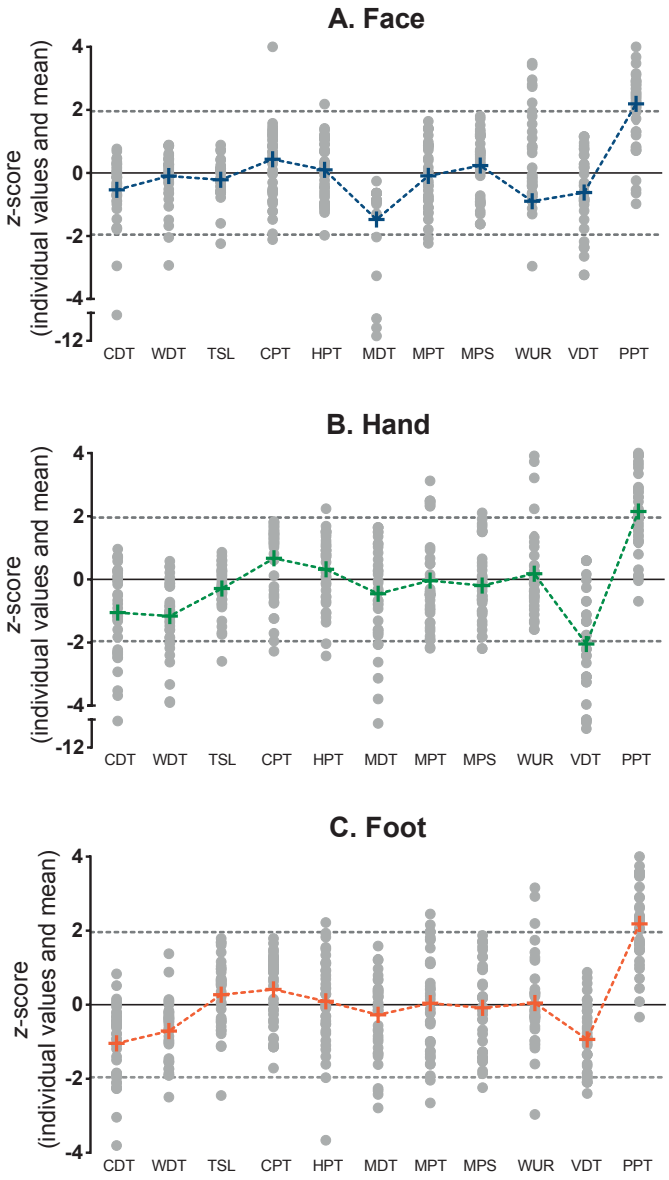


Figure 3. QST profiles of face **(A)**, hand **(B)** and foot **(C)** of patients with fibromyalgia. CDT = cold detection threshold; WDT = warm detection threshold; TSL = thermal sensory limen; CPT = cold pain threshold; HPT = heat pain threshold; MDT = mechanical detection threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; WUR = wind-up ratio; VDT = vibration detection threshold; PPT = pressure pain threshold. The dotted lines indicate ± 1.96*z above or below which values are considered abnormal. Paradoxical heat sensation and dynamic mechanical allodynia were scored dichotomously and are therefore not included in this figure (see Tables 3 and 4). Each grey dot represents the result observed in one patient. The + signs indicate the mean values.

Quantitative Sensory Testing

QST analysis showed that a large number of patients displayed abnormalities with signs of allodynia and hyperalgesia in one or more tested regions (Tables 3 and 4 and Fig. 3). Most important observations include hyperalgesia for mechanical pain (in 21% of patients), wind-up (26%) and pressure pain stimulation (69%) on any of the three test locations. Abnormal sensory detection thresholds were obtained for cold (loss of function), warm (loss of function) and mechanical (loss of function) stimuli in up to 38%, 21% and 23% of patients, respectively. Dynamic mechanical allodynia (gain of function) and paradoxical heat sensations (loss of function) were observed in 13 and 23% of patients, respectively. A loss of vibration sensation was observed in 67% of patients. An overview of the loss and gain of functions in any of the 3 locations is given in Table 3.

Correlations and subgroup analysis

Pearson's r showed a strong significant correlation between the SFNSL and PainDetect questionnaires ($r = 0.77$; $p = 0.00$). No significant correlations were observed between cornea morphology scores and PainDetect, SFNSL or QST scores. For example, CNFL *vs.* PainDetect: $r = -0.11$; $p = 0.52$; CNFL *vs.* SFNSL: $r = -0.12$; $p = 0.47$; CNFL *vs.* CDThand: $r = 0.21$; $p = 0.19$; CNFL *vs.* MDThand: $r = -0.24$; $p = 0.15$; CNFL *vs.* PPThand: $r = 0.15$; $p = 0.37$.

Patients with normal and abnormal CNFL values did not differ with respect to QST parameters on face, hand or foot (CDT $p = 0.51$, WDT $p = 0.43$, TSL $p = 0.99$, PHS $p = 0.99$, CPT $p = 0.49$, HPT $p = 0.99$, MDT $p = 0.99$, MPT $p = 0.99$, MPS $p = 0.99$, ALL $p = 0.15$; WUR $p = 0.72$; VDT $p = 0.99$, and PPT $p = 0.73$), PainDetect ($p = 0.74$) and SFNSL ($p = 0.51$) scores. Similarly, these two populations did not differ in the number of tender points ($p = 0.08$), WPI ($p = 0.21$), SSS ($p = 0.66$), age ($p = 0.86$), BMI ($p = 0.69$) or years with fibromyalgia symptoms ($p = 0.73$).

We defined four subgroups of fibromyalgia patients, based on cornea morphology and signs of central sensitization (as defined by abnormalities in cold pain threshold, mechanical pain threshold, mechanical pain sensitivity, allodynia and/or windup) [27,28]. These four subgroups consisted of a group with normal cornea morphology without ($n = 12$, 31%) and with ($n = 7$, 18%) signs of central sensitization, and a group with abnormal cornea morphology parameters without ($n = 8$, 21%) and with ($n = 12$, 31%) signs of central sensitization (Fig. 4).

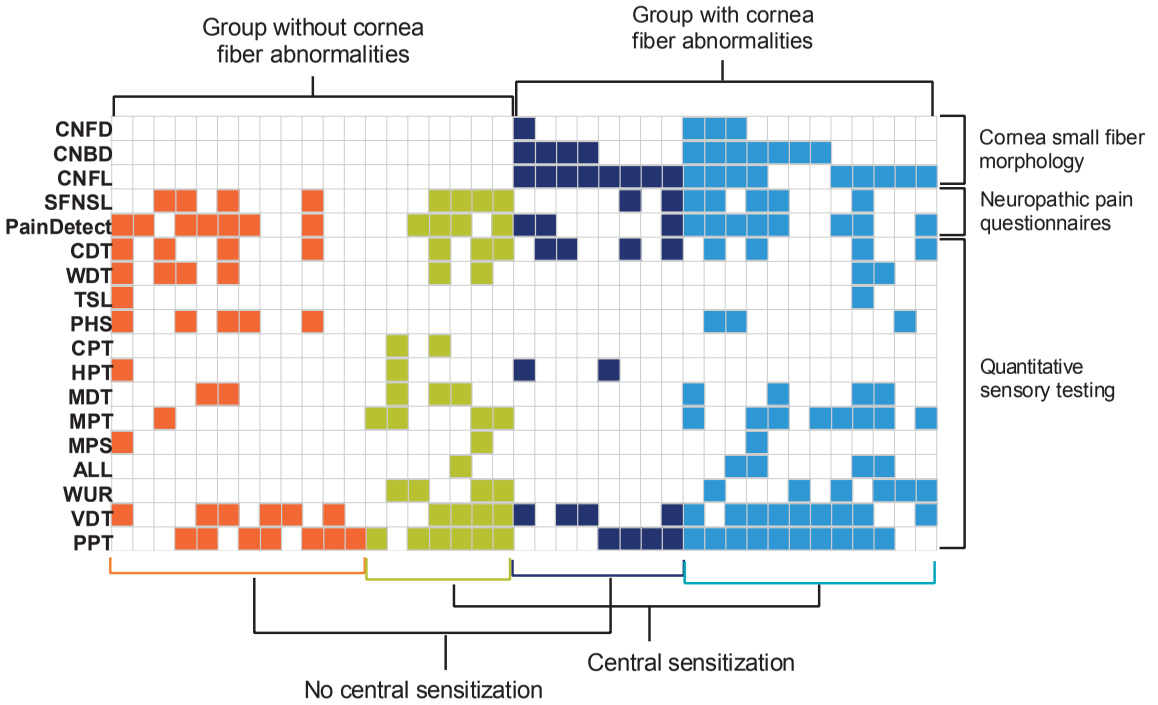


Figure 4. Phenotypes of patients with fibromyalgia based on cornea confocal parameters (CNFD = cornea nerve fiber density; CNBD = cornea nerve branching density; CNFL = cornea nerve fiber length), the small fiber neuropathy screening list (SFNSL), the PainDetect questionnaire and quantitative sensory testing. Columns show normal (white) and abnormal (colored) results per patient. CDT = cold detection threshold; WDT = warm detection threshold; TSL = thermal sensory limen; PHS = paradoxical heat sensation; CPT = cold pain threshold; HPT = heat pain threshold; MDT = mechanical detection threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; ALL = dynamic mechanical allodynia; WUR = wind-up ratio; VDT = vibration detection threshold; PPT = pressure pain threshold. Colored squares (abnormalities in the tests) indicate: for cornea confocal microscopy testing 'values outside the 95% interval of normal reference data'; for QST either a gain- or loss-of-function (< – 1.96*z or > 1.96*z); and for the questionnaires 'values indicative of neuropathic pain'.

DISCUSSION

The main aim of this study was to assess the involvement of small fiber pathology in patients with fibromyalgia as quantified by CCM and to relate cornea morphology results to patient-reported symptoms and standardized QST. We extended observations by Ramirez *et al.* [9] who reported that cornea nerve fiber density was abnormal in their cohort of seventeen patients with fibromyalgia. In our study, CCM analysis revealed at least one significant reduction in one of the small fiber cornea morphology parameters in 51% of fibromyalgia patients when compared to age- and sex-matched reference values. None of the CCM-derived parameter abnormalities were specifically related to age, BMI, questionnaire scores, or QST results.

CCM is a relatively new, non-invasive method to analyze the quantity and quality of small nerve fibers in the cornea. The technique has been validated in several studies involving patients with peripheral neuropathy from various underlying causes, and most studies demonstrate good correlation with intra-epidermal nerve fiber density results from skin biopsies [15,17,29-31]. CCM has proven to be a sensitive and reproducible measure of peripheral neuropathy and because it is non-invasive, it is an attractive alternative to skin biopsies.

In the past 5 years multiple efforts have been made to establish small fiber pathology in patients with fibromyalgia [5,7,8,10,32], but only one study focused on the cornea [9]. Consistent with our results, in all of these studies subgroups of patients with fibromyalgia were identified that displayed small fiber pathology or (indirect) indications of such pathology. However, as in our study these changes often correlated poorly with symptomatology and neurologic or immunologic measurements. Doppler and colleagues [6] recently assessed dermal unmyelinated nerve fiber diameter of skin biopsies of the distal and proximal leg and index finger in patients with fibromyalgia and patients with non-diabetic small fiber neuropathy (SFN). They observed that nerve fiber diameter was reduced in patients with fibromyalgia, but not in patients with SFN. The authors concluded that the pathological mechanism underlying small fiber damage might differ between the two disorders, and that patients with fibromyalgia suffer from small fiber *pathology* rather than SFN. This difference in terminology [13] is a matter of debate and relates to the mechanism of disease; see for example the recent editorial on this topic by Clauw [12] and letter by Üçeyler and Sommer [13]. Rather than considering small fiber neuropathy as the cause of pain and other symptoms in fibromyalgia, these authors contend that small fiber pathology in fibromyalgia should be treated as an adjunct finding since a cause-effect relationship between the small fiber abnormalities and disease symptomatology has not been established. Our results are in agreement with this latter statement, as we observed no correlation between CCM abnormalities and QST, patient reported symptoms, WPI, SSS and disease duration. Additionally, we observed signs of

centrally mediated pain in patients that presented with cornea small fiber pathology, consistent with the idea that central and peripheral pathology coexist in fibromyalgia (Fig. 3). Furthermore, we observed that QST parameters CDT, VDT and PPT were markedly reduced in the majority of patients (Fig. 3), suggesting both small fiber (CDT, PPT) and large fiber (VDT abnormal in 67% of patients) dysfunction. The questionnaire results also indicate that small fiber pathology (SFNSL) or a neuropathic pain component (PainDetect) is highly likely in 38% and 56% of patients. Our results indicate that the fibromyalgia syndrome indeed consists of a heterogeneous group of patients with signs of both central and peripheral small and large nerve fiber pathology. Although the average z-score of the vibration detection test is similar to findings by Klauenberg [33], the large percentage of detected abnormal vibration detection thresholds is not in agreement with earlier findings, where lower percentages of large fiber abnormalities have been described [13,33,34]. At this moment we do not have a satisfying explanation, although it may be related to the small range of normal reference values [25] or differences in the type of recruited patients between our and earlier studies. It is of interest to assess large fiber dysfunction in fibromyalgia patients in further detail by electrophysiological testing.

To better define our patient population, we performed a subgroup analysis to further phenotype the fibromyalgia syndrome based on cornea fiber abnormalities and the presence of central sensitization as suggested by QST parameters cold pain threshold, mechanical pain threshold and mechanical pain sensitivity, allodynia and wind-up (Fig. 4) [27,28,35]. We identified four subgroups based on a distinction between decreased or normal cornea morphology parameters, and a distinction between signs of central sensitization or the lack thereof. The four acquired subgroups consist of a group with normal cornea morphology without and with signs of central sensitization, and a group with abnormal cornea morphology parameters without and with signs of central sensitization. The detection of these four distinct profiles or phenotypes may be related to the mechanism of disease. For example, the symptoms of patients that do not display peripheral nerve pathology in their CCM data are most probably related to pain arising from the central nervous system, either with or without central sensitization. Patients with cornea nerve fiber pathology may have symptoms of peripheral origin, and in 50% of them signs of mixed (peripheral and central) origin are present.

We cannot exclude that in patients with signs of a peripheral origin of pain, central causes of pain may additionally play a role. One of the criteria in the diagnosis of fibromyalgia is widespread pain: the presence of axial pain, bilateral pain, and upper and lower segment pain [19]. In addition, the same comorbidities found in patients with fibromyalgia, such as fatigue, sleep disturbances, irritable bowel syndrome, cognitive deficits and mood disorders also occur in other chronic pain syndromes and central fatigue syndromes [1]. These symptoms all together suggest a role for a central site of origin of fibromyalgia symptoms, including pain. Indeed, several studies found evidence

for central sensitization [36] and dysfunctional pain inhibition [3] in neurophysiological and functional MRI studies. Other neuroimaging studies demonstrated elevated levels of excitatory neurotransmitters in the brains of patients with fibromyalgia [37,38] and structural or functional changes in brain regions involved in pain processing, sleep and mood [39-41]. However, it is not known whether the central changes found in these studies are the cause of the pain, or a consequence of continuous nociceptive input, thereby augmenting pain from a peripheral source in those patients with signs of peripheral nerve pathology [42]. Additional studies are required to elucidate this matter, also taking into account psychological state and trait and the genetic background of patients with fibromyalgia, since these factors are well-known to influence the development of fibromyalgia [43,44]. Finally, we argue that phenotyping patients with fibromyalgia is not only of importance to understand the mechanism of disease but may also be important in the choice of pain medication. For example, we recently showed that patients with sarcoidosis and small fiber neuropathy benefit from ARA290, an erythropoietin analog acting at the innate repair receptor, which restores peripheral nerve morphology and neuropathic symptoms [45]. It may well be that this same compound will be exclusively effective in patients with fibromyalgia and small fiber pathology while centrally acting drugs, such as pregabalin, are required when no signs of peripheral nerve fiber pathology are present. Future studies should address these hypotheses.

In conclusion, in a small cohort of fibromyalgia patients we observed signs of small fiber pathology in 51% of patients as measured by cornea confocal microscopy. Further profiling these patients shows that four distinct phenotypes were present: a group with normal cornea morphology with and without signs of central sensitization, and a group with abnormal cornea morphology parameters with and without signs of central sensitization. These phenotypes indicate possible differences in disease mechanisms and additionally may steer the clinician in his or her choice of treatment of this complex, multi-factorial disorder. Since this and other previous studies were relatively small, larger cohorts of patients with fibromyalgia are needed to come to definite conclusions regarding the existence of subgroups in sensory testing and involvement of smallfiber pathology in the mechanism of disease.

