Advanced quantitative sensory and electrophysiological pain assessment in healthy volunteers and chronic pain patients

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ADVANCED QUANTITATIVE SENSORY AND ELECTROPHYSIOLOGICAL PAIN ASSESSMENT IN HEALTHY VOLUNTEERS AND CHRONIC PAIN PATIENTS

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PhD Thesis

Dissertation submitted for the degree of Doctor of Philosophy

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To my parents, with love.

PUBLICATIONS

This Ph.D. dissertation is based on four original papers that were published to international peer-reviewed journals. The studies are referred thorough this dissertation by roman numerals I-IV.

I. Neziri AY, Curatolo M, Bergadano A, Petersen-Felix S, Dickenson A, Arendt-Nielsen L, Andersen OK.
***New method for quantification and statistical analysis of nociceptive reflex receptive fields in humans*.**
Journal of Neuroscience Methods 2009; 178(1):24-30.
DOI:10.1016/j.jneumeth.2008.11.009

II. Neziri AY, Andersen OK, Petersen-Felix S, Radanov B, Dickenson AH, Scaramozzino P, Arendt-Nielsen L, Curatolo M.
The nociceptive withdrawal reflex: normative values of thresholds and reflex receptive fields.
European Journal of Pain (London, England) 2010; 14(2):134-141.
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III. Neziri AY, Scaramozzino P, Andersen OK, Dickenson A, Arendt-Nielsen L, Curatolo M.
Reference Values of Mechanical and Thermal Pain Tests in a Pain-Free Population.
European Journal of Pain (London, England) Article in press (2010).
DOI:10.1016/j.ejpain.2010.08.011

IV. Neziri AY, Haesler S, Petersen-Felix S, Müller M, Arendt-Nielsen L, Biurrun Manresa J, Andersen OK, Curatolo M.
Generalized expansion of nociceptive reflex receptive fields in chronic pain patients
Pain 2010;151:798 - 805.
DOI:10.1016/j.pain.2010.09.017

PREFACE

The work reported in I-IV have been carried out between March 2006 and December 2009 at the University Department of Anaesthesiology and Pain Therapy, University Hospital of Bern, Bern, Switzerland, and in cooperation with Center for Sensory-Motor Interaction at the Aalborg University, Aalborg, Denmark.

I wish to express my deepest and sincere gratitude to my supervisor Professor Michele Curatolo, for his never failing support, precious scientific guidance, critical reviewing and teaching and for constant and tactful encouragement.

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CONTENTS

LIST OF COMMON ABBREVIATIONS AND DEFINITIONS

NWR	Nociceptive withdrawal reflex
RRF	Reflex receptive field
QST	Quantitative sensory tests
Pdt	Pain detection threshold
Ptt	Pain tolerance threshold
TA	Tibialis anterior muscle
VAS	Visual analogue scale
BDI	Beck depression inventory
STAI	State – Trait anxiety inventory
SF 36	Short-Form 36 questionnaire
AUC	Area under the curve

Pain	"Unpleasant sensory and emotional experience associated with actual or potential tissue damage, ore described in terms of such damage"
Central hypersensitivity	An increase in the excitability of neurons within the central nervous system, so that non-painful or low-intensity painful stimulation are able to induce pain or exaggerated pain, respectively
Psychophysical pain tests	Tests that are based on subjective verbal response to a painful stimulus
Electrophysiological pain tests	Tests that are based on electrophysiological responses to a painful stimulus
QST	Term that includes psychophysical and electrophysiological pain tests
Temporal summation	Increased pain perception during repeated stimulation at constant intensity
Reflex receptive field	Cutaneous area from which a nociceptive stimulus can evoke a reflex in a given muscle

1. INTRODUCION

1.1. EXPERIMENTAL PAIN RESEARCH

Understanding mechanisms of pain is one of the most challenging tasks in clinical practice. Experimental pain research has given a very high contribution to the current understanding of pain mechanisms in humans.

The basic principle in human experimental pain research is to activate the nociceptive system by a well-defined stimulus and then record and quantify the evoked response. The general term that defines this methodology is quantitative sensory pain testing. The response is usually of verbal or electrophysiological character. Quantification of verbal responses to painful stimuli is also denoted as psychophysical pain research. Examples of electrophysiological responses include the nociceptive withdrawal reflex (NWR) and electroencephalographic recordings after nociceptive stimulation.

1.2. PAIN AND CENTRAL HYPERSENSITIVITY

Prolonged afferent nociceptive input induces an increase in the excitability of central sensory neurons and plasticity changes that are responsible for a state of hyperexcitability of the central nervous system (central hypersensitivity) (Woolf and Salter, 2000). The hyperexcitable central nervous system amplifies the nociceptive signal, thereby producing an exaggerated pain response even in the presence of limited tissue damage.

There is evidence that localized tissue damage leads to a state of hyperexcitability that is not confined to the neural structures connected to the site of the lesion, but involves the whole spinal cord and the supraspinal centers (Samad et al., 2001; Suzuki et al., 2002). This phenomenon may be at least partially responsible for a widespread hypersensitivity to peripheral stimulation, with pain being experienced in response to stimulation of tissues that are distant from the site of injury.

1.3. ASSESSMENT OF CENTRAL HYPERSENSITIVITY IN PATIENTS

Central hypersensitivity can be investigated in humans by quantitative sensory tests (Klein et al., 2005; Curatolo et al., 2006). Using these methods, central hypersensitivity has been detected in different chronic musculoskeletal pain syndromes (Curatolo et al., 2006). For instance, patients with chronic low back pain display increased pain sensitivity and enlargement of the areas of referred pain after stimulation of tissues around and at distance from the site of pain (i.e. the leg or the thumb) (Giesecke et al., 2004; Laursen et al., 2005; O'Neill et al., 2007), suggesting that widespread central hypersensitivity is associated with this painful condition.

An investigation that evaluated patients after a whiplash injury in the acute phase and 6 months after injury found that those patients with persistent moderate or severe symptoms at 6 months had displayed, soon after injury, widespread hypersensitivity (Sterling et al., 2003). Therefore, the presence of central hypersensitivity may be an indicator of negative prognosis. An acute peripheral lesion may induce plasticity changes leading to central hypersensitivity in a subset of individuals. Such hypersensitivity would facilitate the transition from acute to chronic pain and disability.

In human pain research a reflex withdrawal reaction can be elicited by transcutaneous electrical stimulation of a sensory peripheral nerve and the electromyographic response may be recorded from the flexor and extensor muscles. Elicited nociceptive withdrawal reflex (NWR) is a poly-synaptic spinal nociceptive reflex, and represents the mechanism of a response in both ipsilateral and contralateral muscle groups for withdrawing an extremity in order to avoid further tissue damage (Sherrington, 1910). The process is initiated by the nociceptive input, but elaboration takes place within the spinal cord. Additional afferent input, descending activity, and the excitability of the neurons in this pathway modulate the generation of the spinal nociceptive reflex.

The NWR and its modulation have been widely used in experimental (Hagbarth, 1960; Kugelberg et al., 1960; Willer and Bathien, 1977; Arendt-Nielsen et al., 2000; Andersen, 2007) and pharmacologic studies (Willer and Bathien, 1977;

Willer, 1985; Arendt-Nielsen et al., 1990; Petersen-Felix et al., 1995; Curatolo et al., 1997; Petersen-Felix et al., 1998; Piguet et al., 1998; Escher et al., 2007) as a noninvasive neurophysiologic tool to objectively assess spinal nociceptive processing.

A phenomenon linked to hypersensitivity is reorganization at the spinal cord level that is manifested by changes in receptive field areas. The receptive field is the size of peripheral tissue that is innervated by a single spinal neuron. An expansion of the receptive fields of individual dorsal horn neurons following peripheral injury has been documented early (McMahon and Wall, 1984) and confirmed in muscle pain: an expansion of the cell population of the dorsal horn that could be excited by input from the inflamed muscle was observed (Hoheisel et al., 1994). The activation of silent synapses leads to the convergence of input from more than one source to the same neurons. These events are likely determinants of hyperalgesia at areas outside the injured region (secondary hyperalgesia) and enlargement of the pain areas, a clinically relevant phenomenon. So far no established method to assess nociceptive receptive fields in humans was available.

1.4. AIMS OF PH.D. PROJECT

Despite the increasing application in clinical research, the usefulness and implementation of quantitative sensory testing in clinical practice as diagnostic tools remains very limited. One important reason is the lack of normative data sets from large population of pain-free individuals. In this respect, knowledge of reference values of the quantitative sensory tests in the normal population is essential to provide clinically useful information on the excitability of central nervous system in individual patients. Furthermore, the concept of expansion of receptive fields did not find applications in clinical research because of the lack of methods to study this mechanism in humans.

The aims of these PhD project were:

1) To establish a model to assess nociceptive reflex receptive field in humans.

2) To determine the reference values of spinal nociceptive reflexes and of the area of the reflex receptive fields (RRF).
3) To determine the reference values of psychophysical measures of nociception, i.e. pain thresholds to electrical, mechanical and thermal stimuli, and withdrawal time for the cold pressor test.
4) To analyze how demographic, psychological and health-related variables influence the quantitative sensory tests in pain-free subjects.
5) To test the hypothesis that patients with chronic pain display enlarged reflex receptive fields compared to pain-free subjects.

The ultimate aim was to provide tools for an application of advanced methods for pain assessment in clinical practice, whereby disturbances in central pain processes are to be detected in individual patients.

2. METHODS USED IN THE EXPERIMENTAL STUDIES

2.1. PAIN FREE SUBJECTS AND CHRONIC PAIN PATIENTS

To determine reference values of psychophysical and electrophysiological measures of nociception and to analyze the influence of demographic, psychological and health-related data on QST and reflex parameters, 300 pain-free subjects participated in study II and III.

In study I, thirty pain-free male subjects (18-35 years) taken from the aforementioned cohort of 300 pain-free subjects and one 39 years old male subject with complete spinal cord injury (SCI) at level T11 were investigated.

Finally, in study IV 20 chronic pain patients with endometriosis and 25 pain-free female subjects (age matched sample taken from 300 pain-free subjects) were analysed.

2.2. DEMOGRAPHIC DATA, PSYCHOLOGICAL AND HEALTH-RELATED VARIABLES (II-IV)

In the studies II-IV, Beck Depression Inventory (BDI), State-Trait-Anxiety-Inventory (STAI), Catastrophizing Scale of the Coping Strategies Questionnaire and Short-Form 36 (SF-36) were used to measure the psychological and health related parameters. Demographic data, i.e. gender, age, height, weight and body mass index (BMI) were recorded. These data were used both for descriptive purposes and as explanatory variables for the reference values of QST.

The BDI is a 21-item self-report measure assessing affective, cognitive and somatic symptoms of depression. Higher scores indicate higher levels of depressive symptoms (Beck et al., 1996).

The STAI is a 40-item self-report questionnaire designed to assess symptoms of anxiety. It consists of two independent scales: a state anxiety scale and a trait anxiety scale, each with 20 items, leading to a score between 20 and 80. Higher

scores indicate greater levels of anxiety. The state and trait scales explore anxiety as a current emotional state and as a personality trait, respectively (Spielberger et al., 1979; Laux et al., 1981).

The 6-item catastrophizing scale of the CSQ was used to assess pain catastrophizing cognitions (Rosenstiel and Keefe, 1983). The subscale score is the mean of all 6 items, and higher scores indicate higher degrees of pain catastrophizing.

The SF-36 questionnaire is a self-administered, 36-item questionnaire that measures health-related functions in eight domains: physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP), vitality (VT), general health perceptions (GH), social functioning (SF), role limitations due to emotional problems (RE) and mental health (MH). These eight domains were grouped into two health dimension scales: physical (PF, RP, BP, VT) and mental (SF, GH, RE, MH) (Ware and Sherbourne, 1992). The total score was also calculated. Each scale ranges from 0 (lowest level of functioning) to 100 (highest level) (Ware et al., 1993).

2.3. ELECTROPHYSIOLOGICAL TESTS (I, II, IV)

In studies I, II and IV, the electrophysiological tests were the main outcomes. However, the studies evaluated also the subjective pain thresholds to the electrical stimuli applied (psychophysical responses). In order to simplify the description of the methodology, these psychophysical responses are described in this chapter.

2.3.1. SINGLE ELECTRICAL STIMULATION

Electrical stimulation was performed through surface electrodes placed caudal to the lateral malleolus, at the innervation area of the sural nerve (Banic et al., 2004). A 25 ms train-of-five square-wave impulses, each lasting 1 ms, was delivered by a computer-controlled constant current stimulator (University of Aalborg, Denmark). The stimulation train is perceived as a single stimulus. Electromyographic (EMG)

reflex responses to electrical stimulation were recorded from the middle of the biceps femoris and the rectus femoris muscles (Ag/AgCl-electrodes).

