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Psychophysical and electrophysiological responses to the experiences of thermal sensation and thermal grill illusion

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Psychophysical and Electrophysiological Responses to the Experiences of Thermal Sensation and Thermal Grill Illusion

Ph.D. Thesis by Xi Li

June 2009

Laboratory for Cortical Plasticity and Human Brain Mapping

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Preface

The present thesis is based on Xi Li's research during her Ph.D. study between July 2006 and June 2009 in the Laboratory for Cortical Plasticity and Human Brain Mapping at the Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University, Denmark.

The following studies are included.

Study I

High resolution topographical mapping of the warm and cold sensitivities

Xi Li, Laura Petrini, Ruth Defrin, Pascal Madeleine, Lars Arendt-Nielsen. Clinical Neurophysiology 2008; vol. 119, nr. 11, s. 2641-6

Study II

The importance of stimulus parameters for the experience of the thermal grill illusion

Xi Li, Laura Petrini, Li Wang, Ruth Defrin, Lars Arendt-Nielsen. Neurophysiologie Clinique 2009; in press.

Study III

Cortical responses to the experience of painful thermal grill illusion

Xi Li, Laura Petrini, Li Wang, Ruth Defrin, Lars Arendt-Nielsen. Experimental brain research; Submitted.

Acknowledgement

Abbreviations

ANOVA	analysis of variance
BMI	body mass index
COLD	cold-specific cells
EEG	electroencephalography
FFT	fast Fourier transform
HPC	noxious heat, pinch and cold responsive cells
NS	nociceptive-specific cells
SD	standard deviation
STT	spinothalamic tract
TG	thermal grill
TGI	thermal grill illusion
TRP	transient receptor potential
TRPV	TRP vanilloid

Abstract

The ÷thermal grill illusionø (or ÷synthetic heatø) describes a paradoxical sensation occurring when someone touches spatially adjacent bars with an alternating pattern of cold and warmth. The fact that simultaneously applied innocuous cold and warm stimuli could possibly elicit painful sensation makes the thermal grill illusion a candidate model to study integration of thermal and pain perception pathways and an interesting research topic to explore its potential connection with pathological pain. However, the elicitation of painful thermal grill illusion and the underlying mechanisms of the thermal grill illusion phenomenon are still not clearly understood. The current Ph.D. study explores thermal grill illusion by means of psychophysical and electrophysiological investigations aiming to control and to understand the thermal grill illusion. Three experimental studies have been conducted on 79 healthy human volunteers in order to investigate the following thermal characteristics: (a) the thermal sensitivity distribution over the glabrous skin in the human hand; (b) the quality of the thermal sensations and the thermal grill illusions; (c) the stimulation influential factors to the thermal grill illusion; and (d) the cortical responses to the thermal and the thermal grill illusions. The thermal sensitivity distribution over the glabrous skin of the hand is found to be highly heterogeneous. The thermal grill stimulation could induce thermal grill illusion in most of the people and the sensation could be painful. Some factors such as the number of stimulation bars and the distance between cold and warm stimulation bars did not affect the elicitation and the sensation of the thermal grill illusion. Painful and non-painful thermal grill sensations had different time courses and subjects who experienced painful thermal grill illusion had different cortical responses from subjects who reported non-painful sensations to thermal grill stimulation. Therefore the presence of painful thermal grill illusion can be assessed quantitatively by changes in the brain oscillations over the frontal area. Psychological and cognitive factors such as the anxiety level or attention and anticipation to pain experience may be of the influential factors that mediating painful thermal grill illusion, which also indicates that thermal grill illusion can be a potential evaluation/diagnosing tool in psychological clinical use.

Dansk resumé

õTermisk grill illusionö (eller õsyntetisk varmeö) beskriver en paradoksal følelse, som opstår, når man rører spatialt nærliggende barer med et vekslende varme-/kuldemønster. Den kendsgerning, at samtidigt påførte uskadelige kolde og varme stimuli muligvis kan fremkalde smertefølelse, gør termisk grill illusion til en egnet model til undersøgelse af integrationen af termiske og smerteopfattende baner samt et interessant forskningsemne til undersøgelse af den potentielle sammenhæng med patologisk smerte. Imidlertid vides det ikke præcis, hvordan termisk grill illusion frembringes, ligesom de underliggende mekanismer af termisk grill illusion heller ikke kendes præcis. Dette Ph.D.-studie undersøger termisk grill illusion ved hjælp af psykofysiske og elektrofysiologiske undersøgelser med formålet at kontrollere og forstå termisk grill illusion. Der er fortaget tre eksperimentelle studier på 79 raske forsøgspersoner til undersøgelse af følgende termiske karakteristika: (a) fordelingen af den termiske sensibilitet over den ubehårede hud i menneskets hånd; (b) kvaliteten af termiske følelser og termisk grill illusion; (c) stimulationsfaktorerne, som påvirker termisk grill illusion; og (d) de kortikale responser på termisk grill illusion. Fordelingen af den termiske sensibilitet over den ubehårede hud i hånden er meget heterogen. Termisk grill stimulation kunne inducere termisk grill illusion i de fleste forsøgspersoner, og den opståede følelse kunne synes smertefuld. Faktorer, som f.eks. antallet af og afstanden mellem kolde og varme stimulationsbarer, påvirkede ikke frembringelsen af følelsen af termisk grill illusion. Smertefuld og ikke-smertefuld termisk grill illusion havde forskellige tidsforløb, og de forsøgspersoner, som oplevede smertefuld termisk grill illusion fik anderledes kortikale responser i forhold til de forsøgspersoner, der rapporterede om ikke-smertefulde sensationer ved termisk grill stimulation. Det vil sige at tilstedeværelsen at smertefuld termisk grill illusion kan måles og kvantificeres via ændringer i kortikale responser over pandelappen. Psykologiske og kognitive faktorer som f.eks. angst, opmærksomhed og forventning om smerte kan have indflydelse på formidlingen af termisk grill illusion. Dette indikere at termisk grill illusion potentielt kan bruges til vurdering og diagnosticering, psykologisk klinisk praksis.

1. Introduction

1.1 Thermal sensations and human physiological pathways

In human skin, cold and warm receptors are specific and located at different areas with different densities. Cold receptors are considered as lying just below the epidermis while warm receptors are lying more in the upper and middle layers of the corium. Cold sensation conveys with a faster velocity than warm sensation and therefore traditionally cold receptors are considered as being supplied mainly by myelinated A nerve fibres while warm receptors by unmyelinated C fibres. The warm receptors usually fire until temperatures up to about 43 ℃ ~ 44 ℃ and the temperatures higher than 43 ℃ ~ 44 ℃ usually trigger a sensation of heat pain. The cold receptors usually fire until about 30 ℃ but some groups of cold receptors have been found to start firing also after around 45 ℃. (Schmidt 1986, Gardner et al. 2000, Green 2004, Meyer et al. 2005) Heat pain and cold pain are perceived by nociceptors in the skin. Moreover, some authors consider heat pain sensation as being conveyed through A nerve fibres where cold pain by C fibres (Gardner et al. 2000). However more commonly, both heat pain and cold pain are considered as can be mediated by both A and polymodal C fibres. The sensation of cold pain is complicated and involves several sensations with different time course, such as sharp pain, ache, cold or prickle sensations (Davis and Pope 2002).

Carrying thermal and nociceptive information from the skin, A and C fibers enter the spinal cord through the lateral division of the dorsal roots entry zone and end on laminae I, II and V in the gray matter, from where the second-order afferent sensory neurons originated. The second-order afferent sensory neurons formed the spinothalamic pathway which includes two separate tracts, the medial (anterior) spinothalamic tract and the lateral spinothalamic tract. Through both spinothalamic tracts, the thermal and nociceptive information is transferred into the brain. In the brain, the two tracts merge with trigeminal afferent fibers from the head and the merged tract terminates in the thalamus. From the thalamus, third-order neurons project their activities to the somatosensory cortex. The journey of thermal and nociceptive information in the brain has not been fully understood yet. Little is known about the processing of thermal information in the brain. But plenty of researches have pointed out a few brain regions forming networks for pain processing, such as prefrontal cortex, somatosensory cortex (SI and SII), anterior cingulated cortex, insula, thalamus, hypothalamus and periaqueductal gray. (Schmidt 1986, Wilils 1996, Peyron et al. 2000, Greestein and Greenstein 2000, Gardner et al. 2000)

Although as frequently experienced sensations, thermal and thermal pain sensations are more complicated and more difficult to understand than people usually think. For instance, cooling may trigger cold sensation but may also induce heat sensation and tactile-like sensations. Heat usually induces heat pain but may also induce cold sensation. Some individuals may fail to report pain at temperatures as low as 0 ℃ but the same person may feel painful to temperatures of around 15 ℃ in another test. Most of

these phenomenons can be explained as due to that the thermal pathways are not specific for processing thermal sensory input. Recent researches have been bringing out clues in identifying at the molecular basis of the temperature-activated ion channels. Most of these channels belong to transient receptor potential (TRP) family and are divided into several subgroups. The more specified classifications and understandings of these ion channels could explain the variety of thermal sensations better than the traditional theories. For instance, TRP vanilloid 1 (TRPV1) was the earliest identified heat-sensitive ion channel and has been found richly located in A and C nerve fibres; TRPM 8 leads a sensation of cold but only react to temperatures of about 30-32 ℃ and has been found almost exclusively existing in a subpopulation of C fibre. Those thermal sensitive ion channels have been found located in the neurons as well as in non-neuronal skin cells. (Mckemy et al. 2002, Perie 2002, Lee and Caterina 2005, Moran et al. 2004, Dhaka et al. 2006, Lumpkin and Caterina 2007)

1.2 The discovery of the thermal grill illusion

In 1896 Thunberg T. built the first thermal grill stimulator in the science history (Figure 1.1). The goal was to understand what would happen when the skin received simultaneously cold and warm stimuli at the same location. The stimulator was made of two coils of spiralled metal tubes with 3 and 1/2 rounds each. The two coils were interlaced with cold coil lying between those of warm coil. The diameter of the tube was 0.3 cm and the contact area was of 0.5 cm wide. The space between tubes was 0.1 cm and the total diameter of the stimulator was 5.5 cm. Temperatures of 24 ℃ and 44 ℃ were used and the cold and warm stimuli were given simultaneously or one after another. Thunberg described his own sensations when he applied the thermal grill stimulations on his thenar and his underarm. The later named ‑thermal grill illusionø was described õas if the temperature was suddenly raised and a feeling of hot appeared coupled with a burning sensation being about to ariseö (Alruz 1898).
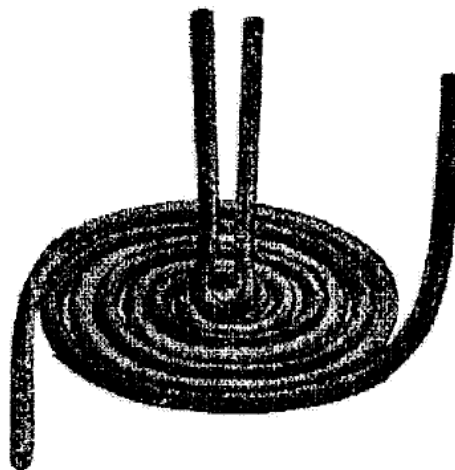

Figure 1.1 Thunbergøs thermal grill stimulator (Cited from Thunberg 1896)

1.3 The significance of the thermal grill illusion and the previous explanations

Soon after its first report, Alruz (1898) used the thermal grill illusion phenomenon to support his view that ‑hotø sensation was not a special sensation but a fuse of cold and warm sensations. Nowadays, a century after Alrutz raised his proposal, the pathways and the molecular basis of thermal sensation are still under investigation. Some of the ion channels in the skin have been identified as possibly involved in certain types of the thermal sensations (Moran et al. 2004, Lumpkin and Caterina 2007) and heat sensation is considered as being modulated differently from warm and cold sensations (Schmidt 1986, Gardner et al. 2000). However, the underlying mechanisms of thermal grill illusion remain unclear.

Thermal grill illusion has been considered as an example of the integration of thermal and pain perception pathways. The fact that simultaneously applied innocuous cold and warm stimuli can elicit painful sensation makes the thermal grill illusion a candidate model to study pathological pain and consequently a potential differential diagnosing tool for pathological pain patients.
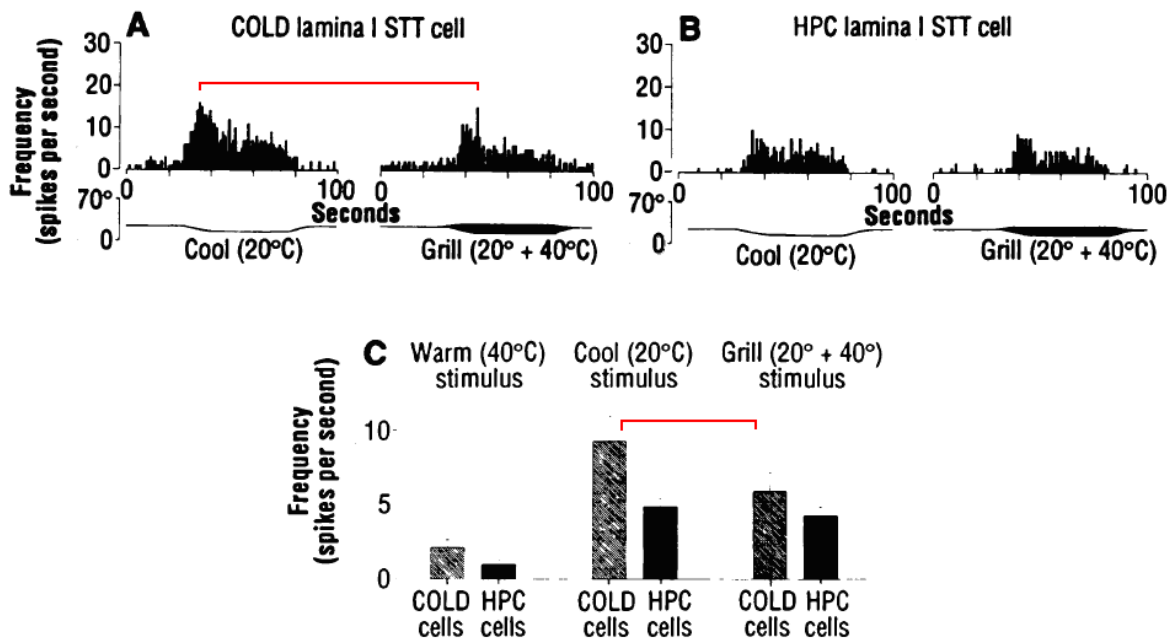
Figure 1.2 A. and B. Single event peristimulus time histograms showing the activity evoked in a COLD lamina I STT cell and in a HPC lamina I STT cell by the cool and thermal grill (Grill) stimuli. C. The averaged discharge rates of COLD (10) and HPC (7) cells, which show thermal grill stimulation induced reduction in COLD cell discharge (Adapted from Fig.3 in Craig and Bushnell 1994)

Before 2006, the best theory to explain thermal grill illusion was proposed in 1994 by Craig and Bushnell. The so-called ‑unmasking theoryø, based on an electrophysiological study with cat, proposed that the painful heat sensation evoked by the thermal grill stimulation reflects the central dis-inhibition of the cold-activated C polymodal nociceptive channel due to a reduction in specific cold activity caused by

the simultaneous warm stimulus. In their study (Craig and Bushnell 1994), three types of cat lamina I spinothalamic tract (STT) neurons were studied: nociceptive-specific cells (NS) that receive inputs from heat nociceptors; cold-specific cells (COLD) that receive input from specific cold receptors; and multimodal cells that are responsive to noxious heat, pinch and cold (HPC) and receive inputs from cold-sensitive C polymodal nociceptors. Three stimuli were given: cool (20 ℃), warm (40 ℃) and Grill (20 ℃ and 40 ℃). NS cells were reported as not activated by any of the stimuli and warm stimuli did not excite any of the three types of neural cells. It was a very interesting finding that the COLD activations were greatly reduced (49%) by thermal grill stimuli in comparison with the activations by cool stimuli (Figure 1.2). However, the activations of HPC cells were confusing since the temperatures of neither of the cool and thermal grill stimuli were in the range that capable to trigger nociceptors. The involvement of the C polymodal nociceptive channel was also questioned (Fruhstorfer et al. 2003, Bouhassira et al. 2005, Defrin et al. 2008).