REFERENCES

1. Clauw DJ: Fibromyalgia: a clinical review. Jama 2014, 311:1547-55.
2. Schmidt-Wilcke T, Clauw DJ: Fibromyalgia: from pathophysiology to therapy. Nature reviews Rheumatology 2011, 7:518-27.
3. Jensen KB, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Giesecke T, Mainguy Y, Gracely R, Ingvar M: Evidence of dysfunctional pain inhibition in Fibromyalgia reflected in rACC during provoked pain. Pain 2009, 144:95-100.
4. Lautenbacher S, Rollman GB: Possible deficiencies of pain modulation in fibromyalgia. The Clinical journal of pain 1997, 13:189-96.
5. Caro XJ, Winter EF: Evidence of abnormal epidermal nerve fiber density in fibromyalgia: clinical and immunologic implications. Arthritis Rheumatol 2014, 66:1945-54.
6. Doppler K, Rittner HL, Deckart M, Sommer C: Reduced dermal nerve fiber diameter in skin biopsies of patients with fibromyalgia. Pain 2015, 156:2319-25.
7. Giannoccaro MP, Donadio V, Incensi A, Avoni P, Liguori R: Small nerve fiber involvement in patients referred for fibromyalgia. Muscle & nerve 2014, 49:757-9.
8. Oaklander AL, Herzog ZD, Downs HM, Klein MM: Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. Pain 2013, 154:2310-6.
9. Ramirez M, Martinez-Martinez LA, Hernandez-Quintela E, Velazco-Casapia J, Vargas A, Martinez-Lavin M: Small fiber neuropathy in women with fibromyalgia. An in vivo assessment using corneal confocal bio-microscopy. Seminars in arthritis and rheumatism 2015, 45:214-9.
10. Uceyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C: Small fibre pathology in patients with fibromyalgia syndrome. Brain : a journal of neurology 2013, 136:1857-67.
11. Serra J, Collado A, Sola R, Antonelli F, Torres X, Salgueiro M, Quiles C, Bostock H: Hyperexcitable C nociceptors in fibromyalgia. Annals of neurology 2014, 75:196-208.
12. Clauw DJ: What is the meaning of "small fiber neuropathy" in fibromyalgia? Pain 2015, 156:2115-6.
13. Uceyler N, Sommer C: Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. Pain 2013, 154:2569.
14. Wopking S, Scherens A, Haussleiter IS, Richter H, Schuning J, Klauenberg S, Maier C: Significant difference between three observers in the assessment of intraepidermal nerve fiber density in skin biopsy. BMC Neurol 2009, 9:13.
15. Petropoulos IN, Alam U, Fadavi H, Asghar O, Green P, Ponirakis G, Marshall A, Boulton AJ, Tavakoli M, Malik RA: Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. Diabetes Care 2013, 36:3646-51.
16. Tavakoli M, Quattrini C, Abbott C, Kallinikos P, Marshall A, Finnigan J, Morgan P, Efron N, Boulton AJ, Malik RA: Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes Care 2010, 33:1792-7.
17. Brines M, Swartjes M, Tannemaat MR, Dunne A, Van Velzen M, Proto P, Hoitsma E, Petropoulos I, Chen X, Niesters M, Dahan A, Malik R, Cerami A: Corneal nerve quantification predicts the severity of symptoms in sarcoidosis patients with painful neuropathy. Technology 2013, 1:1-7.
18. Ziegler D, Papanas N, Zhivov A, Allgeier S, Winter K, Ziegler I, Bruggemann J, Strom A, Peschel S, Kohler B, Stachs O, Guthoff RF, Roden M, German Diabetes Study G: Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. Diabetes 2014, 63:2454-63.

19. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis care & research 2010, 62:600-10.
20. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, *et al.*: The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheumatol 1990, 33:160-72.
21. Chen X, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, Asghar O, Alam U, Marshall A, Fadavi H, Ferdousi M, Azmi S, Tavakoli M, Efron N, Jeziorska M, Malik RA: Small Nerve Fiber Quantification in the Diagnosis of Diabetic Sensorimotor Polyneuropathy: Comparing Corneal Confocal Microscopy With Intraepidermal Nerve Fiber Density. Diabetes Care 2015.
22. Petropoulos IN, Alam U, Fadavi H, Marshall A, Asghar O, Dabbah MA, Chen X, Graham J, Ponirakis G, Boulton AJ, Tavakoli M, Malik RA: Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. Investigative ophthalmology & visual science 2014, 55: 2071-8.
23. Tavakoli M, Ferdousi M, Petropoulos IN, Morris J, Pritchard N, Zhivov A, Ziegler D, Pacaud D, Romanchuk K, Perkins BA, Lovblom LE, Bril V, Singleton JR, Smith G, Boulton AJ, Efron N, Malik RA: Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. Diabetes Care 2015, 38:838-43.
24. Hoitsma E, De Vries J, Drent M: The small fiber neuropathy screening list: Construction and cross-validation in sarcoidosis. Respiratory medicine 2011, 105:95-100.
25. Rolke R, Baron R, Maier C, Tolle TR, Treede RD, Beyer A, Binder A, Birbaumer N, Birklein F, Botefur IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihofner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B: Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain 2006, 123:231-43.
26. Magerl W, Krumova EK, Baron R, Tolle T, Treede RD, Maier C: Reference data for quantitative sensory testing (QST): refined stratification for age and a novel method for statistical comparison of group data. Pain 2010, 151:598-605.
27. Arendt-Nielsen L, Yarnitsky D: Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. The journal of pain : official journal of the American Pain Society 2009, 10:556-72.
28. Woolf CJ: Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011, 152:S2-15.
29. Malik RA, Kallinikos P, Abbott CA, van Schie CH, Morgan P, Efron N, Boulton AJ: Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. Diabetologia 2003, 46:683-8.
30. Tavakoli M, Malik RA: Corneal confocal microscopy: a novel non-invasive technique to quantify small fibre pathology in peripheral neuropathies. J Vis Exp 2011.
31. Tavakoli M, Marshall A, Thompson L, Kenny M, Waldek S, Efron N, Malik RA: Corneal confocal microscopy: a novel noninvasive means to diagnose neuropathy in patients with Fabry disease. Muscle & nerve 2009, 40:976-84.
32. Kosmidis ML, Koutsogeorgopoulou L, Alexopoulos H, Mamali I, Vlachoyiannopoulos PG, Voulgarelis M, Moutsopoulos HM, Tzioufas AG, Dalakas MC: Reduction of Intraepidermal Nerve Fiber Density (IENFD) in the skin biopsies of patients with fibromyalgia: a controlled study. J Neurol Sci 2014, 347:143-7.

33. Klauenberg S, Maier C, Assion HJ, Hoffmann A, Krumova EK, Magerl W, Scherens A, Treede RD, Juckel G: Depression and changed pain perception: hints for a central disinhibition mechanism. Pain 2008, 140:332-43.
34. Blumenstiel K, Gerhardt A, Rolke R, Bieber C, Tesarz J, Friederich HC, Eich W, Treede RD: Quantitative sensory testing profiles in chronic back pain are distinct from those in fibromyalgia. The Clinical journal of pain 2011, 27:682-90.
35. Lang PM, Schober GM, Rolke R, Wagner S, Hilge R, Offenbacher M, Treede RD, Hoffmann U, Irnich D: Sensory neuropathy and signs of central sensitization in patients with peripheral arterial disease. Pain 2006, 124:190-200.
36. Desmeules JA, Cedraschi C, Rapiti E, Baumgartner E, Finckh A, Cohen P, Dayer P, Vischer TL: Neurophysiologic evidence for a central sensitization in patients with fibromyalgia. Arthritis and rheumatism 2003, 48:1420-9.
37. Fayed N, Garcia-Campayo J, Magallon R, Andres-Bergareche H, Luciano JV, Andres E, Beltran J: Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. Arthritis research & therapy 2010, 12:R134.
38. Harris RE, Sundgren PC, Craig AD, Kirshenbaum E, Sen A, Napadow V, Clauw DJ: Elevated insular glutamate in fibromyalgia is associated with experimental pain. Arthritis and rheumatism 2009, 60:3146-52.
39. Gracely RH, Ambrose KR: Neuroimaging of fibromyalgia. Best practice & research Clinical rheumatology 2011, 25:271-84.
40. Jensen KB, Loitoile R, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Mainguy Y, Vitton O, Gracely RH, Gollub R, Ingvar M, Kong J: Patients with fibromyalgia display less functional connectivity in the brain's pain inhibitory network. Molecular pain 2012, 8:32.
41. Napadow V, Harris RE: What has functional connectivity and chemical neuroimaging in fibromyalgia taught us about the mechanisms and management of 'centralized' pain? Arthritis research & therapy 2014, 16:425.
42. Clauw DJ: Diagnosing and treating chronic musculoskeletal pain based on the underlying mechanism(s). Best Pract Res Cl Rh 2015, 29:6-19.
43. Ablin JN, Buskila D: Update on the genetics of the fibromyalgia syndrome. Best practice & research Clinical rheumatology 2015, 29:20-8.
44. Desmeules J, Chabert J, Rebsamen M, Rapiti E, Piguet V, Besson M, Dayer P, Cedraschi C: Central pain sensitization, COMT Val158Met polymorphism, and emotional factors in fibromyalgia. The journal of pain : official journal of the American Pain Society 2014, 15:129-35.
45. Heij L, Niesters M, Swartjes M, Hoitsma E, Drent M, Dunne A, Grutters JC, Vogels O, Brines M, Cerami A, Dahan A: Safety and efficacy of ARA 290 in sarcoidosis patients with symptoms of small fiber neuropathy: a randomized, double-blind pilot study. Mol Med 2012, 18:1430-6.

Chapter 6

Cornea nerve fiber quantification and construction of neuropathic pain phenotypes in patients with diabetes mellitus type 2 and sarcoidosis

LCJ Oudejans, M Niesters, M Brines, A Dahan, M van Velzen.

Submitted.

INTRODUCTION

Small fiber neuropathy (SFN) is a complication of various disorders with inflammatory components such as diabetes mellitus (DM) and sarcoidosis. SNF is often associated with severe chronic pain, which impacts the quality of life significantly. Treatment is generally directed towards the pathogenesis of the underlying disease and the management of pain. Diagnostic and psychophysical tests for neuropathy such as nerve conduction studies, skin biopsies, corneal confocal microscopy (CCM) and quantitative sensory testing (QST) can be used to construct patient-specific neuropathy phenotypes. As we discussed earlier [1], phenotyping may be applied for two specific reasons: first, phenotyping serves as a tool to better understand the disease process mechanistically and in terms of severity, and second, it may be used in decision-making in treatment strategies and consequently shorten the time until adequate pain relief. Currently, treatment is often based on a trial-and-error approach with limited efficacy in a limited number of patients [2-5].

In the past, diagnosis of SFN has relied on assessment of nerve fiber densities in skin biopsies. However, the relatively new technique of CCM is being used increasingly to quantify nerve fibers in the cornea to detect small fiber pathology [6,7]. Earlier, we used CCM and QST to identify phenotypes of fibromyalgia patients and observed a large proportion of patients with small fiber pathology and symptoms of central sensitization [1]. In line with this characterization, the purpose of the current study was to assess whether distinct phenotypes exist in patients with DM type 2 and sarcoidosis. In both syndromes SFN is prevalent in a large proportion of patients. In DM 8-25% of patients suffer from SFN [8-10]. With the growing prevalence of diabetes [4,11], diabetic painful neuropathy will be increasingly diagnosed. Recent studies indicate that pain in patients with chronic sarcoidosis is related to SFN in up to 70% of patients [12-15].

In this study, cornea confocal microscopy measurements and quantitative sensory testing according to the protocol of the German research network on neuropathic pain [16] were performed in patients with chronic pain diagnosed with DM type 2 or sarcoidosis. CCM abnormalities and signs of central sensitization were used to identify distinct phenotypes.

METHODS

Patients

Study protocols were approved by the local Ethics Committee (Leiden University Medical Center, Leiden, the Netherlands), and study procedures were conducted according to GCP guidelines and adhered to the tenets of the Declaration of Helsinki. All patients gave verbal and written informed consent before the start of the study.

The following inclusion criteria applied: (i) age between 18 and 70 years, (ii) body mass index (BMI) < 40 kg/m^2, (iii) a spontaneous pain level ("pain now" or "average daily pain") of 5 or greater on an 11-point numerical rating scale (0 = no pain, 10 = worst pain imaginable), (iv) pain defined as distal pain/discomfort plus one of the following: dysesthesia, burning/ painful feet worsening at night, or intolerance of sheets or clothes touching the legs or feet, (v) a score > 22 on the small fiber neuropathy screening list (SFNSL). In the sarcoidosis population a spontaneous pain level less than five was acceptable with an SFNSL score >37. The SFNSL is a validated questionnaire that is used to detect the presence of SFN in the Dutch population [17].

Quantitative Sensory Testing

Thermal detection and pain thresholds, mechanical detection and pain thresholds, pressure pain and vibration detection were evaluated on the face, hand and foot. Cold pain threshold (CPT), mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), dynamic mechanical allodynia (ALL) and wind-up ratio (WUR; temporal summation) were assessed as signs of central sensitization. In several studies hypersensitivity to cold and static (punctate) and dynamic mechanical stimuli have been associated with central sensitization [18-23]. All measurements were performed according to the protocol of the German research network on neuropathic pain and data were expressed accordingly as transformed z-scores where values < −1.96 (loss of function) or > +1.96 (gain of function) are considered abnormal [16]. Dynamic mechanical allodynia and paradoxical heat sensations were scored dichotomously as present or not present. Pain scoring was adapted in the protocol from a 0-100 point scale to a 0-10 point scale to simplify scoring and because the Dutch population is used to 10-point scoring systems in general (*e.g.* in the clinic). Normative data were obtained from Magerl *et al.* [24]. Patients were considered to have abnormal sensations when at least one of the three locations deviated from normal values.

Cornea Confocal Microscopy

Cornea nerve fiber density (CNFD), cornea nerve fiber length (CNFL) and cornea nerve branching density (CNBD) were determined using the Rostock Cornea Module with the Heidelberg Retina Tomograph III (Heidelberg, Germany). For a full description of CCM methodology see reference [25]. In short, after topical anesthesia of the eyes, the microscope was placed at the surface of the cornea apex. Confocal images were acquired with a field of view of 400 x 400 µm and automatically quantified using ACCmetrics software (provided by the faculty of Medical and Human Sciences of the University of Manchester, United Kingdom). Five to ten representative, high-quality images per eye were selected from a total of at least 50 images. CNFD, CNFL and CNBD were quantified by an investigator who was blinded for subject demographics and disease state. Because a good correlation has been demonstrated between semi-automated (CCmetrics) and

automated (ACCmetrics) corneal nerve fiber quantification [26,27], data from the current study were compared with semi-automatically quantified reference values of healthy volunteers from Tavakoli *et al.* [28].

Statistical analysis

Statistical analyses were performed using SPSS statistics version 23 (IBM SPSS Statistics, Armonk, NY) and GraphPad Prism 6 (GraphPad Software, San Diego, CA). Comparisons between groups were evaluated with the Student's ttest or Mann Whitney U test (DM *versus* sarcoidosis) or Kruskal-Wallis test (differences among phenotypes). P-values < 0.05 were considered significant. Data are presented as average ± 95% confidence interval or range, unless otherwise indicated.

RESULTS

Patients

A total of 107 patients were included in this study: 49 patients with diabetes (20 female) and 58 patients with sarcoidosis (27 female). Patient characteristics are presented in Table 1. In the DM cohort, mean age was 63 (range 46-74) years, BMI 31 (21-40) kg/m^2 and disease duration 12 (1-31) years. In the sarcoidosis cohort, age was 50 (26-68) years, BMI 26 (18-33) kg/m^2 and disease duration 9 (1-37) years. DM patients were older ($p = 0.001$), had a higher BMI ($p = 0.001$) and a longer disease duration ($p = 0.03$) than sarcoidosis patients.

Table 1. Patient characteristics

	Diabetes	Sarcoidosis
Number of patients, n (male/female)	49 (29/20)	58 (31/27)
Age in years, mean (range)	63 (46-74)	50 (26-68)*
Body mass index, kg/m^2, mean (range)	30.7 (20.5-40)	25.8 (18.4-32.7)*
Years since diagnosis, mean (range)	12 (1-31)	8.7 (1-37)*

* Significant difference between diabetes and sarcoidosis patients: age $p = 0.00$; BMI $p = 0.00$; years since diagnosis $p = 0.03$

Quantitative Sensory Testing

The QST results are summarized in Figure 1 (except PHS and ALL since these were scored dichotomously) and Table 2. In both patient groups, cold detection threshold (CDT) and warm detection threshold (WDT) were the most prominent parameters with significantly reduced values in at least one body location. Specifically, in the DM cohort CDT and WDT were reduced in 86% and 92% of patients; in the sarcoidosis cohort CDT and WDT were reduced in 76% and 72% of patients. Two other parameters that were

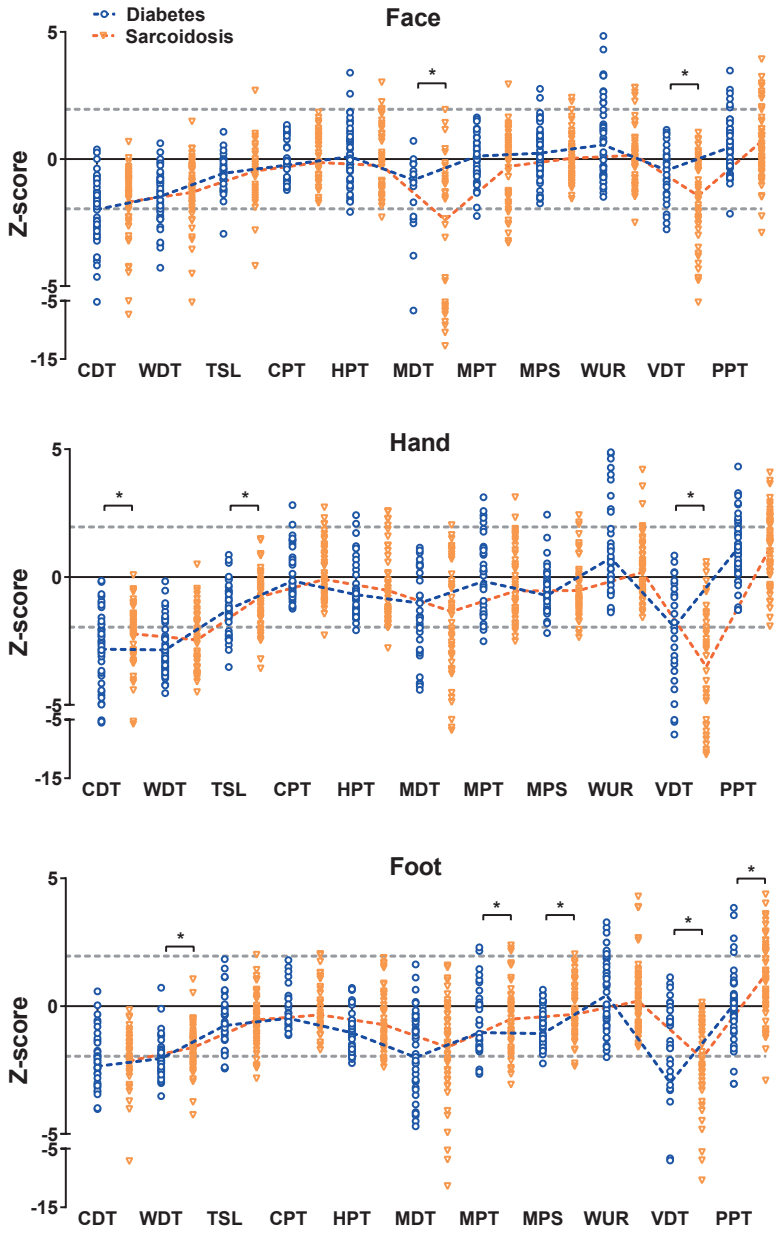


Figure 1. Quantitative sensory test results on three locations for diabetes (blue circles) and sarcoidosis (orange triangles) patients. Grey dotted lines represent the 95% confidence interval of the normalized Z-scores. Blue and orange dotted lines connect the mean values per test. CDT: cold detection threshold; WDT: warm detection threshold; TSL: thermal sensory limen; MDT: mechanical detection threshold; MPT: mechanical pain threshold; WUR: wind-up ratio; VDT: vibration detection threshold; PPT: pressure pain threshold.

significantly reduced in more than 50% of patients in both cohorts were: mechanical detection threshold (MDT) in 63% of patients with DM and 55% of patients with sarcoidosis and vibration detection threshold (VDT) in 67% of DM and 71% of sarcoidosis patients.