The current intensity was increased from 1 mA in steps of 0.5 mA until: 1) a reflex with an amplitude exceeding 20 μV for at least 10 ms in the 70-150 ms post-stimulation interval was detected (single stimulus reflex threshold); and 2) a pain sensation was evoked (single stimulus pain threshold). The program delivered the impulses at random time intervals (between 8 and 12 s), so that the subject was not aware of when the stimulus was applied.

2.3.2. Repeated electrical stimulation (temporal summation)

The stimulus burst used for single stimulus was repeated five times with a frequency of 2 Hz, at constant intensity (Arendt-Nielsen et al., 1994). EMG recordings were similar as for single stimulation. The current intensity of the five constant stimuli was increased from 1 mA in steps of 0.5 mA until: 1) an increase in the amplitude of the last two or three reflexes above a fixed limit of 20 μV for at least 10 ms in the 70-150 ms post-stimulation interval was observed (temporal summation reflex threshold); and 2) the subjects felt pain during the last 2 to 3 of the 5 electrical bursts (temporal summation pain threshold).

2.3.3. Reflex receptive fields

To evaluate reflex receptive fields (RRF), a procedure, which is widely described in chapter 3, was employed. Ten surface electrodes (15 × 15 mm, type 700, Ambu A/S, Denmark) were mounted on the sole of the foot (see fig 1). A common anode (50 × 90 mm electrode, type Synapse, Ambu A/S, Denmark) was placed on the dorsum of the foot. A computer-controlled electrical relay delivered a stimulus to one of the 10 electrodes in a randomized sequence and double-blind manner. Each stimulus consisted of a constant current pulse train of five individual 1 ms pulses delivered at 200 Hz (Stimulator Noxitest IES 230, University of Aalborg, Denmark). This train of stimuli is felt as single stimulus.

The EMG was recorded with surface electrodes (type 720, Ambu A/S, Denmark) over the belly of the tibialis anterior muscle with an inter-electrode distance of 2 cm. The EMG signals were amplified (up to 50 000 times), filtered (5–500 Hz, 2nd order), sampled (2000 Hz), displayed on the computer screen, and stored on computer disk. The EMG signals were stored from 200 ms before stimulation until 1000 ms after stimulation onset.

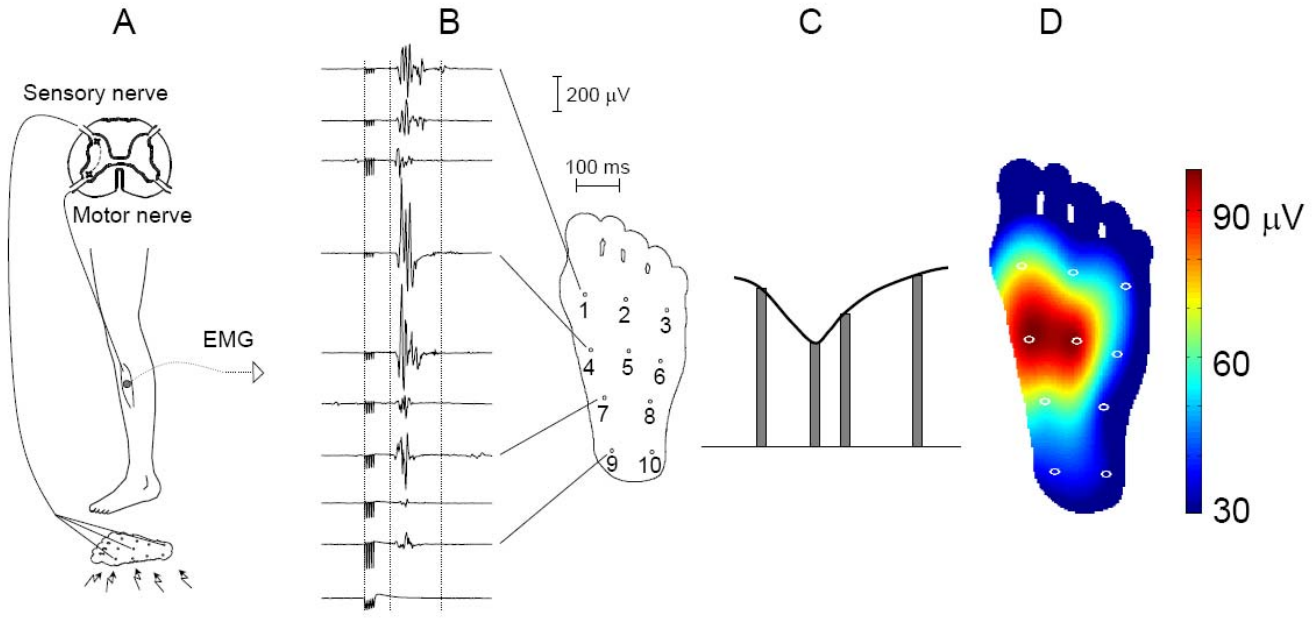

Fig. 1. The general method for determining reflex receptive fields is depicted. A. Reflex responses were evoked by distributed electrical stimulation on the sole of the foot using surface electrodes. A common electrode was placed on the dorsum of the foot. The reflex responses were recorded by surface EMG. B. Four stimuli were delivered at all sites in randomized sequence, and the EMG signals were averaged for every stimulation site. The reflex size was quantified in the 60-180 ms time interval (indicated by the middle and right vertical lines). Stimulus onset is also indicated by the left vertical line. C. The reflex size detected at the ten electrodes is interpolated and extrapolated demanding that the curve fitting to pass through the actual recordings at the specific electrode positions (see method section for the interpolation technique). D. The two-dimensional interpolation map is then superimposed onto a map of the foot for depicting the reflex sensitivity in a particular muscle. The position of the electrodes is illustrated by white circles.

First, the pain thresholds were determined for each of the 10 stimulation sites. Then a stimulus intensity equal to 1.5 times higher that the individual pain threshold was delivered. The EMG responses for each stimulation site were recorded from the tibialis anterior muscle. The perceived pain intensity was rated on a 10 cm electronic visual analogue scale (VAS) (Aalborg University, Denmark),

whereby 0 = no pain and 10 = the worst pain imaginable. Each electrical stimulus was scored by the subject and stored on the computer.

The area of the RRF was calculated using the procedure presented above in data analyses. It is expressed as the area of the foot from which a reflex from a given muscle can be elicited. The volume of the RRF was calculated by integration of the EMG activity in the identified RRF area by calibrating to a standard foot size of 25×10 cm and expressed as μV*mm².

Chemical activation of the nociceptors by capsaicin (the pungent extract of chilli-pepper) has been used in study I to induce an experimental state of clinical hyperalgesia. The chemonociceptor was supposed to respond vigorously to capsaicin and thereby induce (and maintain) the state of central sensitization. 10μg capsaicin dissolved in a volume of 10μl was injected into the flexor digitorum brevis muscle via the sole of the foot (Fig. 9).

2.3.4. EMG RECORDINGS

The electromyogram (EMG) was recorded with surface electrodes (type 720, Ambu A/S, Denmark) over the belly of the tibialis anterior (TA) muscle with an inter-electrode distance of 2 cm. Before attachment of the electrodes, the skin was lightly abraded and cleaned with isopropyl alcohol. The EMG signals were amplified (up to 50 000 times), filtered (5–500 Hz, 2nd order), sampled (2000 Hz), displayed on the computer screen, and stored on computer disk. The EMG signals were stored from 200 ms before stimulation until 1000 ms after stimulation onset.

2.4. PSYCHOPHYSICAL TESTS (III)

2.4.1. PRESSURE PAIN STIMULATION

Pain detection and tolerance thresholds were measured with an electronic pressure algometer (Somedic, Sweden) using a probe with 1 cm^2 surface. The pressure was increased from 0 at a rate of 30 kPa/s to a maximum pressure of 1000 kPa. Pain detection threshold was defined as the point at which the pressure sensation turned

to pain. Pain tolerance threshold was defined as the point at which the subject felt the pain as intolerable. The subjects were instructed to press a button when these points were reached. The algometer displayed the pressure intensity at which the button was pressed. If the subjects did not press the button at 1000 kPa, this value was considered as threshold.

The test was performed at three locations, in a randomized order: 1) in the middle of a horizontal line drawn between the posterior border of the acromion and the spinous process of the 7th cervical vertebra (suprascapular) 2) in the middle of a horizontal line drawn between the upper border of the iliac crest and the corresponding spinous process (low back); 3) the center of the pulp of the ipsilateral 2nd toe (toe).

2.4.2. THERMAL PAIN STIMULATION – HEAT AND COLD

Thermal stimulation is a natural modality to activate warm and cold receptors and nociceptors in the skin. Thermal polymodal nociceptors are innervated by both Ad- and C-afferents (Meyer et al. 1994). In the present study (III) activation of the thermal nociceptors was achieved by contact thermodes. A contact peltier-based thermode of the dimensions 30x30 mm of thermo-sensory stimulator (Medoc TSA-II; Medoc Ltd, Ramat Yishai, Israel) was used in III for estimating the heat and cold pain thresholds.

To estimate heat pain thresholds, the temperature of the thermode was continuously increased from 30 ºC to a maximum of 50.5 ºC at a rate of 1.5 ºC/s.

To estimate cold pain thresholds, the temperature of the thermode was continuously decreased from 30 ºC to a minimum of 0 ºC at a rate of 1.5 ºC/sec. Pain detection and tolerance threshold were defined as for pressure stimulation. Once the threshold was detected, the temperature of the probe returned to baseline.

The test was performed at 3 locations, in a randomized order: 1) in the middle of a horizontal line drawn between the posterior border of the acromion and the spinous process of the 7th cervical vertebra (suprascapular); 2) in the middle of a horizontal line drawn between the upper border of the iliac crest and the

corresponding spinous process (low back); 3) the lateral aspect of the leg, midway between the knee and the lateral malleolus (leg).

2.4.3. COLD PRESSOR TEST (ICE WATER STIMULATION)

The hand was immersed in ice saturated water (0.7±0.1 °C) for a maximum of 2 minutes. The subject was instructed to withdraw the hand when they felt the pain as intolerable and the time of hand immersion was recorded. If the hand was not withdrawn at 2 minutes, this time was recorded for data analyses. Perceived pain intensity was continuously rated with an electronic visual analogue scale (scaled from 0 – no pain to 100 mm – intolerable pain) and the recorded by computer. The area under the pain intensity/time curve was determined. If the hand was withdrawn before the end of the 2 minutes, the pain intensity was considered to be maximal until the end of the period.

2.5. DATA ANALYSIS

2.5.1. METHOD TO DETERMINE REFLEX RECEPTIVE FIELD PARAMETERS (I)

To analyse data in paper I, the size of the reflexes were quantified by the root mean square (RMS) amplitude of the individual reflexes. The reflex sizes for each stimulation position were averaged. The RMS was calculated in the 80-180 ms post-stimulus window (Andersen, 2007). In order to illustrate the reflex receptive field, two-dimensional interpolation was calculated of the grand mean reflex size (mean of all subjects and all stimuli) for all stimulation sites using a custom made Matlab program. To be able to perform statistical analysis on the measured RRF, a number of features were extracted but only from the interpolated image (see fig. 2) to avoid basing the findings on extrapolated values. The interpolated image is the part of the image encompassed by the electrodes whereas the extrapolated values refer to the fringe of the image, i.e. the edges of the foot not covered by the electrodes. The features were designed to quantify the size and location of the RRF. The area of the RRF was assessed in a two step procedure. First, the fraction of the interpolated image with a Z-score higher than 2.58 (corresponding to a α-level of 0.01) based

on the pre-stimulus EMG activity was determined. The Z-score is calculated for each pixel in the image by subtracting mean pre-stimulus EMG activity and subsequently dividing by the standard deviation of the pre-stimulus activity. The distribution of the pre-stimulus activity (mean and standard deviation) was determined from all sweeps. This threshold corresponds to likelihood for significant EMG activity of 99%. However, often an increase in the EMG tone is seen in response to the stimulus which is not equal to a significant reflex activity. Hence, the standard deviation of the identified map with Z-scores above 2.58 was calculated. The RRF area was subsequently defined as that fraction of the sole of the foot with EMG activity higher than peak EMG minus 2 times the calculated standard deviation as illustrated in fig. 2.