1.4 EEG brain mapping technique

Electroencephalography (EEG) records the cerebral electrical potentials and synaptic potentials are the most important source. Pyramidal cells in layers of cortical neurons are the major contributor of the synaptic potentials that recorded by extracranial EEG. EEG signals represent pooled synchronous electrical activity generated by large populations of neurons. (Nunez and Srinivasan 2006, Tatum et al. 2008)

EEG has a higher temporal resolution than most of other brain mapping techniques, which makes EEG the best tool to study real time and dynamical changes of the brain activity (Figure 1.3). From the practical point of view, EEG recording is less costly and easier to apply in experiments. Its portability also allows EEG to be combined with many tasks, especially when there is movement involved.
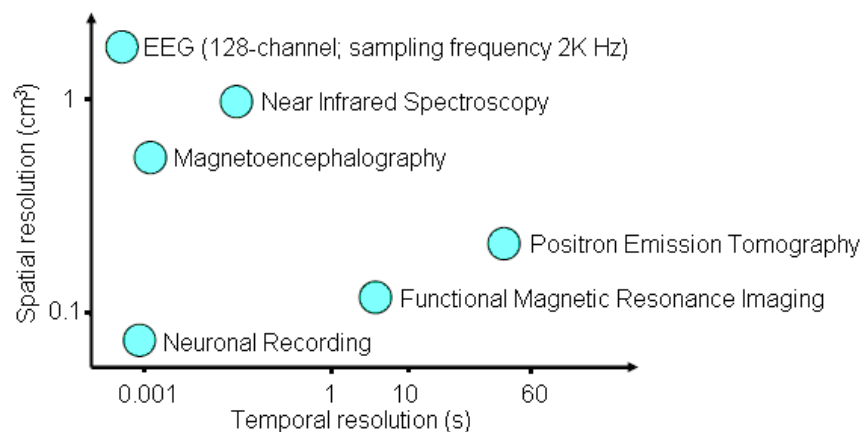
Figure 1.3 EEG has a higher temporal resolution and is less costly and easier in application than other technologies for assessing brain function

1.5 The structure and the aims of this Ph.D. study
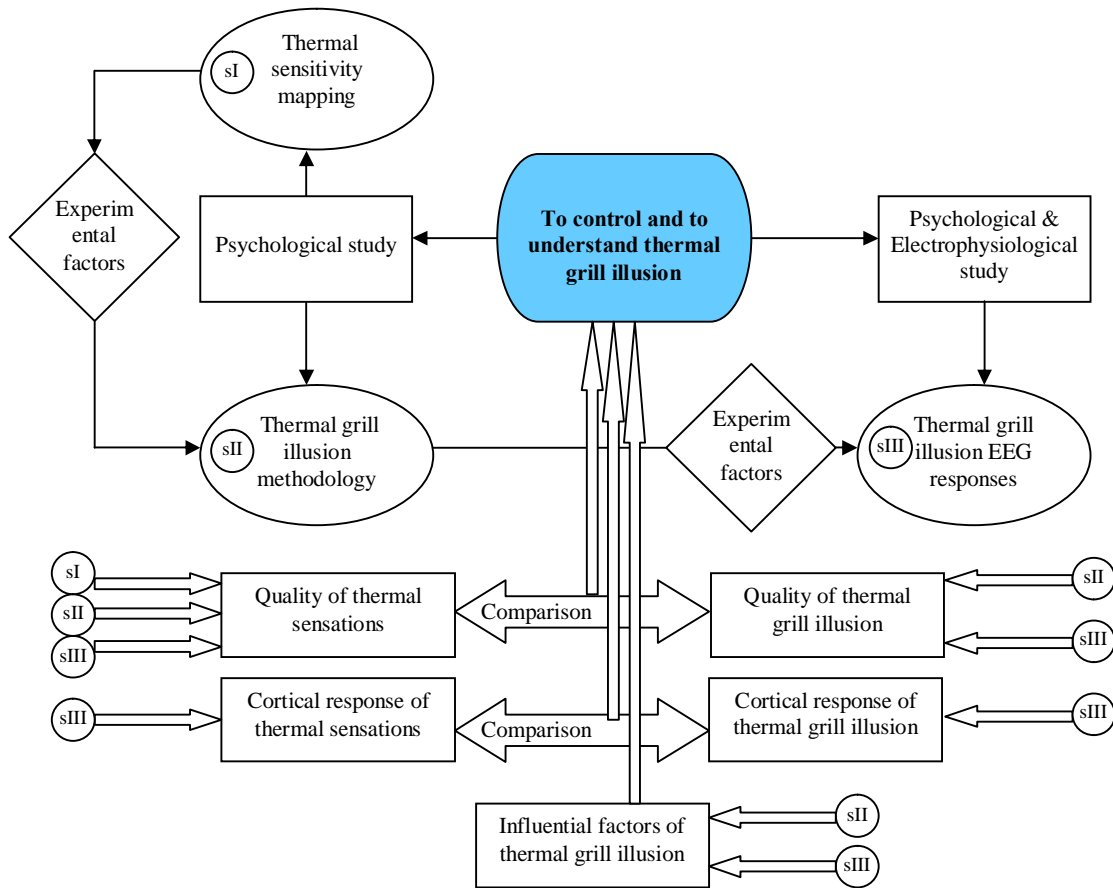
Figure 1.4 A diagram illustrating the structure of this Ph.D. study (study I, II and III are presented as (sI)(sII) and (sIII))

The main aim of this Ph.D. project was to study and compare thermal sensation and thermal grill illusion by means of psychophysical and EEG investigations with the ultimate goal to contribute to the control and the understanding of the thermal grill illusion. The cool and warm sensations and the heterogeneity of the sensitivity distributions on the skin of human's inner hand were studied in study I. The experimental factors that influence the excitation of the thermal grill illusion have not been sufficiently studied previously. Study II aimed to investigate the variable qualities of the thermal grill illusion and to validate systematically the importance of certain stimulus parameters to the perception of thermal grill illusion. The ongoing electroencephalographic (EEG) brain activity during the experience of thermal and thermal grill illusion was evaluated in study III.

2. Methods and Materials

2.1 Subjects

Eighty-four right-handed healthy volunteers participated in three studies and 79 of them completed the experiments. Data from 71 subjects (32 females, age: 24.5 ± 3.5 years; weight: 67.1 ± 12.1 kg; height: 172.2 ± 10.7 cm) were included into the analysis distributed in three studies. The reasons for the exclusion of the data were due to e.g. outlier (1 case in study I), thresholds not detectable (5 cases for cold sensitivity analysis and 1 case for warm sensitivity analysis in study I), abnormal sensations (1 case in study I, 1 case in study II and 4 cases in study III) or the quality of the EEG data was too bad to use (1 case in study III).

All experiments were conducted in the Laboratory for cortical plasticity and human brain mapping in the Center for sensory-motor interaction (SMI) at Aalborg University, Denmark. The lab was in a quiet, cozy and well facilited room. All participants gave their informed consent prior to the experiments. All studies were conducted in conformity with the Declaration of Helsinki and all the experimental procedures were approved by the local ethics committee (VN-2006/0026 and N-20070055).

2.2 Stimulations

The thermal grill stimulation apparatus is not commercially available. Building such a device for experimental use is one of the tasks in this Ph.D. study. There have been mainly two kinds of thermal grill devices used by a number of research groups, with running water or with electrical controlled Peltier elements. A system employing peltier elements was at first developed but was very unstable. The problem was due to unsuccessful removal of the heat that generated during heating up which disturbed the stability. The peltier elements were fragile and expensive and they may still work but in disorder once they were malfunctioned. For instance, all subjects from the first a few pilot tests reported heat pain to thermal grill stimulation which turned out to be caused by a hardly noticeable malfunction of one of the sixteen pieces of peltier elements. Therefore, an alternative device that facilited by running water was built.

This thermal grill system was capable to give thermal stimulations of different temperatures simultaneously. The thermode was flexible with respect to variable stimulating areas, adjustable distance between stimulations of different temperatures and flexible mounting for application on the body surface. The thermal stimulation thermode was composed of 6 hollow brass bars perfused with warm or cold water in preset temperatures (Figure 2.1). The size of each bar was 10 mm x 120 mm. Thermosensors attached beneath each bar provided continuous temperature feedback which ensured the required temperatures. The temperature feedbacks were collected through A/D board and were managed by a specially developed software using Labview.
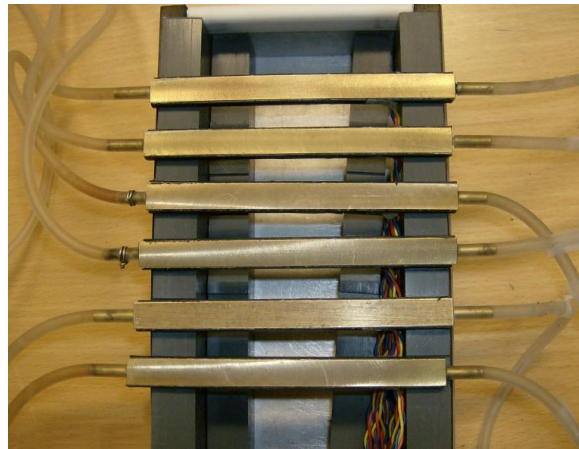
Figure 2.1 Six thermal grill stimulation bars spaced with 1 cm

With different temperature combinations, the thermal grill system was able to give static stimulations that induce sensations such as the non-painful cold sensation, non-painful warm sensation, cold pain, heat pain and thermal grill illusion to the subjects. In study II and III, the cold and warm temperatures employed in thermal grill stimulation were the same as those in non-painful cold and non-painful warm stimulations to the same subject. In study II, to induce thermal grill illusion, the temperatures of 20 °C ± 1°C and 40 °C ± 1°C were used. In study III, temperatures were individually adjusted according to the subjectøs thermal thresholds. Warm temperature was set as about 2 degrees lower than the subjectøs heat pain threshold. In order to trigger the painful thermal grill illusion at the maximal possibilities, non-painful cold were set as about 20 degrees lower than warm (Bouhassira et al. 2005). Cold pain and heat pain were 4 degrees lower or higher than the subjectøs cold pain threshold and heat pain threshold, respectively.

Besides temperatures, different spatial arrangements and combinations were used for different stimulations. Figure 2.2 displays the various stimulation configurations applied in study II when the effects of the number of, and the distance between the stimulation bars were studied.

Duration of the thermal stimulations affects the sensations. Thermal grill stimulations of both short and long durations were studied. In study II the stimulation duration was different and individually determined (within limited range of a few seconds) by the subjects in the way that the subjects placed their hand on the stimulating bars and lifted the hand when their initiative sensations were certain. The resulted averaged length of all the stimulations was 2.3 ± 0.9 seconds. In study III prolonged stimulations as long as 180 seconds were studied.
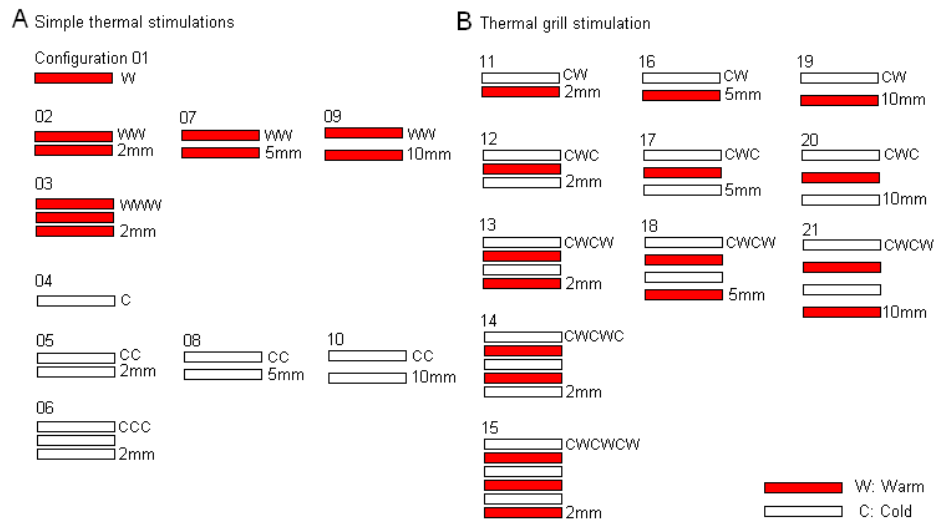
Figure 2.2 Twenty-one stimulation configurations applied in study II, including A. simple thermal stimuli (10 configurations) and B. thermal grill stimuli (11 configurations). The placement of the bars and the distances between bars are labelled

All experiments were in double-blinded design and all stimulations and measurements were performed in randomized order. Experiments usually followed a training session when the subjects were getting familiar with experimental procedures and their tasks. Possible sensations were informed to the subjects, including cold, warm, mixed cold and warm, and pain. All stimulations were applied on the glabrous skin in the right hand of the subjects, in the palm area (study II and III) or on the fingers (study II) (Figure 2.3).
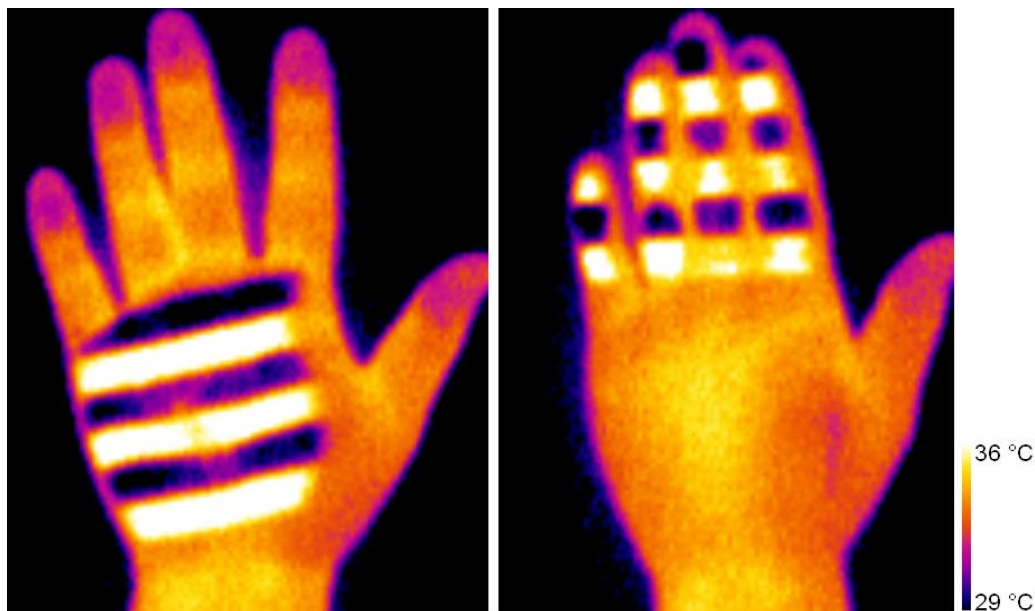
Figure 2.3 Thermographs taken immediately after the application of the thermal grill stimulation on the palm (left) and on the fingers (right)

2.3 Psychophysical data measurements

Measurements of thermal thresholds

The thermal thresholds were measured by using a PATHWAY system (Medoc, Ramat Yishai, Israel) with the model of ATS (Advanced Thermal Stimulator). The temperature limit of the system was between 0°C and 55°C. The size of the thermode was 3 cm x 3 cm. The Method of Limits was used for the threshold detection. Subjects received a series of thermal stimuli that continually increased or decreased from baseline and were asked to press a button when they perceived the onset of one of the cold, warm, cold pain or heat pain sensations in correspondence to the measurements of cold threshold, warm threshold, cold pain threshold or heat pain threshold, respectively. When subjects pressed the button the temperature stopped increasing/decreasing and then the temperature was recorded. Such tests were repeated for three times and the average of those measured thresholds (if the variance of the three measurements was less than 0.8) was recorded as thermal sensation threshold. The baseline temperature was set to 32°C and the temperature change rate was set to 2°C/sec for heating up/cooling down from the baseline temperature and 8°C/sec for returning back to the baseline temperature.