QST values of sarcoidosis patients were significantly worse than those of DM patients on the face for MDT ($p = 0.01$) and VDT ($p = 0.001$), on the hand for VDT ($p = 0.002$) and on the foot for WDT ($p = 0.01$), MPT ($p = 0.04$), MPS ($p = 0.000$), VDT ($p = 0.04$) and pressure pain threshold (PPT) ($p = 0.000$). DM patients showed significantly worse values than sarcoidosis patients on the hand for CDT ($p = 0.02$), thermal sensory limen (TSL) ($p =$

Table 2. Average quantitative sensory testing scores and number & percentage of patients with dysesthesias, compared to age- and sex- matched reference values [7]

	Abnormal function diabetes patients, n (%)		Abnormal function sarcoidosis patients, n (%)	
	Loss of function	Gain of function	Loss of function	Gain of function
Cold detection threshold	42 (86%)		44 (76%)	
Warm detection threshold	45 (92%)		42 (72%)	
Thermal sensory limen	16 (33%)		11 (19%)	2 (3%)
Paradoxical heat sensations	25 (51%)		22 (38%)	
Cold pain threshold		2 (4%)	2 (3%)	4 (7%)
Heat pain threshold	6 (12%)	2 (4%)	9 (16%)	7 (12%)
Mechanical detection threshold	31 (63%)		32 (55%)	
Mechanical pain threshold	10 (20%)	7 (14%)	15 (26%)	5 (9%)
Mechanical pain sensitivity	4 (8%)	2 (4%)	4 (7%)	3 (5%)
Vibration detection threshold	33 (67%)		41 (71%)	
Pressure pain threshold	4 (8%)	14 (29%)	3 (5%)	30 (52%)
Temporal summation (wind-up)		19 (39%)		12 (21%)
Dynamic mechanical allodynia		14 (29%)		14 (24%)

The prevalence of paradoxical heat sensations and dynamic mechanical allodynia was recorded in a dichotomous fashion. Measurements were taken from 3 locations: on the cheekbone (face), and the dorsal surface of hand and foot.

Table 3. Quantification of corneal nerve fibers by cornea confocal microscopy in diabetes and sarcoidosis patients

	Average (95% CI)		Range (min-max)		Significantly decreased, n (%)	
	Diabetes	Sarcoidosis	Diabetes	Sarcoidosis	Diabetes	Sarcoidosis
CNFD (n/mm^2)	20.6 (19.6-21.6)	22.1 (21.3-22.9)	6.2-34.4	9.4-34.4	10 (20%)	5 (9%)
CNFL (mm/mm^2)	12.6 (12.1-13.1)	13.6 (13.1-14.1)	4.8-20.7	6.8-21.3	25 (51%)	25 (43%)
CNBD (n/mm^2)	31.1 (28.6-33.6)	32.5 (29.8-35.2)	3.1-90.6	3.1-84.4	11 (22%)	16 (28%)

CI = confidence interval; CNFD = Cornea nerve fiber density; CNBD = cornea nerve branching density; CNFL = cornea nerve fiber length.

0.01) and paradoxical heat sensations (PHS) (p = 0.02). Abnormal QST parameters indicative of central sensitization (CPT, MPT, MPS, ALL, WUR) were present in more patients with DM than sarcoidosis, with 73% of DM patients and 52% of sarcoidosis patients with an abnormality in at least one of these parameters.

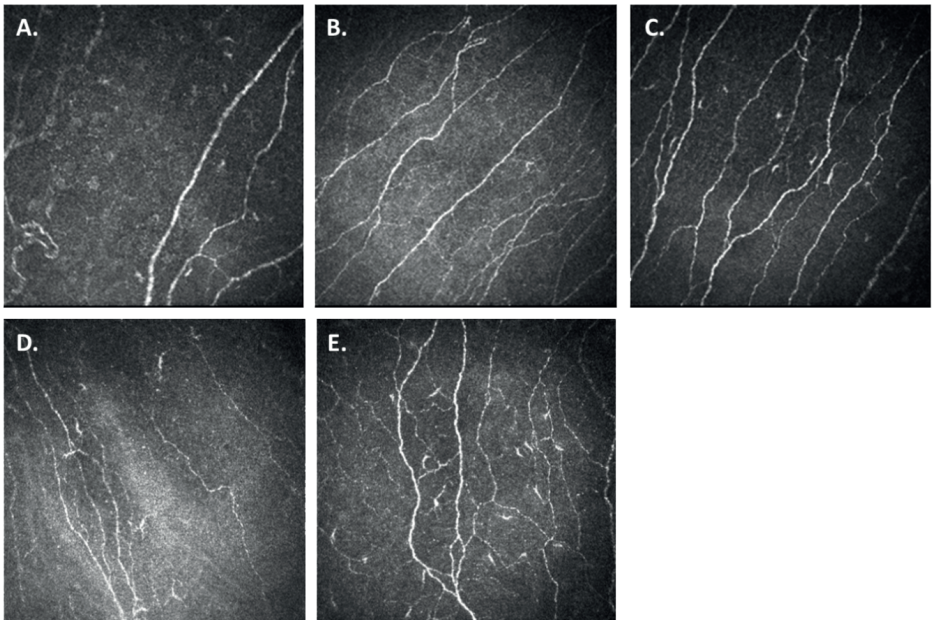


Figure 2. Confocal microscopy images of corneal nerve fibers. **(A)** 56 years-old male diabetes patient with abnormal nerve fiber state. **(B)** 53 years-old male diabetes patient with normal nerve fiber state. **(C)** 53 years-old healthy male volunteer with normal nerve fiber state. **(D)** 52 years-old male sarcoidosis patient with abnormal nerve fiber state. **(E)** 53 years-old male sarcoidosis patient with normal corneal nerve fiber state. All images were acquired with a field of view of 400x400 µm.

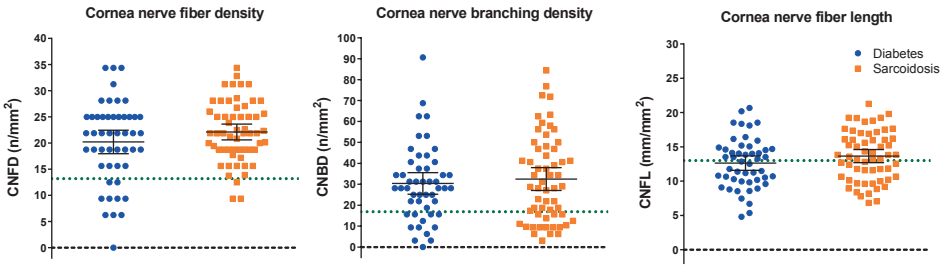
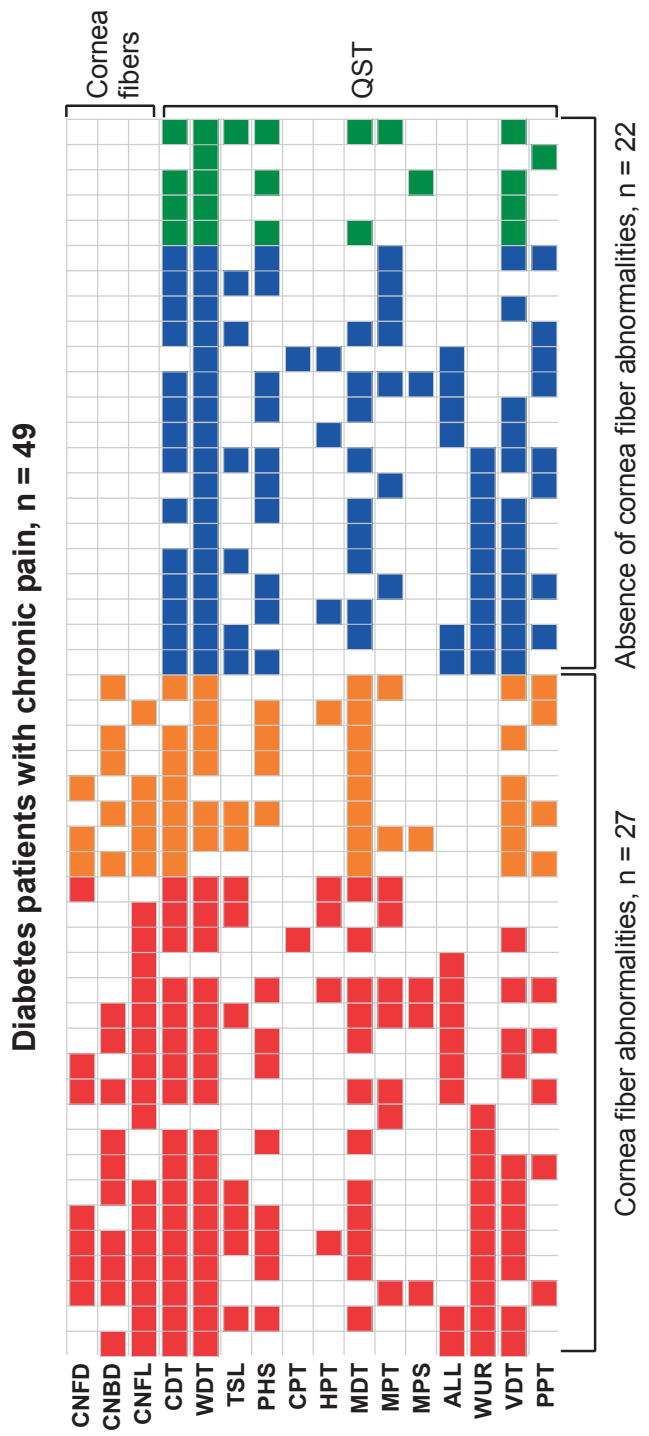


Figure 3. Average (95% confidence interval) corneal nerve fiber parameters from diabetes (blue dots) and sarcoidosis patients (orange squares). The dotted line represents the 5th percentile below which values are considered abnormal for the age category (46-65) of the average age of the patients. CNFD: corneal nerve fiber density; CNBD: corneal nerve branching density; CNFL: corneal nerve fiber length.


Diabetes patients with chronic pain, n = 49
Cornea fibers
QST
CNFD
CNBD
CNFL
CDT
WDT
TSL
PHS
CPT
HPT
MDT
MPT
MPS
ALL
WUR
VDT
PPT
Cornea fiber abnormalities, n = 27
Absence of cornea fiber abnormalities, n = 22

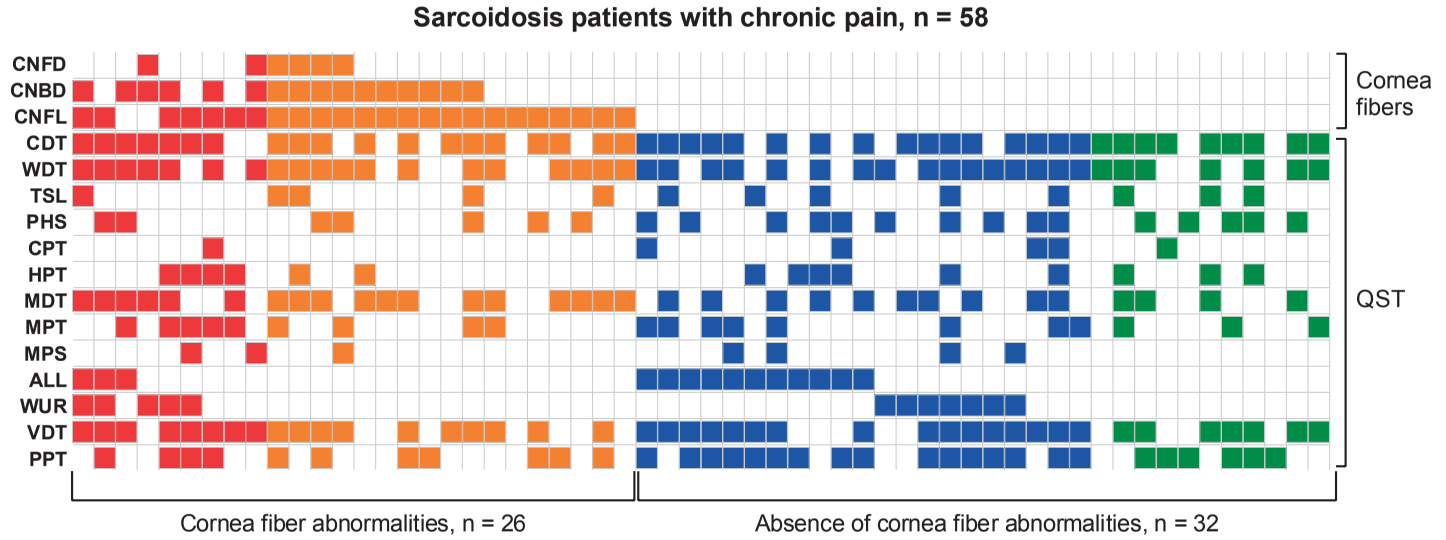


Figure 4. Neuropathic pain phenotypes for diabetes patients (upper panel) and sarcoidosis patients (lower panel). Four phenotypes were constructed based on the presence of corneal nerve fiber (CCM) abnormalities and the presence or absence of central sensitization. **Red**: abnormal cornea morphology and signs of central sensitization; **Orange**: abnormal cornea morphology without signs of central sensitization; **Blue**: normal cornea morphology with signs of central sensitization; **Green**: normal cornea morphology without signs of central sensitization. CNFD = corneal nerve fiber density; CNBD = corneal nerve branching density; CNFL = corneal nerve fiber length; CDT: Cold detection threshold; WDT: Warm detection threshold; TSL: thermal sensory limen; MDT: Mechanical detection threshold; MPT: Mechanical pain threshold; WUR: Wind-up ratio; VDT: Vibration detection threshold; PPT: Pressure pain threshold, QST: Quantitative sensory testing. Note that in groups without central sensitization, abnormalities in CPT, MPT and MPS may still be present. In these cases a loss of function is present as opposed to a gain of function as a sign of central sensitization.

Table 4. Number of patients per phenotype group of the diabetes and sarcoidosis cohort and in both cohorts (overall)

		Diabetes	Sarcoidosis	Overall
1) CCM abnormal	CS pos	19 (39%)	9 (16%)	28 (26%)
2) CCM abnormal	CS neg	8 (16%)	17 (29%)	25 (23%)
3) CCM normal	CS pos	17 (35%)	21 (36%)	38 (36%)
4) CCM normal	CS neg	5 (10%)	11 (19%)	16 (15%)

CCM = cornea confocal microscopy; CS = central sensitization

Cornea Confocal Microscopy

Since CCM data of the left and right eye were similar, these data were averaged per patient. Mean CNFD, CNFL and CNBD values are given in Table 3. Examples of patients with abnormal and normal cornea morphology and a healthy male control are shown in Figure 2. Compared with reference values of normal controls, at least one abnormality in cornea nerve fiber morphology was observed in 55% of DM patients and in 45% of sarcoidosis patients. In the DM cohort CNFD, CNFL and CNBD values were below the 5th percentile of reference values in 20%, 51% and 22% of patients, respectively. In the sarcoidosis cohort the corresponding percentages were 9% (CNFD), 43% (CNFL) and 28% (CNBD). Individual scores of CCM parameters are illustrated in Figure 3; the dotted line represents the lower 5th percentile of the 56-65 age category (averaged for men and women) of healthy controls [28]. There were no differences in distributions of CNFD ($p = 0.15$), CNFL ($p = 0.15$) or CNBD ($p = 0.80$) between the diabetes and sarcoidosis cohorts.

Neuropathic pain phenotypes

We defined four subgroups of patients in both the DM group and the sarcoidosis group, based on cornea morphology, and signs of central sensitization (by CPT, MPT, MPS, WUR and/or ALL in QST). The four subgroups consisted of a group with abnormal cornea morphology with and without signs of central sensitization, and a group with normal cornea morphology parameters with and without signs of central sensitization. In the DM cohort, the distribution over the phenotypes was: (1) 39% (n=19) abnormal cornea morphology and signs of central sensitization; (2) 16% (n=8) abnormal cornea morphology without signs of central sensitization; (3) 35% (n=17) normal cornea morphology and signs of central sensitization; (4) 10% (n=5) normal cornea morphology without signs of central sensitization. For the sarcoidosis cohort, the distribution over the 4 groups was 16% (n=9); 29% (n=17); 36% (n=21); and 19% (n=11) (Table 4).

CCM parameters and all QST parameters were implemented in fire plots to visualize the phenotypes in DM and sarcoidosis (Figure 4). Subgroup analysis revealed no differences among phenotypes in age ($p = 0.16$), BMI ($p = 0.27$), disease duration ($p = 0.27$), average daily pain score ($p = 0.08$) or QST parameter values.

DISCUSSION

To characterize DM type 2 and sarcoidosis patients with neuropathic pain we performed sensory tests and small nerve fiber quantification. We determined the presence of distinct phenotypes based on cornea nerve fiber morphology and the presence or absence of signs of central sensitization as determined from QST. The four phenotypes were equally distributed across patient populations, irrespective of the underlying disease (*i.e.* DM or sarcoidosis).