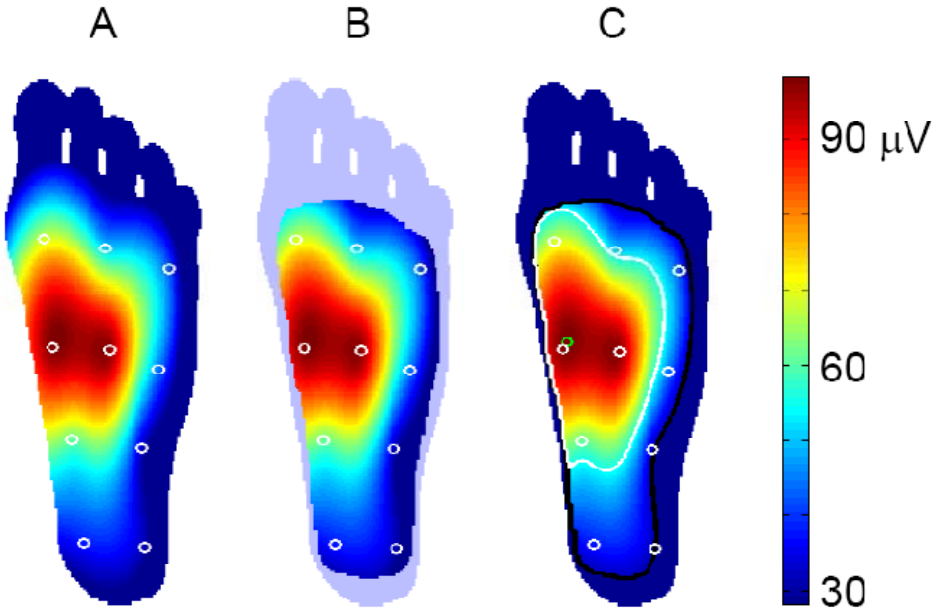

Fig. 2. A. Illustration of the mean reflex receptive field of the 30 healthy volunteers. This RRF includes both interpolated and extrapolated values. In particular the extrapolated values must be treated with caution. The determination of the RRF size in every individual volunteer was therefore only based on the interpolated values (illustrated in B). C. The border of the RRF is outlined by the white line (see methods section for details). The black line illustrates the part of the RRF with EMG level higher than the pre-stimulus EMG level ($P<0.001$).

The volume (RRF area × reflex size) of the RRF was calculated by integration of the EMG activity in the identified RRF area by calibrating to a standard foot size of 25×10 cm (Andersen et al., 2001). The location of the peak of the interpolated EMG was identified and marked in the interpolated image, see fig. 3. In addition the Center of Gravity (CoG) was calculated for the identified RRF and indicated on the

RRF. The CoG was included in case the distribution of the RRF is skewed and hence the peak is not located near the center of the RRF. The CoG is calculated as the cumulative sum of the reflex size (pixel value) multiplied by the distance and subsequently divided by the cumulative reflex size. Both peak and CoG were calculated with reference to the top left corner of the image (arbitrary). The location of these values is expressed as percentage of the width/length of the image relative to the top left corner (fig. 3).

Onset latency was detected using the same method as used in (Andersen et al., 2001). In short, the onset latencies were determined by the first signal component 5 times larger than the background noise for a period of more than 7 ms with the constraint that no component earlier than 60 ms was detected. The background noise was calculated by the RMS of the pre-stimulus.

2.5.2. QUANTIFICATION OF REFERENCE VALUES (II/III)

In order to analyse the effect of the independent variables on the quantitative sensory tests (electrophysiological and psychophysical), backward stepwise multiple regression analyses were conducted on each test.

The multiple regression analyses were performed on the following electrophysiological dependent variables: single stimulus reflex threshold, single stimulus pain threshold, temporal summation reflex threshold, temporal summation pain threshold, area of RRF and volume of RRF. For the psychophysical tests, the individual tests for each sensory modality (pressure, heat, cold) were summarized by the principal component of the standardized measurement variables. For instance, pressure pain threshold was analyzed as a single dependent variable by pooling pain detection and pain tolerance thresholds at the three body sites (6 variables).

Because a very high proportion of subjects had normal health status, BDI, STAI, catastrophizing and SF-36 were not analyzed as continuous variables but were dichotomized as described below. The cut off values for each of these variables were chosen to best distinguish normal from abnormal values for this specific sample. In all the regressions, the following independent (explanatory) variables

were analyzed: gender, age, gender-age interaction, BMI, body side of testing (right vs. left and dominant vs. non-dominant), BDI (cut off 11), STAI state scale (cut off 35), STAI trait scale (cut off 35), catastrophizing (cut off 3), SF-36 physical dimension (cut off 90), SF-36 mental dimension (cut off 90) and SF-36 total score (cut off 90). Concerning body side, the regression analyses for the psychophysical tests were more significant when right vs. left was used, whereas for the electrophysiological test the regressions were more significant when dominant vs. non-dominant was used.

A P value < 0.05 was considered as significant. In the final regression models all the variables with a $P<0.1$ were included in order to provide information on the variables that were only marginally statistically insignificant.

In paper II (electrical pain tests), the confidence intervals were calculated and presented as reference values. In paper III (mechanical and thermal pain), the method to determine the reference values was further developed. Namely, quantile regression analyses were conducted on each sensory test in order to set up reference values for assessing central hypersensitivity. The 5^{th}, 10^{th} and 25^{th} percentiles for each test were estimated with bootstrapped standard errors (1'000 replications for each estimation). The percentiles were first estimated for the whole sample, and then stratified by gender and age. The age groups were 18 – 49 and 50 – 80 years. We defined two groups for ages in order to have an adequate sample size for a precise estimation of the reference values.

2.5.3. COMPARING RRF OF CHRONIC PAIN PATIENTS AND PAIN-FREE SUBJECTS (IV)

The main endpoint according to the study hypothesis was the assessment of reflex receptive fields. Secondary endpoints were subjective pain thresholds and parameters of spinal cord nociceptive excitability (nociceptive withdrawal reflexes to single and repeated electrical stimulation).

To calculate the sample size in the absence of data on the quantitative meaning of expansion of receptive fields, we chose a value of one third of the expected mean area of the reflex receptive field as the minimum difference between patients and controls. At the time of study plan, pilot experiments of a running study on healthy

volunteers yielded a mean area of 0.432 (fraction of foot sole) and a standard deviation of 0.145. The target difference was therefore 0.432 / 3 = 0.144. Setting α = 0.05 and β = 0.8, and using the standard deviation of 0.145, a significant difference of 0.144 in reflex receptive field area among groups would be detected by a sample size of 17 subjects per group. To minimize the likelihood of insufficient power due to unexpected higher variability, we decided to study 20 patients and to select as many as possible (but least 20) control subjects from the above described cohort of pain-free volunteers. This resulted in 25 control subjects as described above.

RRF areas between groups were compared using the unpaired t test (for normally distributed data). Pain and reflex thresholds to single and repeated electrical stimulation were compared between groups by the Mann-Whitney rank sum test (for non-normally distributed data). P-values < 0.05 were considered as significant.

3. RESULTS

3.1. DEMOGRAPHICAL AND PSYCHOLOGICAL CHARACTERISTICS OF PAIN-FREE SUBJECTS

Table 1 shows the demographic, psychological and health-related data of the 300 pain-free individuals.

	Mean	SD	95% CI	Range
Age (yr)	47	16	45 – 49	20 – 77
Height (cm)	174	8	173 – 175	152 – 198
Weight (kg)	73.3	12.5	72 – 75	46 – 130
BMI (kg/m^2)	24.1	3.2	23.7 – 24.5	17.6 – 50.8
BDI (score 0 – 63)	2.2	3.1	1.9 – 2.6	0 – 27.0
STAI State (score 20-80)	31.2	6.4	30.4 – 31.9	20.0 – 63.0
STAI Trait (score 20-80)	28.2	7.5	27.4 – 29.1	20.0 – 67.0
CSQ Catastrophizing (score 0-6)	2.1	0.9	2.0 – 2.2	1.0 – 5.5
SF 36 (score 0-100)				
Total	91.5	7.5	90.6 – 92.3	50.2 – 100
Physical Function	97.5	5.3	96.9 – 98.1	65.0 – 100
Role-Physical	97.4	10.6	96.2 – 98-6	0 – 100
Bodily Pain	95.6	12.1	94.2 – 97.0	22.0 – 100
General Health	88.5	13.4	87.0 – 90.1	27.0 – 100
Vitality	75.9	12.7	74.4 – 77.3	25.0 – 100
Social Functioning	96.7	10.7	95.4 – 97.9	0 – 100
Role Emotional	96.2	15.7	94.4 – 98.0	0 – 100
Mental Health	84.3	11.3	83.0 – 85.6	36.0 – 100
Dimension Physical Health	91.0	7.2	90.1 – 91.8	48.8 – 100
Dimension Mental Health	88.3	9.5	87.2 – 89.3	33.4 – 100

Tab. 1. Demographic, psychological and health-related variables. For gender, 148 females and 152 males were studied. Scale Bodily Pain of SF-36 was excluded from one female subject because of menstrual pain (visual analogue scale: 8) three weeks before test. SD: standard deviation. CI: confidence interval. BMI: body-mass index. BDI: Beck Depression Inventory. STAI: State Trait Anxiety Inventory. CSQ: Coping Strategies Questionnaire. SF: short-form.

3.2. MODEL FOR THE ASSESSMENT OF REFLEX RECEPTIVE FIELDS (I)

3.2.1. Quantification of RRF

The reflex receptive fields could be determined in all 30 participants. The stimulus intensity needed for detecting the pain threshold depended strongly on stimulation site. The pain thresholds followed roughly skin thickness as the stimulus intensities needed to quantify the RRF were highest in areas with thick skin (heel and central pads). Thus stimulation on the heel needed 111% higher stimulus intensities than

in the arch of the foot. The mean pain intensity evoked by the electrical stimuli was 4.3±2.2 but with lower VAS ratings on the heel (RM ANOVA P<0.001, site 9 and 10 compared to all other sites, P<0.05) despite higher stimulus intensities.

The RRF detected for the TA muscle exhibited highest reflex sensitivity in the arch of the foot and distal towards the hallux (fig. 2). The mean area of the RRF covered a 0.57±0.06 fraction of the foot while the mean RRF volume was 0.46±0.08 $mm^2 \times \mu V$.

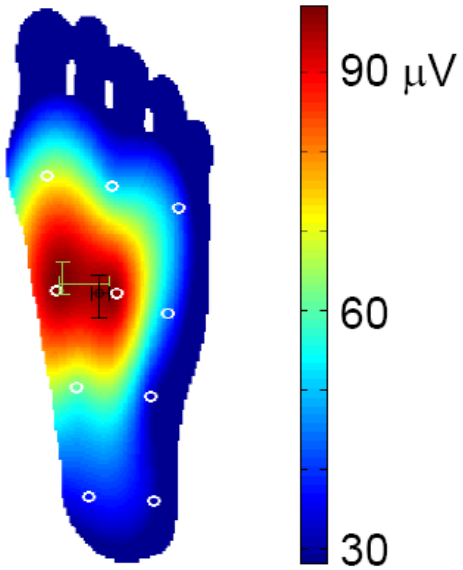

Fig. 3. The median location of the peak RRF (white) and the center of gravity (black) depicted on the mean RRF of the 30 healthy volunteers. The 25% and 75% quartiles are illustrated.

The peak of the RRF was located between stimulation sites four and five (figs. 2 and 3) with coordinates of 31.8, 47.0 (percentage of the width, percentage of the length of the image). In fig. 3, the variation in location of the peak is depicted. The center of gravity is located a bit more lateral on the image (47.8 by 54.4, also percentages of the width and length, respectively), see fig. 5. The onset latency was shortest in the arch of the foot (80.5±1.4 ms, site 4) compared to reflexes detected at the lateral forefoot (91.7±3.8 ms, site 3) and heel (83.4±2.2 ms, site 10). Further, onset latencies were detected in 26/30 volunteers at site 4 while only in 18/30 at site 3. ANOVA analysis was not possible because the number of detected latencies varied.

3.2.2. MODULATION OF RRF BY CAPSAICIN

Injection of capsaicin in the flexor digitorum brevis in one SCI subject resulted in expansion of the RRF (from 0.33 to 0.75 of the foot) and a shift of the location of the peak towards the injection site while the center of gravity did not move . The capsaicin injection itself evoked brief, tonic reflexes/shaking lasting no more than 30 s. After sixty minutes, the size of the RRF area was still large (0.66), i.e. larger than the baseline recording (0.33). The CoG did not move after the capsaicin injection as the RRF covered a large part of the sole of the foot. In addition, variation in RRF volume reflects the capsaicin injection, i.e. 0.04 $mm^2 \times \mu V$, 0.13 $mm^2 \times \mu V$, and 0.07 $mm^2 \times \mu V$ before, during and after the pause.

3.3. REFERENCE VALUES OF PAIN TESTS

3.3.1. ELECTROPHYSIOLOGICAL TESTS (II)

Consistent with the description of the methods, this section includes also the psychophysical assessments for the electrical pain tests, since they have been studied in paper II.

Descriptive statistics of demographic, psychological and health-related data for the 300 subjects are presented in table 1. The different levels of CI across the pain tests revealed very modest differences. Hence, the 80%, 90% and 95% CI for electrical single stimulation pain detection for the 300 subjects were 10.7-11.2, 10.7-11.2 and 10.6-11.3 mA, respectively. The same result was observed for the other variables. Consistent with most of the medical literature, we chose the 95% CI as a guide for the reference values.

Descriptive statistics and regression models for the tests analyzed are presented in tab. 2-4.