In study I cold and warm thresholds were measured in 23 locations on the volar side of the hand with limited contact area of 1 cm x 2 cm (Figure 2.4). In study III, cold, warm, cold pain, heat pain thresholds were determined in an area of 30mm x 30mm located at the proximal end of the hypothenar where it is one of the most sensitive (both to cold and to warmth) parts in the palm area (paper I: Li et al. 2008). In study II these thresholds were determined (test area 3 cm x 3 cm) for the palm at the location of the middle part of the hypothenar; and for the fingers on the proximal phalanges of the index, middle and ring fingers, which areas were within the stimulated area by all the stimulation configurations.
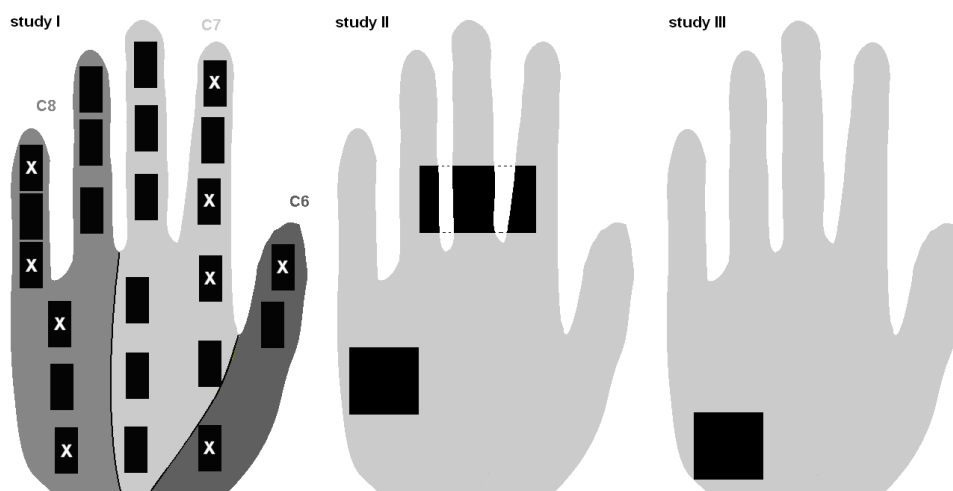

Figure 2.4 The measured locations for thermal thresholds in study I, II and III. Black rectangle marks the location for threshold test (thermode size: 1 cm x 2 cm in study I; 3 cm x 3 cm in study II and III), and

with 'X' in white colour marks the measured locations for epidermis thickness in study I. Different colours indicate dermatomes C6, C7 and C8 for study I (defined according to Keegan and Garrett, 1948)

Measurements of hand temperature

Hand temperatures were measured and monitored in all the studies. Measurements were made before, in the middle of and after the experiment for study I; and before applying each stimulus in study II and III by using thermography (Agema ™ Thermovision 900, FLIR Systems Ltd, Ontario, Canada). Averaged temperature values over an area of about 3 cm x 3 cm in the centre of the palm or fingers were recorded.

Measurements of hand area

In study I, the area of the subjects' volar hand skin was measured (ACECAD, D9000+ digitizer, Taipei, Chinese Taipei) and was included in the analysis. The relative hand area was also calculated as hand area divided by subject's height.

Measurements of epidermis thickness

B-mode ultrasound skin scanning was performed on the hand glabrous skin with a 50 MHz ultrasound scanner (DermaScan C, Cortex Technology, Hadsund, Denmark), producing cross-sectional images of the skin down to a depth of 3mm with an axial resolution of 25 m and a lateral resolution of 60 m. The distance between the surface of the skin and the epidermis-dermis interface was defined as epidermis thickness. Nine locations were measured on the right hand, shown in Figure 2.4 with white crosses.

Measurements of subjective sensations: ratings and verbal descriptors

Subjects reported their sensations using pre-defined numerical ratings and selected verbal descriptors. Different rating scales were used for different studies shown as in Figure 2.5. These scales were placed in front of the subjects during the experiments in order for them to easily remember and report.

After each stimulus in study II, subjects reported their sensations by using a scale (0-neutral sensation; 2-cold/warm threshold; 3~9-cold/warm with different intensities; 10-cold/heat pain threshold) and verbal descriptors (warm; burning pain; cold; freezing pain; tingling; pricking pain; stinging; pressing; itchy; annoying and others). In study III, in every 15 seconds during the stimulations, the subjects reported their sensation intensity (0-neutral; 1-slight cool/warm; 2-cool/warm; 3-cold/warm, no pain; 4-slight pain; 5-mild pain; 6-moderate pain; 7-moderate-strong pain; 8-strong pain; 9-severe pain; 10-unbearable pain) and their sensation distress level (0-neutral; 1-barely discomforting; 2- discomforting; 3-unpleasant; 4-irritating; 5-distressing; 6-miserable; 7-awful; 8-horrible; 9-agonising; 10-excruciating) (Chang et al.

2002). After every stimulus, they also reported the quality of their sensations (cold, warm, cold pain, heat pain or others).
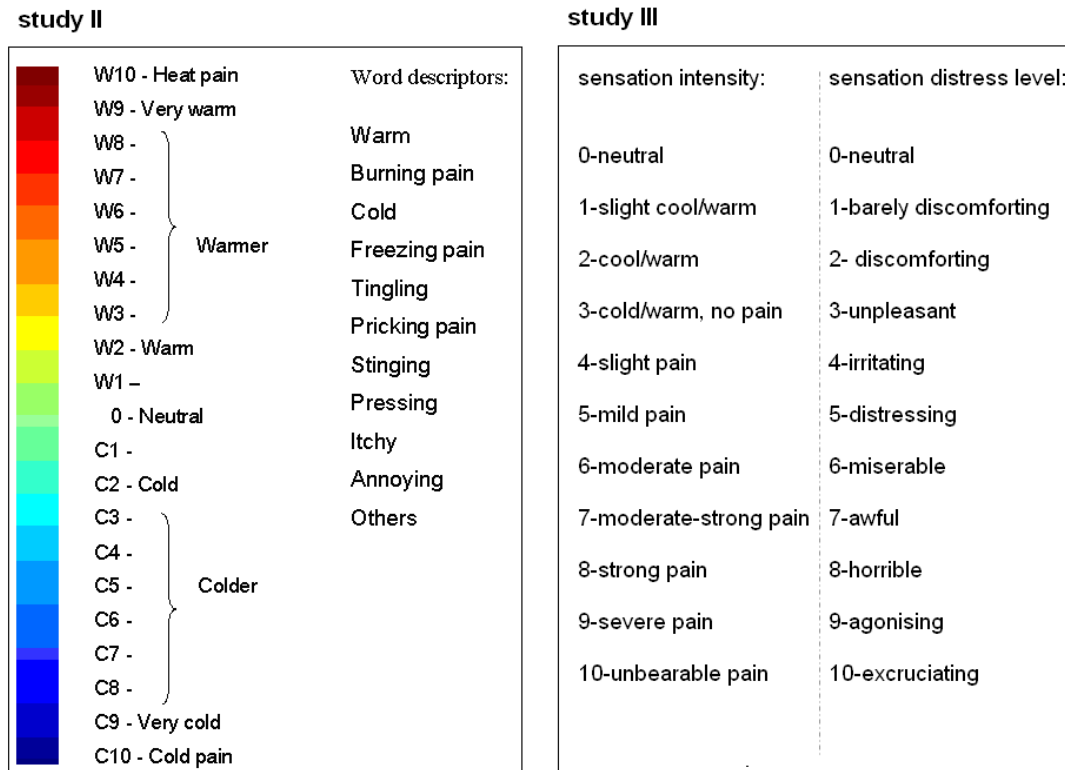
study II

W10 - Heat pain	Word descriptors:
W9 - Very warm	
W8 - ⎫	Warm
W7 - ⎪	Burning pain
W6 - ⎬	Cold
W5 - ⎪ Warmer	Freezing pain
W4 - ⎪	Tingling
W3 - ⎭	Pricking pain
W2 - Warm	Stinging
W1 –	Pressing
0 - Neutral	Itchy
C1 -	Annoying
C2 - Cold	Others
C3 - ⎫	
C4 - ⎪	
C5 - ⎬ Colder	
C6 - ⎪	
C7 - ⎪	
C8 - ⎭	
C9 - Very cold	
C10 - Cold pain	

study III

sensation intensity:	sensation distress level:
0-neutral	0-neutral
1-slight cool/warm	1-barely discomforting
2-cool/warm	2- discomforting
3-cold/warm, no pain	3-unpleasant
4-slight pain	4-irritating
5-mild pain	5-distressing
6-moderate pain	6-miserable
7-moderate-strong pain	7-awful
8-strong pain	8-horrible
9-severe pain	9-agonising
10-unbearable pain	10-excruciating

Figure 2.5 Thermal perception ratings and word descriptors used in study II and III

2.4 Electrophysiological data (EEG) measurements and processing

In study III, EEG was recorded simultaneously with the stimulations. The EEG data were recorded from 128 surface electrodes mounted on an EEG cap (WaveGuard [TM], Advanced Neuro Technology, Enschede, Netherlands) employing the 10-5 system (Oostenveld and Praamstra, 2001) (Figure 2.6). The data were recorded with EEProbe [TM] software (Advanced Neuro Technology, Enschede, Netherlands) with sampling frequency at 2 K Hz. Left mastoids was used as reference for recording.

After recording, the EEG data were re-referenced using both mastoids and band-pass filtered between 0.5 Hz and 50 Hz. The EEG data were cleaned by automatic artefact detection (± 75 V) followed by manually removing artefacts with the assistant of ASA software (ASA 3.0, Advanced Neuro Technology, Enschede, Netherlands). The EEG data were then cut into epochs of 2-minute long and FFT (with window length of 2 minutes) were calculated with the valid epochs (that contained no artefact). EEG power densities were calculated in 7 different frequency bands: Delta (0.5-3.5 Hz), Theta (4-7 Hz), Alpha1 (7.5-9.5 Hz), Alpha2 (10-12 Hz), Beta1 (13-23 Hz), Beta2 (24-34 Hz) and Gamma (35-45 Hz).
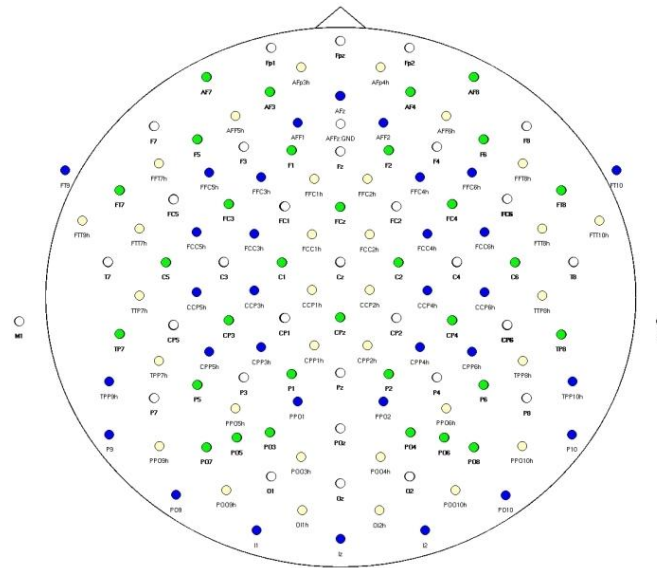
Figure 2.6 Layout of 128 surface electrodes for EEG recording employing the 10-5 system (provided by Advanced Neuro Technology, Enschede, Netherlands)

EEG data from a focal area represent a more credible and precise measurement of cortical activity than data from a single channel. Focal areas were defined as including focal maximal channels and their surrounding channels (Egsgaard et al. 2009). In study III of this Ph.D. project focal areas were defined according to the significant power changes to the stimulations from the baseline. Specifically, baseline frequency power density was subtracted from each condition at each channel and the differences in each frequency band were subjected to t-test in order to define focal areas for further study (study III).

2.5 Data analysis

Different statistical analyses were performed to data in different situations, including t-test, chi-square test, one-way-ANOVA, one-way repeated-measures ANOVA, two-way-ANOVA, two-way repeated-measures ANOVA and one-within and two-between ANOVA. Post-hoc pair-wise comparisons were performed with Tukey HSD and Bonferroni corrected t-test. The level of significance was set to $P < 0.05$. Linear regression analysis was used to calculate correlations between factors in different cases.

3. Results and discussions

3.1 Factors that affect thermal thresholds

From measurements in all three studies, the averaged thermal thresholds were: cold threshold: 27.7 ± 2.2 ℃; warm threshold: 36.2 ± 2.4 ℃, cold pain threshold: 16.8 ± 4.4 ℃ and heat pain threshold: 42.9 ± 1.8 ℃.

Many factors may influence the thermal thresholds:

Location: *The thermal perception sensitivity varies in the glabrous skin on human hand*

It has been well known that the sensitivities to cold and warmth are not uniformly distributed across the body regions, the extremities being the least sensitive (Meh and Denislic 1994, Stevens and Choo 1998, Hilz et al. 1999, Hagander et al. 2000, Defrin et al. 2006). In study I of this Ph.D. project, the cold and warm perception sensitivities were examined over the skin of a small area, the human hand. Data were collected from measurements from 23 spots with an area of 1 cm x 2 cm each located in the glabrous skin (Figure 2.4) in human hands of 25 healthy volunteers. Warm threshold data from 23 subjects and cold threshold data from 19 subjects were used for analysis and contributed to the sensitivity maps shown in Figure 3.1.
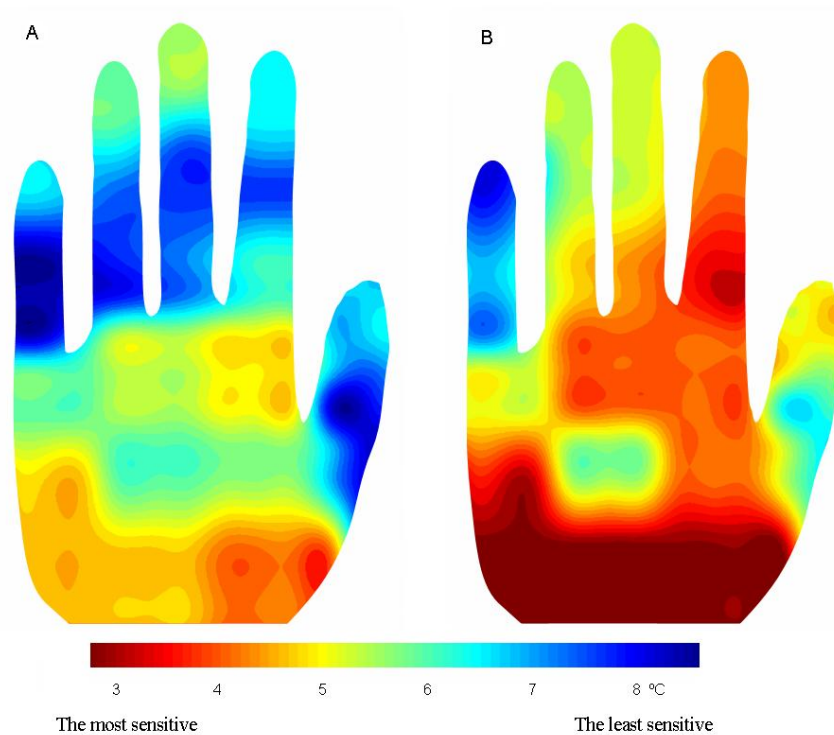

Figure 3.1 Topographical maps of the thermal sensitivities on the hand (palmar view). A. a mean map of the sensitivity to warmth (N = 23 subjects); B. a mean map of the sensitivity to cold (N = 19 subjects). The baseline temperature was 32°C. Based on data collected in study I

It was confirmed in this study that the thermal sensitivity differed significantly even within a relatively small body area as the volar part of the hand. The proximal part of the palm was the most sensitive part in the hand to both cold and warm stimuli when in general the palm was more sensitive than fingers. The variability of the cold and warm sensitivity reflected the inhomogeneities in thermal receptorsø distribution that cold and warm receptors are located separately in the skin with different densities. The cold and warm sensitivity variations were not bounded by dermatome territory and may also vary across dermatomes, e.g. C7 appeared more sensitive than C8 to cold stimuli. These findings regarding the variation of the cold and warm sensitivities within a small body area and within the same dermatome demanded that testing locations should be strictly and precisely determined for thermal sensory examinations in clinical and research applications.