We used CCM to complement phenotyping based on QST measurements. Where QST provides functional assessment of nerve fibers, CCM allows quantification of small nerve fibers. CCM is a relatively new, rapid, non-invasive tool and as such, CCM is suitable to monitor disease progression and assess treatment efficacy. Previous studies using CCM in patients with diabetic neuropathy showed that CCM measures correlate with severity of neuropathy, especially CNFD and CNFL, and that CCM is an appropriate method to assess small fiber pathology [29-32].

In this study two systemic diseases with distinct etiologies were evaluated. DM is a metabolic disorder with SFN related to hyperglycemia, while sarcoidosis is an inflammatory disease with SFN presumably related to peripheral inflammation. Here we show that these distinct diseases present with similar chronic pain phenotypes with homogeneous patterns of somatosensory symptoms and peripheral (cornea) nerve pathology. Cornea nerve fiber abnormalities were present in 55% of DM patients and 45% of sarcoidosis patients. Signs of central sensitization were present in 73% of DM patients and 52% of sarcoidosis patients. The phenotypes demonstrate that patients may present with isolated presence of small nerve fiber pathology or central sensitization, with neither, or with both, while symptoms such as reduced thermal thresholds may be present in all phenotypes. We previously showed that another chronic pain state with a much more complex and less-well understood pathophysiological mechanism, fibromyalgia, can be described according to these same four phenotypes with similar distributions among somatosensory symptoms and peripheral nerve damage [1].

Neuropathic pain phenotypes were constructed based on CCM abnormalities and the presence or absence of central sensitization. The latter represents an enhancement of signal propagation of neurons in nociceptive pathways that can be caused by increases in membrane excitability and synaptic efficiency and/or reduced inhibition. As such, it is a major contributor to chronic neuropathic pain and an important factor in patient stratification and pharmacological targeting. To identify central sensitization, we considered all QST tests that have been frequently described to be associated with central sensitization: CPT, MPT, MPS, ALL and WUR (temporal summation). However, it is often difficult to dissociate peripheral from central factors in the chronification of pain as there are many mechanisms that play a role in the development of hyperalgesia and allodynia [33]. Similar

to our approach, in a review discussing the pathophysiology of pain in PHN, Fields *et al.* defined patient subgroups according to central mechanisms of pain [34]. The authors made a distinction between patients with irritable nociceptors and patients with deafferentation with or without allodynia. The irritable nociceptor group was defined by allodynia in the absence of sensory loss, while the deafferentation groups were defined by impairment of nociceptive and thermal sensations with accompanying allodynia, or presence of spontaneous pain without hyperalgesia or allodynia. Although we did not include spontaneous pain in our phenotypes, the deafferentation group with or without accompanying allodynia are analogous to our groups with CCM abnormalities with and without signs of central sensitization. In both studies, subgroups with peripheral and central mechanisms are present in isolation or in combination. This creates a clear distinction in pathophysiological mechanisms involved in pain generation and possible related treatment options between subgroups of patients. In a recent study by Vaegter *et al.* [35] central mechanisms were further divided to explore the existence of subgroups based on central modulation phenotype in 400 patients with pain of various etiologies. Four distinct groups were formed: patients with impaired endogenous pain modulation (as measured by conditioned pain modulation [CPM]) with or without facilitated temporal summation (TS); and patients with normal CPM with or without normal TS. The group with deficiencies in both CPM and TS had more painful body regions and higher clinical pain scores than other phenotypes indicating that both mechanisms play an important role in widespread pain. Groups with decreased CPM responses showed lower pain thresholds suggesting that impaired CPM is implicated in widespread hyperalgesia. This study indicates that several mechanisms underlie central sensitization and that these can further distinguish between symptoms and phenotypes.

Additional studies which have stratified chronic pain patients according to QST and neuropathic pain questionnaires have identified homogenous response groups comparable to our findings. In a study that aimed to identify whether subgroups with distinct patterns of sensory signs and symptoms exist in various neuropathic pain disorders [36], similar profiles were present in chronic pain patients from all etiologies (central post-stroke pain, posttraumatic peripheral pain, painful HIV neuropathy, and painful diabetic peripheral neuropathy). In that study, patients were stratified according to responses to the Neuropathic Pain Symptom Inventory (NPSI) questionnaire and QST. This yielded 4 clusters with distinct pain characteristic profiles based on NPSI-derived pain dimensions provoked, deep, and pinpoint pain and QST-derived pain dimensions cold-evoked pain and touch-evoked pain. Despite the different determinants for subgroup identification, the results are in agreement with our finding that homogeneous patterns of symptoms and pathology could be identified irrespective of the underlying disease. Similarly, in a large survey of 2100 patients with diabetic painful neuropathy (DPN) or postherpetic neuralgia (PHN), patient reported outcomes from the PainDetect questionnaire were

used to identify homogeneous subgroups of patients [37]. In patients with both DPN and PHN, 5 subgroups were identified with notable differences in quality of pain. Distinction between subgroups was made based on (i) the quality of pain such as burning, prickling, and the occurrence of attacks, (ii) pain responses to thermal or pressure stimuli and (iii) the presence of allodynia and numbness. Although the distribution of subgroups differed between PHN and DPN, all of the subgroups showed relevant numbers of patients in both disease entities. Taken together, these results demonstrate that defining somatosensory phenotypes identifies subgroups of patients with homogeneous signs and symptoms across different diseases. Since the presence of these sensory symptoms is likely related to specific pain-generating pathophysiological mechanisms, these subgroups may show differential responses to analgesic treatment.

A number of studies have addressed treatment effects in identified subgroups of neuropathic pain patients [38-41]. For example, in patients with painful diabetic neuropathy Campbell *et al.* [38] showed that the topical application of the α_2-adrenergic receptor agonist clonidine has greater analgesic efficacy in patients that showed prior sensitivity to capsaicin. Response to capsaicin is a sign of the presence of functional nociceptors. The authors therefore concluded that the efficacy of clonidine depends on the level of functionality of nociceptors in the skin. Demant *et al.* [39] observed that oxcarbazepine, an anticonvulsant which blocks sodium channels, is more efficacious in neuropathic pain of various etiologies when patients have an irritable nociceptor phenotype. Irritable nociceptors were here defined by normal thermal detection thresholds indicative of preserved small fiber function, in combination with reduced mechanical detection thresholds, reduced pain thresholds or allodynia. A different approach was taken by Martini *et al.* [42], who classified patients with postherpetic neuralgia based on their response profile to treatment with capsaicin, and subsequently identified specific patient characteristics including baseline pain scores and efficacy of lidocaine pretreatment as predictors of treatment efficacy. Finally, Holbech and colleagues [43] recently reported a retrospective analysis of 7 placebo-controlled clinical trials of various analgesic drugs for phenotype-specific effects in patients with painful polyneuropathy of various etiologies. Phenotypes were based on presence of numbness and quality of pain (tingling/prickling, burning, paroxysms), mechanical and cold allodynia, thermal detection thresholds and other QST abnormalities indicative of either small or large fiber deafferentation. Of the studied drugs (venlafaxine, St. John's wort, escitalopram, valproic acid, levetiracetam, oxcarbazepine, imipramine and pregabalin), only the TCA imipramine and the calcium channel α2δ ligand pregabalin showed higher pain reduction effects in a particular phenotype. Imipramine showed a larger effect in a phenotype characterized by gain of sensory function, and pregabalin in a phenotype with preserved large fiber function. It was concluded that somatosensory phenotyping has limited usefulness for individualized treatment. However, as this was a retrospective analysis, a prospective study

design selecting specific drugs corresponding to pain generating mechanisms based on the phenotypes might yield better phenotype-specific results.

In the current study we do not report treatment efficacy in the identified patient phenotypes. We may however speculate that patients with small nerve fiber pathology and signs of central sensitization would benefit especially from treatment aimed at restoration of small fiber damage and stabilization of the central nervous system inflammatory response to peripheral nerve damage, irrespective of their underlying disease. One such treatment is ARA290, an erythropoietin analog acting at the innate repair receptor, which restores peripheral nerve defects, inhibits spinal cord inflammation and reduces neuropathic symptoms. Indeed, we showed previously that ARA290 is effective in sarcoidosis and DM patients with neuropathic pain [25]. Alternatively ketamine could be used in patients with small nerve fiber pathology and signs of central sensitization. Ketamine it is an NMDA receptor antagonist that effectively counteracts central sensitization, has central anti-inflammatory properties and causes long-term reduction of neuropathic symptoms [44,45]. Especially patients with an increased wind-up ratio may benefit from ketamine treatment [46,47].

There are some limitations to our study. (1) We included patients with an advanced disease profile. Consequently our phenotypes are limited to individuals with these more advanced disease characteristics. Including a more diverse group of patients in terms of disease progression might have offered a better understanding of the development of nerve fiber dysfunction and morphological changes. (2) Since the pain symptoms of our patients were severe, their pain medication was maintained. This might have affected some of the QST results, especially the pain thresholds. (3) Where other studies make a distinction between affected and non-affected areas, we applied all stimuli on the same three locations. Consequently, for some patients the test location was an affected area, but for others it was not. As the distribution in a group of patients with widespread pain from fibromyalgia was similar to the distributions of DM and sarcoidosis patients here, we do not think that our approach affected the subgroup distribution significantly.

In conclusion, we identified four somatosensory phenotypes in diabetes and sarcoidosis patients based on normal or abnormal cornea morphology and the presence or absence of signs of central sensitization. The four identified phenotypes constitute groups of patients with more homogeneous patterns of somatosensory symptoms. Notably, the distribution of somatosensory profiles was similar between the two patient cohorts, suggesting that these patients may benefit from phenotype-based pharmacological interventions unrelated to the underlying disease.

REFERENCES

1. Oudejans L, He X, Niesters M, Dahan A, Brines M, van Velzen M: Cornea nerve fiber quantification and construction of phenotypes in patients with fibromyalgia. Scientific reports 2016, 6:23573.
2. Dahan A, Olofsen E, Niesters M: Pharmacotherapy for pain: efficacy and safety issues examined by subgroup analyses. Pain 2015, 156 Suppl 1:S119-26.
3. Dworkin RH, O'Connor AB, Audette J, Baron R, Gourlay GK, Haanpaa ML, Kent JL, Krane EJ, Lebel AA, Levy RM, Mackey SC, Mayer J, Miaskowski C, Raja SN, Rice AS, Schmader KE, Stacey B, Stanos S, Treede RD, Turk DC, Walco GA, Wells CD: Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. Mayo Clinic proceedings 2010, 85: S3-14.
4. Javed S, Alam U, Malik RA: Burning through the pain: treatments for diabetic neuropathy. Diabetes, obesity & metabolism 2015, 17:1115-25.
5. Watson JC, Dyck PJ: Peripheral Neuropathy: A Practical Approach to Diagnosis and Symptom Management. Mayo Clinic proceedings 2015, 90:940-51.
6. Brines M, Swartjes, M., Tannemaat M.R., Dunne, A., van Velzen, M., Proto, P., Hoitsma, E., Petropoulos, I., Chen, X., Niesters, M., Dahan, A., Malik R., Cerami, A. : Corneal nerve quantification predicts the severity of symptoms in sarcoidosis patients with painful neuropathy. Technology 2013, 1:1-7.
7. Papanas N, Ziegler D: Corneal confocal microscopy: Recent progress in the evaluation of diabetic neuropathy. Journal of diabetes investigation 2015, 6:381-9.
8. Abbott CA, Malik RA, van Ross ER, Kulkarni J, Boulton AJ: Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K. Diabetes care 2011, 34:2220-4.
9. Callaghan BC, Price RS, Feldman EL: Distal Symmetric Polyneuropathy: A Review. Jama 2015, 314: 2172-81.
10. Van Acker K, Bouhassira D, De Bacquer D, Weiss S, Matthys K, Raemen H, Mathieu C, Colin IM: Prevalence and impact on quality of life of peripheral neuropathy with or without neuropathic pain in type 1 and type 2 diabetic patients attending hospital outpatients clinics. Diabetes & metabolism 2009, 35:206-13.
11. Peltier A, Goutman SA, Callaghan BC: Painful diabetic neuropathy. Bmj 2014, 348:g1799.
12. Drent M, Strookappe B, Hoitsma E, De Vries J: Consequences of Sarcoidosis. Clinics in chest medicine 2015, 36:727-37.
13. Heij L, Niesters M, Swartjes M, Hoitsma E, Drent M, Dunne A, Grutters JC, Vogels O, Brines M, Cerami A, Dahan A: Safety and efficacy of ARA 290 in sarcoidosis patients with symptoms of small fiber neuropathy: a randomized, double-blind pilot study. Molecular medicine 2012, 18:1430-6.
14. Hoitsma E, Marziniak M, Faber CG, Reulen JP, Sommer C, De Baets M, Drent M: Small fibre neuropathy in sarcoidosis. Lancet 2002, 359:2085-6.
15. Tavee J, Culver D: Sarcoidosis and small-fiber neuropathy. Current pain and headache reports 2011, 15:201-6.
16. Rolke R, Baron R, Maier C, Tolle TR, Treede RD, Beyer A, Binder A, Birbaumer N, Birklein F, Botefur IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihofner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B: Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain 2006, 123:231-43.
17. Hoitsma E, De Vries J, Drent M: The small fiber neuropathy screening list: Construction and cross-validation in sarcoidosis. Respiratory medicine 2011, 105:95-100.

18. Arendt-Nielsen L, Yarnitsky D: Experimental and Clinical Applications of Quantitative Sensory Testing Applied to Skin, Muscles and Viscera. J Pain 2009, 10:556-72.
19. LaMotte RH, Shain CN, Simone DA, Tsai EF: Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. Journal of neurophysiology 1991, 66:190-211.
20. Price DD, Hu JW, Dubner R, Gracely RH: Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. Pain 1977, 3:57-68.
21. Woolf CJ: Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011, 152:S2-15.
22. Woolf CJ, Mannion RJ: Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 1999, 353:1959-64.
23. Yarnitsky D, Ochoa JL: Release of Cold-Induced Burning Pain by Block of Cold-Specific Afferent Input. Brain 1990, 113:893-902.
24. Magerl W, Krumova EK, Baron R, Tolle T, Treede RD, Maier C: Reference data for quantitative sensory testing (QST): refined stratification for age and a novel method for statistical comparison of group data. Pain 2010, 151:598-605.
25. Dahan A, Dunne A, Swartjes M, Proto PL, Heij L, Vogels O, van Velzen M, Sarton E, Niesters M, Tannemaat MR, Cerami A, Brines M: ARA 290 improves symptoms in patients with sarcoidosis-associated small nerve fiber loss and increases corneal nerve fiber density. Molecular medicine 2013, 19:334-45.
26. Chen X, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, Asghar O, Alam U, Marshall A, Fadavi H, Ferdousi M, Azmi S, Tavakoli M, Efron N, Jeziorska M, Malik RA: Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. Diabetes care 2015, 38:1138-44.
27. Petropoulos IN, Alam U, Fadavi H, Marshall A, Asghar O, Dabbah MA, Chen X, Graham J, Ponirakis G, Boulton AJ, Tavakoli M, Malik RA: Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. Investigative ophthalmology & visual science 2014, 55: 2071-8.
28. Tavakoli M, Ferdousi M, Petropoulos IN, Morris J, Pritchard N, Zhivov A, Ziegler D, Pacaud D, Romanchuk K, Perkins BA, Lovblom LE, Bril V, Singleton JR, Smith G, Boulton AJ, Efron N, Malik RA: Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. Diabetes care 2015, 38:838-43.
29. Ahmed A, Bril V, Orszag A, Paulson J, Yeung E, Ngo M, Orlov S, Perkins BA: Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. Diabetes care 2012, 35:821-8.
30. Malik RA, Kallinikos P, Abbott CA, van Schie CH, Morgan P, Efron N, Boulton AJ: Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. Diabetologia 2003, 46:683-8.
31. Petropoulos IN, Alam U, Fadavi H, Asghar O, Green P, Ponirakis G, Marshall A, Boulton AJ, Tavakoli M, Malik RA: Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. Diabetes care 2013, 36:3646-51.
32. Tavakoli M, Quattrini C, Abbott C, Kallinikos P, Marshall A, Finnigan J, Morgan P, Efron N, Boulton AJ, Malik RA: Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes care 2010, 33:1792-7.
33. Jensen TS, Finnerup NB: Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms. The Lancet Neurology 2014, 13:924-35.

34. Fields HL, Rowbotham M, Baron R: Postherpetic neuralgia: irritable nociceptors and deafferentation. Neurobiology of disease 1998, 5:209-27.
35. Vaegter HB, Graven-Nielsen T: Pain modulatory phenotypes differentiate subgroups with different clinical and experimental pain sensitivity. Pain 2016.
36. Freeman R, Baron R, Bouhassira D, Cabrera J, Emir B: Sensory profiles of patients with neuropathic pain based on the neuropathic pain symptoms and signs. Pain 2014, 155:367-76.
37. Baron R, Tolle TR, Gockel U, Brosz M, Freynhagen R: A cross-sectional cohort survey in 2100 patients with painful diabetic neuropathy and postherpetic neuralgia: Differences in demographic data and sensory symptoms. Pain 2009, 146:34-40.
38. Campbell CM, Kipnes MS, Stouch BC, Brady KL, Kelly M, Schmidt WK, Petersen KL, Rowbotham MC, Campbell JN: Randomized control trial of topical clonidine for treatment of painful diabetic neuropathy. Pain 2012, 153:1815-23.
39. Demant DT, Lund K, Vollert J, Maier C, Segerdahl M, Finnerup NB, Jensen TS, Sindrup SH: The effect of oxcarbazepine in peripheral neuropathic pain depends on pain phenotype: a randomised, double-blind, placebo-controlled phenotype-stratified study. Pain 2014, 155:2263-73.
40. Tesfaye S, Wilhelm S, Lledo A, Schacht A, Tolle T, Bouhassira D, Cruccu G, Skljarevski V, Freynhagen R: Duloxetine and pregabalin: high-dose monotherapy or their combination? The "COMBO-DN study"--a multinational, randomized, double-blind, parallel-group study in patients with diabetic peripheral neuropathic pain. Pain 2013, 154:2616-25.
41. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y: Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 2012, 153:1193-8.
42. Martini CH, Yassen A, Krebs-Brown A, Passier P, Stoker M, Olofsen E, Dahan A: A novel approach to identify responder subgroups and predictors of response to low- and high-dose capsaicin patches in postherpetic neuralgia. European journal of pain 2013, 17:1491-501.
43. Holbech JV, Bach FW, Finnerup NB, Jensen TS, Sindrup SH: Pain phenotype as a predictor for drug response in painful polyneuropathy A retrospective analysis of data from controlled clinical trials. Pain 2016.
44. De Kock M, Loix S, Lavand'homme P: Ketamine and peripheral inflammation. CNS neuroscience & therapeutics 2013, 19:403-10.
45. Tawfic QA: A review of the use of ketamine in pain management. Journal of opioid management 2013, 9:379-88.
46. Davies SN, Lodge D: Evidence for involvement of N-methylaspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat. Brain research 1987, 424:402-6.
47. Graven-Nielsen T, Aspegren Kendall S, Henriksson KG, Bengtsson M, Sorensen J, Johnson A, Gerdle B, Arendt-Nielsen L: Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. Pain 2000, 85:483-91.