For single stimulus thresholds, age and BDI were significant predictors of pain threshold, whereas body side significantly predicted reflex threshold (tab. 4). BMI had a P value of 0.064 for reflex threshold (tab. 2). The regression models for temporal summation pain and reflex threshold were virtually identical: age was the

only significant predictor; BMI, SF-36 physical and mental dimensions had a P value of less than 0.1 (tab. 3).

	Coefficient	Robust SE	95% CI	P
Pain threshold				
Age (yr)	0.0463	0.0111	0.0245 – 0.0681	0.000
BDI (1 if ≥11, 0 if <11)	-2.5943	0.8026	-4.1739 – -1.0147	0.001
Constant	8.8051	0.5198	7.7822 – 9.8280	0.000
Reflex threshold				
Side (Dominant=1, Non dominant=0)	-0.9696	0.4251	-1.8063 – -.1330	0.023
BMI (kg/cm^2)	0.1575	0.0871	-0.0139 – 0.3288	0.072
Constant	12.8695	2.0886	8.7595 – 16.9803	0.000

Tab.2. Regression model for single stimulus pain and reflex threshold, including only the predictors with P<0.1. Pain threshold: R-squared = 0.08, Root MSE = 2.86. Reflex threshold: R-squared = 0.03, Root MSE = 3.67. SE: standard error. CI: confidence interval. BDI: Beck Depression Inventory. . BMI: body-mass index.

	Coefficient	Robust SE	95% CI	P
Pain threshold				
Age (yr)	-0.0183	0.0087	-0.0353 – -0.0012	0.036
BMI (kg/cm^2)	0.1457	0.0788	-0.0083 – 0.3018	0.063
SF-36 Physical (1 if ≥90, 0 if <90)	-0.6262	0.3682	-1.3508 – 0.0983	0.090
SF-36 Mental (1 if ≥90, 0 if <90)	0.5497	0.3258	-0.0910 – 1.1909	0.093
Constant	5.9624	1.6830	2.6502 – 9.2745	0.000
Reflex threshold				
Age (yr)	-0.0184	0.0087	-0.0355 – -0.0013	0.035
BMI (kg/cm^2)	0.1459	0.0786	-0.0079 – 0.3016	0.063
SF-36 Physical (1 if ≥90, 0 if <90)	-0.6253	0.3678	-1.3591 – 0.0886	0.085
SF-36 Mental (1 if ≥90, 0 if <90)	0.5525	0.3260	-0.0892 – 1.1941	0.091
Constant	5.9755	1.6789	2.6723 – 9.2806	0.000

Tab.3. Regression models for temporal summation pain and reflex thresholds, including only the predictors with P<0.1. R-squared = 0.06, Root MSE = 2.13 for both regressions.

	Coefficient	Robust SE	95% CI	P
Area				
Age (yr)	-0.0024	0.0006	-0.0036 – -0.0011	0.000
SF-36 Total (1 if ≥90, 0 if <90)	-0.0577	0.0273	-0.1114 – -0.0039	0.036
STAI state (1 if ≥35, 0 if <35)	-0.0459	0.0269	-0.0988 – 0.0071	0.086
Constant	0.4913	0.0348	0.4228 – 0.5598	0.000
Volume				
Age (yr)	-0.0031	0.0012	-0.0054 – -0.0007	0.011
SF-36 Mental (1 if ≥90, 0 if <90)	-0.0681	0.0381	-0.1431 – 0.0069	0.075
Constant	0.4452	0.0636	0.3200 – 0.5704	0.000

Tab. 4. Regression models of reflex receptive field area and volume for the muscle tibialis anterior, including only the predictors with P<0.1. Area: R-squared = 0.07, Root MSE = 0.27. Volume: R-squared = 0.06, Root MSE = 0.49.

	Mean	SD	95% CI	Range
Single stimulus pain threshold	10.9	3.0	10.6 – 11.3	5.0 – 38.0
Single stimulus reflex threshold	16.2	3.7	15.8 – 16.6	5.3 – 31.3
Temporal summation pain threshold	8.5	2.2	8.3 – 8.8	4.0 – 20.7
Temporal summation reflex threshold	8.5	2.2	8.3 – 8.8	4.0 – 20.7
Reflex receptive field area	0.33	0.17	0.31 – 0.35	0.04 – 0.77
Reflex receptive field volume	0.26	0.31	0.22 – 0.30	0 – 1.62

Tab. 5. Descriptive statistics for single and repeated (temporal summation) electrical stimulation in mA. Descriptive statistics for reflex receptive field area (proportion of foot area) and volume (μV*mm²) of the tibialis anterior muscle.

The RRF could be measured in all 300 participants. The pain thresholds varied strongly depending on the stimulation site and were higher at areas with the thickest skin, i.e. the heel and central pads (data not presented). Descriptive statistics and reference values of RRF area and volume are presented in tab. 5. Age and the total score of SF-36 were significant predictors of RRF area, while the STAI state scale had a P value of 0.086 (tab. 6). For volume, age was a significant predictor, whereas the mental health dimension of the SF-36 had a P value of 0.075 (tab. 4).

3.3.2. PSYCHOPHYSICAL TESTS (III)

Regression models for the tests analyzed are shown in table 6. Gender, the gender–age interaction, the SF-36 total score and the physical health dimension of the SF-36 were significant predictors of pressure pain thresholds. Catastrophizing had a p value of 0.084.

Gender, age and body side were found to be significantly related to heat pain thresholds. The gender–age interaction had a p value of 0.077. Gender, age, gender–age interaction, BMI and SF-36 physical health dimension were significant predictors of cold pain thresholds.

The reference values for the tests analyzed are shown in tab. 7. The estimates for the pain thresholds to cold are not presented, because most of the observed measurements had the value of zero. This was the result of the cut off of 0°C, the lower limit allowed by the device. As a result, a precise estimation of the percentiles was not possible. The interaction of gender with age for pressure pain is illustrated in fig. 4. Gender displayed a p value of 0.068 for hand withdrawal time and 0.017 for area under the curve, with no other significant parameter for the cold pressor test.

	Coefficient	Robust SE	95% CI	p
Pressure pain thresholds				
Gender (female=1, male=0)	-3.6276	0.5271	(-4.6653, -2.5899)	0.000
Gender*age (yr) (female=1, male=0)	0.0477	0.0096	(0.0287, 0.0667)	0.000
Catastrophizing (1 if ≥3, 0 if <3)	-0.4930	0.2840	(-1.0521, 0.0662)	0.084
SF-36 Physical (1 if ≥90, 0 if <90)	-0.8878	0.3108	(-1.4997, -0.2760)	0.005
SF-36 Total (1 if ≥90, 0 if <90)	0.6537	0.3229	(0.0180, 1.2895)	0.044
Constant	0.8723	0.2548	(0.3708, 1.3739)	0.001
Heat pain thresholds				
Gender (female=1, male=0)	-2.0487	0.8750	(-3.7712, -0.3262)	0.020
Age (yr)	-0.0315	0.0097	(-0.0505, -0.0124)	0.001
Gender*age (yr) (female=1, male=0)	0.0284	0.0160	(-0.0031, 0.0599)	0.077
Side (left=1, right=0)	-0.5489	0.2127	(-0.9677, -0.1301)	0.010
Constant	2.1561	0.5362	(1.1010, 3.2116)	0.000
Cold pain thresholds				
Gender (female=1, male=0)	2.0906	1.0603	(0.0332, 4.1779)	0.050
Age (yr)	-0.0451	0.0147	(-0.0741, -0.0162)	0.002
Gender*age (yr) (female=1, male=0)	-0.0430	0.0220	(-0.0863, -0.0003)	0.052
BMI (kg/cm2)	-0.1768	0.0682	(-0.3111, -0.0425)	0.010
SF-36 total (1 if ≥90, 0 if <90)	-1.3199	0.4211	(-2.1488, -0.4910)	0.002
Constant	6.1452	1.6757	(2.8463, 9.4441)	0.000
Cold pressor test – hand withdrawal time				
Gender (female=1, male=0)	-4.7441	2.5939	(-9.8491, 0.3610)	0.068
Constant	39.4342	1.7703	(35.9500, 42.9184)	0.000
Cold pressor test – area under the curve				
Gender (female=1, male=0)	268.5162	111.3783	(49.3286, 487.7038)	0.017
Constant	9900.349	75.1780	(9752.402, 10048.3)	0.000

Tab. 6. Principal component regression models for pressure, heat, and cold pressor test. Only the predictors with p<0.1 are included. Pressure: R-squared = 0.22, Root mean square = 1.65. Heat: R-squared = 0.08, Root mean square = 1.82. Cold: R-squared = 0.10, Cold pressor: R-squared = 0.01, Root mean square = 22.20. SE: heteroskedasticity-robust standard error. CI: confidence interval. SF Physical: SF physical dimension. SF total: SF total score. BMI: body-mass index.

Fig. 4. Interaction gender – age for pressure pain detection and tolerance threshold. Mean and SD are presented.

Age		Females Mean (SD)	Females Reference values for hyper-sensitivity (p^5, p^{10}, p^{25})	Females Reference values for hypo-sensitivity (p^{75}, p^{90}, p^{95})	Males Mean (SD)	Males Reference values for hyper-sensitivity (p^5, p^{10}, p^{25})	Males Reference values for hypo-sensitivity (p^{75}, p^{90}, p^{95})
Pressure pain thresholds (kPa)							
p. detection toe	20 – 49	215 (82)	(104, 122, 158)	(262, 328, 377)	275 (127)	(120, 125, 169)	(348, 425, 489)
	50 – 80	179 (67)	(90, 105, 125)	(219, 299, 322)	188 (59)	(106, 116, 138)	(226, 272, 301)
p. tolerance toe	20 – 49	471 (138)	(289, 305, 371)	(510, 680, 769)	600 (215)	(227, 351, 424)	(767, 909, 957)
	50 – 80	431 (148)	(230, 256, 303)	(502, 646, 702)	429 (141)	(254, 275, 317)	(502, 683, 729)
p. detection back	20 – 49	260 (121)	(119, 143, 179)	(300, 379, 528)	398 (141)	(200, 227, 299)	(494, 597, 653)
	50 – 80	353 (98)	(194, 224, 290)	(408, 488, 505)	389 (116)	(259, 274, 309)	(435, 505, 538)
p. tolerance back	20 – 49	618 (221)	(317, 366, 429)	(755, 998, 1000)	804 (210)	(468, 503, 625)	(1000, 1000, 1000)
	50 – 80	733 (163)	(465, 524, 629)	(829, 1000, 1000)	754 (158)	(515, 578, 644)	(889, 1000, 1000)
p. detection scap	20 – 49	212 (74)	(118, 123, 153)	(258, 309, 360)	313 (94)	(150, 168, 243)	(395, 428, 451)
	50 – 80	314 (80)	(199, 210, 250)	(379, 417, 451)	355 (105)	(223, 246, 279)	(415, 474, 544)
p. tolerance scap	20 – 49	684 (107)	(556, 573, 607)	(752, 857, 917)	777 (102)	(606, 632, 700)	(869, 891, 927)
	50 – 80	832 (83)	(688, 713, 785)	(885, 930, 974)	851 (78)	(736, 763, 798)	(904, 966, 1018)
Heat pain thresholds (°C)							
p. detection leg	20 – 49	42.9 (2.9)	(38.2, 39.6, 41.5)	(45.2, 45.8, 46.5)	44.0 (3.0)	(39.1, 40.0, 42.3)	(46.7, 48.1, 49.0)
	50 – 80	42.1 (2.5)	(38.4, 39.1, 40.7)	(43.7, 45.2, 48.3)	42.9 (2.2)	(39.9, 40.2, 41.2)	(45.7, 46.6, 48.0)
p. tolerance leg	20 – 49	49.1 (1.3)	(47.3, 47.7, 48.2)	(50.5, 50.5, 50.5)	49.7 (1.1)	(48.2, 48.3, 49.7)	(50.5, 50.5, 50.5)
	50 – 80	49.2 (1.2)	(47.7, 48.0, 48.4)	(50.5, 50.5, 50.5)	49.5 (1.1)	(46.9, 47.9, 48.6)	(50.5, 50.5, 50.5)
p. detection back	20 – 49	40.3 (2.6)	(36.7, 38.1, 38.8)	(42.4, 44.4, 45.9)	41.5 (2.5)	(37.5, 38.3, 40.0)	(43.9, 45.5, 46.0)
	50 – 80	40.2 (1.7)	(37.5, 38.5, 38.9)	(41.4, 42.7, 45.3)	40.6 (1.6)	(38.6, 38.9, 39.4)	(41.0, 42.4, 43.9)
p. tolerance back	20 – 49	47.6 (2.2)	(44.3, 46.2, 46.7)	(50.0, 50.3, 50.4)	48.3 (2.0)	(44.5, 46.6, 47.9)	(50.0, 50.5, 50.5)
	50 – 80	47.9 (1.3)	(46.4, 46.8, 46.9)	(49.2, 50.1, 50.5)	47.7 (1.4)	(45.0, 46.0, 46.9)	(48.7, 49.9, 50.1)
p. detection scap	20 – 49	40.4 (2.5)	(38.1, 38.5, 39.1)	(41.9, 43.8, 44.4)	41.2 (2.4)	(38.6, 39.0, 39.5)	(45.1, 46.3, 46.3)
	50 – 80	39.8 (1.8)	(36.8, 38.3, 38.9)	(40.6, 41.7, 44.5)	40.1 (1.5)	(38.0, 38.6, 39.4)	(40.6, 42.3, 42.6)
p. tolerance scap	20 – 49	47.6 (1.8)	(45.2, 46.0, 46.6)	(48.8, 49.4, 49.5)	48.2 (1.6)	(45.7, 46.1, 47.6)	(49.8, 50.5, 50.5)
	50 – 80	47.4 (1.6)	(45.1, 46.3, 46.8)	(48.8, 49.1, 50.2)	47.4 (1.0)	(45.5, 46.2, 46.5)	(48.4, 49.3, 49.9)
Cold pressor test							
hand withdrawal time (sec)		35 (23)	(15, 17, 21)	(40, 56, 75)	39 (22)	(17, 20, 24)	(50, 59, 79)
			(p^{95}, p^{90}, p^{75})	**(p^5, p^{10}, p^{25})**		**(p^{95}, p^{90}, p^{75})**	**(p^5, p^{10}, p^{25})**
AUC (mm*sec)		10169 (1000)	(10815, 11048, 11118)	(8475, 8766, 9389)	9900 (927)	(10543, 10872, 10543)	(8607, 9213, 9759)