Subjects were found more sensitive to cold than to warmth reflecting the anatomical difference of a higher cold receptor density with respect to warm receptors in the skin, where e.g. in hand surface there are 1-5 cold receptors/cm^2 but only 0.4 warm receptors/cm^2 (Schmidt 1986, Green et al. 2008). Sensitivities to cold stimuli and warm stimuli were highly correlated in the palm supporting, and further defining it into a smaller skin area, the view that the more sensitive a body region is to cold, the more sensitive it is to warmth (Stevens and Choo 1998). The correlation may also imply that although the cold and warm receptors are distributed in different densities, they may exist with proportional numbers and cold and warm sensations may share a common ascending system (Green and Akirav 2007). The relation between warm and cold sensitivities over the fingers was more complicated than that in the palm.

Gender: Females are more sensitive than males

There have been contradictories among studies regarding the gender effects on the thermal sensitivities. Stevens and Choo (1998) reported that thermal sensitivity is age dependent but not gender dependent whereas Meh and Denislic (1994) reported that women showed greater sensitivity for small temperature changes at a few body regions including the thenar region. Defrin et al. (2006) reported that females were more sensitive than males to heat pain at the hand (the mid dorsal surface). Thermal thresholds were measured in all three studies in this Ph.D. project using the same device (Medoc, Ramat Yishai, Israel) under similar conditions (except the size of the measured area). Analysis in study I was based on data from 23 locations from the hand of 23 subjects and suggested gender difference. However no gender difference was shown in study II or III. Comparing to study I, one possible reason to the lack of the significance might be due to the small sample size in these two studies.

Cold and warm perception thresholds were measured in all studies and cold pain and heat pain thresholds were measured in study II and III. All these data were accumulated and the gender difference was studied. Two-way ANOVA was applied to cold and warm perception thresholds with factors of

gender and detection area (2 cm^2 vs. 9 cm^2). One-way ANOVA was applied to cold and heat pain thresholds with gender as the factor.

The results concluded that females were significantly more sensitive than males to cold (F =7.0, P < 0.05), warm (F = 8.3, P < 0.05) and heat pain (F = 5.6, P < 0.05) stimuli however not to cold pain stimuli (Figure 3.2).
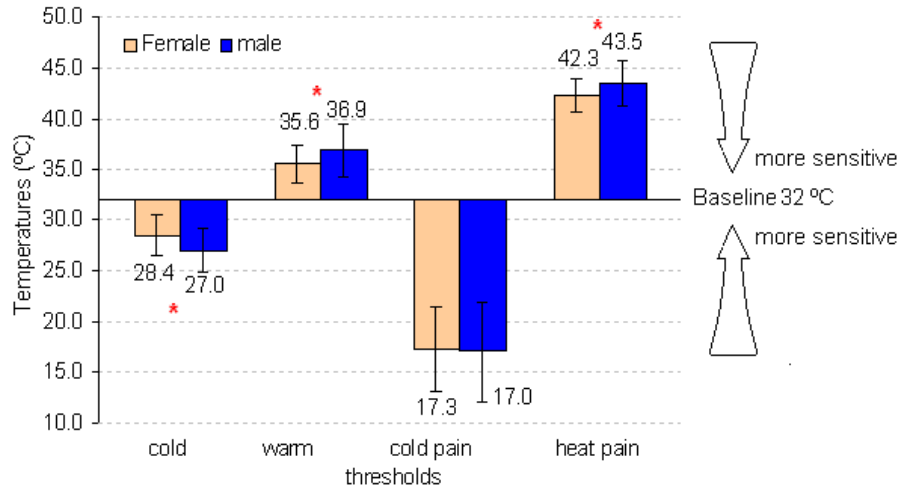

Figure 3.2 Gender differences with cold threshold, warm threshold, cold pain threshold and heat pain threshold (mean ± SD) based on data collected in study I, II and III. * indicates the significance (P < 0.05)

To exclude the interaction by the size of the areas on the gender difference to warm and cold thresholds, one-way ANOVA was applied with threshold data from study II and study III (both with the same size of detection area of 3 cm x 3 cm) with gender as a factor. The results accorded with above findings that females were more sensitive than males to cold (trend: F = 3.2, P = 0.082) and warm (F = 5.1, P < 0.05) stimuli.

Epidermis thickness

Skin thickness is one of the factors that may influence thermal sensitivities. In human skin, warm and cold receptors are specific and located at different areas with different densities. Cold receptors lie just below the epidermis while warm receptors lie more in the upper and middle layers of the corium (Schmidt 1986). Skin thickness may affect the heat conduction in the skin and hereby the thermal sensations.

Epidermis thickness was measured in a few locations on the hand in study I (Figure 3.3A). No correlations were shown between epidermis thickness and the cold and warm thresholds. However epidermis thickness was different between genders. Males have thicker epidermis than females (Figure 3.3C). It is presumable that such difference is related to the gender difference that shown with the thermal

sensitivities. Epidermis thickness was also found thicker in the glabrous skin on fingers than that on the palm (Figure 3.3B).
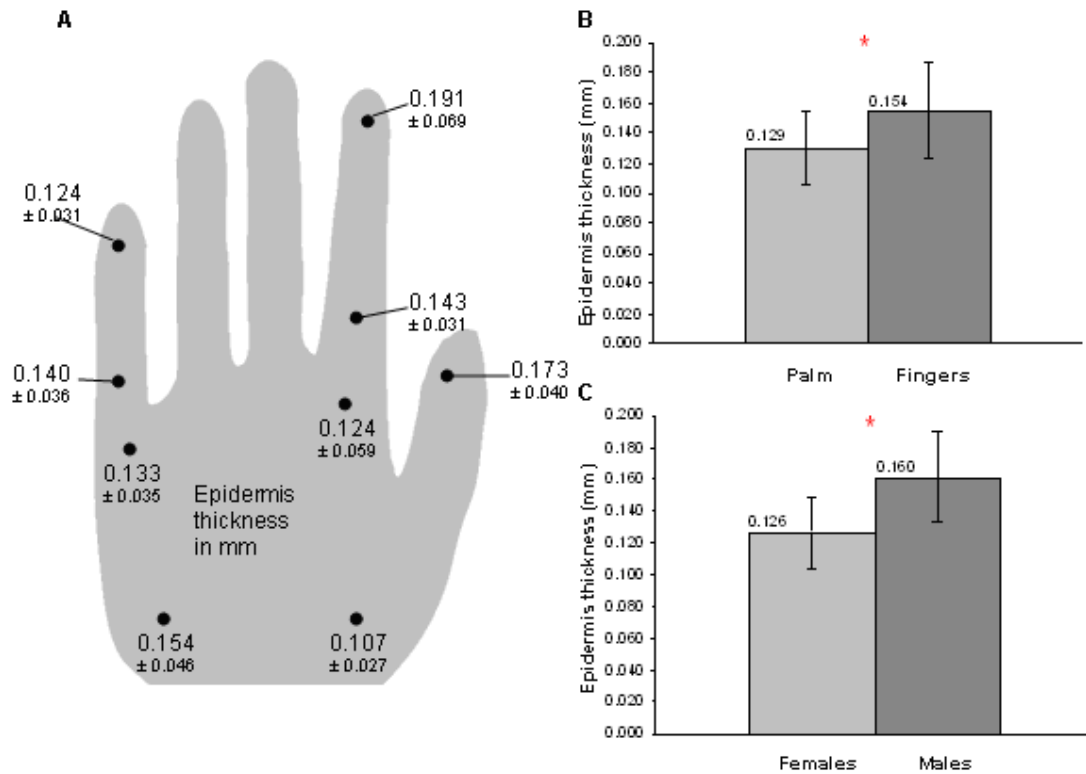

Figure 3.3 Epidermis thickness (mm) measurements collected in study I. A. mean (± SD) epidermis thickness (N = 8 subjects) at various anatomical sites (palmar view); B. mean (± SD) epidermis thickness on palm and fingers (N = 8 subjects); C. mean (± SD) epidermis thickness for females (N = 4 subjects) and males (N = 4 subjects). ÷*ø indicates statistically significant difference between groups (P < 0.05)

Body dimensions

Greenspan and Kenshalo (1985) suggested body size should be considered as a factor when studying spatial effect on thermal sensations. In study I, the effect of the subjectsø hand size on thermal thresholds was studied. Neither the size of the hand or its ratio to height was correlated to the subjectsø warm threshold. A dependence of cold sensitivity on the subjectøs hand area and a tendency of the dependence on the ratio between the hand area and the height were shown.

The hand area was not measured in study II and III. However, the body mass index (BMI) can be used as an indicator of the effect of body dimension on the thermal thresholds. BMI is defined as weight (kilogram) divided by the square of the height (meter). Two-way ANOVA suggested that BMI did not differ between genders or among studies. Cold and warm perception thresholds were correlated to BMI (Figure 3.4). Therefore, body dimensions may be one of the factors that influence thermal thresholds.
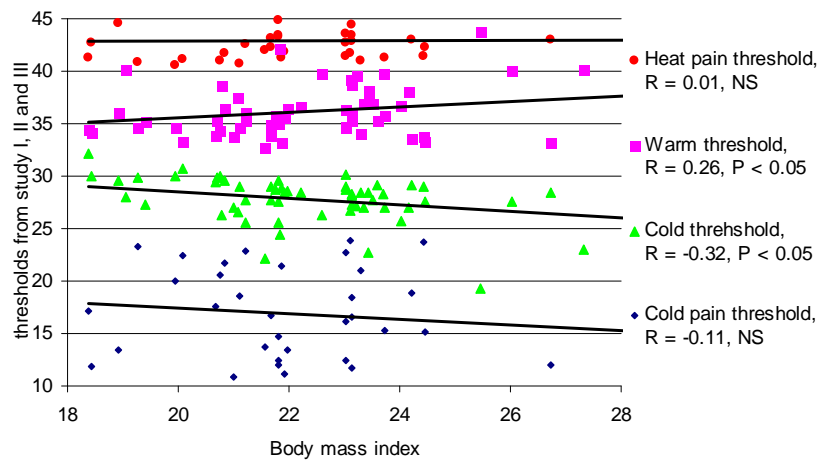
Figure 3.4 Cold and warm thresholds showed correlation with body mass index, based on data collected in study I, II and III

Size of detection area: *Thermal perception sensitivity increases with the size of the detection area*

There is no doubt that thermal threshold test is affected by the size of stimulated skin area. The sensitivities to cooling and warming of large areas are higher than when small areas are stimulated due to central spatial summation effect (Schmidt 1986). Cold and warm thresholds were measured in areas of 2 cm^2 (1 cm x 2 cm) in study I and 900 cm^2 (30 cm x 30 cm) in study II and study III. The size effect was studied by applying two-way ANOVA to cold and warm perception thresholds with factors of gender and detection area. The results were in line with the well accepted view that the larger the stimulated area the higher the sensitivities to cold and warm perception thresholds (Figure 3.5).
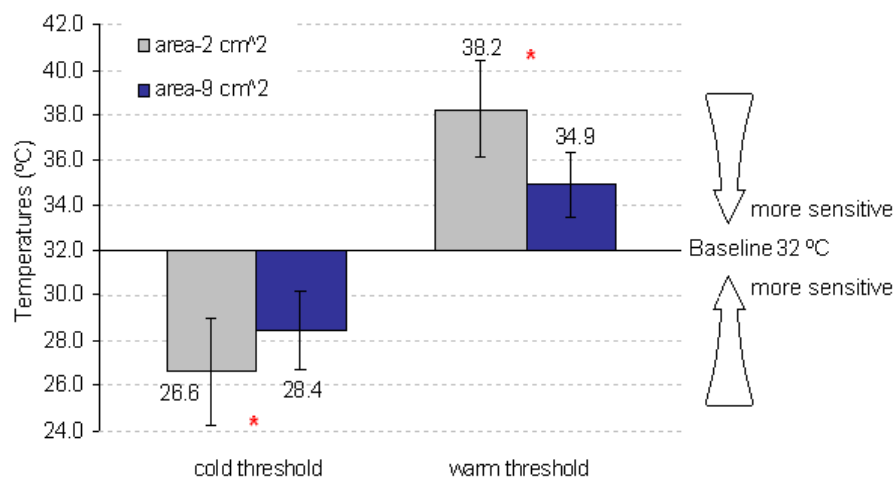

Figure 3.5 The size of the detection areas affects the cold and warm thresholds (mean ± SD): the larger the stimulated area the higher the sensitivities to cold and warm perception thresholds, based on data collected in study I, II and III. * indicates the significance (P < 0.05)

Baseline skin temperature

Baseline skin temperature is a factor that influences thermal sensations and thus thermal threshold measurement (Schmidt 1986). Therefore, in this project, the measurements of the thermal thresholds and the application of the stimuli started only when the subjectsø baseline hand temperatures were at about 30 -36 ℃ (considered as the neutral temperature). Time-breaks were arranged if the temperature was not in that range or if the subjects had abnormal sensations. No pre-warming or pre-cooling was applied to the subjects.

Applied on accumulated data from all three studies, one-way ANOVA suggested that the baseline skin temperatures did not differ between genders or among studies. However, no correlation was shown between baseline skin temperature and any of the thermal thresholds (Figure 3.6).
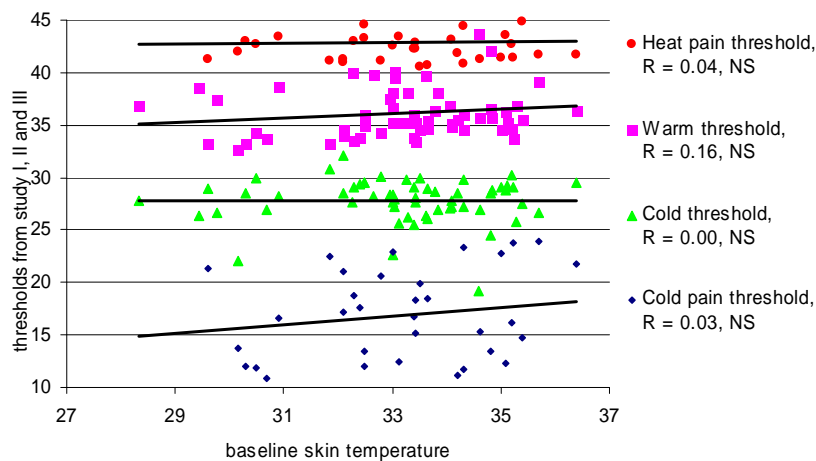
Figure 3.6 No correlation was shown between baseline skin temperature and the measured thermal thresholds, based on data collected in study I, II and III

Ambient temperature

A study (Strigo et al. 2000) has shown that ambient temperature affects the skin temperatures and thermal perceptions. Cool ambient temperature reduced the ratings to cold and heat stimuli. Cool and warm ambient temperatures increased the unpleasantness to cold stimuli and warm stimuli, respectively. Therefore ambient temperature is one of the factors that influence human thermal perceptions and needs to be controlled during such tests.