Chapter 7

Summary, general discussion and conclusions

SUMMARY

Pain is a complicated sensation that is influenced by prior exposures, expectations, attention, mood, genetic make-up, nervous system physiology and neurochemical and anatomical variation. The pain pathway consist of peripheral nerves that connect to second order neurons in the spinal cord, which further convey the pain signal to several brain regions involved in pain perception. From these brain regions, descending pathways send signals back to the spinal cord, where incoming pain signals are modulated at the level of the dorsal horn. Both pain inhibition and pain facilitation may take place and are known as descending inhibition and descending facilitation [1-5]. Chronic pain may arise as a consequence of inadequate descending inhibition or enhanced facilitation often complicated by central sensitization and related to sustained afferent nociceptive input. The latter may be caused by a focal lesion or systemic disease that may concurrently cause large and/or small fiber pathology. Hence, often both peripheral and central mechanisms contribute to chronification of pain.

In clinical and experimental settings, acute and chronic pain can be evaluated by a number of instruments, which include neurological testing, questionnaires and quantitative sensory testing (QST). Static tests such as pinprick sensitivity or thermal detection and pain thresholds may be performed, as well as dynamic tests that give an indication of the state of the endogenous pain modulatory system. Examples of dynamic tests are conditioned pain modulation (CPM) and offset analgesia (OA). CPM is performed by application of a second nociceptive stimulus on a remote location from the first. This second stimulus inhibits the primary nociceptive stimulus in healthy subjects. OA is performed by slightly reducing a nociceptive heat stimulus, which causes an unproportionately large reduction in pain perception. Additionally, the structure and function of large and small nerve fibers can be assessed by neurophysiological testing (function of large fibers) and corneal confocal microscopy and skin biopsies (quantity and morphology of small fibers). Corneal confocal microscopy (CCM) visualizes small nerve fibers that innervate the cornea. From skin biopsies the intraepidermal nerve fiber density can be derived.

Combining the results of the tests discussed above enables us to characterize chronic pain patients and to construct a neuropathic pain phenotype of an individual patient. The heterogeneous neuropathic pain population can be divided into more homogeneous cohorts, which allows evaluating subgroups separately in terms of pathophysiological mechanisms and treatment options. In this thesis studies are described that used the aforementioned technologies to characterize the nociceptive state of both healthy volunteers and chronic pain patients with fibromyalgia, diabetes mellitus type 2 and sarcoidosis. Performed tests include CPM, OA, QST, CCM, skin biopsies and questionnaires.

In **Chapter 2** we assessed the ability of acute and chronic pain patients to grade pain using the 11-point Numerical Rating Scale (NRS) with '0' indicating 'no pain' and '10' indicating 'worst pain imaginable'. Number-based assessment tools such as the NRS are frequently used to evaluate pain perception in patients and determine the effect of pain management. We focused on patients with acute pain following major surgery and patients with fibromyalgia, a disorder of unknown etiology mainly defined by widespread pain and fatigue. We developed a technique in which subjects were exposed to random heat pain (Hp) and electrical pain (Ep) stimuli between pain threshold and pain tolerance. The subject's response to each stimulus was scored on the 11-point NRS. After obtaining baseline values the effect of opioid treatment on pain rating was assessed. The data were analyzed using a penalty score system, based on the assumption that stimuli of higher intensity are scored with a greater NRS. The data were stratified into cohorts corresponding to "good", "mediocre" and "poor" scoring. Healthy controls were well able to score pain with 73% (Hp) and 81% (Ep) of subjects classified into cohort "good". Fibromyalgia patients heat pain scores, but not electrical pain scores were significantly worse with 45% (Hp, p=0.03 *vs.* controls) and 67% (Ep) of patients in cohort "good". In controls scoring deteriorated during opioid administration leaving just 40% (Hp, p=0.015 *vs.* baseline) and 70% (Ep) of subjects in the cohort "good". Similar observations were made in fibromyalgia patients (Hp p=0.02) but not in surgical patients with postoperative pain. Our findings are in agreement with those of others, and indicate that the NRS is a valid tool to describe acute pain and pain relief in healthy subjects. We concluded that consistency to grade pain using an NRS is high in healthy volunteers but deteriorates in chronic pain patients and during opioid administration to volunteers and chronic pain patients but not to acute pain patients.

In **chapter 3** the presence of offset analgesia (OA) was evaluated in fibromyalgia patients and compared with healthy age- and sex-matched controls. OA was induced by noxious thermal stimulation on the arm, causing an electronic visual analogue score (eVAS) of about 50 mm (on an electronic scale of 0-100 mm, from left indicating 'no pain' to right indicating 'worst pain imaginable'), followed by a 1°C temperature decrease. The offset analgesia response is defined by the reduction in visual analogue score induced by the 1°C stimulus decrease. To assess whether the OA response could be enhanced two more tests were applied: repetition of the OA paradigm and 1°C temperature downward steps after an initial OA test (downward steps test). To assess whether OA affects onset of pain, offset analgesia steps at increasing temperatures (upward OA steps test) were applied and compared to a continuously increasing temperature test. Fibromyalgia patients showed reduced OA responses with a reduction in eVAS of 65.3% ± 4.5 (mean ± SD) *versus* controls 97.8% ± 4.6 ($p<0.001$). Decreased OA responses were not enhanced or restored by repeating the offset analgesia paradigm or by the downward steps test.

Defective engagement of OA had a significant effect on pain onset, as observed from the upward OA steps test, illustrated by the earlier onset of pain in fibromyalgia patients compared with healthy volunteers, relative to the ramp test. We concluded that fibromyalgia patients showed less pain inhibition as measured by the offset analgesia paradigm, which influenced both the onset and offset of pain.

Conditioned pain modulation (CPM) is a paradigm that is used to evaluate the endogenous ability of the body to modulate incoming pain signals. However, comparisons across studies using CPM paradigms are virtually impossible because of the large variation in methodology. There is a pressing need for standardized methods and materials that are easily operated in both research and clinical settings. The aim of **chapter 4** was to explore whether a device carrying two contact heat thermodes, the Q-sense CPM device (with which both the test and conditioning stimuli are applied), could induce a similarly large CPM effect as the frequently used test with cold water immersion as conditioning stimulus. To assess CPM effects, we applied a classic 30 second CPM paradigm (CPM30) and a 3 x 10 second CPM paradigm (CPM10), with the conditioning stimulus both ipsilateral and contralateral to the test stimulus. In contrast to cold water immersion, the Q-sense CPM device did not induce significant CPM responses in any of the paradigms. CPM effects (reduction in peak pain scores) were 10% (p=0.20) and 7% (p=0.34) (contralateral conditioning stimulus, CPM30 and CPM10) and -4% (p=0.86) and -0.2% (p=0.48) (ipsilateral conditioning stimulus, CPM30 and CPM10). For water immersion these reductions were 17% (p=0.01) and 25% (p=0.04), and 19% (p=0.00) and 14% (p=0.045) respectively. For future application of CPM paradigms we recommend the use of cold water as conditioning stimulus at the contralateral site of the test stimulus. The main conclusion of this chapter is that the use of two contact probes to induce CPM may be feasible when the Q-sense CPM device is adjusted to carry contact probes with a larger surface area and the ability to give noxious cold stimuli.

The aim of **chapter 5** was to quantify the morphological features of small nerve fibers using cornea confocal microscopy (CCM) in patients with fibromyalgia and to phenotype patients using these results and standardized quantitative sensory testing (QST) results. Small fiber pathology was detected in 51% of patients: nerve fiber length was significantly decreased in 44% of patients compared with age- and sex-matched reference values; nerve fiber density and branching were significantly decreased in 10% and 28% of patients. The combination of the CCM parameters and sensory tests for central sensitization from QST (cold pain threshold, mechanical pain threshold, mechanical pain sensitivity, allodynia and wind-up), yielded four phenotypes of fibromyalgia patients in a subgroup analysis: a group with normal cornea morphology without (group 1) and with (group 2) signs of central sensitization, and a group with abnormal cornea morphol-

ogy parameters without (group 3) and with (group 4) signs of central sensitization. In conclusion, half of the tested fibromyalgia population demonstrates signs of small fiber pathology as measured by CCM. The four distinct phenotypes suggest possible differences in disease mechanisms and may require different treatment approaches.

In **chapter 6** we performed CCM and QST in 107 patients with diabetes mellitus type 2 (DM) or sarcoidosis to assess presence of small fiber pathology and to construct phenotypes of neuropathic pain. Small fiber neuropathy is a complication of sarcoidosis and DM and may cause severe pain in a subgroup of patients. Abnormalities in cornea nerve fiber morphology were observed in 55% of patients with DM and in 45% of patients with sarcoidosis. The distribution of QST abnormalities was similar between the diabetes and sarcoidosis cohort. QST parameters indicative of central sensitization were abnormal in 73% of DM patients and 52% of sarcoidosis patients. Based on the presence of abnormal CCM values and signs of central sensitization we identified four distinct phenotypes similar to those described in chapter 5 for fibromyalgia. The distribution of patients over the four phenotypes was similar for the diabetes and sarcoidosis cohort.

The results of Chapters 5 and 6 indicate that patients with distinct uniform phenotypes may be defined with homogeneous patterns of somatosensory symptoms. These phenotypes are identical in various disease states of different etiologies and have similar distributions.

GENERAL DISCUSSION

Assessment of pain perception

Perception of pain is influenced by many factors and is difficult to assess, as there is no objective measurement instrument to register pain. The numerical rating scale (NRS), visual analogue scale (VAS) and other pain scales have been studied extensively (**chapter 2**), but adequacy of pain evaluation remains a matter of debate. Our findings of good consistency in pain rating using the NRS are in agreement with those of others [6,7] and indicate that the NRS is a valid tool to describe pain and pain relief in healthy subjects. However, chronic pain patients are less able to grade random noxious stimuli (chapter 2). In a separate study we recently showed that obese individuals have a reduced ability to grade pain as well [8]. For both populations this may be related to peripheral nerve damage causing a reduced ability to discriminate between stimuli of different intensities, or to diminished cognitive function, which has been demonstrated in both chronic pain and obese populations [7,9-11] . In addition, we showed that opioids negatively influence pain rating in healthy subjects and chronic pain patients. Thus, for populations such as chronic pain patients or otherwise cognitively impaired patients, and patients receiving

opioid treatment, additional pain scoring systems might be necessary to obtain an accurate assessment of pain intensity, or evaluate the psychological and functional impact of chronic pain. In the postsurgical setting where decisions on analgesic treatments rely on the pain NRS, an additional inquiry may be the mere question whether more analgesics are desired, because patients may interpret the NRS differently than health care professionals [12]. For chronic pain, examples of additional pain scoring systems may be an 'irritation factor scale', to give an indication of the psychological effect of ongoing pain, or a quality of life questionnaire. It is important to realize that in evaluations of analgesic treatments, the pain intensity after weeks of treatment might be at the same level as prior to the treatment, while the activity level of the patient has increased. Thus, with the same level of pain, the patient is able to perform more tasks or enjoy more activities. This might indicate that the analgesic treatment is effective, even though this would not be concluded from assessing pain levels only. Activity level assessment methods and quality of life questionnaires have been developed and validated [13-15] and should be used in combination with pain level assessments by NRS to gain a more complete understanding of an individual's pain perception and to evaluate analgesic treatments.

Phenotyping neuropathic pain patients

The difficulty of measuring pain and interpreting the test results are illustrated by the effort it takes to determine the mechanism of altered pain perception and altered pain modulation in patients with chronic neuropathic pain. Static and dynamic QST, neuropathic pain questionnaires and CCM yield information on the structure and function of nerve fibers that hint towards the underlying pathophysiology of pain generation. However, distinct measurements indicative of the same pathology often demonstrate a lack of correlation, impeding firm conclusions on somatosensory pathology in individual patients (**chapter 5**) [16,17]. Therefore, it is useful to construct phenotypes from the information gained from sensory testing, questionnaires and nerve morphology measurements and to subgroup patients accordingly. The ultimate goal is to link the phenotypes to one or more pain mechanisms to treat patients with that phenotype with mechanism-based therapies. One of the difficulties of this approach is to define on which determinants the phenotypes need to be based. Thus far, phenotyping has been performed based on many different test outcomes. Examples include distinctions based on (1) small fiber pathology and signs of central sensitization (this thesis, chapters 5 and 6); (2) the pain dimensions provoked, deep, and pinpoint pain derived from the Neuropathic Pain Symptom Inventory (NPSI) and the pain dimensions cold-evoked pain and touch-evoked pain derived from QST [16]; (3) the quality of pain in the PainDetect questionnaire as burning, prickling or coming in attacks, pain from thermal or pressure stimuli and the presence of allodynia and numbness [18]; (4) the presence or absence of small fiber pathology [19]; (5) functionality of cutaneous nociceptors assessed by pain rat-

ing responses to topical capsaicin [20]; (6) the presence of wind-up as a sign of central pain facilitation [21]; (7) sensory loss [22], sensory loss with allodynia [23] or numbness without allodynia [21] defining the deafferentation phenotype; and (8) tingling/prickling sensations with burning pain and paroxysm without numbness, defining the 'irritable nociceptor' phenotype [21]. Other authors defined the irritable nociceptor phenotype by "preserved small-fibre function (cold, warm, and pinprick sensitivity) together with hyperalgesia" [22] or "normal thermal detection thresholds indicative of preserved small fiber function, in combination with reduced mechanical detection thresholds, reduced pain thresholds or allodynia" [24] or "allodynia in the absence of sensory loss" [23]. Although the variety in methods and identified phenotypes may partly lie in the variety of underlying diseases that were studied, the plethora of phenotype determinants indicate the difficulty to match signs and symptoms with pathophysiological mechanisms of pain.

A similar concern exists in the assessment of small fiber pathology. The assessment of intraepidermal nerve fiber density (IENFD) in skin biopsies from the thigh or calf used to be the sole method to confirm the diagnosis of small fiber neuropathy in patients with neuropathic pain [25]. However, during the past decade, CCM has been developed to image the corneal sub-basal nerve plexus in Bowman's layer and detect abnormalities in small nerve fibers, specifically in nerve fiber density, nerve fiber branching and nerve fiber length, in a non-invasive manner [26-28]. Both methods have been described to have a good correlation with neuropathy severity [26,29,30], although for CCM parameters a lack of correlation has also been reported [31]. Few studies have compared IENFD and CCM parameters directly, and while some studies found a clear correlation between IENFD and CCM indices [32,33], others did not [34]. An additional analysis of available CCM and IENFD data from our population of diabetes and sarcoidosis patients (unpublished analysis) demonstrated that CCM and skin biopsy partly identified the same, and partly identified different patients with reduced small nerve fibers. A regression analysis showed a lack of correlation between the two measures in sarcoidosis patients and a limited correlation in diabetes patients (Figure 1). A possible explanation for this discrepancy may be the patchy nature in which neuropathy can sometimes be present, especially in sarcoidosis. Another explanation may be that nerves innervating the eye may be affected differentially or with a different time course than nerves innervating the skin, as the eye has a specific local environment and is less well vascularized [35]. Notwithstanding, both IENFD and CCM parameters indicate pathology of small nerve fibers. Corresponding and non-corresponding measurements in distinct patients possibly reflect different pathophysiological mechanism, or different disease stages. Therefore, rather than being an alternative, CCM might be an addition to skin biopsies in the range of available tools to evaluate small fiber pathology.

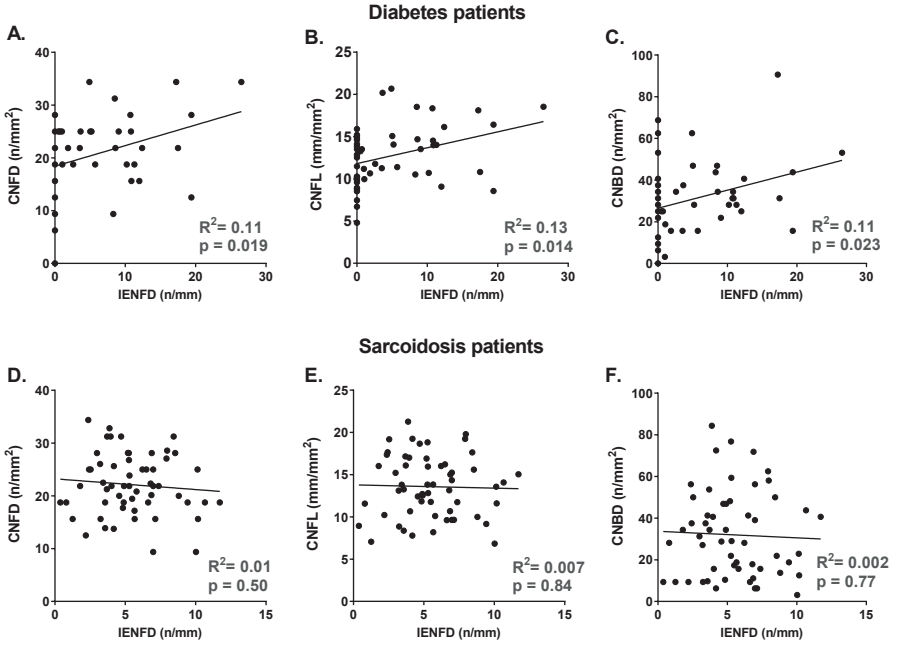


Figure 1. Correlations between cornea confocal microscopy (CCM) parameters cornea nerve fiber density (CNFD), cornea nerve fiber length (CNFL), cornea nerve branching density (CNBD) and intraepidermal nerve fiber density (IENFD) assessed in skin biopsies for **(A, B and C)** diabetes patients, and **(D, E, and F)** sarcoidosis patients.