Tab. 7. Reference values of pain thresholds for pressure and heat stimulation, and cold pressor test. The normal values for heat pain thresholds refer to the right side and for the left side the values should be corrected by the regression coefficient -0.5489. (Toe- 2nd toe, Back – low back, Scap- Suprascapular region. AUC – area under the curve; p^5 – 5th percentile (0.05 quantile), p^{10} – 10th percentile (0.10 quantile), p^{25} – 25th percentile (0.25 quantile), p^{75} – 75th percentile (0.75 quantile), p^{80} – 80th percentile (0.80 quantile), p^{95} – 95th percentile (0.95 quantile).

3.4. REFLEX RECEPTIVE FIELDS IN CHRONIC PAIN PATIENTS WITH ENDOMETRIOSIS (IV)

The descriptive variables in the two groups, patients and controls, are presented in tab. 8. Compared with the pain-free subjects, the group of patients displayed higher scores for depression, anxiety and catastrophizing, as well as lower scores of SF-36 parameters. This was expected and is consistent with findings of previous studies on chronic pain patients (Banic et al., 2004; Herren-Gerber et al., 2004; Laursen et al., 2005). The two groups were comparable for all the other descriptive variables.

	Patients (n=20)	Controls (n=25)	
	Median (25-75 percentiles)	Median (25-75 percentiles)	P-value
Age (yr)	33 (30-36)	27 (24-37)	0.266
Height (cm)	163 (158-169)	168 (165-170)	0.036
Weight (kg)	59 (54-64)	60 (55-64)	0.968
BMI (kg/m^2)	21.6 (19.8-25.3)	21.6 (20.4-23.5)	0.332
BDI (score 0 – 63)	9 (5-16)	0 (0-3)	<0.001
STAI State (score 20-80)	53 (43-58)	34 (30-35)	<0.001
STAI Trait (score 20-80)	47 (35-51)	31 (28-37)	<0.001
CSQ Catastrophizing (score 0-6)	3.8 (2.5-4.2)	1.5 (1.3-2.3)	<0.001
SF 36 (score 0-100)			
Total	60 (53-77)	91 (87-94)	<0.001
Physical Function	90 (75-100)	100 (100-100)	0.001
Role-Physical	75 (50-81)	100 (100-100)	<0.001
Bodily Pain	41 (32-72)	100 (100-100)	<0.001
General Health	53 (34-82)	92 (72-97)	<0.001
Vitality	45 (35-56)	70 (60-75)	<0.001
Social Functioning	75 (62-87)	100 (100-100)	0.018
Role Emotional	83 (33-100)	100 (100-100)	0.008
Mental Health	62 (48-73)	84.0 (80-88)	<0.001
Dimension Physical Health	57 (44-74)	90.4 (88-94)	<0.001
Dimension Mental Health	61 (53-76)	87.0 (79-91)	<0.001
Pain at time of testing (VAS)	4.2 (3.1-4.5)	---	---
Maximal pain ever experienced (VAS)	8.0 (7.1-9.2)	---	---

Tab. 8. Demographic, psychological and health-related variables of chronic pain patients with endometriosis and healthy subjects.

	Patients (n=20)	Controls (n=25)	P-value
	Median (25-75 percentiles)	Median (25-75 percentiles)	
Reflex receptive field area (fraction of area of foot sole)	0.48 (0.38-0.54)	0.33 (0.27-0.39)	0.008
Single electrical stimulation - pain threshold (mA)	6.0 (5.0-8.0)	8.0 (8.0-12.0)	<0.001
Single electrical stimulation - reflex threshold (mA)	9.0 (7.0-10.0)	15.0 (11.0-19.0)	<0.001
Repeated electrical stimulation - pain threshold (mA)	5.0 (4.0-5.5)	9.0 (7.0-10.0)	<0.001
Repeated electrical stimulation - reflex threshold (mA)	5.0 (4.0-6.0)	9.0 (7.0-10.0)	<0.001

Tab. 9. Area of reflex receptive fields and thresholds after single and repeated (temporal summation) electrical stimulation.

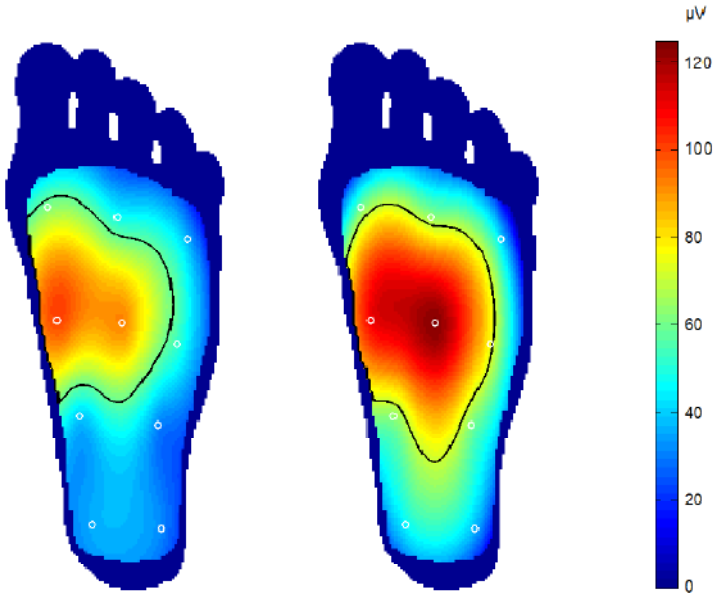

Fig. 5. Mean reflex receptive fields (RRF) for controls (left) and endometriosis patients (right). The white dots indicate the stimulation sites. The black line represents the contour of the RRF area. The colours indicate the reflex amplitude. P=0.008 for the RRF area.

Patients were characterized by larger RRF areas than pain-free subjects (main endpoint of the study). This is reflected by the enlargement of the area of the foot sole from which a nociceptive reflex in the tibialis anterior muscle can be elicited (box plots of fig. 6 and black line of fig. 5). Furthermore, the reflex amplitude was higher in patients than in pain-free subjects, as shown in the colour map of fig. 5.

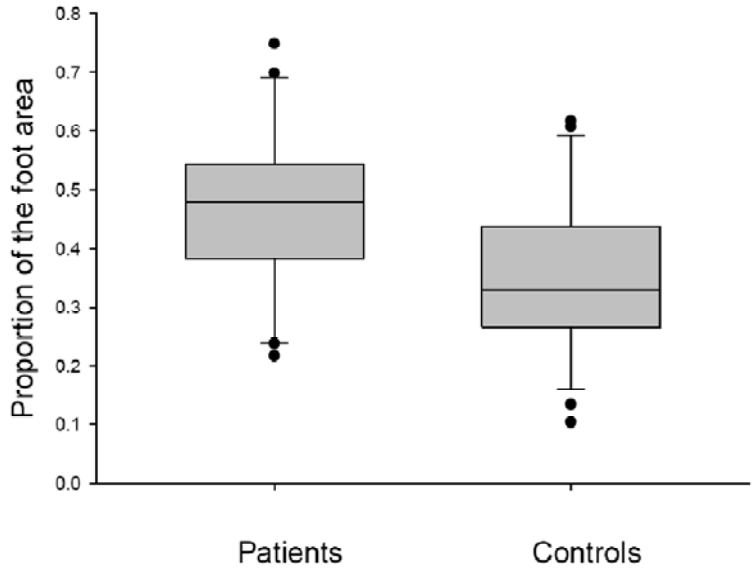

Fig. 6. Area of nociceptive withdrawal reflex receptive fields, expressed as fraction of the sole of the foot from which a reflex in the tibialis anterior muscle was elicited. Data are presented as median, 10^{th}, 25^{th}, 75^{th} and 90^{th} percentiles. The black dots represent the values that lie outside the 10^{th} and 90^{th} percentiles. P=0.008.

Concerning the secondary endpoints, the subjective pain threshold and the threshold to evoke a nociceptive reflex after a single electrical stimulus were lower in patients, compared to the pain-free subjects (tab, 9 and fig. 7). The same was observed with repeated electrical stimulation evoking temporal summation: both the threshold to induce the subjective feeling of increasing pain sensation and the threshold that evokes a nociceptive reflex during repeated stimulation were lower in patients, compared to the pain-free subjects (tab. 9 and fig 8).

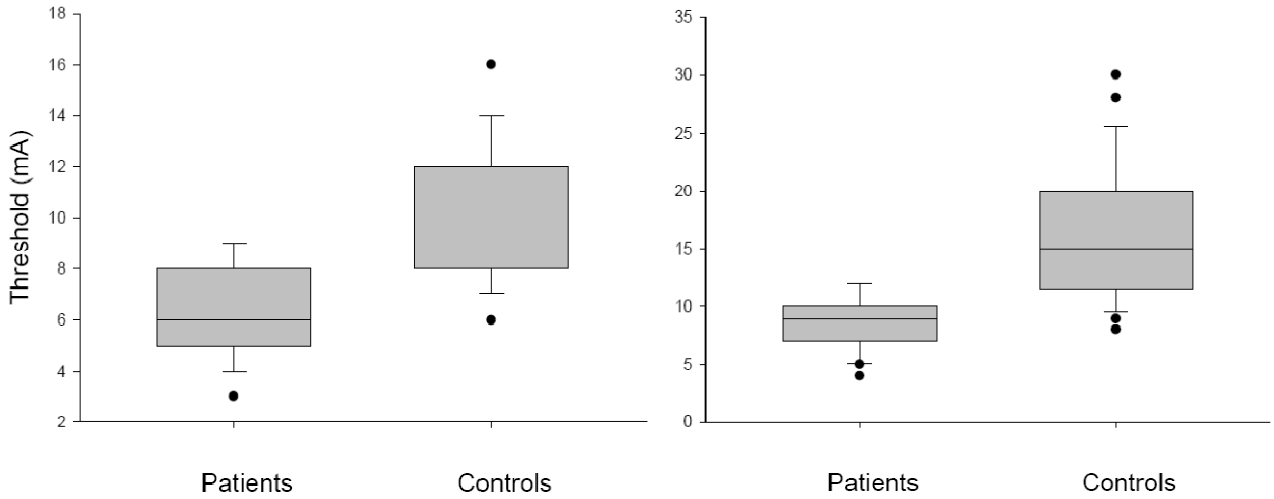

Fig 7. Pain (left) and nociceptive reflex (right) thresholds for single electrical stimulation. Data are presented as median, 10th, 25th, 75th and 90th percentiles. The black dots represent the values that lie outside the 10th and 90th percentiles. For the pain threshold of the control group (bottom graph), median and 25th percentile overlap. P<0.001.