Ambient temperatures were controlled in all studies and therefore did not affect the threshold measurements in this Ph.D. project. The averaged room temperatures were 23.6 ± 0.9 ℃ (23.7 ± 1.0 ℃, 23.5 ± 0.7 ℃ and 23.6 ± 0.9 ℃ in study I, II and III, respectively).

Detection method

There are mainly two groups of test algorithms for quantitative test of thermal sensitivities: reaction time inclusive tests, including Method of Limits and Thermal Sensitivity Limen, where subjective decisions are made during the stimulation temperature changing; and reaction time exclusive tests, including Method of Force Choice, Method of Levels and Staircase, where the subjective decisions are made after the stimulation (Yarnitsky and Sprecher 1994). Each method has both advantages and disadvantages. In contrast to reaction-time-exclusive methods, using Method of Limits may induce individual reaction time bias to the measurements but has the advantage of ease in application (Yarnitsky 1997). Threshold values obtained using Method of Limits were higher than, but meanwhile well correlated to the thresholds measured using Method of Levels (Yarnitsky and Sprecher 1994, Defrin et al., 2006). Claus et al (1990) reported that Method of Force Choice took 6 times longer than the Method of Limits while the results were comparable between two methods. Comparing with Method of Levels and Staircase, Yarnitsky and Sprecher (1994) reported that Method of Limits took slightly shorter time. In addition, the repeatability of the Method of Limits is acceptable (Zwart and Sand 2002), although not as high as the Method of Levels and Staircase (Yarnitsky and Sprecher 1994, Palmer and Martin 2005).

Method of Limits was adopted to evaluate thermal perception and thermal pain thresholds in all studies in this Ph.D. study. During the tests, all subjects were requested to give their full concentration to the tasks to minimize the reaction time bias. Each threshold test was repeated for three times. A threshold was calculated as the average of three valid measurements that satisfy the limit of variance of 0.8. The tests were randomized across locations (in study I) and across modalities with sufficient break time (study II and III).

Two parameters may influence the thermal thresholds when using Method of Limits: the starting temperature and the rate of temperature change. The starting temperature has been found having significant influence on warm and cold thresholds but less effect on the neutral zone (difference between cold and warm thresholds) (Ruffell and Griffin 1995). The rate of temperature change has been reported as having little effect on the warmth and cold thresholds, as long as the change was more rapid than 0.1 ºC/s (Schmidt 1986). However, when using Method of Limits the rate of the temperature change in conjunction with reaction time may have larger effects to the thermal thresholds. Higher rate of temperature change may result higher thresholds (Pertovaara and Kojo 1985, Hilz et al. 1999). The sane starting temperature of 32 ºC and the same temperature change rate was 2ºC/s were used in all studies in this Ph.D. project and the results were therefore comparable across studies.

3.2 The time courses and the qualities of thermal sensations

In study III, non-painful cold, warm, painful cold and painful heat stimuli were applied to the subjects for 180 seconds and their sensation intensity ratings and distress levels were recorded every 15 seconds. Data were collected from 21 subjects and their averaged thermal thresholds were: cold perception

threshold: 29.1 ± 1.1 ℃; warm perception threshold: 34.9 ± 1.3 ℃; cold pain threshold: 17.0 ± 4.0 ℃ and heat pain threshold: 42.7 ± 1.7 ℃. The temperatures used for stimulations were determined individually according to subjectsø sensation thresholds. The temperature used for non-painful cold stimulation was set as about 20 degrees lower than that of warm which was set as about 2 degrees lower than the subjectøs heat pain threshold. The temperatures used to elicit painful cold and painful heat stimulations were 4 degrees lower or higher than the subjectøs cold pain and heat pain thresholds, respectively. Therefore the temperatures to elicit each type of perceptions were: non-painful cold: 21.0 ± 1.7 ℃; non-painful warm: 40.6 ± 1.3 ℃; painful cold: 11.9 ± 2.6 ℃ and painful heat: 46.5 ± 1.5 ℃.

During the 180 seconds, the averaged intensity ratings were well correlated with the distress levels for all the stimulation modalities ($R > 0.74$, $P < 0.001$). Averaged ratings of non-painful cold, cold pain, heat pain sensations were increased over time ($R > 0.87$, $P < 0.001$) suggesting effects of temporal summation. Averaged ratings of warm sensation declined over time ($R < -0.72$, $P < 0.05$) which suggested an effect of habituation to non-painful warm stimulations (Figure 3.7).
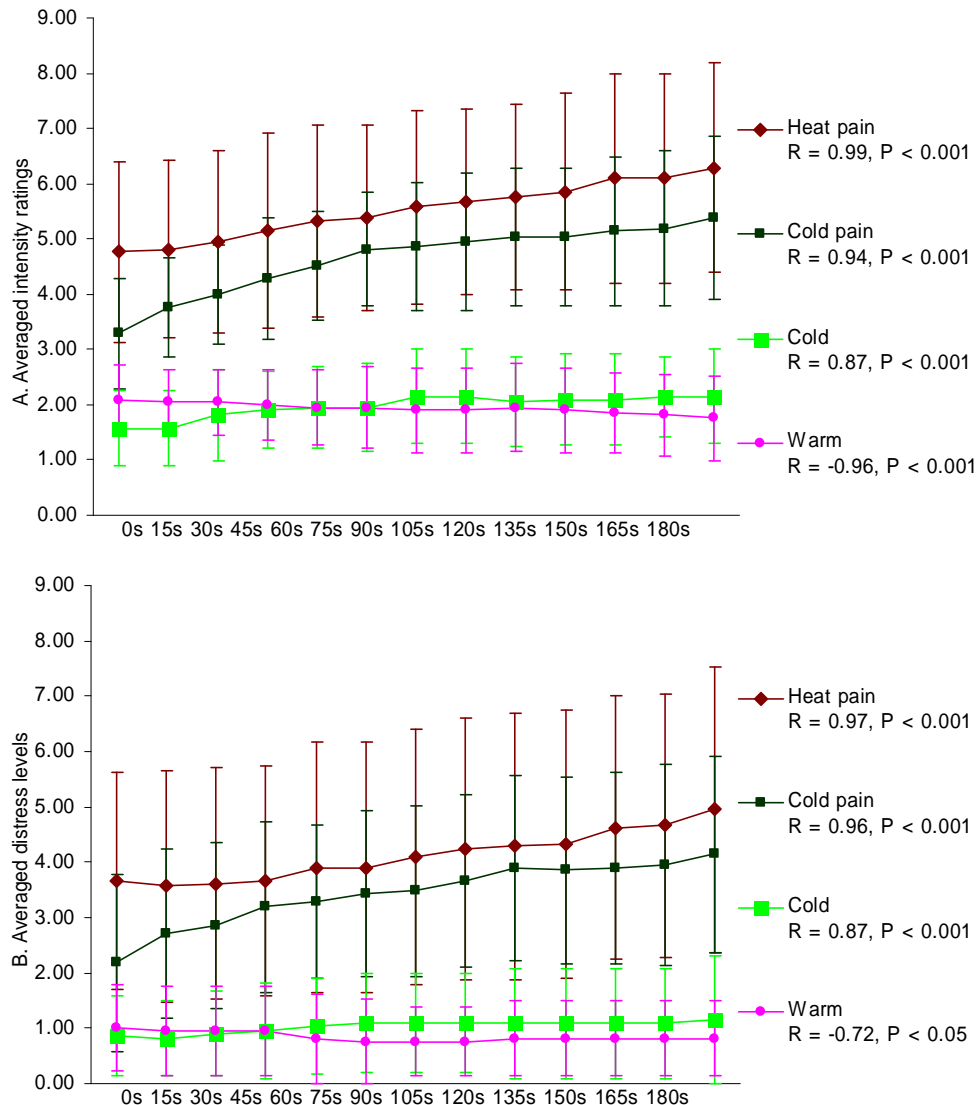
Figure 3.7 The averaged intensity ratings (A.) were well correlated with the distress levels (B.) and both increased over time in heat pain, cold pain, non-painful cold and non-painful warm sensations (mean ± SD), based on data collected in study III

The summation effects of cold pain and heat pain are believed to be modulated by C polymodal nociceptive afferents at the spinal level (Mendell 1966, Price et al. 1978, Kenshalo et al. 1979, Hashmi and Davis 2008) and possibly also by integration network at thalamic or higher levels. The responses of thalamic cells to noxious stimuli are more prolonged than the responses of spinothalamic neurons (Kenshalo et al. 1979) and than the responses of spinal cord cells (Dong et al. 1978). Spinal wind-up effect of A- fibres is also believed being involved in temporal summation, although not as well pronounced as C fibres (Andersen et al. 1994).

The mechanisms underlying temporal summation effect of non-painful cold sensation and the adaptation effect of warm sensation are not well documented. Some spinothalamic neurons showed rapid adaptation following the maximum response of the cell in a study using however noxious heat as stimuli (Kenshalo et al. 1979). A possible mechanism may occur in the peripheral level involving the heat/cold conduction and thermoregulatory compensation.

3.3 The qualities and the time courses of thermal grill sensations

Elicited by thermal grill stimulation containing non-painful cold and non-painful warm stimuli, any sensations that are beyond cold or warm are illusory sensations and should be considered as thermal grill illusion. In study II where 22 (11 in the palm and 11 on the fingers) different kinds of thermal grill stimuli (all with 20.0 ± 1.0 °C and 40.0 ± 1.0 °C) were applied to 19 subjects, the terms used by the subjects to describe the qualities of the thermal grill illusion were: hot burning pain, cold freezing pain, pricking pain, tingling, itching, annoying, unpleasant, confusing, surprising and strange. Meanwhile, the subjects reported perceptions of both warm and cold sensations in 96.55 % of the cases. The rest of the cases were reported as either warm or cold instead of a mixture of both; while in one case neither cold nor warm were perceived. The stimulation durations were individually determined by the subjects in the way that they removed their hand from the thermode when their initiative sensations were certain. The averaged duration was 2.3 ± 1.0 seconds and it was 2.5 ± 1.0 seconds when thermal grill illusion was reported and 2.1 ± 0.9 seconds when thermal grill illusion was not reported. In addition, females (2.3 ± 0.9 seconds) took longer time to determine the sensations than males (1.9 ± 0.9 seconds) (P Ö0.001). Either/both in the palm or/and on the fingers, 7 subjects experienced painful thermal grill illusion and all 7 subjects reported heat burning pain when 3 of them could also perceive cold pain and 2 of them experienced pain but not specifically due to heat or cold.

Out of 21 subject participated in study III where thermal grill stimuli of 180-second were applied to the subjects, 10 subjects (6 females) experienced pain (painful-TGI-responders) and 11 subjects (6 females) reported non-painful sensation (non-painful-responders). In stead of using fixed temperatures for all subjects, in this study temperatures were determined individually according to subjectsø sensation thresholds. The cold (about 20 degrees lower than that of warm) and warm (about 2 degrees lower than the subjectøs heat pain threshold) temperatures used for thermal grill stimuli were 21.3 ± 1.8 ºC and 40.3 ± 1.3 ºC. Out of the 10 subjects who experienced painful thermal grill illusion, 8 subjects reported heat pain; 1 subject reported cold pain and 1 subject reported a mixture of both heat pain and cold pain. Sixteen subjects reported as perceiving both cold and warm sensations when the rest of the subjects reported either only cold or only warm.

During the 180 seconds, painful and non-painful thermal grill sensations showed different time courses. The averaged pain ratings and distress levels of painful thermal grill illusion (averaged among subjects who perceived painful thermal grill illusion) were well correlated and both increased over time ($P < 0.001$) (Figure 3.8). The pain ratings and the distress levels of the painful thermal grill illusion were significantly higher than the intensity ratings of non-painful cold at all time points ($P < 0.05$) and than the intensity ratings of warm at all time points ($P < 0.05$) except at 0-second ($P < 0.09$). The averaged intensity ratings and distress levels of non-painful thermal grill sensations (averaged among subjects who did not report pain to thermal grill stimulations) were not correlated and both showed no significant trend over time (Figure 3.9). The intensity ratings of non-painful thermal grill sensations were significantly higher than the intensity ratings of non-painful cold sensation in the first 30 seconds ($P < 0.05$) and higher than the intensity ratings to warm stimuli in the last 30 seconds ($P < 0.05$). The distress levels to non-painful thermal grill sensation were not significantly higher than the distress levels to non-painful cold stimuli or those to warm stimuli except at a few time points (Figure 3.9).
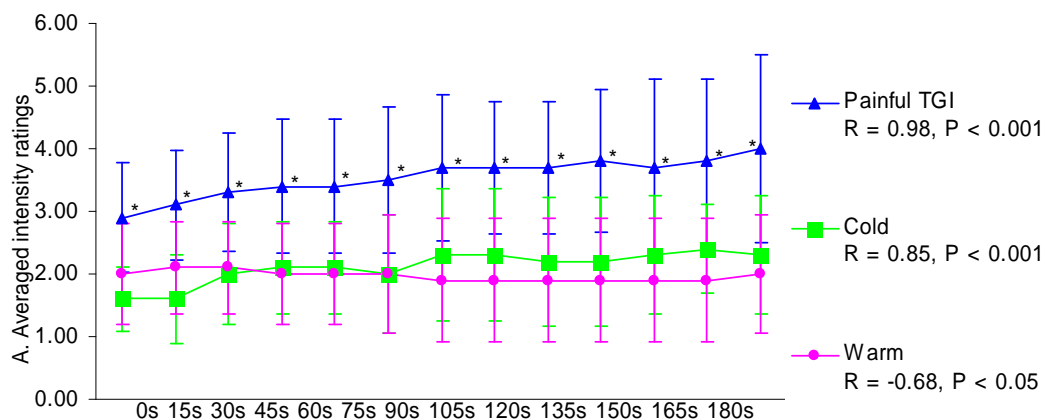
Figure 3.8 Sensation intensity ratings (A.) and distress levels (B.) of each condition averaged among subjects who perceived painful thermal grill illusion (mean ± SD). The intensity ratings and distress levels of painful thermal grill illusion were correlated and both increased over time. * marks when the ratings of painful thermal grill illusion were significantly higher than the ratings of non-painful cold or warm sensations. Based on data that were collected in study III
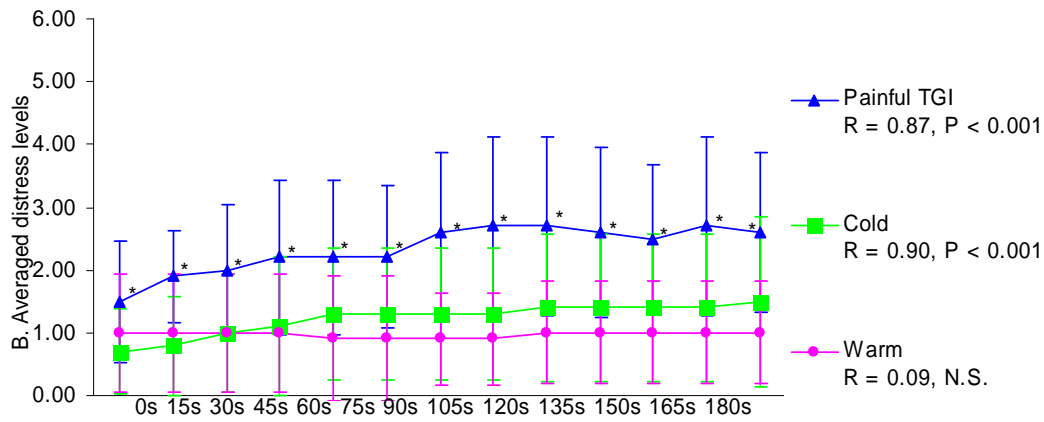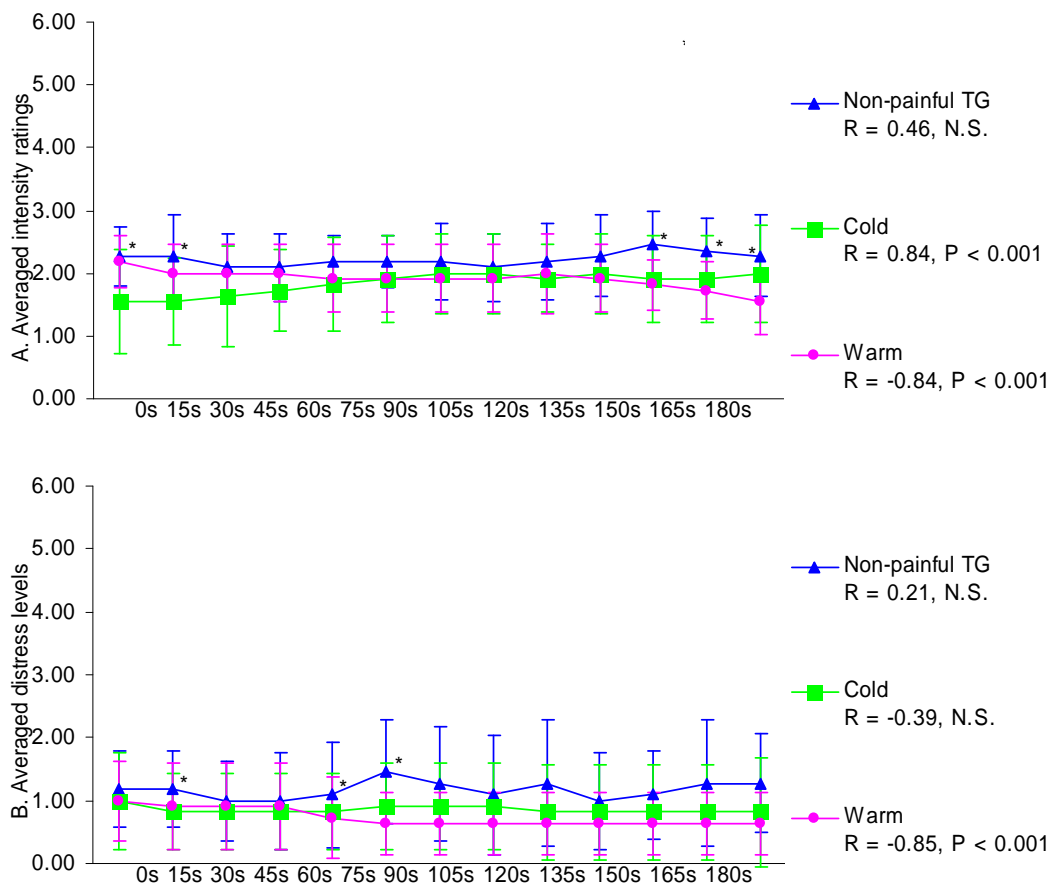