The many ways in which functional and morphological nerve qualities are used to construct phenotypes illustrates the complex nature of neuropathic pain and suggests that multiple pathological processes may converge in individual patients. We applied a straightforward phenotyping strategy using only signs of small fiber pathology combined with signs of central sensitization (or the absence of those signs). In the aforementioned phenotyping studies, several other strategies were employed that yielded similar phenotypes to ours. Possibly four general categories might suffice to produce relevant subgroups that are homogenous in symptoms and mechanism of disease: (1) pain from peripheral, central or both etiologies; (2) presence or absence of small fiber pathology / sensory loss; (3) Aβ, Aδ and/or C fiber dysfunction; (4) presence or absence of hyperalgesia or allodynia. All etiologies of pain generation can be incorporated in these four categories, regardless of the source of information (questionnaires, QST, etc.). Individual patients can be described according to each of these categories, resulting in a clear, comprehensive phenotype. For example, the following phenotype might emerge: Pain from central etiology without small fiber pathology, but with sensory loss due to Aβ dysfunction, with presence of allodynia. As an alternative method to categorize patients

in specific phenotypes, the drug target could serve as the reference point. This is attractive especially for evaluation of treatment efficacy. This method has been used in the study of Campbell *et al.,* [20] who evaluated functionality of cutaneous nociceptors by assessment of pain rating responses after topical application of capsaicin, and showed that clonidine efficacy was related to functionality of the nociceptors.

FUTURE PERSPECTIVES

To meet the need for novel therapies that will improve overall treatment efficacy in diverse neuropathic pain conditions, clinical trial design should be optimized to identify compounds directed against mechanism-based molecular targets. This may be realized by performing clinical trials based on neuropathic pain phenotypes instead of clinical trials based on the underlying disorder. A number of important issues need to be addressed. First, studies should be prospective and well-designed with large groups of patients providing enough power to detect significant effects. This might be easier to achieve when patients of multiple disorders with similar phenotypes are included. Second, phenotyping patients should be more standardized according to the above mentioned criteria as this will yield subgroups that may specifically benefit from particular classes of drugs. In addition, when a central component of pain generation is present, dynamic tests (CPM, OA and temporal summation) may be used to further specify which central mechanisms play a role in the generation of pain. For example, a patient with a pain modulatory phenotype with reduced pain inhibition as assessed by CPM, rather than a pain facilitation phenotype as assessed by the temporal summation (wind-up) test in QST, may benefit more from noradrenaline reuptake inhibitors than patients with other phenotypes [36,37]. However, for CPM to be more useful, the large variation in CPM methodology needs to be addressed and standardized protocols need to be implemented allowing reduction of within- and between-subject variability as well as between-study variability. Third, CCM and skin biopsies can be used in combination to assess small fiber pathology.

CONCLUSIONS FROM THIS THESIS

- The numerical rating scale is a valid tool to describe pain and pain relief in healthy subjects. However, for populations such as chronic pain patients or patients receiving opioid treatment additional pain evaluation systems are necessary to improve the assessment of pain and the assessement of the impact of chronic pain.

- To be clinically useful, conditioned pain modulation methodology needs to be more consistent across studies. A conditioned pain modulation paradigm using cold water as conditioning stimulus at the contralateral side of the test stimulus is recommended for future studies.

- Pain perception in patients with fibromyalgia is augmented by multiple pathophysiological mechanisms illustrated by hyperalgesia to heat, electrical and mechanical stimuli, the presence of small fiber pathology, signs of central sensitization and reduced pain inhibition. Reduced pain inhibition can be measured by the offset analgesia paradigm and influences the onset and offset of pain.

- Patients with fibromyalgia are heterogeneous in their signs and symptoms and can be assigned to four distinct phenotypes. The different phenotypes suggest alternative disease mechanisms and may require different treatment approaches.

- Cornea confocal microscopy and skin biopsy partly identify different patients with small fiber pathology, therefore, cornea confocal microscopy may be used as an addition to skin biopsy in phenotyping patients with painful neuropathy.

- Phenotyping of neuropathic pain patients allows the separation of heterogeneous chronic pain populations into subgroups with more homogeneous patterns of somatosensory symptoms that may be expected to show differential responses to pain mechanism-based medication.

REFERENCES

1. Ossipov MH, Dussor GO, Porreca F: Central modulation of pain. The Journal of clinical investigation 2010, 120:3779-87.
2. Price DD, Hayes RL, Ruda M, Dubner R: Spatial and temporal transformations of input to spinothalamic tract neurons and their relation to somatic sensations. Journal of neurophysiology 1978, 41: 933-47.
3. Price DD, Hu JW, Dubner R, Gracely RH: Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. Pain 1977, 3:57-68.
4. Wall PD: The laminar organization of dorsal horn and effects of descending impulses. The Journal of physiology 1967, 188:403-23.
5. Woolf CJ: Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011, 152:S2-15.
6. DeLoach LJ, Higgins MS, Caplan AB, Stiff JL: The visual analog scale in the immediate postoperative period: intrasubject variability and correlation with a numeric scale. Anesthesia and analgesia 1998, 86:102-6.
7. Wolrich J, Poots AJ, Kuehler BM, Rice AS, Rahman A, Bantel C: Is number sense impaired in chronic pain patients? British journal of anaesthesia 2014, 113:1024-31.
8. Torensma B, Oudejans, L.C.J., van Velzen, M., Olofsen, E., Niesters, M., Swank, D., Dahah, A.: Pain sensitivity and pain grading in morbid obesity. Pain Reports submitted.
9. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR: Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. The Journal of neuroscience : the official journal of the Society for Neuroscience 2004, 24:10410-5.
10. Bocarsly ME, Fasolino M, Kane GA, LaMarca EA, Kirschen GW, Karatsoreos IN, McEwen BS, Gould E: Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function. Proceedings of the National Academy of Sciences of the United States of America 2015, 112: 15731-6.
11. Prickett C, Brennan L, Stolwyk R: Examining the relationship between obesity and cognitive function: a systematic literature review. Obesity research & clinical practice 2015, 9:93-113.
12. van Dijk JF, van Wijck AJ, Kappen TH, Peelen LM, Kalkman CJ, Schuurmans MJ: Postoperative pain assessment based on numeric ratings is not the same for patients and professionals: a cross-sectional study. International journal of nursing studies 2012, 49:65-71.
13. EuroQol G: EuroQol--a new facility for the measurement of health-related quality of life. Health policy 1990, 16:199-208.
14. Guyatt GH, Sullivan MJ, Thompson PJ, Fallen EL, Pugsley SO, Taylor DW, Berman LB: The 6-minute walk: a new measure of exercise capacity in patients with chronic heart failure. Canadian Medical Association journal 1985, 132:919-23.
15. Ware JE, Jr., Sherbourne CD: The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Medical care 1992, 30:473-83.
16. Freeman R, Baron R, Bouhassira D, Cabrera J, Emir B: Sensory profiles of patients with neuropathic pain based on the neuropathic pain symptoms and signs. Pain 2014, 155:367-76.
17. Tampin B, Briffa NK, Slater H: Self-reported sensory descriptors are associated with quantitative sensory testing parameters in patients with cervical radiculopathy, but not in patients with fibromyalgia. European journal of pain 2013, 17:621-33.

18. Baron R, Tolle TR, Gockel U, Brosz M, Freynhagen R: A cross-sectional cohort survey in 2100 patients with painful diabetic neuropathy and postherpetic neuralgia: Differences in demographic data and sensory symptoms. Pain 2009, 146:34-40.
19. Giannoccaro MP, Donadio V, Incensi A, Avoni P, Liguori R: Small nerve fiber involvement in patients referred for fibromyalgia. Muscle & nerve 2014, 49:757-9.
20. Campbell CM, Kipnes MS, Stouch BC, Brady KL, Kelly M, Schmidt WK, Petersen KL, Rowbotham MC, Campbell JN: Randomized control trial of topical clonidine for treatment of painful diabetic neuropathy. Pain 2012, 153:1815-23.
21. Holbech JV, Bach FW, Finnerup NB, Jensen TS, Sindrup SH: Pain phenotype as a predictor for drug response in painful polyneuropathy A retrospective analysis of data from controlled clinical trials. Pain 2016.
22. Themistocleous AC, Ramirez JD, Shillo PR, Lees JG, Selvarajah D, Orengo C, Tesfaye S, Rice AS, Bennett DL: The Pain in Neuropathy Study (PiNS): a cross-sectional observational study determining the somatosensory phenotype of painful and painless diabetic neuropathy. Pain 2016, 157: 1132-45.
23. Fields HL, Rowbotham M, Baron R: Postherpetic neuralgia: irritable nociceptors and deafferentation. Neurobiology of disease 1998, 5:209-27.
24. Demant DT, Lund K, Vollert J, Maier C, Segerdahl M, Finnerup NB, Jensen TS, Sindrup SH: The effect of oxcarbazepine in peripheral neuropathic pain depends on pain phenotype: a randomised, double-blind, placebo-controlled phenotype-stratified study. Pain 2014, 155:2263-73.
25. Lauria G, Hsieh ST, Johansson O, Kennedy WR, Leger JM, Mellgren SI, Nolano M, Merkies IS, Polydefkis M, Smith AG, Sommer C, Valls-Sole J, European Federation of Neurological S, Peripheral Nerve S: European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. European journal of neurology 2010, 17:903-12, e44-9.
26. Malik RA, Kallinikos P, Abbott CA, van Schie CH, Morgan P, Efron N, Boulton AJ: Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. Diabetologia 2003, 46:683-8.
27. Oliveira-Soto L, Efron N: Morphology of corneal nerves using confocal microscopy. Cornea 2001, 20:374-84.
28. Tavakoli M, Quattrini C, Abbott C, Kallinikos P, Marshall A, Finnigan J, Morgan P, Efron N, Boulton AJ, Malik RA: Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes care 2010, 33:1792-7.
29. Ahmed A, Bril V, Orszag A, Paulson J, Yeung E, Ngo M, Orlov S, Perkins BA: Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. Diabetes care 2012, 35:821-8.
30. Petropoulos IN, Alam U, Fadavi H, Asghar O, Green P, Ponirakis G, Marshall A, Boulton AJ, Tavakoli M, Malik RA: Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. Diabetes care 2013, 36:3646-51.
31. Maier C, Krumova, E., Mainka, T., Schargus, M.: Quantitative sensory testing and confocal corneal microscopy: Indications, methodology, interpretation, and pitfalls. Washington, D.C.: IASP Press, 2014.
32. Brines M, Swartjes, M., Tannemaat M.R., Dunne, A., van Velzen, M., Proto, P., Hoitsma, E., Petropoulos, I., Chen, X., Niesters, M., Dahan, A., Malik R., Cerami, A. : Corneal nerve quantification predicts the severity of symptoms in sarcoidosis patients with painful neuropathy. Technology 2013, 1:1-7.

33. Quattrini C, Tavakoli M, Jeziorska M, Kallinikos P, Tesfaye S, Finnigan J, Marshall A, Boulton AJ, Efron N, Malik RA: Surrogate markers of small fiber damage in human diabetic neuropathy. Diabetes 2007, 56:2148-54.
34. Ziegler D, Papanas N, Zhivov A, Allgeier S, Winter K, Ziegler I, Bruggemann J, Strom A, Peschel S, Kohler B, Stachs O, Guthoff RF, Roden M, German Diabetes Study G: Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. Diabetes 2014, 63:2454-63.
35. Eghrari AO, Riazuddin SA, Gottsch JD: Overview of the Cornea: Structure, Function, and Development. Progress in molecular biology and translational science 2015, 134:7-23.
36. Vaegter HB, Graven-Nielsen T: Pain modulatory phenotypes differentiate subgroups with different clinical and experimental pain sensitivity. Pain 2016.
37. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y: Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 2012, 153:1193-8.

Hoofdstuk 8

Nederlandse samenvatting, algemene discussie en conclusies

SAMENVATTING

Pijn is een ingewikkelde sensatie die beïnvloed wordt door factoren als eerdere ervaringen, verwachtingen, aandacht, gemoedstoestand, genetische achtergrond, fysiologie van het zenuwstelsel en neurochemische en anatomische variatie. Het pijnsysteem bestaat uit perifere zenuwen die connecties maken met zenuwen in het ruggenmerg, die vervolgens het pijnsignaal verder geleiden naar verschillende breinregio's die betrokken zijn bij de waarneming van pijn. Vanuit deze breinregio's worden via dalende zenuwbanen signalen teruggestuurd naar het ruggenmerg, waar binnenkomende pijnsignalen worden gemoduleerd in de dorsale hoorn. Zowel pijn inhibitie als pijn facilitatie kan plaatsvinden en wordt 'dalende inhibitie' en 'dalende facilitatie' genoemd [1-5]. Chronische pijn kan ontstaan als gevolg van inadequate dalende inhibitie of versterkte facilitatie, wordt vaak verder versterkt door centrale sensitisatie en is gerelateerd aan aanhoudende afferente nociceptische input. Deze aanhoudende input kan optreden door een plaatselijke laesie of door een systemische ziekte die dikke en/of dunne vezel pathologie veroorzaakt. Op deze manier dragen vaak zowel perifere als centrale mechanismen bij aan de chronificatie van pijn.

In de kliniek en tijdens experimenteel onderzoek kunnen acute en chronische pijn worden onderzocht met behulp van een aantal instrumenten, waaronder neurologische testen, vragenlijsten en kwantitatief sensorisch testen (QST). Statische testen zoals gevoeligheid voor scherpe pin prikken, warmtedetectie en pijndrempels kunnen worden uitgevoerd, evenals dynamische testen die een indicatie geven van de staat van het endogene pijnstillingssysteem. Voorbeelden van dynamische testen zijn geconditioneerde pijn modulatie (CPM) en 'offset analgesie' (OA). CPM wordt uitgevoerd door het toedienen van een tweede nociceptische stimulus op een andere locatie dan de eerste. Bij gezonde proefpersonen remt deze tweede stimulus de primaire nociceptische stimulus. OA wordt uitgevoerd door een nociceptische hittestimulus met eén graad Celsius te verlagen, wat een onproportioneel grote reductie in pijnperceptie tot gevolg heeft. Verder kan de structuur en functie van dikke en dunne zenuwvezels worden bepaald door neurofysiologische testen (functie van dikke vezels) en cornea confocal microscopy en huidbiopten. Cornea confocal microscopy (CCM) visualiseert de dunne zenuwvezels die de cornea innerveren. Uit huidbiopten kan de intra-epidermale zenuwvezeldichtheid worden bepaald.

Het combineren van bovenstaande testresultaten maakt het mogelijk om chronische pijn patiënten te karakteriseren en om een neuropatische pijn fenotype van een individuele patiënt te construeren. De heterogene neuropatische pijn populatie kan zodoende verdeeld worden in meer homogene cohorten, waardoor het mogelijk wordt subgroepen apart te evalueren op het gebied van pathofysiologische mechanismen en behandelingsmogelijkheden. In dit proefschrift worden studies beschreven waarin de

voorgenoemde technologieën zijn toegepast om de nociceptische staat te kenschetsen van zowel gezonde vrijwilligers als chronische pijn patiënten met fibromyalgie, diabetes mellitus type 2 en sarcoïdose. De testen die zijn uitgevoerd zijn CPM, OA, en QST; huidbiopten en vragenlijsten zijn afgenomen.

In **hoofdstuk 2** hebben we het vermogen bepaald van acute en chronische pijn patiënten om een cijfer te geven aan pijn op een 11-punts numerieke schaal (NRS) waarbij '0' 'geen pijn' betekent, en '10' 'de ergst denkbare pijn'. Instrumenten die gebaseerd zijn op getallen zoals de NRS worden vaak gebruikt om pijnperceptie en het effect van pijninterventies te bepalen. Onze focus lag op patiënten met acute pijn na een chirurgische ingreep en patiënten met fibromyalgie, een aandoening met onbekende oorzaak voornamelijk getypeerd door wijdverbreide pijn en vermoeidheid. We hebben een techniek ontwikkeld waarbij proefpersonen gerandomiseerd hittepijn- (Hp) en elektrische pijn- (Ep) stimuli kregen toegediend die tussen de pijndrempel en de pijntolerantie lagen. De respons van de proefpersoon werd gescoord op een 11-punts NRS. Na het meten van de basiswaarden werd het effect van behandeling met opioïden op het scoren van pijn bepaald. De data werden geanalyseerd door een penalty-score systeem, gebaseerd op de veronderstelling dat een stimulus met een hogere intensiteit gescoord wordt met een hogere NRS. De data werden gestratificeerd in cohorten die overeenkomen met "goed", "middelmatig" en "slecht" scoren. Gezonde controles waren goed in staat om pijn te scoren met 73% (Hp) en 81% (Ep) van de proefpersonen gecategoriseerd in het cohort "goed". De hittepijn scores, maar niet de elektrische pijn scores van fibromyalgie patiënten waren significant slechter met 45% (Hp, p=0.03 *vs.* controles) en 67% (Ep) van de patiënten in het cohort "goed". Bij gezonde vrijwilligers verslechterde het scoren tijdens het toedienen van opioïden, waardoor er slechts 40% (Hp, p=0.015 *vs.* basis waarden) en 70% (Ep) van de proefpersonen in het cohort "goed" overbleven. Vergelijkbare resultaten werden gezien bij fibromyalgie patiënten (Hp p=0.02) maar niet bij postoperatieve pijn patiënten. Onze bevindingen komen overeen met die van anderen en geven aan dat de NRS een valide instrument is om acute pijn en pijnverlichting in gezonde personen te beschrijven. De conclusie van ons onderzoek is dat de consistentie in het scoren van pijn met behulp van een NRS hoog is in gezonde vrijwilligers maar vermindert in chronische pijn patiënten en tijdens het toedienen van opioïden aan gezonde vrijwilligers en chronische pijn patiënten, maar niet aan acute pijn patiënten.