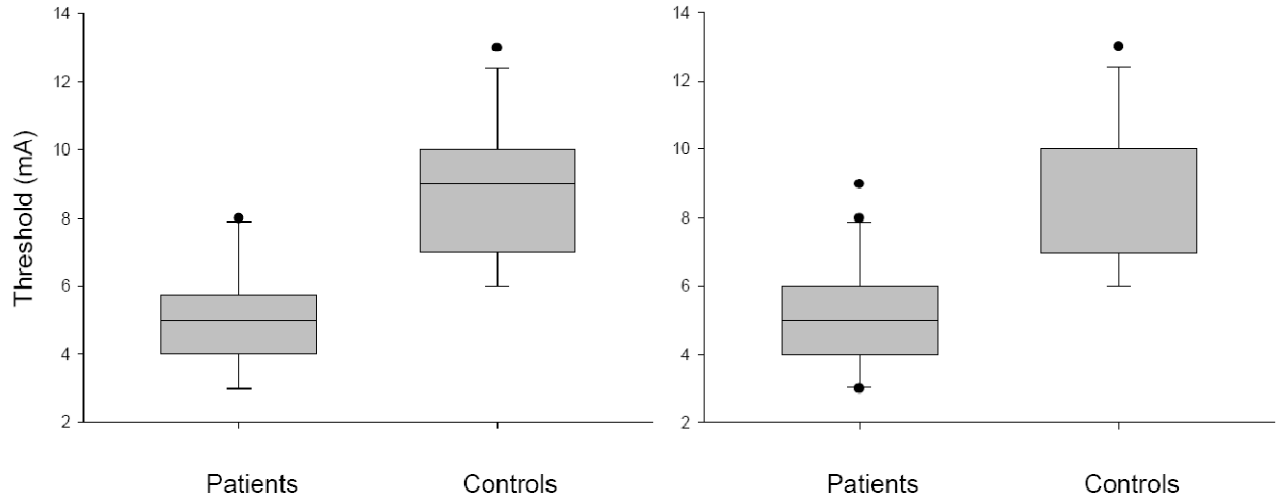

Fig. 8. Pain (left) and nociceptive reflex (right) thresholds for repeated electrical stimulation (5 stimuli at 2 Hz). Data are presented as median, 10th, 25th, 75th and 90th percentiles. The black dots represent the values that lie outside the 10th and 90th percentiles. P<0.001.

4. DISCUSSION

4.1. QUANTIFICATION OF REFLEX RECEPTIVE FIELDS (I)

A method for quantifying reflex receptive fields was developed, based on non-invasive measures of the nociceptive withdrawal reflex in humans. The paper describes both laboratory procedures and data analysis methods for extracting relevant parameters describing the RRF size and location allowing relevant statistical analysis. Such quantitative methods are needed for assessing the excitability of the spinal nociceptive system in relation to experimental and chronic pain studies and also for assessing efficacy of new centrally acting compounds.

4.1.1. STIMULATION METHOD

Nociceptive withdrawal reflexes have been elicited by electrical stimulation in many human experimental pain studies (Hugon, 1973;Willer, 1977;Petersen-Felix et al., 1996;Andersen, 2007;France et al., 2007). This is a very efficient stimulus for evoking withdrawal reflexes even though it is non-natural. Heat stimulation has been attempted but the level needed for evoking spinal reflexes in an experimental setting is often associated with mild tissue damage (reddening) and large reflex variability (Andersen et al., 2006). Care must be taken with positioning of the stimulating electrodes in order to avoid stimulation of nerve trunks and ensure that very local sensations are evoked. Stimulation of nerve trunks activates axons innervating large areas and hence might cover both excitatory and inhibitory reflex receptive fields (Weng and Schouenborg, 1996;Sonnenborg et al., 2000) resulting in ambiguous assessments of the RRF. Habituation is often seen with electrical stimulation (Dimitrijevic et al., 1972) but by constantly changing the stimulation site the problem is minimised (Fuhrer, 1973;Carstens and Ansley, 1993). Blinding of the subjects as to position and timing of the next stimulation improves the quality of the recordings as the subject has less chance of modulating the withdrawal voluntarily.

A critical methodological aspect is detection of the pain thresholds as this is the method for ensuring even input to the spinal cord irrespective of stimulation site. Often subjects find that the quality of the sensations evoked at the different sites varies, which is probably related to skin thickness. Hence, stimulation at the heel is less sharp compared to stimulation in the arch of the foot, most likely due to larger spread of the current through thick epidermal layers. There is a strong correlation between electrode impedance and pain thresholds (Andersen et al., 2004). Furthermore, it is imperative to familiarise the volunteer before assessing the pain thresholds to avoid gradual adaptation to the electrical stimulations. Randomisation in the sequence the pain thresholds are detected is important and further direct comparisons between a 'control' site (site 5) helps to ensure that the intensity of the stimuli is comparable across stimulation sites. The lower VAS ratings at the heel could be explained by the less sharp quality of the electrical stimuli. Furthermore, the pain intensity stimulus-response curves might very well be less steep at skin sites with thick epidermal layers so multiplying the stimulus intensity at all sites with a fixed factor is not optimal. A future alternative could be to evoke the reflexes at stimulus intensities that produce similar pain intensity scores for all stimulation sites.

4.1.2. RRF ESTIMATION TECHNIQUE

The interpolation method applied in the present paper is based on non-uniformly based data points in two dimensions, i.e. the location of the electrodes is according to anatomical landmarks and not in a uniform grid. The interpolation surface-map is further constrained to go through the actual recordings (see fig. 1) at the ten electrodes sites and is based on an inverse distance weighting method (Shepard, 1968;Sandwell, 1987) for interpolation implemented in Matlab. One important precaution is not to base any further statistical analysis on the extrapolated values, as steep gradients towards the border of the interpolated map (fig. 2) will result in biomechanically distorted values in the extrapolated regions. The surface-map is then modulated onto a binary image of a foot in Matlab to derive the images (see fig. 1 for the assessment procedure).

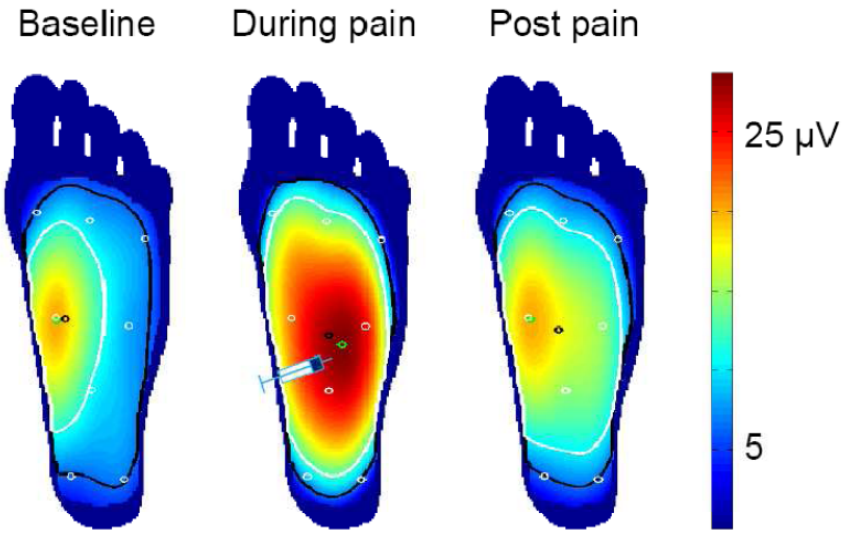

Fig. 9. Modulation of RRF by an intramuscular injection of capsaicin into the flexor digitorum brevis muscles. The RRF before, during and after (60 min) intramuscular injection of capsaicin in a single subject with complete spinal cord injury are illustrated. The detected RRF areas, peak reflex response, and CoG are illustrated. The injection site is depicted by the syringe. During muscle pain, the RRF area expanded and the peak reflex response moved towards the injection site. After a break of 60 min where the nociceptive activity from the flexor digitorum brevis muscle most likely vanished, the RRF almost returned to baseline values. The CoG showed a marginal posterior move immediately after capsaicin injection and returned near to a pre-injection location after the muscle nociceptive afferent activity ceased.

From the surface-map the RRF is extracted based on estimations of the level and variability of the background EMG activity prior to the stimulus. The area of the RRF is then calculated via statistical evaluation of the part of the surface map with significant EMG activity. Hence, the present method is more robust than a method based on simple, fixed thresholds (Andersen et al., 2001). Two other features to describe the location of the RRF are suggested in the present paper, the location of the peak of the RRF and the center of gravity of the detected RRF. Based on the observations in the capsaicin experiment, the peak of the RRF seems to be more sensitive to describe changes in the location of the RRF (fig. 9). The RRF of the tibialis anterior muscle in spinal cord injured subjects covers a large fraction of the sole of the foot compared to spinal intact volunteers (Andersen et al., 2004), and therefore the main change observed with the capsaicin injection is *within* the RRF. This suggests that the RRF volume and location of the peak appear to be the most sensitive measures.

The shape and position of the detected RRF depicted in fig. 3 resembles previous reports (Andersen et al., 1999;Andersen et al., 2001) based on substantially lower

samples. This is also the situation for the distribution of the onset latencies (fig. 4), with gradually longer onset latencies toward the border of the RRF (Andersen et al., 1999), which is also in agreement with observations in rats (Schouenborg and Kalliomäki, 1990). These similarities with previous findings suggest that the RRF may be a stable measure from experiment to experiment.

4.1.2 THE RRF AS A QUANTITATIVE MEASURE OF CENTRAL HYPERSENSITIVITY IN HUMANS

Widely accepted experimental models of spinal central sensitisation in humans are all based on psychophysical measures of cutaneous allodynia, hyperalgesia or referred pain associated with experimental induction of pain in deep structures (Klein et al., 2005). In contrast, even robust noxious conditioning stimuli leading to accepted psychophysical signs of central sensitisation have very limited effects on the nociceptive withdrawal reflex. Topical capsaicin has been shown to produce enhanced reflexes, but only while the volunteers perceived ongoing pain from the treated skin site (Grönross and Pertovaara, 1993) or when concurrent pain was evoked from the skin sites with allodynia/hyperalgesia (Andersen et al., 1995). Deep pain evoked by i.m. injection of hypertonic saline had only marginal effects on withdrawal reflex sizes (Andersen et al., 2000). This lack of evidence for central manifestations might be related to minor changes in reflex gain associated with the humans models despite the substantial changes in reflex excitability in animal models (Woolf, 1983;Xu et al., 1995;Tabo et al., 1998;Harris and Clarke, 2003). Alternatively, it could also be related to insufficient sensitivity of the reflex methods developed for human studies.

In chronic musculoskeletal pain patients, lower withdrawal reflex thresholds have been identified (Desmeules et al., 2003;Banic et al., 2004). Expansion of receptive field size is accepted as one of the most robust measures of central sensitisation in animal models (Cook et al., 1987;Hoheisel and Mense, 1989;Dubner, 1991). The encoding of the spinal reflex receptive fields is assumed to involve dorsal horn neurons located in deep lamina. Hence, wide dynamic range (WDR) neurons with receptive fields resembling the RRF for specific muscles have been identified (Schouenborg et al., 1995) which therefore are putative encoders of the RRF. These

neurons do not have ascending collaterals indicating they are spinal reflex pathway interneurons. WDR neurons in the same part of the dorsal horn show prolonged firing following repetitive stimulation of C fibres (wind-up) (Schouenborg and Sjölund, 1983) and the firing is linked to gradual increases in withdrawal reflexes (You et al., 2003). Wind-up is closely associated with central sensitisation and hence assessment of RRF in humans could provide a unique and robust view of spinal nociceptive processing in human subjects. The participants tolerated the electrical stimulation well which was also the case in a similar study in chronic pain patients (Banic et al., 2004).

Intramuscular injection of capsaicin has been shown to produce signs of central sensitisation in human volunteers in the form of referred pain (Witting et al., 2000). The pain evoked by capsaicin lasted 38±5 minutes in the latter experiment for a dose of 100 µg in a volume of 1 ml injected into the brachioradial muscle. However, injection into the same foot muscle as in the present experiment (flexor digitorum brevis) did not modulate the RRF (Andersen, 2007) despite robust pain for ten minutes (average VAS rating above 3 on a 0-10 scale). This might be related to descending inhibition triggered by the capsaicin injection, and hence the pilot findings presented in this paper were obtained from a volunteer with complete spinal cord injury. Recordings from more subjects are clearly needed to decisively determine if descending modulation is a key factor controlling the reflex pathway excitability in experimental chronic pain models or not. In animal models, the reflex excitability is substantially increased in spinal models compared to spinal intact animals (Carstens and Douglass, 1995;Gozariu et al., 1997;Clarke et al., 2002), in particular during central sensitisation (Harris and Clarke, 2003). The expansion of receptive fields of dorsal horn nociceptive neurons is further highly dependent on descending activity (Laird and Cervero, 1990;Yu and Mense, 1990;Schouenborg, 2002). The RRF in human spinal cord injured subjects is expanded compared to spinally intact subjects, indicating that descending control is essential for maintaining biomechanically functionally relevant RRF (Andersen et al., 2004).

4.1.3 CONCLUSIONS (I)

Paper I described a new method for acquiring and quantifying reflex receptive fields in humans based on electrical stimuli presented to several electrode sites in random order. The detected reflex EMG responses were interpolated and modulated onto an image of a foot. From the interpolated image, a number of features were extracted to quantify the size and location of the RRF. The assessment of the RRF may become an important method for evaluating mechanisms of central sensitisation in chronic pain patients.

4.2 REFERENCE VALUES OF QUANTITATIVE SENSORY TESTS (II-III)

Reflex responses to single stimuli, assessment of temporal summation and of the size of receptive fields reflect mechanisms of spinal nociception that have great importance in the pathophysiology of pain states (Woolf and Salter, 2000; D'Mello and Dickenson, 2008). Therefore, their evaluation may provide relevant information on the nociceptive system not only for research purposes, but also in individual patients. The present project defined normative data in a large pain-free population that can be used as reference values when the nociceptive system is explored in individual patients (95% CI, table 2), provided that exactly the identical assessment procedures that we described are used.