Figure 3.9 Sensation intensity ratings (A.) and distress levels (B.) of each condition averaged among subjects who perceived non-painful thermal grill sensations (mean ± SD). * marks when the ratings of

non-painful thermal grill sensations were significantly higher than the ratings of non-painful cold or warm sensations. Based on data that were collected in study III

The sensation intensity ratings and the distress levels were higher in the subjects who perceived painful thermal grill illusions than those who perceived non-painful thermal grill sensations, however except at the beginning of the stimulation (0-second). There were trends showing that painful-TGI-responders had higher cold pain thresholds ($P = 0.06$) and lower warm perception thresholds ($P = 0.07$) than the non-painful-responders. There were no significant differences in cold perception thresholds or heat pain thresholds between the two groups.

3.4 The cortical oscillatory changes to thermal and thermal grill stimulations

EEG data were recorded for 180 seconds during the stimulations of non-painful cold, non-painful warm, painful cold, painful heat and thermal grill. As written in section 2.4, focal areas (Figure 3.10) were defined before performing EEG power analysis.
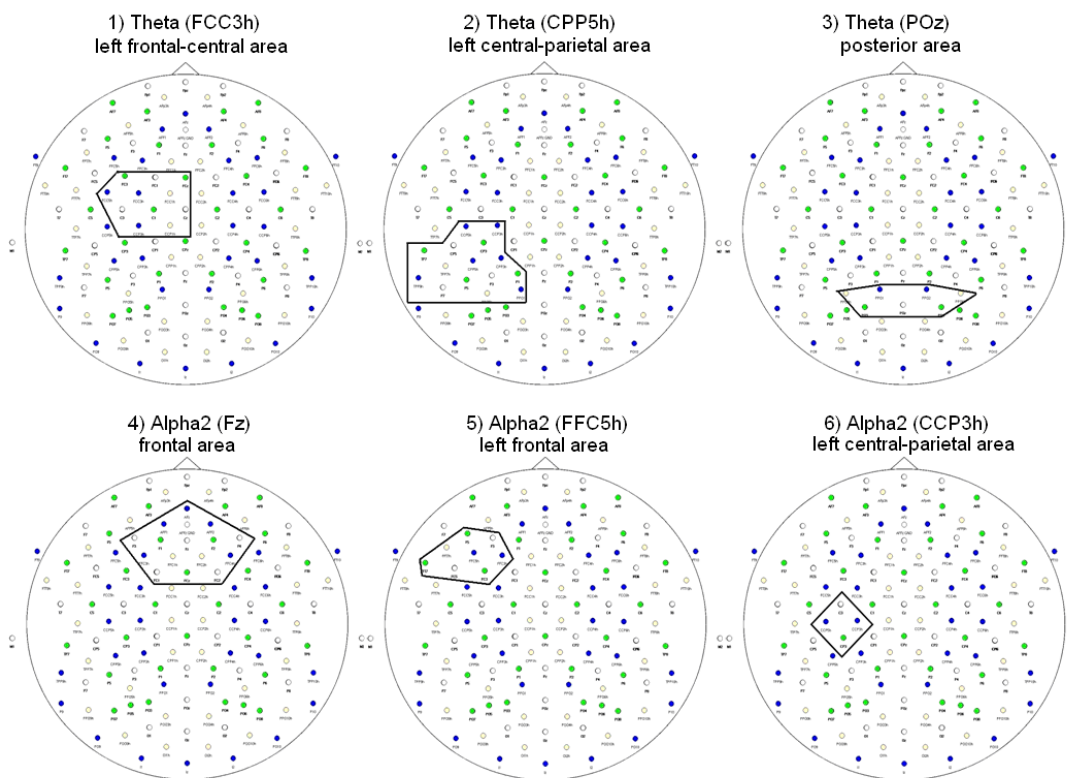

Figure 3.10 Adjacent channels were grouped into a focal area for further study when the EEG power of these channels changed significantly from baseline in response to stimuli. Six focal areas were defined in Theta and Alpha2 frequency bands and nominated as –frequency band (central electrode)ø based on data collected in study III

EEG power changes (declined from baseline) in Theta and Alpha2 frequency bands were found in response to all modalities of the given stimuli and most of these changes occurred in the contralateral hemisphere to the stimulated site (Figure 3.11).
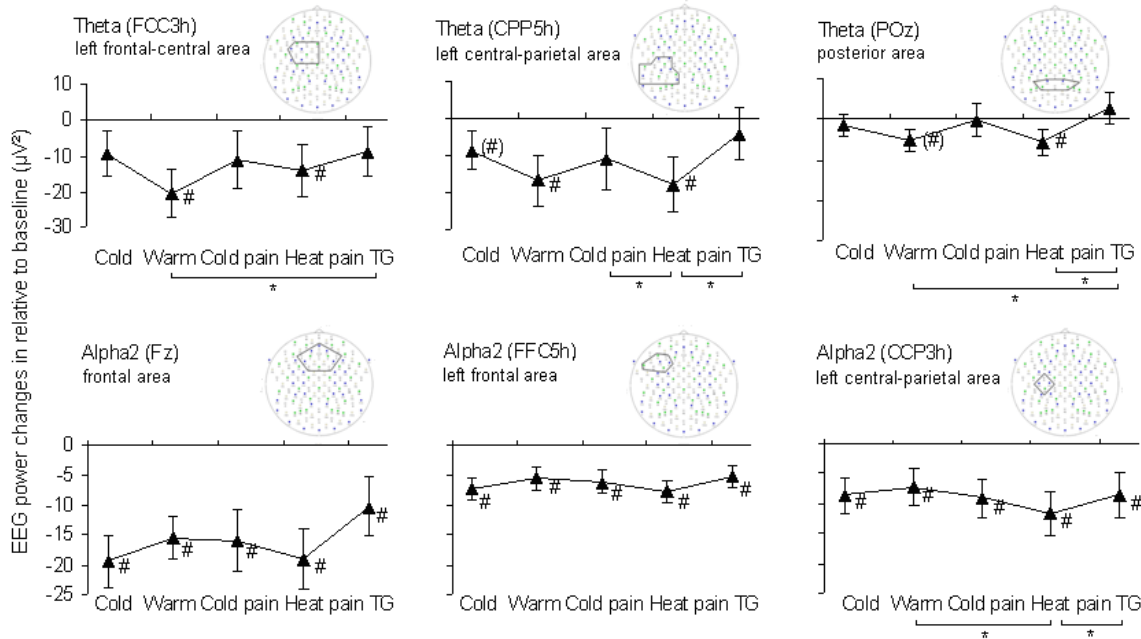

Figure 3.11 Mean EEG power (estimated marginal mean ± SD) of every condition in every focal area (baseline subtracted). * marks the significant difference (P < 0.05) between conditions; # marks the significant difference (P < 0.05) from baseline (abscissa); (#) marks the trend of significant difference (0.05 < P < 0.07) from baseline. Based on data collected in study III

Decreased activation in Theta band has been found in the frontal-central area, in central-parietal and posterior regions and these changes appeared to be more significant to warm and heat pain comparing with other thermal stimuli (Figure 3.11). These deactivations may be related to motivational regulation and networks between brain regions to produce habituation or alertness effects for thermal stimulations.

To all stimulation modalities applied, the EEG power decreased significantly in Alpha2 frequency band over central and left lateral frontal lobe and over the left motor and somatosensory cortices located approximately over the area representing the hand (Penfield and Rasmussen 1950) (Figure 3.11). The decrease of Alpha power has been previously referred as ÷Alpha blockingø and has been seen as a response to pain (Chang et al. 2002, Babiloni et al. 2008, Egsgaard et al. 2009) and reflect excitatory brain process (Klimesch et al. 2007). As presented in study III, the alpha blocking was not specific to pain but to all the thermal stimuli. The Alpha co-activations may present a network over regions of frontal lobe and left motor and somatosensory cortices in central processing of attention system, e.g. in sensory-discriminative aspect, during a variety of thermal inputs (Laurent et al. 2000, Peyron et al. 2000).

3.5 The cortical oscillatory changes of painful-TGI-responders vs. non-painful-responders

Figure 3.12 shows the topographical EEG power changes relative to baseline for every condition in Theta and Alpha2 frequency bands with painful-TGI-responders and non-painful-responders.
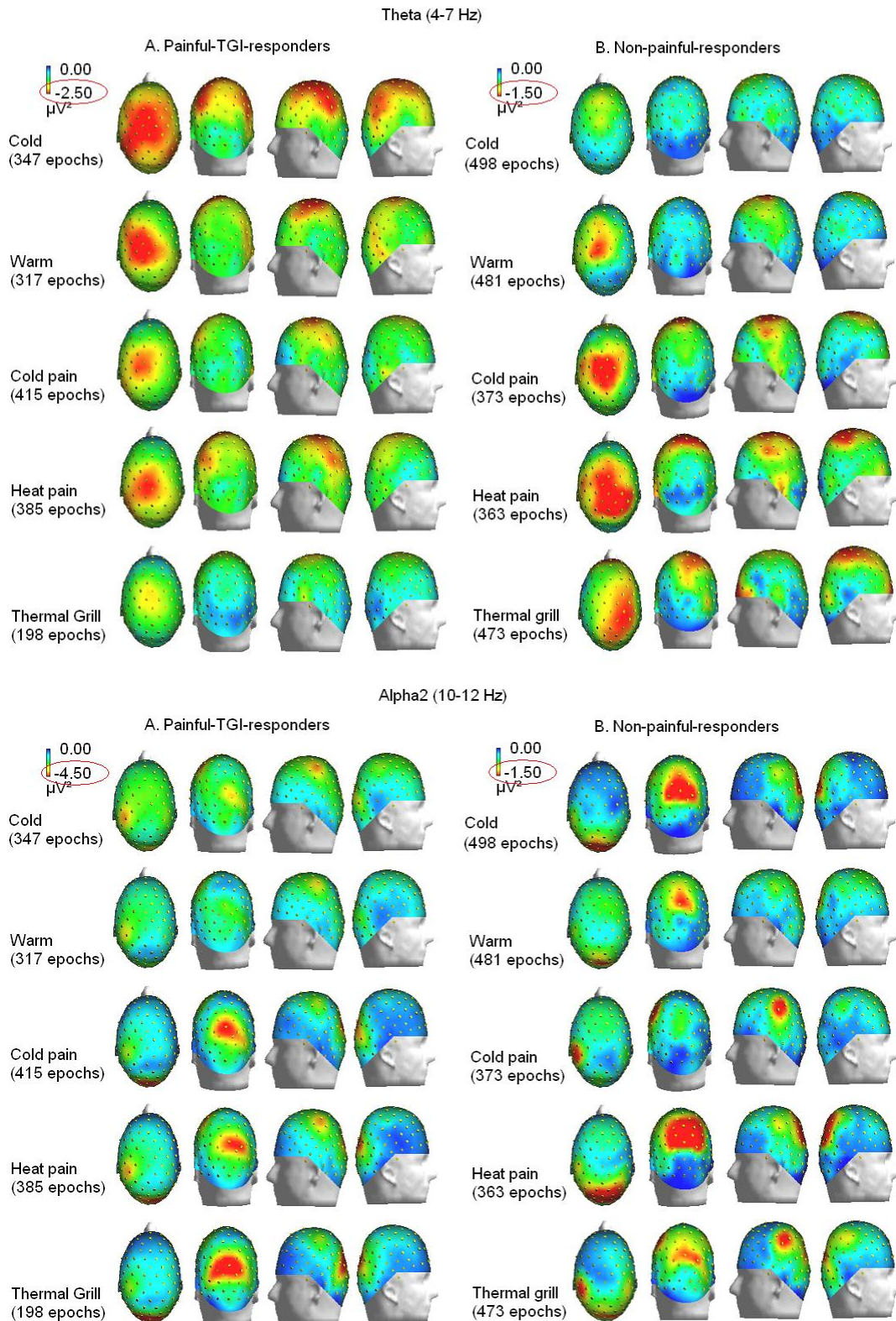
Figure 3.12 The EEG topography (A. averaged among painful-TGI-responders and B. averaged among non-painful-responders) for every condition in Theta and Alpha2 frequency bands (baseline subtracted). Note the different scales. Based on data collected in study III