In **hoofdstuk 3** wordt de aanwezigheid van offset analgesie (OA) in fibromyalgie patiënten geanalyseerd en vergeleken met gezonde geslacht- en leeftijd-gepaarde vrijwilligers. OA werd geïnduceerd door een pijnlijke hitte-stimulus op de arm toe te dienen die een elektronische visuele analoge score (eVAS) van ongeveer 50 mm veroorzaakte (op een elektronische schaal van 0-100 mm, geheel links = 'geen pijn', geheel rechts

= 'ergst denkbare pijn'), gevolgd door een verlaging van de temperatuur met 1°C. De offset analgesie respons wordt gedefinieerd door de afname van de visuele analoge pijn score die door de verlaging van de stimulus temperatuur met 1°C wordt veroorzaakt. Om te bepalen of de OA respons verbeterd kon worden werden nog 2 testen uitgevoerd: herhaling van het OA model en neerwaartse stappen van 1°C na een initiële OA test (downward steps test). Om te bepalen of OA de 'onset' van pijn beïnvloedt, werden er OA stappen van oplopende temperatuur toegediend (upward OA steps test), en vergeleken met een test met een continu oplopende temperatuur. Fibromyalgie patiënten hadden een verminderde OA respons met een reductie in eVAS van 65.3% ± 4.5 (gemiddelde ± SD) versus gezonde vrijwilligers 97.8% ± 4.6 ($p<0.001$). Verminderde OA responsen verbeterden of herstelden niet door herhaling van het OA model of door de downward steps test. Het verminderde vermogen om OA te activeren had een significant effect op de onset van pijn, zoals blijkt uit de resultaten van de upward steps test. Dit wordt geïllustreerd door de vervroegde onset van pijn in fibromyalgie patiënten vergeleken met gezonde vrijwilligers, in relatie tot de ramp test. We concluderen dat fibromyalgie patiënten minder pijn inhibitie tonen gemeten door het OA model, en dat dit zowel de onset als de offset van pijn beïnvloedt.

Geconditioneerde pijn modulatie (CPM) is een model dat gebruikt wordt voor het evalueren van het vermogen van het lichaam om binnenkomende pijnsignalen te moduleren. Echter, het vergelijken van studies die CPM modellen gebruiken is nagenoeg onmogelijk vanwege de grote variatie in methodologie. Er is grote behoefte aan gestandaardiseerde methoden en materialen die praktisch zijn in gebruik zowel in de kliniek als voor onderzoeksdoeleinden. Het doel van **hoofdstuk 4** was om te onderzoeken of een apparaat met 2 contact-warmte thermodes, de Q-sense CPM (waarmee zowel de test stimulus als de conditionerende stimulus wordt gegeven), een even groot CPM effect kon induceren als het vaak gebruikte koud-water-bad als conditionerende stimulus. Om CPM effecten te bepalen werden een klassiek 30 seconden CPM model (CPM30) en een 3x10 seconden CPM model (CPM10) gebruikt. De conditionerende stimulus werd in beide modellen zowel aan de ipsilaterale als aan de contralaterale zijde van de test stimulus geplaatst. In tegenstelling tot het koud-water-bad, induceerde de Q-sense CPM significante CPM responsen in geen enkele van de modellen. CPM effecten (vermindering in pijn scores) waren 10% ($p=0.20$) en 7% ($p=0.34$) (contralaterale conditionerende stimulus, CPM30 en CPM10) en -4% ($p=0.86$) en -0.2% ($p=0.48$) (ipsilaterale conditionerende stimulus, CPM30 en CPM10). Voor het koud-water-bad waren deze effecten respectievelijk 17% ($p=0.01$) en 25% ($p=0.04$), en 19% ($p=0.00$) en 14% ($p=0.045$). Voor toekomstig CPM onderzoek raden wij een CPM model aan met gebruik van een koud-water-bad als conditionerende stimulus aan de contralaterale zijde van de test stimulus. De voornaamste conclusie van dit hoofdstuk is dat het gebruik van 2 contact thermodes om CPM te induceren zou

kunnen slagen als de Q-sense CPM wordt aangepast met contact thermodes met een groter oppervlak en de mogelijkheid om koude pijn stimuli te kunnen geven.

Het doel van **hoofdstuk 5** was het kwantificeren van de morfologische kenmerken van dunne zenuwvezels met behulp van cornea confocal microscopy (CCM) in patiënten met fibromyalgie, en om patiënten met de resultaten hiervan en de resultaten van gestandaardiseerd kwantitatief sensorische testen (QST) te fenotyperen. Dunne vezel pathologie werd gevonden in 51% van de patiënten: zenuwvezellengte was significant verlaagd in 44% van de patiënten in vergelijking met geslacht- en leeftijd-gepaarde referentiewaarden; zenuwvezeldichtheid en –vertakkingen waren significant verlaagd in 10% en 28% van de patiënten. De combinatie van de CCM parameters en sensorische testen voor centrale sensitisatie uit de QST testbatterij (koude pijndrempel, mechanische pijndrempel, mechanische pijn sensitiviteit, allodynie en wind-up), brachten vier fenotypen fibromyalgie patiënten voort in een subgroep analyse: een groep met *normale* cornea morfologie zonder (groep 1) en met (groep 2) tekenen van centrale sensitisatie, en een groep met *abnormale* cornea morfologie zonder (groep 3) en met (groep 4) tekenen van centrale sensitisatie. Concluderend heeft de helft van de geteste fibromyalgie populatie tekenen van dunne vezel pathologie gemeten m.b.v. CCM. De vier fenotypen impliceren eventuele verschillen in ziektemechanismen en patiënten met verschillende fenotypen hebben mogelijk verschillende behandelingen nodig.

In **hoofdstuk 6** hebben we CCM en QST uitgevoerd bij 107 patiënten met diabetes mellitus type 2 (DM) of sarcoïdose om de aanwezigheid van dunne vezel pathologie vast te stellen en om neuropatische pijn fenotypen te construeren. Dunne vezel neuropathie is een complicatie van sarcoïdose en DM en kan ernstige pijn veroorzaken in subgroepen patiënten. Afwijkingen in cornea zenuwvezel morfologie werden geobserveerd in 55% van DM patiënten en 45% van patiënten met sarcoïdose. De distributie van afwijkingen in QST resultaten waren vergelijkbaar tussen het diabetes en sarcoïdose cohort. QST parameters die een indicatie zijn van centrale sensitisatie waren afwijkend in 73% van DM patiënten en 52% van patiënten met sarcoïdose. Op basis van afwijkende CCM waarden en tekenen van centrale sensitisatie werden vier fenotypen geïdentificeerd, gelijk aan de vier fenotypen voor fibromyalgie zoals beschreven in hoofdstuk 5. De distributie van DM patiënten en patiënten met sarcoïdose over de vier fenotypen was vergelijkbaar.

De resultaten van hoofdstuk 5 en 6 duiden aan dat patiënten met specifieke uniforme fenotypen kunnen worden geïdentificeerd die homogene patronen van somatosensorische symptomen vertonen. Deze fenotypen zijn identiek in aandoeningen of ziekten met verschillende oorzaken en hebben vergelijkbare distributies.

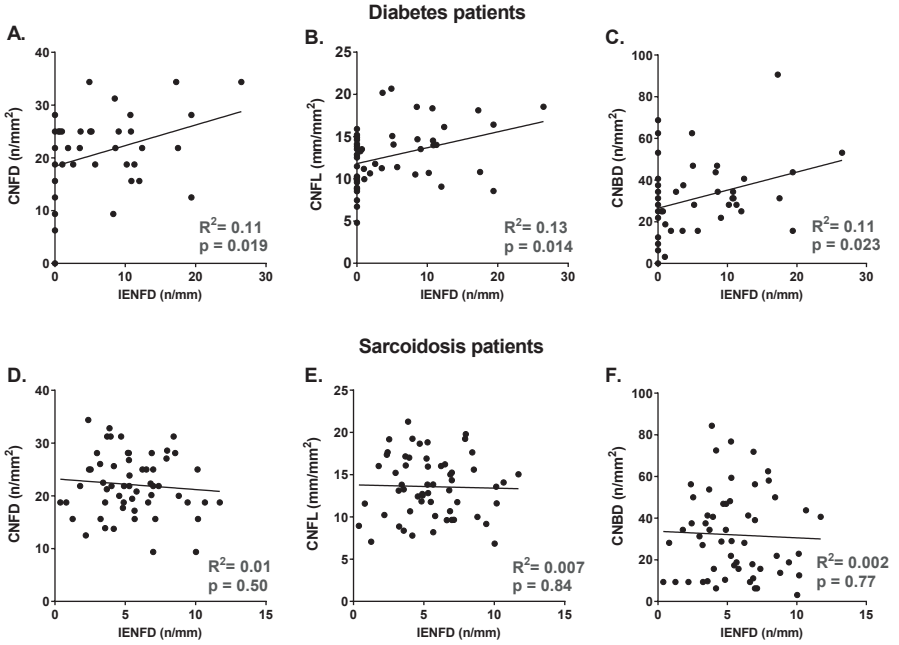
ALGEMENE DISCUSSIE

Meten van pijnperceptie

De perceptie van pijn wordt beïnvloed door vele factoren en is moeilijk om te meten aangezien er geen objectief meetinstrument bestaat om pijn te registreren. De numerieke schaal (NRS), visuele analoge schaal (VAS) en andere pijnschalen zijn uitgebreid onderzocht (**hoofdstuk 2**), maar de doelmatigheid van het evalueren van pijn blijft een veelbesproken kwestie. Onze bevinding van goede consistentie in het scoren van pijn bij gebruik van de NRS komt overeen met dat van anderen [6,7] en geeft aan dat de NRS een valide instrument is om pijn en pijnverlichting in gezonde proefpersonen te beschrijven. Echter, chronische pijn patiënten zijn minder goed in staat om gerandomiseerde pijnlijke prikkels te scoren (**hoofdstuk 2**). In een afzonderlijke studie hebben we recentelijk aangetoond dat personen met obesitas ook minder goed in staat zijn om pijn te scoren [8]. Dit is voor beide populaties mogelijk gerelateerd aan perifere zenuwschade dat een verminderd vermogen veroorzaakt om onderscheid te maken tussen stimuli van verschillende intensiteit. Het kan echter ook gerelateerd zijn aan verminderde cognitieve functie, wat in beide populaties is aangetoond [7,9-11]. Verder laten we zien dat opioïden het scoren van pijn negatief beïnvloeden in gezonde proefpersonen en in chronische pijn patiënten. Voor populaties zoals chronische pijn patiënten of andere patiënten met verminderde cognitieve functie, en voor patiënten die behandeld worden met opioïden zal er dus een extra pijnscoringssysteem nodig zijn om pijnintensiteit accuraat vast te stellen, of om de psychologische en functionele gevolgen van chronische pijn te beoordelen. In de postoperatieve setting waar beslissingen over pijnbehandelingen afgestemd worden op de pijn NRS, kan additionele informatie verkregen worden door de simpele vraag of meer pijnstillende middelen gewenst zijn, want patiënten kunnen de NRS soms anders interpreteren dan professionals in de gezondheidszorg [12]. Additionele pijnscoringssystemen voor chronische pijn zouden bijvoorbeeld kunnen zijn: een 'irritatie factor schaal', om een indicatie te geven van het psychologische effect van de constante aanwezigheid van pijn, of een kwaliteit-van-leven vragenlijst. Voor het evalueren van pijnbehandelingen is het belangrijk om zich te realiseren dat wanneer de pijnintensiteit na een wekenlange behandeling op hetzelfde niveau is als vóór de behandeling, het activiteitsniveau van de patiënt daarbij eventueel wèl gestegen kan zijn. Dus, bij hetzelfde pijnniveau kan de patiënt meer taken uitvoeren of meer genieten van dagelijkse activiteiten. Mogelijkerwijs is dan de pijnbehandeling wel effectief, terwijl dit niet geconcludeerd zou worden uit het meten van alleen het pijnniveau. Methoden om activiteitsniveau en kwaliteit-van-leven te meten zijn reeds ontwikkeld en gevalideerd [13-15] en zouden gebruikt moeten worden in combinatie met NRS pijnscores om een completer begrip te krijgen van de pijnperceptie van een individu, en om pijnbehandelingen te evalueren.

Het fenotyperen van neuropatische pijn patiënten

De moeilijkheid van het meten van pijn en het interpreteren van de testresultaten wordt geïllustreerd door de inspanningen die het kost om de mechanismen van een veranderde pijnperceptie en veranderde pijnmodulatie bij chronische pijn patiënten te bepalen. Statische en dynamische QST, neuropatische pijn vragenlijsten en CCM leveren informatie op over de structuur en het functioneren van zenuwvezels, wat indicaties geeft voor de onderliggende pathofysiologische processen van de oorzaak van pijn. Echter, resultaten van verschillende meetmethoden die indicatief zijn voor eenzelfde pathologie correleren vaak niet waardoor definitieve conclusies over de somatosensorische pathologie in individuele patiënten niet getrokken kunnen worden [16,17] (**hoofdstuk 5**). Het is daarom zinvol om fenotypen te creëren met de informatie verkregen uit sensorische testen, vragenlijsten en zenuwmorfologie metingen, en om patiënten te groeperen volgens deze fenotypen. Het uiteindelijke doel is om de fenotypen te koppelen aan eén of meerdere pijnmechanismen om vervolgens patiënten met dat fenotype te behandelen met op het pijnmechanisme gebaseerde therapieën. Een van de moeilijkheden van deze benadering is dat het lastig is vast te stellen op welke determinanten de fenotypen gebaseerd moeten worden. Tot op heden zijn fenotyperingen gebaseerd op veel verschillende testresultaten. Onderscheid in fenotypen is bijvoorbeeld gemaakt op basis van (1) dunne vezel pathologie en tekenen van centrale sensitisatie (dit proefschrift, hoofdstuk 5 en 6); (2) de pijndimensies opgewekte, diepe en scherpe prik pijn uit de Neuropathic Pain Symptom Inventory (NPSI) vragenlijst en de pijndimensies koudepijn en pijn door aanraking uit de QST [16]; (3) beschrijving van pijn in de PainDetect vragenlijst als brandend, prikkend of aanvalsgewijs, temperatuur- of drukpijn en de aanwezigheid van allodynie of een doof gevoel [18]; (4) de aan- of afwezigheid van dunne vezel pathologie [19]; (5) functionaliteit van nociceptoren in de huid bepaald aan de hand van pijnresponsen na applicatie van capsaïcine op de huid [20]; (6) de aanwezigheid van wind-up als teken van centrale pijn facilitatie [21]; (7) verlies van sensorische functie [22], verlies van sensorische functie in combinatie met allodynie [23] of een doof gevoel in combinatie met allodynie [21] (het deafferentiatie fenotype); en (8) tintelend/prikkend gevoel met brandende pijn en paroxismen zonder doof gevoel, de definitie voor het 'geïrriteerde nociceptor' fenotype [21]. Andere auteurs definieerden het geïrriteerde nociceptor fenotype met "functionele dunne vezels (koude-, warmte- en scherpe prik sensitiviteit aanwezig) in combinatie met hyperalgesie" [22] of "normale temperatuur detectie drempels als indicatie voor functionele dunne vezels, in combinatie met verlaagde mechanische detectie drempels, verlaagde pijndrempels of allodynie" [24] of "allodynie zonder verlies van sensorische functie" [23]. Hoewel de diversiteit in methoden en geïdentificeerde fenotypen deels kan liggen aan de verschillen in onderliggende ziektebeelden die bestudeerd werden, geeft het plethora aan fenotype-determinanten



Figuur 1. Correlaties tussen cornea confocal microscopy (CCM) parameters cornea zenuwvezeldichtheid (CNFD), cornea zenuwvezellengte (CNFL), cornea zenuwvertakkingsdichtheid (CNBD) en zenuwvezel -dichtheid in de epidermis (IENFD) gemeten in huidbiopten voor **(A, B and C)** diabetes patienten, en **(D, E, and F)** sarcoïdosis patienten.

aan hoe moeilijk het is om klachten en symptomen aan pathofysiologische mechanismen van pijn te koppelen.

Een vergelijkbaar probleem bestaat in het vaststellen van dunne vezel pathologie. Het bepalen van intra-epidermale zenuwvezeldichtheid (IENFD) uit huidbiopten genomen uit het dijbeen of de kuit, waren altijd de enige methode om de diagnose 'dunne vezel neuropathy' te bevestigen in patiënten met neuropatische pijn [25]. Echter, gedurende de afgelopen 10 jaar is CCM ontwikkeld als techniek waarmee de sub-basale plexus in Bowman's layer in de cornea in beeld kan worden gebracht. Hierdoor kunnen afwijkingen in dunne zenuwvezels worden gedetecteerd op een non-invasieve manier [26-28], met name zenuwvezeldichtheid, zenuwvezel vertakkingen en zenuwvezellengte. Voor beide methoden is een goede correlatie met de ernst van neuropathie beschreven [26,29,30], hoewel voor CCM parameters ook een gebrek aan correlatie is gerapporteerd [31]. Maar weinig studies hebben IENFD en CCM direct vergeleken, en terwijl sommige studies een duidelijke correlatie vonden tussen IENFD en CCM parameters[32,33], vonden andere die niet [34]. Een analyse van beschikbare CCM en IENFD data van onze populaties DM en sarcoïdosis patiënten (niet-gepubliceerde analyse) liet zien dat CCM en IENFD deels in

dezelfde, deels in andere patiënten verlaagde dunne vezel dichtheid aantoonden. Uit een regressie analyse bleek dat de twee meetwaarden van patiënten met sarcoïdose niet gecorreleerd waren, en dat er een kleine correlatie bestond tussen de waarden van DM patiënten (Figuur 1). Een mogelijke verklaring voor deze discrepantie zou kunnen zijn dat neuropathie soms pleksgewijs (patchy) voorkomt, vooral in sarcoïdose. Een andere verklaring zou kunnen zijn dat zenuwen die het oog innerveren op een andere wijze aangetast worden, of met een ander tijdsverloop dan zenuwen in de huid, omdat het oog een specifiek lokaal mileu heeft en minder goed gevasculariseerd is [35]. Desondanks zijn zowel verlaagde IENFD als CCM parameters een indicatie voor pathologie van dunne zenuwvezels. Overeenkomstige of juist niet-overeenkomstige bevindingen in specifieke patiënten weerspiegelen mogelijkerwijs verschillende pathologische mechanismen, of verschillen in stadia waarin ziekten zich bevinden. Om die reden zou CCM eerder een aanvulling op IENFD kunnen zijn dan een alternatief binnen het geheel aan beschikbare meetinstrumenten om dunne vezel pathologie vast te stellen.