The threshold for evoking reflexes was higher than the pain threshold after single stimulus (table 7). Previous studies have found identical thresholds (Willer, 1977; Chan and Dallaire, 1989), while in other studies the reflex threshold was lower than the pain threshold (Bromm and Treede, 1980; Micalos et al., 2008). This is probably related to different test sites and/or different definitions of the reflex threshold (Rhudy and France, 2007). In paper II, a demand of fairly long lasting EMG burst might explain the relatively higher reflex thresholds to single electrical stimulation. On the other hand, the pain and reflex thresholds to repeated electrical stimulation were almost identical, in agreement with previous observations (Banic et al., 2004).

For mechanical and thermal psychophysical tests (paper III), the different levels of quantile analyses, i.e. 5^{th}, 10^{th} and 25^{th} percentiles, represent the limits to categorize patients with lower pain thresholds as hypersensitive. The same applies to the 75^{th}, 90^{th} and 95^{th} percentiles to categorize patients with higher thresholds as hyposensitive. As for the electrical pain tests (paper II), the normative data provided in paper III can be used as reference values when alterations in pain sensitivity is explored in individual patients. The most straightforward application is the use of the lower bounds of the percentiles to assess central pain hypersensitivity. In this respect, the choice of 5^{th}, 10^{th} or 25^{th} percentile as cut off for normal values (table 4) depends on how conservative the estimation should be for each particular patient. Choosing the 5^{th} percentile would categorize few patients as having central hypersensitivity, whereas using 10^{th} or 25^{th} percentile increases the number of patients who would be identified as having central sensitization. While values below the 5^{th} percentile can be considered as abnormal with a high confidence, increasing degrees of caution are required for values that lye above the 5^{th} and below the 25^{th} percentile. In cervical or low back pain, regional and generalized central sensitization can be assessed by applying the stimuli at the cervical/low back region and the lower extremity, respectively.
Less evident applications arise from the use of the upper bounds of the percentiles, whereby patients whose values are higher than the 75^{th}, 90^{th} or 95^{th} percentiles would be categorized as pain-hyposensitive. In neuropathic pain conditions, abnormally high pain thresholds can be a sign of nerve damage. In other chronic pain conditions the incidence and meaning of hyposensitivity to mechanical and thermal painful stimuli are at present unclear.

4.3 INFLUENCE OF DEMOGRAPHIC VARIABLES

4.3.2 GENDER, AGE AND INTERACTION OF GENDER WITH AGE

Previous investigations have shown that pain thresholds are lower in women than in men across various stimulus modalities (Chesterton et al., 2003a; Ge et al., 2004).

The influence of age seems to be strongly dependent on the stimulus modality (Gibson and Farrell, 2004; Lautenbacher et al., 2005). However, the influence of age on pain sensitivity is still controversial and the mechanisms underlying the correlation are poorly understood. So far, the interaction of gender with age was not investigated.

In paper II, gender was not a predictor of any outcome measure, whereas age was related with different assessment modalities (tables 2-4). In previous investigations, the nociceptive reflex threshold to single stimulus was either not affected by gender (Willer, 1990) or lower in women than in men (France and Suchowiecki, 1999). The temporal summation reflex threshold was slightly lower in women than in men (Serrao et al., 2004). Unlike these investigations, the finding on the lack of gender effect resulted from the analysis of a large sample size and was consistent across the different tests, suggesting that electrical tests are probably insensitive to gender differences.

When the tests are used for clinical purposes, not only the statistical significance but also the quantitative impacts of the explanatory variables are important. The quantitative impact is determined by the regression coefficients (tables 2-4 and 6) and provide indications on the magnitude of clinical relevance of the correlations.

The highest quantitative impact was observed for age with the single stimulus pain threshold, with a correlation coefficient of 0.0463. This means that for an increase in 10 years of age the threshold increases by 0.463 mA, i.e. by 4.2% in relation to the mean value of the threshold. For the temporal summation assessments, the correlation was negative, but the quantitative impact was negligible: for an increase in 10 years of age the threshold increases by 0.183 and 0.184 mA for pain and reflex thresholds, respectively. The same negligible quantitative impact was observed for area and volume of RRF. The generally low quantitative influence of age on the assessments probably explains the inconsistent findings of previous investigations on the nociceptive reflex, which were conducted on smaller sample sizes and did not cover the whole range of age (Sandrini et al., 2005). A less efficient endogenous inhibitory control has been detected in elderly compared with young subjects, which may partly explain the increased pain sensitivity that we found with single stimulus pain threshold (Edwards et al., 2003). For practical

purposes, we suggest that the confidence intervals presented in table 5 are used as reference values independent of age.

In paper III, women displayed lower pressure and thermal pain thresholds than men, although the influence of gender decreased with increasing age (Figure 4). Previous investigations on the influence of gender on pressure pain thresholds found either lower pain thresholds in women than in men (Otto and Dougher, 1985; Buchanan and Midgley, 1987; Fischer, 1987; Brennum et al., 1989; Jensen et al., 1992; Riley et al., 1998; Fillingim, 2000; Chesterton et al., 2003b) or no differences between genders (Sandrini et al., 1994; Isselee et al., 1997). Findings in paper III are consistent with the results of thermal tests of most studies conducted on healthy subjects (Arendt-Nielsen and Bjerring, 1988; Feine et al., 1991; Fillingim et al., 1998; Sheffield et al., 2000). Data on age are quite contradictory, suggesting that pain sensitivity increases, decreases or remains unchanged with age (Gibson and Helme, 2001).

The most challenging finding in paper III is that the difference in pain sensitivity between men and women may disappear or be quantitatively modest for older age groups. This challenges the general view that women are generally more pain sensitive than men. Previous studies on this subject were probably limited by the fact that mostly young subjects were investigated (Ellermeier and Westphal, 1995; Chesterton et al., 2003b; Fillingim et al., 2005; Komiyama and De Laat, 2005; Garcia et al., 2007).

Gender differences in pain have been attributed to many factors, including gonadal hormones (Riley et al., 1999; Fillingim, 2000; Fillingim and Ness, 2000; Craft, 2007; Li et al., 2009; Mensah-Nyagan et al., 2009) and differences in central pain modulation (Staud et al., 2003; Martin, 2009; Mensah-Nyagan et al., 2009). A recent meta-analysis (Martin, 2009) concluded that fluctuations of ovarian hormones in the course of the menstrual cycle may be associated with a mild to moderate effect on pain response. Of 19 studies, seven studies reported decreased pain thresholds during late-luteal or early-follicular phases (hormonal milieu of low and declining serum concentrations of estrogen and progesterone); five studies reported decreased pain thresholds during the late follicular and early luteal phases (hormonal milieu of high serum estrogen concentrations and rising progesterone

concentrations); The other studies analyzed in the review found no differences between the phases of the menstrual cycle.

Post-menopause is characterised by low serum concentration of estrogens and very low serum concentration or lack of progesterone. Thus, the higher pain thresholds that we observed in older ages may be supported indirectly by the studies that found a correlation between high pain thresholds and low hormonal level during the menstrual cycle (Hapidou and De Catanzaro, 1988; Bajaj et al., 2001; Drobek et al., 2002). On the other hand, the studies showing lower thresholds during low serum concentration of estrogen do not support the view that hormonal changes account for the interaction of gender with age that was observed on this project (Rao et al., 1987; Fillingim et al., 1997; Isselee et al., 2001; Gazerani et al., 2005). Further studies explained the gender differences as the result of differences in central pain modulation, with females having less effective central inhibitory mechanisms than men (Staud et al., 2003; Ge et al., 2007; Martin, 2009; Mensah-Nyagan et al., 2009).

4.3.3 BMI AND BODY SIDE

In paper III, BMI influenced the cold pain thresholds (table 6). The correlation coefficient was -0.1768, implying a reduction in pain threshold (i.e. lower pain sensitivity) with increasing BMI. For instance, an increase in BMI by 5 results in a decrease in the cold pain threshold by 0.88 °C. This suggests that studies using cold pain thresholds should take into consideration the BMI, e.g. when comparing groups.

The body side was related significantly with reflex threshold to single stimulus. Measurements on the dominant side had a threshold lower than on the non-dominant side by 0.9696 mA, i.e. 6.0% lower in relation to the mean value of the threshold. A study on non-nociceptive reflexes revealed no side differences, but because only 11 subjects were investigated the study probably did not have sufficient power to detect differences (Sakamoto et al., 2006). We are not aware of studies analyzing the effect of body side on nociceptive reflex parameters. In the absence of such investigations, explanations for our finding remain speculative. Differences in sensory and motor conduction velocities of peripheral nerves

between dominant and non-dominant arm have been documented (Colak et al., 2004): it can be postulated that the preferential use of the dominant limb may lead to a subclinical sensitization that is reflected by lowered reflex thresholds. A further possible explaining factor is the greater strength and muscle mass of the dominant side, leading perhaps to a lower activation threshold of the muscles.

In paper III, the body side was related significantly with heat pain thresholds. Measurements on the left body side had a threshold lower than on the right side by 0.5489 °C (table 6). A previous study on reference values of quantitative sensory tests (Rolke et al., 2006) found no significant left-right differences for heat pain threshold. We do not find a clear reason for this discrepancy. Perhaps a possible explanation is the different rate of temperature increase during testing (1.5 °C/s and 1.0 °C/s in the present and Rolke et al 2006, respectively). Our finding suggests that caution should be taken when one side is used as control for the other side, as it is often the case in clinical studies.

4.4 INFLUENCE OF PSYCHOLOGICAL AND HEALTH-RELATED VARIABLES

The analyses on psychological and health-related parameters should be evaluated under the consideration that we studied almost only healthy subjects. Only a small number of them displayed disturbances in the investigated dimensions, so that the variables had to be dichotomized in order to measure their potential role. This implies that the effects of the variable under consideration are only significant beyond a critical threshold of the psychological and health-related parameters.

The importance of depression in pain syndromes is well-known, but it is still unclear whether depression is a determinant or a cause of pain (Angst et al., 2008). There are few and inconsistent data on the influence of depressive symptoms on pain thresholds.

In paper II, BDI was a predictor only of the single stimulus electrical pain threshold, with a correlation coefficient of -2.5943. This means that subjects with depression scores ≥11 having an estimated pain thresholds 2.5943 lower than those subjects with scores <11. This reflects a 23.8% decrease in relation to the mean value of the threshold. The fact that depression affected only a subjective pain threshold

and not the reflex assessments suggests that pure spinal nociceptive processes may be independent of the influence of depression. The same can be said for the subjective pain threshold to repeated stimulation (temporal summation), which was not affected. This model may therefore reflect spinal integrative mechanisms, rather than supraspinal pain processing. In an early study on chronic pain patients, BDI was not related to any experimental pain modality including the nociceptive reflex (Boureau et al., 1991).

In previous studies, depression as assessed by the BDI affected pressure (Petzke et al., 2003) and heat pain (de Zwaan et al., 1996). Findings in paper III showed that depression levels did not affect the experimental pain measures, which is in accordance with previous studies on healthy volunteers (Klauenberg et al., 2008) and chronic pain patients (Skevington, 1983; Boureau et al., 1991). However, other studies on pain-free subjects found either increased (Adler and Gattaz, 1993; Lautenbacher et al., 1994) or decreased pain thresholds with increasing depression levels (Chiu et al., 2005). While some investigations on chronic pain patients indicated that depressed subjects have higher pain thresholds than non depressed controls (Adler and Gattaz, 1993; Lautenbacher et al., 1994; Dickens et al., 2003; Bar et al., 2005), other studies found that pain thresholds are reduced in depression (Frank et al., 1988; Summers et al., 1988; Chiu et al., 2005)

The relation between catastrophizing and pain has been studied using different pain modalities and in different patient groups, including mixed chronic pain (Sullivan and D'Eon, 1990), low back pain (Flor et al., 1993), rheumatoid arthritis (Keefe et al., 1989), and whiplash injuries (Sullivan et al., 2002). Those studies found that catastrophizing is associated with increased pain. This project found that catastrophizing did not affect any quantitative sensory test in pain-free subjects. Possibly, the influence of catastrophizing on pain is not accompanied by enhanced pain sensitivity as assessed by thermal, mechanical and electrical pain tests. Our findings confirm the lack of correlation between catastrophizing and nociceptive reflex threshold in both healthy volunteers (France et al., 2002; Rhudy et al., 2007) and patients with neck pain after whiplash injury (Sterling et al., 2008).

In a previous study, inducing anxiety experimentally in healthy volunteers decreased heat pain thresholds. In contrast, anxiety did not affect the nociceptive

reflex threshold after single electrical stimulation (French et al., 2005). In an early study, anxiety influenced electrical pain tolerance, but not pain detection threshold (Robin et al., 1987). In papers II and III, state and trait scales of STAI were not significantly correlated with any test, indicating that anxiety is not a relevant contributor of quantitative sensory tests in pain-free subjects.