In Alpha2 band, baseline EEG had higher power in painful-TGI-responders as compared with the non-painful-responders (F = 6.66, P < 0.05) (Figure 3.13).
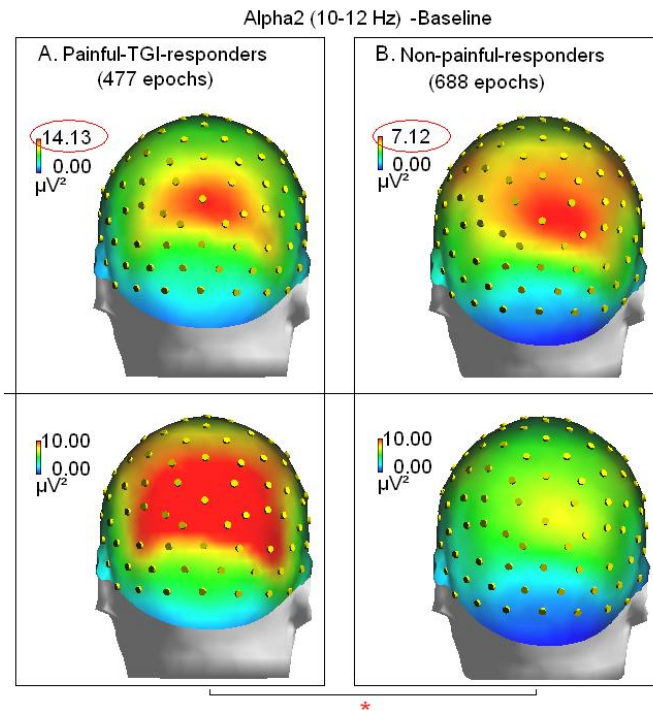
Figure 3.13 The EEG topography of baseline (a. averaged among painful-TGI-responders and b. averaged among non-painful-responders) in Alpha2 frequency band displayed in different scales (upper figures in automatic scales; lower figures in the same scale). * marks the significant difference (P < 0.05). Based on data collected in study III

In response to stimuli, the EEG power of Alpha2 on the left frontal area (FFC5h) decreased from baseline in a larger extend in painful-TGI-responders than non-painful-responders (Figure 3.14). Therefore, the presence of painful thermal grill illusion can be assessed quantitatively by changes in the brain oscillations over the frontal area.
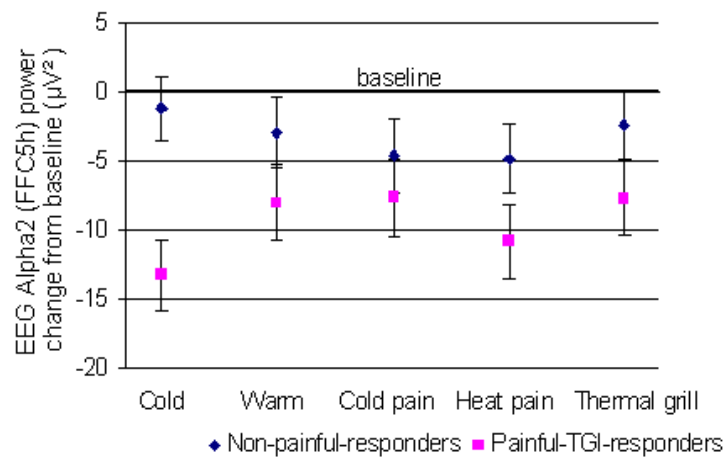
Figure 3.14 Mean EEG power (estimated marginal mean ± SD) in Alpha2 (FFC5h) decreased from baseline (abscissa) in a larger extend with painful-TGI-responders than non-painful-responders (P < 0.05), based on data collected in study III

3.6 Psychological factors may influence TGI experience

The baseline EEG power of alpha rhythms can be different between individuals (Chen 1991, Egsgaard et al. 2009) and may be related to psychological differences such as anxiety sensitivity, attentions and anticipations. For instance, studies have shown that subjects in higher anxiety showed higher baseline alpha power and higher reactivity of alpha rhythms (Knyazev et al. 2005, Knyazev et al. 2006). It is known that psychological factors moderate the perception of pain. For example people who were fearful of pain or under threaten to health or life tend to report more negative pain experiences (Harrison and Davis 1999, Keogh et al. 2001). Attention and anticipation may also modulate pain experience (Petrovic et al. 2000, Babiloni et al. 2006, Babiloni et al. 2008). Therefore, the difference of baseline and the reactivity of Alpha2 rhythms between painful-TGI-responders and non-painful-responders may reflect the difference of the state of anxiety, attention and the anticipation to pain experience.

3.7 The possible underlying mechanisms of the thermal grill illusion

A possible peripheral mechanism which may mediate thermal grill illusion is a spatial summation of warm-activated warm receptors and cold-activated warm receptors (Defrin et al. 2008). It is believed that low-threshold warm receptors exist and that those receptors can be activated by cold, which has been previously considered as the underlying mechanism of paradoxical heat and innocuous cold nociception (Torebjörk et al. 1984, Green et al. 2008). Besides low-threshold warm receptors, cold receptors could also play a role in mediating thermal grill illusion. Some cold receptors, which have been cooled, continue to discharge during warming ó õat first even at an increasing rateö (Schmidt 1986). These cold receptors change their response characteristics while adapting rapidly to a considerably lower temperature in a way that their steady-state response is suppressed and that a burst of impulses occurs on rewarming

(Kenshalo and Duclaux 1977). Furthermore, repetition in short intervals may increase the response (Long 1977). Therefore, certain cold receptors activated by the concurrent and continuous (considered as repetitions in infinite short interval) cold and warmth, could also play a role in the perception of the painful thermal grill illusion. The activation of the cold receptors could be the reason why some of the painful thermal grill illusions were perceived as cold pain. However, in study II, the insignificant effects of the number of bars and the distance between bars on TGI showed a lack of spatial summation, either peripherally or centrally.

The central role involved in the thermal grill illusion has previously been addressed by a few studies. Craig and Bushnell (Craig and Bushnell 1994, Craig et al. 1996) reported the ÷unmaskingø hypothesis to explain the phenomenon of the thermal grill illusion, which have been the first and only electrophysiological studies before this Ph.D. study. In their hypothesis, the burning pain excited by thermal grill was due to a reduction of cold-induced inhibition in nociceptive pathway, the reduction being caused by the concurrent warm simulation. More recent studies from Bouhassiraøs group (Kern wt al. 2008a, Kern et al. 2008b) have interestingly displayed that the thermal grill illusion can be modulated pharmacologically. The effect of morphine on reducing normal thermal pain and the thermal grill pain suggested possible correlations between these two types of pain (Kern et al. 2008b). Interestingly, ketamine showed a selective effect on reducing thermal grill pain (Kern et al. 2008a). A central involvement was suggested by the authors. However, it is not clear that the responsible NMDA receptors that ketamine affected were those in the peripheral level or those in the central level.

Found in study III, the difference of baseline Alpha2 rhythms between painful-TGI-responders and non-painful-responders may indicate that the state of anxiety, attention and the anticipation to pain experience may have influence on the experience of painful thermal grill illusion.

3.8 The experimental considerations for the research of the thermal grill illusion

Thermal grill illusion may be elicited by simultaneously applied warm and cold stimuli by means of mainly two types of stimulators in previous studies: water running through metal bars (Thunberg 1896, Craig and Bushnell 1994, Craig et al. 1996, Fruhstorfer et al. 2003, Leung et al. 2005, Li et al. in press) and electrically controlled Peltier thermoelectric modules (Green 2002, Bouhassira et al. 2005, Defrin et al. 2008, Kern et al. 2008a, Kern et al. 2008b). It has been concluded in study II that stimulation parameters such as the number of the stimulating bars and the distance between and cold warm bars have no effects on experiencing painful thermal grill illusion. A previous study gave a similar conclusion that the distance between warm and cold bars was not affective to the experience of painful thermal grill illusion as long as the distance was shorter than 30 mm (Defrin et al. 2008).

The stimulation duration may have influence on the experience of painful thermal grill illusion. In study III, approximately 48% of the subjects experienced pain during the 180-second administrations of

the thermal grill stimulations. In previous studies, thermal grill stimuli of 50-second duration may induce painful thermal grill illusion to 91% (10 out of 11) of the subjects (Craig and Bushnell 1994) while 30-second thermal grill stimuli may induce painful thermal grill illusion to 46% of the subjects (Bouhassira et al. 2005). In study II, about 30% of the subjects could experience painful thermal grill illusion to short stimuli duration of about 2 seconds; whereas in some studies sensations that were elicited by thermal grill stimuli of short duration (< 5 seconds) were described as non-painful (Green 2002) or that the reports of painful sensation were negligible (Fruhstorfer et al. 2003).

The influence of the stimulation temperatures appears not to be as important as other factors. Correlations between thermal thresholds and the experience of painful thermal grill illusion were found in study III while a previous study (Bouhassira et al. 2005) showed that the experience of painful thermal grill illusion does not depend on thermal thresholds. The difference between warm and cold temperatures may influence the thermal grill sensations. Stimuli with a difference of 20-degree (or 25-degree if the temperatures do not surpass painful thresholds) trigger painful thermal grill illusion at the highest probability comparing with those with lower temperature differences (Bouhassira et al. 2005).

In addition, it seems that the experience of painful thermal grill illusion depends on how the temperatures are applied: statically (the temperatures are preset before applying) or dynamically (the temperatures change when the skin is in contact with the stimulator). Based on previous studies (Green 2002, Fruhstorfer et al. 2003), the dynamic stimuli (with short duration of less than 5 seconds) may be linked to the absence of the painful thermal grill illusion.

The thermal grill stimulation has not been applied in many body parts except in the hand (glabrous skin) in most studies and on the forearm (hairy skin) (Defrin et al. 2008). The influences by the locations on the body and by the different skin types have not been sufficiently studied. The repeatability of thermal grill illusion was not good. During our pilot experiments, we have found that a subject could have non-painful sensation to the same thermal grill stimuli to which he/she previously reported as painful. This is not surprising after we concluded that psychological factors such as attention and anticipation to pain experience may influence the experience of the painful thermal grill illusion. This could also be account for the low presence of painful thermal grill illusion in study II where many thermal grill stimuli (22 on the palm and on the fingers) were applied.

3.9 Future perspective

More research should be conducted to explore the thermal grill illusion, with the aims to clearly understand and to control the phenomenon and eventually to use it as a model or a tool to study human thermal sensations, thermal-pain integration, neuropathic pain and possibly diagnosis. Based on what have been done in this Ph.D. project and other previous studies, the future human studies on thermal grill illusion could use simpler experimental designs with respect to generating painful thermal grill illusion.

Furthermore, new directions can be explored in order to understand the phenomenon of the thermal grill illusion, such as pharmacological modulations, integration with other modalities, and the induction of thermal grill illusion on different body parts. As a naive experience to many subjects, thermal grill stimulation can also be used in studies where attention and anticipation of pain experience are factors, such as in the study of mirror box induced illusions. More electrophysiological animal studies similar to what Craig and Bushnell (1994) have done can be extremely valuable to the understanding of thermal grill illusion. More studies should be conducted to examine the application of thermal grill illusion with the aims to use the thermal grill illusion for evaluation, diagnosis or even rehabilitation in neuropathic pain patients and patients/individuals who might suffer psychological problems, such as pain catastrophizing.

4. Conclusion

The thermal grill illusion describes a paradoxical sensation occurring when someone touches spatially adjacent bars with an alternating pattern of cold and warmth. The main aim of this Ph.D. project was to study and compare thermal sensation and the thermal grill illusion by means of psychophysical and electrophysiological (EEG) investigations aiming for better control and better understanding to the thermal grill illusion. Three studies were included and the main findings were: The cold and warm sensitivity distribution over the glabrous skin of the hand was found to be highly heterogeneous calling for necessity to strictly define testing body regions and locations when studying and evaluating thermal dysfunctions; many factors that may influence the measurements of thermal thresholds were discussed; the cold and warm sensitivities in the skin over palm were different from those in the skin over fingers; the time course of the thermal sensations were different between painful and non-painful sensations and between non-painful cold and warm; the thermal grill illusion could be painful; the factors such as the distance between cold and warm stimulation bars did not affect the elicitation and the sensation of the thermal grill illusion; painful and non-painful thermal grill sensations had different time courses; and the presence of painful thermal grill illusion can be assessed quantitatively by changes in the brain oscillations over the frontal area, which meanwhile implied that psychological and cognitive factors such as the anxiety level or attention and anticipation to pain experience may be of the influential factors that mediating painful thermal grill illusion. Therefore, this Ph.D. study suggested for the first time that, besides for neuropathic pain patients, thermal grill illusion could be a potential tool for psychological clinical use. For example, it could be possible to use thermal grill illusion to examine the possibility of pain catastrophizing, without intervention which may lead to chronic pain and disability over time.

References

Andersen OK, Jensen LM, Brennum J, Arendt-Nielsen L. Evidence for central summation of C and A delta nociceptive activity in man. Pain 1994;59(2):273-80. doi:10.1016/0304-3959(94)90080-9

Alrutz S. The sensation 'hot' Mind 1898;7:141-4.

Babiloni C, Brancucci A, Del Percio C, Capotosto P, Arendt-Nielsen L, Chen AC, Rossini PM. Anticipatory electroencephalography alpha rhythm predicts subjective perception of pain intensity. J Pain 2006;7(10):709-17. doi:10.1016/j.jpain.2006.03.005

Babiloni C, Capotosto P, Brancucci A, Del Percio C, Petrini L, Buttiglione M, Cibelli G, Romani GL, Rossini PM, Arendt-Nielsen L. Cortical alpha rhythms are related to the anticipation of sensorimotor interaction between painful stimuli and movements: a high-resolution EEG study. J Pain 2008;9(10):902-11. doi:10.1016/j.jpain.2008.05.007

Basar E, Schurmann M. Alpha rhythms in the brain: Functional correlates. News Physiol Sci 1996;11:90-6.

Belmonte C, Brock JA, Viana F. Converting cold into pain. Exp Brain Res 2009;196(1):13-30. doi: 10.1007/s00221-009-1797-2

Bouhassira D, Kern D, Rouaud J, Pelle-Lancien E, Morain F. Investigation of the paradoxical painful sensation ('illusion of pain') produced by a thermal grill. Pain 2005;114:160-7. doi:10.1016/j.pain.2004.12.014

Buzsaki G. Rhythms of the Brain. Oxford University Press, New York, USA, 2006.

Chang PF, Arendt-Nielsen L, Chen AC. Dynamic changes and spatial correlation of EEG activities during cold pressor test in man. Brain Res Bull 2002;57(5):667-75. doi:10.1016/S0361-9230(01)00763-8

Chen AC. Individual differences in human topographic EEG power: hemodynamic and psychological predictors. Int J Neurosci 1991;59(4):271-80.

Claus D, Hilz MJ, Neundörfer B. Thermal discrimination thresholds: a comparison of different methods. Acta Neurol Scand 1990;81(6):533-40. doi: 10.1111/j.1600-0404.1990.tb01015.x

Craig AD. Insights from the Thermal Grill on the Nature of Pain and Hyperalgesia in Humans. Hyperalgesia: Molecular Mechanisms and Clinical implications, Progress in Pain Research and Management 2004;30:311-27.

Craig AD, Andrew D. Responses of spinothalamic lamina I neurons to repeated brief contact heat stimulation in the cat. J Neurophysiol 2002;87:1902-14. doi:10.1038/82924

Craig AD, Bushnell MC. The thermal grill illusion: unmasking the burn of cold pain. Science 1994;265:252-5. doi:10.1126/science.8023144

Craig AD, Chen K, Bandy D, Reiman EM. Thermosensory activation of insular cortex. Nat Neurosci 2000;3:184-90. doi:10.1038/72131

Craig AD, Reiman EM, Evans A, Bushnell MC. Functional imaging of an illusion of pain. Nature 1996;384:258-60. doi:10.1038/384258a0

Darian-Smith I, Johnson KO. Thermal sensibility and thermoreceptors. Journal of Investigative Dermatology 1977;69:14653. doi:10.1111/1523-1747.ep12497936

Davis KD. Cold-induced pain and prickle in the glabrous and hairy skin. Pain 1998;75(1):47-57. doi:10.1016/S0304-3959(97)00203-0

Davis KD, Pope GE. Noxious cold evokes multiple sensations with distinct time courses. Pain 2002;98:179-85. doi:10.1016/S0304-3959(02)00043-X

Davis KD, Pope GE, Crawley AP, Mikulis DJ. Neural correlates of prickle sensation: a percept-related fMRI study. Nat Neurosci 2002;5(11):1121-2. doi:10.1038/nn955

Defrin R, Benstein-Sheraizin A, Bezalel A, Mantzur O, Arendt-Nielsen L. The spatial characteristics of the painful thermal grill illusion. Pain 2008;138(3):577-86. doi:10.1016/j.pain.2008.02.012

Defrin R, Ohry A, Blumen N, Urca G. Sensory determinants of thermal pain. Brain 2002;125:501-10. doi: 10.1093/brain/awf055

Defrin R, Shachal-Shiffer M, Hadgadg M, Peretz C. Quantitative somatosensory testing of warm and heat-pain thresholds: the effect of body region and testing method. Clin J Pain 2006;22(2):130-6.