De vele manieren waarop functionele en morfologische karakteristieken van zenuwvezels worden gebruikt om fenotypen te construeren, illustreert de complexe aard van neuropatische pijn en suggereert dat meerdere pathologische processen zich voordoen in individuele patiënten. Wij hebben een relatief simpele strategie toegepast waarin alleen aanwijzingen voor dunne vezel pathologie werden gecombineerd met tekenen van centrale sensitisatie (of de afwezigheid van deze tekenen). In de bovengenoemde fenotyperingstudies werden diverse andere strategieën gebruikt waarmee fenotypen gevonden werden vergelijkbaar met de onze. Mogelijk volstaan vier algemene categorieën om relevante subgroepen te vormen die homogeniteit vertonen in symptomen en ziektemechanismen: (1) pijn van perifere, centrale of beider oorsprong; (2) aan- of afwezigheid van dunne vezel pathologie / verlies van sensorische functie; (3) een Aβ, Aδ en/of C-vezel stoornis; (4) aan- of afwezigheid van hyperalgesie of allodynie. Alle oorzaken van het genereren van pijn passen in deze vier categorieën, onafhankelijk van de bron van informatie (bijv. vragenlijsten of QST). Individuele patiënten kunnen beschreven worden aan de hand van elk van deze categorieën, wat resulteert in een duidelijk en uitgebreid fenotype. Het volgende fenotype zou daar bijvoorbeeld uit kunnen voortkomen: Pijn van centrale oorsprong zonder dunne vezel pathologie maar met verlies van sensorische functie door een Aβ-vezel stoornis, met aanwezigheid van allodynie. Een mogelijk alternatief om patiënten in fenotypen in te delen, vooral als het gaat om het evalueren van behandelingsmethoden, zou kunnen zijn om het aangrijpingspunt van een medicijn als uitgangspunt te nemen. Dit is bijvoorbeeld gedaan in de studie van Campbell *et al.* [20] in relatie tot de effectiviteit van clonidine, door de functie van nociceptoren in de huid te evalueren aan de hand van gescoorde pijn na toediening

van capsaïcine op de huid. De effectiviteit van clonidine bleek te zijn gerelateerd aan de functionaliteit van de nociceptoren.

TOEKOMSTPERSPECTIEVEN

Om te kunnen voorzien in de behoefte aan nieuwe therapieën ter verhoging van de effectiviteit van behandelingen voor neuropatische pijn, moeten onderzoeksontwerpen voor klinisch onderzoek geoptimaliseerd worden om stoffen te identificeren die gericht zijn tegen moleculaire mechanismen van pijn. Dit kan gerealiseerd worden door klinisch onderzoek uit te voeren dat zich richt op neuropatische pijn fenotypen in plaats van op de onderliggende ziektebeelden. Een aantal belangrijke punten dienen daarbij in acht te worden genomen. Ten eerste zouden prospectieve, goed ontworpen studies moeten worden uitgevoerd met grote groepen patiënten om genoeg statistische power te hebben om significante effecten te kunnen vinden. Dit kan wellicht makkelijker worden bereikt door patiënten met verschillende aandoeningen maar vergelijkbare fenotypen te includeren. Ten tweede moet het fenotyperen van patiënten meer gestandaardiseerd worden volgens bovenstaande criteria omdat met deze fenotypen subgroepen zullen ontstaan die baat kunnen hebben bij specifieke klassen van medicatie. Bovendien kunnen dynamische tests (CPM, OA en temporal summation (wind-up)) gebruikt worden wanneer er een centrale pijn component aanwezig is, zodat gespecificeerd kan worden welke centrale mechanismen een rol spelen bij de oorzaak van pijn. Een patiënt met een pijnmodulatie fenotype 'verminderde pijn inhibitie' gemeten met CPM, zal bijvoorbeeld meer baat hebben bij noradrenaline heropnameremmers dan een patiënt met het pijnmodulatie fenotype 'pijn facilitatie' gemeten door temporal summation [36,37]. Echter, om CPM goed te kunnen gebruiken, moet eerst de grote variatie in CPM-methodologie verminderd worden, en moeten gestandaardiseerde protocollen worden toegepast om variabiliteit binnen - en tussen proefpersonen, en tussen studies te verminderen. Ten derde kunnen CCM en huidbiopten gecombineerd gebruikt worden om dunne vezel pathologie vast te stellen.

CONCLUSIES UIT DIT PROEFSCHRIFT

- De numerieke schaal is een valide instrument om pijn en pijnverlichting te beschrijven in gezonde vrijwilligers. Echter, voor populaties zoals chronische pijn patiënten of patiënten die behandeld worden met opioïden zijn additionele pijn beoordelingssystemen nodig om het meten van pijn en van de impact van chronische pijn te verbeteren.

- Voor praktisch gebruik in de kliniek moet geconditioneerde pijn modulatie methodologie consistenter worden tussen verschillende studies. Een geconditioneerde pijn modulatie model met koud water als conditionerende stimulus aan de contralaterale zijde van de test stimulus wordt geadviseerd voor toekomstige studies.

- Pijnperceptie in patiënten met fibromyalgie wordt verhoogd door meerdere pathofysiologische mechanismen, geïllustreerd door hyperalgesie voor hitte, elektrische en mechanische stimuli, de aanwezigheid van dunne vezel pathologie, tekenen van centrale sensitisatie en verminderde pijn inhibitie. Verminderde pijn inhibitie kan gemeten worden met het offset analgesie model en beïnvloedt de onset en offset van pijn.

- Patiënten met fibromyalgie zijn heterogeen in hun klachten en symptomen en kunnen worden ingedeeld in vier specifieke fenotypen. De verschillende fenotypen suggereren verschillen in ziektemechanismen en vereisen mogelijk verschillende behandelingsmethoden.

- Cornea confocal microscopy en huidbiopten identificeren deels verschillende patiënten met dunne vezel pathologie. Cornea confocal microscopy zou daarom gebruikt kunnen worden als aanvulling op huidbiopten voor het fenotyperen van patiënten met pijnlijke neuropathie.

- Het fenotyperen van neuropatische pijn patiënten maakt het mogelijk om de heterogene chronische pijn populatie te verdelen in subgroepen met meer homogene patronen van somatosensorische symptomen, waarvan verwacht kan worden dat ze verschillend reageren op medicatie gebaseerd op pijnmechanismen.

1. Ossipov MH, Dussor GO, Porreca F: Central modulation of pain. The Journal of clinical investigation 2010, 120:3779-87.
2. Price DD, Hayes RL, Ruda M, Dubner R: Spatial and temporal transformations of input to spinothalamic tract neurons and their relation to somatic sensations. Journal of neurophysiology 1978, 41: 933-47.
3. Price DD, Hu JW, Dubner R, Gracely RH: Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. Pain 1977, 3:57-68.
4. Wall PD: The laminar organization of dorsal horn and effects of descending impulses. The Journal of physiology 1967, 188:403-23.
5. Woolf CJ: Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011, 152:S2-15.
6. DeLoach LJ, Higgins MS, Caplan AB, Stiff JL: The visual analog scale in the immediate postoperative period: intrasubject variability and correlation with a numeric scale. Anesthesia and analgesia 1998, 86:102-6.
7. Wolrich J, Poots AJ, Kuehler BM, Rice AS, Rahman A, Bantel C: Is number sense impaired in chronic pain patients? British journal of anaesthesia 2014, 113:1024-31.
8. Torensma B, Oudejans, L.C.J., van Velzen, M., Olofsen, E., Niesters, M., Swank, D., Dahah, A.: Pain sensitivity and pain grading in morbid obesity. Pain Reports submitted.
9. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR: Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. The Journal of neuroscience : the official journal of the Society for Neuroscience 2004, 24:10410-5.
10. Bocarsly ME, Fasolino M, Kane GA, LaMarca EA, Kirschen GW, Karatsoreos IN, McEwen BS, Gould E: Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function. Proceedings of the National Academy of Sciences of the United States of America 2015, 112: 15731-6.
11. Prickett C, Brennan L, Stolwyk R: Examining the relationship between obesity and cognitive function: a systematic literature review. Obesity research & clinical practice 2015, 9:93-113.
12. van Dijk JF, van Wijck AJ, Kappen TH, Peelen LM, Kalkman CJ, Schuurmans MJ: Postoperative pain assessment based on numeric ratings is not the same for patients and professionals: a cross-sectional study. International journal of nursing studies 2012, 49:65-71.
13. EuroQol G: EuroQol--a new facility for the measurement of health-related quality of life. Health policy 1990, 16:199-208.
14. Guyatt GH, Sullivan MJ, Thompson PJ, Fallen EL, Pugsley SO, Taylor DW, Berman LB: The 6-minute walk: a new measure of exercise capacity in patients with chronic heart failure. Canadian Medical Association journal 1985, 132:919-23.
15. Ware JE, Jr., Sherbourne CD: The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Medical care 1992, 30:473-83.
16. Freeman R, Baron R, Bouhassira D, Cabrera J, Emir B: Sensory profiles of patients with neuropathic pain based on the neuropathic pain symptoms and signs. Pain 2014, 155:367-76.
17. Tampin B, Briffa NK, Slater H: Self-reported sensory descriptors are associated with quantitative sensory testing parameters in patients with cervical radiculopathy, but not in patients with fibromyalgia. European journal of pain 2013, 17:621-33.
18. Baron R, Tolle TR, Gockel U, Brosz M, Freynhagen R: A cross-sectional cohort survey in 2100 patients with painful diabetic neuropathy and postherpetic neuralgia: Differences in demographic data and sensory symptoms. Pain 2009, 146:34-40.

19. Giannoccaro MP, Donadio V, Incensi A, Avoni P, Liguori R: Small nerve fiber involvement in patients referred for fibromyalgia. Muscle & nerve 2014, 49:757-9.
20. Campbell CM, Kipnes MS, Stouch BC, Brady KL, Kelly M, Schmidt WK, Petersen KL, Rowbotham MC, Campbell JN: Randomized control trial of topical clonidine for treatment of painful diabetic neuropathy. Pain 2012, 153:1815-23.
21. Holbech JV, Bach FW, Finnerup NB, Jensen TS, Sindrup SH: Pain phenotype as a predictor for drug response in painful polyneuropathy A retrospective analysis of data from controlled clinical trials. Pain 2016.
22. Themistocleous AC, Ramirez JD, Shillo PR, Lees JG, Selvarajah D, Orengo C, Tesfaye S, Rice AS, Bennett DL: The Pain in Neuropathy Study (PiNS): a cross-sectional observational study determining the somatosensory phenotype of painful and painless diabetic neuropathy. Pain 2016, 157: 1132-45.
23. Fields HL, Rowbotham M, Baron R: Postherpetic neuralgia: irritable nociceptors and deafferentation. Neurobiology of disease 1998, 5:209-27.
24. Demant DT, Lund K, Vollert J, Maier C, Segerdahl M, Finnerup NB, Jensen TS, Sindrup SH: The effect of oxcarbazepine in peripheral neuropathic pain depends on pain phenotype: a randomised, double-blind, placebo-controlled phenotype-stratified study. Pain 2014, 155:2263-73.
25. Lauria G, Hsieh ST, Johansson O, Kennedy WR, Leger JM, Mellgren SI, Nolano M, Merkies IS, Polydefkis M, Smith AG, Sommer C, Valls-Sole J, European Federation of Neurological S, Peripheral Nerve S: European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. European journal of neurology 2010, 17:903-12, e44-9.
26. Malik RA, Kallinikos P, Abbott CA, van Schie CH, Morgan P, Efron N, Boulton AJ: Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. Diabetologia 2003, 46:683-8.
27. Oliveira-Soto L, Efron N: Morphology of corneal nerves using confocal microscopy. Cornea 2001, 20:374-84.
28. Tavakoli M, Quattrini C, Abbott C, Kallinikos P, Marshall A, Finnigan J, Morgan P, Efron N, Boulton AJ, Malik RA: Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes care 2010, 33:1792-7.
29. Ahmed A, Bril V, Orszag A, Paulson J, Yeung E, Ngo M, Orlov S, Perkins BA: Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. Diabetes care 2012, 35:821-8.
30. Petropoulos IN, Alam U, Fadavi H, Asghar O, Green P, Ponirakis G, Marshall A, Boulton AJ, Tavakoli M, Malik RA: Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. Diabetes care 2013, 36:3646-51.
31. Maier C, Krumova, E., Mainka, T., Schargus, M.: Quantitative sensory testing and confocal corneal microscopy: Indications, methodology, interpretation, and pitfalls. Washington, D.C.: IASP Press, 2014.
32. Brines M, Swartjes, M., Tannemaat M.R., Dunne, A., van Velzen, M., Proto, P., Hoitsma, E., Petropoulos, I., Chen, X., Niesters, M., Dahan, A., Malik R., Cerami, A. : Corneal nerve quantification predicts the severity of symptoms in sarcoidosis patients with painful neuropathy. Technology 2013, 1:1-7.
33. Quattrini C, Tavakoli M, Jeziorska M, Kallinikos P, Tesfaye S, Finnigan J, Marshall A, Boulton AJ, Efron N, Malik RA: Surrogate markers of small fiber damage in human diabetic neuropathy. Diabetes 2007, 56:2148-54.

34. Ziegler D, Papanas N, Zhivov A, Allgeier S, Winter K, Ziegler I, Bruggemann J, Strom A, Peschel S, Kohler B, Stachs O, Guthoff RF, Roden M, German Diabetes Study G: Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. Diabetes 2014, 63:2454-63.
35. Eghrari AO, Riazuddin SA, Gottsch JD: Overview of the Cornea: Structure, Function, and Development. Progress in molecular biology and translational science 2015, 134:7-23.
36. Vaegter HB, Graven-Nielsen T: Pain modulatory phenotypes differentiate subgroups with different clinical and experimental pain sensitivity. Pain 2016.
37. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y: Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 2012, 153:1193-8.

Addenda

CURRICULUM VITAE

Linda Oudejans was born on the 29th of May 1977 in Alkmaar, the Netherlands. After obtaining her VWO degree in 1995 and working in Paris as an au-pair she studied at the school for Physical Education (Academie voor Lichamelijke Opvoeding) at the Hogeschool van Amsterdam and completed this program three years later. She then started the study of Science of Human Movement at the Vrije Universiteit in Amsterdam. She performed her internship on the decline and recovery of the fitness level of soccer players at H.F.C. Haarlem and A.F.C. Ajax.

After graduating in 2004 Linda began working as a teacher at the ROC school of 'Sport en Bewegen'. In 2007 she also became a lecturer at the Hogeschool van Amsterdam at the school of 'Sport, Management en Ondernemen'. In 2010 she decided to take on another study, Biomedical Sciences at the Leiden University. She performed an internship on Natural Killer cell-activation against osteosarcoma and Ewing's sarcoma. Her internship on the effects of deafferentation from spinal anesthesia on pain sensitivity and resting-state functional brain connectivity at the department of anesthesiology at the LUMC, led to the admittance to her PhD trajectory at the same department in 2012 under the supervision of Prof. dr. Albert Dahan. This thesis, Pain perception and modulation in acute and chronic pain states, is the result of that PhD trajectory.

LIST OF PUBLICATIONS

Niesters M, Sitsen E, Oudejans L, Vuyk J, Aarts LPHJ, Rombouts AARB, de Rover M, Khalili-Mahani N, Dahan A. Effect of deafferentation from spinal anesthesia on pain sensitivity and resting-state functional brain connectivity in healthy male volunteers. Brain connectivity 2014: 4(6); 404-416.

Oudejans LCJ, Smit JM, van Velzen M, Dahan A, Niesters M. The influence of offset analgesia on the onset and offset of pain in fibromyalgia patients. Pain 2015: 156(12); 2521–2527.

Oudejans L, van Velzen M, Olofsen E, Beun R, Dahan A, Niesters M. Translation of random painful stimuli into numerical responses in fibromyalgia and perioperative patients. Pain 2016: 157(1); 128–136.

Oudejans LCJ, He X, Niesters M, Dahan A, van Velzen M. Cornea nerve fiber quantification and construction of phenotypes in patients with fibromyalgia. Scientific Reports 2016: 6, 23573; doi: 10.1038/srep23573

Oudejans LCJ, van Velzen M, Dahan A. Ketamine Analgesia. The Neuropathology Of Drug Addictions And Substance Misuse, Volume 2, 1st edition. Edited by Preedy V. Academic Press, 2016. pp. 541-550.

Oudejans LCJ, Niesters M, Brines M, Dahan A, van Velzen M. Cornea nerve fiber quantification and construction of phenotypes in patients with sarcoidosis and diabetes mellitus type 2. Submitted.

Oudejans LCJ, van Velzen M, Dahan A, Niesters M. Evaluation of a novel contact heat device (Q-sense CPM) for conditioned pain modulation testing in healthy volunteers. Submitted.

Torensma B*, Oudejans LCJ*, van Velzen M, Olofsen E, Niesters M, Swank D, Dahan A. Pain sensitivity and pain scoring in morbid obesity. Submitted. *contributed equally.

Sitsen ME, Dahan A, Nuninga JO, Oudejans LCJ, Tracey I, de Rover M, Niesters M. Effect of spinal anesthesia on resting state insular networks. In preparation.

Sitsen ME, Oudejans LCJ, Nuninga JO, Niesters M, Ramlakan K, Tracey I, Dahan A, Niesters M. Effects of spinal anesthesia on insular networks in relation to acute pain. In preparation.