There is a lack of investigations to correlate parameters of the SF-36 or similar scales with pain thresholds. In this project, the only statistical significance for electrophysiological tests (II) among the different SF-36 parameters was observed on the area of the RRF for mental health. The correlation was negative, reflecting a decrease in pain sensitivity for scores ≥90: the RRF area decreases by 0.0577, which represents 17.5% of the mean value of RRF area. This finding suggests a possible modest influence of general health status on spinal nociceptive processes, but the fact that only one parameter was affected render an interpretation of this result difficult. For the psychophysical tests, the only statistical significance was observed on the pressure and cold thresholds for physical health and on pressure for total scale of SF-36. However, descriptive analyses revealed only very modest quantitative impacts of these variables on the pain thresholds.

Overall, we found only limited influence of the different variables analyzed on the quantitative sensory tests. This was particularly true for the electrophysiological pain tests (paper II). The limited influence of the predictors on the electrical tests that we analyzed can be considered in two ways. The lack of effect of factors that are known to influence pain sensitivity, such as gender or certain psychological factors indicates that such electrical tests explore only part of the complex sensory and affective experience of pain. On the other hand, the relative robustness of the tests may be used advantageously when the influence of confounding parameters is unwanted. This may be the case for pharmacological studies conducted on small samples, in which it may be difficult to control for confounding factors. In a clinical setting, the evaluation of nociceptive processes that are unaffected by demographic and psychological factors may be useful in different situations: for instance, to make inferences on central plasticity processes leading to generalized central hypersensitivity, independent of the influences of higher cognitive and affective components.

4.5 CONCLUSIONS (II/III)

Reference values of parameters related to the spinal nociceptive reflex, electrical pain thresholds (paper II), pressure, heat and cold pain stimuli (paper III) were determined. These data can be used to detect central hypersensitivity in individual patients.

Demographic, psychological and health-related factors have modest influences on psychophysical electrical tests and nociceptive spinal reflexes. For most psychophysical tests, the values must be stratified according to gender and age. In general, women displayed lower pain thresholds than men. However, the influence of gender decreased with increasing age, with no or minimal gender difference in elderly subjects. These interactions depended on the type of painful stimulus applied.

The findings are expected to provide tools for the application of quantitative sensory tests in clinical practice and for a better use of the models in clinical research.

4.6 EXPANSION OF REFLEX RECEPTIVE FIELDS IN CHRONIC PAIN PATIENTS (IV)

Previous animal and human studies using the withdrawal reflex paradigm indicated that the reflex is organized in a modular fashion: each muscle or synergistic muscle group has a well-defined coetaneous receptive field, the reflex receptive field (RRF) (Schouenborg and Kalliomaki, 1990; Andersen et al., 1999). Nociceptive input applied to that area evokes a withdrawal reflex in the muscle, while stimulation outside the area has no effect (Sonnenborg et al., 2000). The reflex receptive field is probably encoded by wide-dynamic range (WDR) neurons located in the deep dorsal horn (Schouenborg et al., 1995). Receptive field expansion has been demonstrated in WDR projection neurons in this part of the dorsal horn (Dubner, 1991). The present project provides the first evidence that a chronic human pain condition is associated with expansion of nociceptive reflex receptive fields.

4.6.2 NWR AND PAIN THRESHOLDS

The reflex threshold after application of a single electrical stimulus was lower in patients than in controls (see tab. 9 and fig. 7). Because the site of stimulation is outside the area of pain, this finding indicates that patients display generalized spinal cord hypersensitivity. Accordingly, the reflex threshold after application of repeated electrical stimulation was lower in patients than in controls (see tab. 9 und fig. 8), indicating generalized facilitated temporal summation. Temporal summation probably reflects neuronal integration processes that can lead to neuronal hyperexcitability (Price, 1972; Arendt-Nielsen et al., 1994). The results on single and repeated electrical stimulation are consistent with observations in chronic neck pain and fibromyalgia patients (Desmeules et al., 2003; Banic et al., 2004).

4.6.3 ENLARGED AREAS OF RRF IN CHRONIC PAIN

The enlarged area of RRF observed (tab. 9, fig. 5-6) indicates that such a generalized spinal cord hyperexcitability is associated with an expansion of the nociceptive receptive fields in the spinal cord. This suggests that the modular organization of the pathways responsible for the nociceptive withdrawal reflex may undergo reorganization under pathological conditions.

Expansion of receptive fields following tissue damage has been observed by several animal investigations. For instance, appearance of new receptive fields of spinal cord neurons could be induced by intramuscular injection of bradykinin in rats, suggesting that silent synaptic connections within the spinal cord are activated (Hoheisel et al., 1993). However, this phenomenon has been investigated in regions of the spinal cord that correspond to the site of tissue damage. In contrast, finding in paper IV demonstrated that expansion of receptive fields occurs at an area far distant from the site of expected tissue damage. To date, animal research provides only indirect support to explain this finding. An early investigation found that blocking descending pathways by cooling the thoracic spinal cord of cats produced expansion of receptive fields at L7 level, suggesting that such widespread expansion of receptive fields may result from changes in descending modulation (Zieglgansberger and Herz, 1971). Later investigations showed that peripheral

inflammation can lead to widespread spinal cord hyperexcitability via activation of descending facilitatory pathways that involve the spinal 5-HT3 receptor (Suzuki et al., 2002). Tissue damage has been shown to produce generalized expression of COX-2 in the spinal cord, mediated by the humoral release of inflammatory mediators from the damaged tissue (Samad et al., 2001).

The above data from animal experiments suggest that humoral factors and/or changes in descending modulatory influences may play a role in the widespread expansion of receptive fields that we observed. However, the results of human studies on descending modulation are not univocal. In a study on healthy volunteers, rapid and slow distension of the rectum induced facilitation and inhibition of the nociceptive reflex, respectively (Bouhassira et al., 1998). The former finding would support the hypothesis that clinical pain arising from visceral structures, in our case from the pelvis, can lead to widespread spinal cord hypersensitivity. On the other hand, inhibition of the nociceptive reflex by slow distension of the rectum indicates that spinal hyperexcitability can undergo heterotopic inhibition via descending modulation.

A well-known method to study endogenous modulation in humans is the assessment of diffuse noxious inhibitory control: under normal conditions, pain after application of a test nociceptive stimulus is attenuated by the application of an additional "conditioning" noxious stimulus to a remote body region, reflecting diffuse endogenous inhibition (Chitour et al., 1982; Ge et al., 2004). A study that applied this model to neuropathic pain patients revealed a complex picture: the effect of conditioning stimuli on spinal nociception depended on the type of stimulus applied and the pathophysiological mechanisms underlying the pain condition (Bouhassira et al., 2003). A study investigating the efficacy of coping skills training in patients with arthritis of the knee found an increase in nociceptive reflex threshold, suggesting that spinal nociceptive reflexes may be influenced by descending modulation (Emery et al., 2006). On the other hand, techniques to induce expectancy-mediated analgesia reduced subjective pain, but not nociceptive reflex thresholds in patients with fibromyalgia (Goffaux et al., 2009).

Noteworthy, the few available human studies have used different methods of assessing descending modulation and have been conducted on patients with

different types of pain conditions. This renders the interpretation of the data difficult. Based on the available literature, spinal cord hypersensitivity that leads to generalized expansion of nociceptive receptive fields may be the result of multiple factors, including tissue damage via neural and humoral mediators, as well as influences from higher centres mediated by descending pathways. The present project will hopefully stimulate further research on the determinants of this phenomenon in pain patients.

4.6.4 CONCLUSIONS (IV)

Paper IV provided the first evidence for widespread expansion of spinal nociceptive receptive fields in a human chronic pain condition. This finding contributes to elucidate the mechanisms that underlie central hypersensitivity in chronic pain. Reverting the expansion of nociceptive receptive fields may become a target of clinical research.

5. SUMMARY

The aims of this project were: 1) to establish a new method to quantify reflex receptive fields in humans; 2) to determine the reference values of psychophysical and electrophysiological pain tests; and 3) to study whether widespread expansion of receptive fields is present in chronic pain patients.

In paper I, a method for quantifying nociceptive withdrawal reflex receptive fields (RRF) in pain-free subjects and patients was described. Electrical stimuli were applied to the sole of the foot evoking reflexes in the tibialis anterior muscle. The method is based on random stimulations presented in a blinded sequence to the ten stimulation sites. A set of features describing the size and location of the RRF was presented based on statistical analysis of the sensitivity map within every subject. The features include RRF area, volume, peak location and center of gravity.

Reference values of parameters related to the spinal nociceptive reflex, electrical pain (paper II), pressure, heat and cold pain stimuli (paper III) were determined. This allows their clinical application for assessing central hyperexcitability in individual patients. In paper II, age had a statistically and quantitatively significant influence on the subjective pain threshold to single electrical stimuli. Depression had a negative impact on the subjective pain threshold to single electrical stimuli. All the other factors had either no statistically significant influence or a quantitatively insignificant impact of the electrical tests. Thus, the electrical pain tests, and in particular the reflex assessments, explore aspects of sensitization processes that are largely independent of demographic characteristics, cognitive and affective factors.

In paper III gender, age and/or the interaction of age with gender were the only variables that consistently affected the pain measures. Women were more pain sensitive than men. However, the influence of gender decreased with increasing age. The data indicate that the reference values of these tests have to be stratified by gender and age.

In paper IV, patients with chronic endometriosis pelvic pain displayed a larger area of RRF, compared with pain-free subjects. Pain and reflex thresholds after sural

nerve stimulation (secondary endpoints) were significantly lower in patients than in controls.

In conclusion, the present project provided data for an application of advanced pain assessments to detect aspects of central hypersensitivity in individual patients. Furthermore, it detected for the first time widespread expansion of nociceptive receptive fields in chronic pain patients. This phenomenon may underlie central hypersensitivity in human chronic pain conditions and may become a target for the development of future therapeutic interventions.

6. DANSK SAMMENFATNING

Formålet med dette project var at: 1) udvikle en ny metode to at måle refleks receptive felter hos menesker; 2) bestemme referenceværdier for psykofysiske og elektrofysiologiske smertetests; 3) udforske om patienter med kroniske smerter har en udtalt udvidelse af deres refleks receptive arealer.

I artikel I. beskrives en metode til at kvantificere nociceptive afværge receptive refleks arealer (reflex receptive fields: RRF) hos frivillige forsøgspersoner og patienter. Elektriske stimuli, som udløser reflekser i tibialis anterior musklen, blev apliceret via 10 elektroder placeret under foden. Stimuli blev apliceret i en blindet og randomiseret rækkefølge via alle 10 elektroder. Baseret på en statistisk analyse af sensiviteten indenfor stimulations området af de enkelte individer, kunne en række deskriptive egenskaber, som beskriver størrelsen og lokalisationen af RRF bestemmes. Disse egenskaber omfatter RRF arealet, RRF volumen, lokalisationen af den største refleks i RRF og placeringen af RRF's tyngdepunkt Referenceværdier for disse parametre relateret til den spinale nociceptive refleks (artikel II) og til smertefulde tryk, varme og kulde stimuli (artikel III) blev bestemt. Derved kan disse parametre bruges klinisk til at bedømme graden af central sensibilisering hos individuelle patienter. I artikel II viste alderen sig at have en statistisk og kvantitativ signifikant indflydelse på den subjektive smertetærskel for enkelte elektriske stimuli. Derimod havde depression en negativ inflydelse. Alle andre faktorer havde enten ingen statistisk signifikant inflydelse eller en kvantitativ ubetydelig indflydelse på smerten induceret af elektrisk stimulation. Således viser det sig, at de elektriske smertetests, og specielt refleks bestemmelser, kan udforske aspekter af sensibiliseringsprocesser, som stort set er uafhængige af demografiske karakteristika og af kognitive og affektive faktorer.

I artikel III viste køn, alder og/eller interaktionen alder med køn sig at være de eneste variable som konsistent havde en indflydelse på smertemålinger. Kvinder viste større sensitivitet for smerter end mænd, men forskellen aftog med stigende alder. Resultaterne antyder, at referenceværdierne bør stratifiseres after alder og køn.

Artikel IV viste, at patienter med kroniske endometriose betingede bækkensmerter havde større RRF arealer sammenlignet med smertefrie kvinder. Smerte- og reflekstærskler efter stimulation af nervus suralis var signifikant lavere hos smertepatienter end hos de raske kontrolpersoner.

Sammenfattende etablerede dette projekt metoder og data, som muliggør en avanceret bedømmelse af visse aspekter af central sensibilisering hos individuelle smerte patienter. Desuden demonstrerede projektet for første gang en udvidelse af nociptive refleks receptive arealer hos kroniske smertepatienter. Dette er sandsynligvis en vigtig komponent i den centrale hypersensitivitet hos patienter med kroniske smerter. Dette kan blive et mål for udviklingen af fremtidige therapeutiske interventioner.

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