Dhaka A, Viswanath V, Patapoutian A. TRP ion channels and temperature sensation. Annu Rev Neurosci 2006;29:135-61. doi: 10.1146/annurev.neuro.29.051605.112958

Dyck PJ, Karnes JL, Gillen DA, O'Brien PC, Zimmerman IR, Johnson DM. Comparison of algorithms of testing for use in automated evaluation of sensation. Neurology 1990;40(10):1607-13.

Egsgaard LL, Wang L, Arendt-Nielsen L. Volunteers with high versus low alpha EEG have different pain-EEG relationship: a human experimental study. Exp Brain Res 2009;193(3):361-9. doi: 10.1007/s00221-008-1632-1

Eysel UT. Illusions and Perceived Images in the Primate Brain. Science 2003;302: 789-91. doi: 10.1126/science.1091065

Fruhstorfer H, Harju EL, Lindblom UF. The significance of A-delta and C fibres for the perception of synthetic heat. Eur J Pain 2003;7:63-71.

Gardner EP, Martin JH, Jessel TM. The bodily senses. In: Kandel ER, Schwartz JH, Jessell TM, eidtors. Principles of neural science. 4th edition. McGraw-Hill, USA, 2000. p. 430-50.

Granot M, Granovsky Y, Sprecher E, Nir RR, Yarnitsky D. Contact heat-evoked temporal summation: tonic versus repetitive-phasic stimulation. Pain 2006;122(3):295-305. doi:10.1016/j.pain.2006.02.003

Granovsky Y, Matre D, Sokolik A, Lorenz J, Casey KL. Thermoreceptive innervation of human glabrous and hairy skin: a contact heat evoked potential analysis. Pain 2005;115(3):238-47. doi:10.1016/j.pain.2005.02.017

Green BG. Synthetic heat at mild temperatures. Somatosens Mot Res 2002;19:130-8. doi:10.1080/08990220220220131524

Green BG. Temperature perception and nociception. Journal of Neurobiology 2004;61(1):13-29. doi: 10.1002/neu.20081

Green BG, Akirav C. Individual differences in temperature perception: evidence of common processing of sensation intensity of warmth and cold. Somatosens Mot Res 2007;24(1-2):71-84. doi:10.1080/08990220701388117

Green BG, Pope JV. Innocuous cooling can produce nociceptive sensations that are inhibited during dynamic mechanical contact. Exp Brain Res 2003;148(3):290-9. doi: 10.1007/s00221-002-1280-9

Green BG, Roman C, Schoen K, Collins H. Nociceptive sensations evoked from ٜspotsø in the skin by mild cooling and heating. Pain 2008;135(1-2):196-208. doi:10.1016/j.pain.2007.11.013

Green BG, Zaharchuk R. Spatial variation in sensitivity as a factor in measurements of spatial summation of warmth and cold. Somatosens Mot Res 2001;18(3):181-90. doi:10.1080/01421590120072178

Greenberg SA. The history of dermatome mapping. Arch Neurol 2003;60(1):126-31.

Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB, et al. Studying sex and gender differences in pain and analgesia: a consensus report. Pain 2007;132 Suppl 1:S26-45. doi:10.1016/j.pain.2007.10.014

Greenspan JD, Kenshalo DR Sr. The primate as a model for the human temperature-sensing system: 2. Area of skin receiving thermal stimulation (spatial summation). Somatosens Res 1985;2(4):315-24.

Greenspan JD, Taylor DJ, McGillis SL. Body site variation of cool perception thresholds, with observations on paradoxical heat. Somatosens Mot Res 1993;10(4):467-74.

Greestein B and Greenstein A. Color atlas of neuroscience. Thieme, Stuttgart, USA, 2000.

Hagander LG, Midani HA, Kuskowski MA, Parry GJG. Quantitative sensory testing: effect of site and pressure on vibration thresholds. Clin Neurophysiol 2000;111(6):1066-9(4). doi:10.1016/S1388-2457(00)00278-9

Harrison JL, Davis KD. Cold-evoked pain varies with skin type and cooling rate: a psychophysical study in humans. Pain 1999;83(2):123-35. doi:10.1016/S0304-3959(99)00099-8

Hashmi JA, Davis KD. Effect of static and dynamic heat pain stimulus profiles on the temporal dynamics and interdependence of pain qualities, intensity, and affect. J Neurophysiol 2008;100(4):1706-15. doi: 10.1152/jn.90500.2008

Heavner JE, Calvillo O, Racz GB. Thermal grill illusion and complex regional pain syndrome type I (reflex sympathetic dystrophy). Reg Anesth 1997;22:257-9. doi:10.1016/S1388-2457(00)00278-9

Hilz MJ, Stemper B, Axelrod FB, Kolodny EH, Neundörfer B. Quantitative thermal perception testing in adults. J Clin Neurophysiol 1999;16(5):462-71.

Huang YP, Zheng YP, Leung SF, Choi AP. High frequency ultrasound assessment of skin fibrosis: clinical results. Ultrasound Med Biol 2007;33(8):1191-8. doi:10.1016/j.ultrasmedbio.2007.02.009

Huber MT, Bartling J, Pachur D, Woikowsky-Biedau S, Lautenbacher S. EEG responses to tonic heat pain. Exp Brain Res 2006;173(1):14-24. doi: 10.1007/s00221-006-0366-1

Hutchison WD, Davis KD, Lozano AM, Tasker RR, Dostrovsky JO. Pain-related neurons in the human cingulate cortex. Nat Neurosci 1999;2:403-5. doi:10.1038/8065

Iggo A. Cutaneous thermoreceptors in primates and sub-primates. J Physiol 1969;200(2):403-30.

Kauer JA, Gibson HE. Hot flash: TRPV channels in the brain.Trends Neurosci 2009;32(4):215-24. doi:10.1016/j.tins.2008.12.006

Keegan JJ, Garrett FD. The segmental distribution of the cutaneous nerves in the limbs of man. The Anatomical Record 1948;102(4):409-37.

Kenshalo DR, Duclaux R. Response characteristics of cutaneous cold receptors in the monkey. J Neurophysiol 1977;40(2):319-32.

Kenshalo DR Jr, Leonard RB, Chung JM, Willis WD. Responses of primate spinothalamic neurons to graded and to repeated noxious heat stimuli. J Neurophysiol 1979;42(5):1370-89.

Keogh E, Ellery D, Hunt C, Hannent I. Selective attentional bias for pain-related stimuli amongst pain fearful individuals. Pain 2001;91(1-2):91-100. doi:10.1016/S0304-3959(00)00422-X

Kern D, Pelle-Lancien E, Luce V, Bouhassira D. Pharmacological dissection of the paradoxical pain induced by a thermal grill. Pain 2008a;135(3):291-9. doi:10.1016/j.pain.2007.12.001

Kern D, Plantevin F, Bouhassira D. Effects of morphine on the experimental illusion of pain produced by a thermal grill. Pain 2008b;139:653-9. doi:10.1016/j.pain.2008.07.001

Klimesch W. EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. Brain Res Rev 1999;29(2-3):169-95. doi:10.1016/S0165-0173(98)00056-3

Klimesch W, Sauseng P, Hanslmayr S. EEG alpha oscillations: the inhibition-timing hypothesis. Brain Res Rev 2007;53(1):63-88. doi:10.1016/j.brainresrev.2006.06.003

Knyazev GG, Savostyanov AN, Levin EA. Anxiety and synchrony of alpha oscillations. Int J Psychophysiol 2005;57(3):175-80. doi:10.1016/j.ijpsycho.2005.01.004

Knyazev GG, Savostyanov AN, Levin EA. Alpha synchronization and anxiety: implications for inhibition vs. alertness hypotheses.Int J Psychophysiol 2006;59(2):151-8. doi:10.1016/j.ijpsycho.2005.03.025

Lautenbacher S, Strian F. Sex differences in pain and thermal sensitivity: the role of body size. Percept Psychophys 1991;50(2):179-83.

Lautenbacher S, Strian F. The role of body size in somatosensory testing. Electromyogr Clin Neurophysiol 1993;33(2):113-8.

Lee DK, McGillis SL, Greenspan JD. Somatotopic localization of thermal stimuli: I. A comparison of within-versus across-dermatomal separation of innocuous thermal stimuli. Somatosens Mot Res 1996;13(1):67-71.

Lee H, Caterina MJ. TRPV channels as thermosensory receptors in epithelial cells. Pflugers Arch 2005;451(1):160-7. doi: 10.1007/s00424-005-1438-y

Leung AY, Wallace MS, Schulteis G, Yaksh TL. Qualitative and quantitative characterization of the thermal grill. Pain 2005;116:26-32. doi:10.1016/j.pain.2005.03.026

Li X, Petrini L, Defrin R, Madeleine P, Arendt-Nielsen L. High resolution topographical mapping of warm and cold sensitivities. Clin Neurophysiol 2008; 119(11):2641-6. doi:10.1016/j.clinph.2008.08.018

Li X, Petrini L, Wang L, Defrin R, Arendt-Nielsen L. The importance of stimulus parameters for the experience of the thermal grill illusion. Neurophysiol Clin 2009; 39(6):275-82. doi: 10.1016/j.neucli.2009.06.006

Long RR. Sensitivity of cutaneous cold fibers to noxious heat: paradoxical cold discharge. J Neurophysiol 1977;40(3):489-502.

Lumpkin EA, Caterina MJ. Mechanisms of sensory transduction in the skin. Nature 2007;445(7130):858-65. doi:10.1038/nature05662

McKemy DD. How cold is it? TRPM8 and TRPA1 in the molecular logic of cold sensation. Mol Pain 2005; 1:16. doi:10.1186/1744-8069-1-16

McKemy DD, Neuhausser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002;416(6876):52-8. doi:10.1038/nature719

Meh D, Denislic M. Quantitative assessment of thermal and pain sensitivity. J Neurol Sci 1994;127(2):164-9. doi:10.1016/0022-510X(94)90069-8

Mendell LM. Physiological properties of unmyelinated fiber projection to the spinal cord. Exp Neurol 1966;16(3):316-32. doi:10.1016/0014-4886(66)90068-9

Meyer RA, Ringkamp M, Campbell JN, Raja SN. Peripheral mechanisms of cutaneous nociception. In: McMahon S, Koltzenburg M, editors. Wall and Melzack's Textbook of Pain. 5th edition. Elsevier/Churchill Livingstone, 2005. p. 3-12.

Moran MM, Xu H, Clapham DE. TRP ion channels in the nervous system. Curr Opin Neurobiol 2004;14(3):362-9. doi:10.1016/j.conb.2004.05.003

Morin C, Bushnell MC, Luskin MB, Craig AD. Disruption of thermal perception in a multiple sclerosis patient with central pain. Clin J Pain 2002;18(3):191-5.

Mountcastle VB. The sensory hand: neural mechanisms of somatic sensation. Harvard University Press, Massachusetts, USA, 2005.

Nielsen J, Arendt-Nielsen L. Spatial summation of heat induced pain within and between dermatomes. Somatosens Mot Res 1997;14(2):119-25.

Numazaki M, Tominaga M. Nociception and TRP Channels. Curr Drug Targets CNS Neurol Disord 2004;3(6):479-85. doi: 10.2174/1568007043336789

Nunez PL, Srinivasan R. Electric Fields of the Brain: The Neurophysics of EEG. 2nd edition. Oxford University Press, New York, USA, 2005.

Nygaard OP, Mellgren SI. The function of sensory nerve fibers in lumbar radiculopathy. Use of quantitative sensory testing in the exploration of different populations of nerve fibers and dermatomes. Spine 1998;23(3):348-52.

Oostenveld R, Praamstra P. The five percent electrode system for high-resolution EEG and ERP measurements. Clin Neurophysiol 2001;112(4):713-9. doi:10.1016/S1388-2457(00)00527-7

Palmer ST, Martin DJ. Thermal perception thresholds recorded using method of limits change over brief time intervals. Somatosens Mot Res 2005;22(4):327-34. doi:10.1080/08990220500420731

Paulson PE, Minoshima S, Morrow TJ, Casey KL. Gender differences in pain perception and patterns of cerebral activation during noxious heat stimulation in humans. Pain 1998;76(1-2):223-9. doi:10.1016/S0304-3959(98)00048-7

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A. A TRP channel that senses cold stimuli and menthol. Cell 2002;108(5):705-15. doi:10.1016/S0092-8674(02)00652-9

Penfield W, Rasmussen T. The Cerebral Cortex of Man: A Clinical Study of Localization of Function. MacMillan, New York, USA, 1950.

Pertovaara A, Kojo I. Influence of the rate of temperature change on thermal thresholds in man. Exp Neurol 1985;87(3):439-45. doi:10.1016/0014-4886(85)90174-8

Petrovic P, Petersson KM, Ghatan PH, Stone-Elander S, Ingvar M. Pain-related cerebral activation is altered by a distracting cognitive task. Pain 2000;85(1-2):19-30. doi:10.1016/S0304-3959(99)00232-8

Peyron R, Laurent B, García-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin 2000;30(5):263-88. doi:10.1016/S0987-7053(00)00227-6

Price DD, Hayes RL, Ruda M, Dubner R. Spatial and temporal transformations of input to spinothalamic tract neurons and their relation to somatic sensations. J Neurophysiol 1978;41(4):933-47.

Robinson ME, Wise EA, Gagnon C, Fillingim RB, Price DD. Influences of gender role and anxiety on sex differences in temporal summation of pain. J Pain 2004;5(2):77-82. doi:10.1016/j.jpain.2003.11.004

Ruffell CM, Griffin MJ. Effect of starting temperature on the repeatability of thermotactile thresholds. Cent Eur J Public Health 1995;3 Suppl:81-4.

Schiff E, Eisenberg E. Can quantitative sensory testing predict the outcome of epidural steroid injections in sciatica? A preliminary study. Anesth Analg 2003;97(3):828-32. doi: 10.1213/01.ANE.0000078583.47735.69

Schmidt RF. Somatovisceral sensibility. In: Schmidt RF, editor. Fundamentals of sensory physiology. 3rd edition. Springer-Verlag, Berlin, Germany, 1986. p. 30-61.

Sivilotti LG, Thompson SW, Woolf CJ. Rate of rise of the cumulative depolarization evoked by repetitive stimulation of small-caliber afferents is a predictor of action potential windup in rat spinal neurons in vitro. J Neurophysiol 1993;69(5):1621-31.

Spicarová D, Palecek J. The role of spinal cord vanilloid (TRPV1) receptors in pain modulation. Physiol Res 2008;57 Suppl 3:S69-77.

Spray DC. Cutaneous temperature receptors. Annu Rev Physiol 1986;48:625-38. doi: 10.1146/annurev.ph.48.030186.003205

Stancák A, Polácek H, Vrána J, Mlynár J. Cortical oscillatory changes during warming and heating in humans. Neuroscience 2007;147(3):842-52. doi:10.1016/j.neuroscience.2007.04.055

Stevens JC. Variation of cold sensitivity over the body surface. Sens Processes 1979;3(4):317-26.

Stevens JC, Choo KK. Temperature sensitivity of the body surface over the life span. Somatosens Mot Res 1998;15(1):13-28. doi:10.1080/08990229870925

Strigo IA, Carli F, Bushnell MC. Effect of Ambient Temperature on Human Pain and Temperature Perception. Anesthesiology 2000;92(3):699-707.

Tatum WO, Husain AM, Benbadis SR, Kaplan PW. Handbook of EEG Interpretation. Demos Medical Publishing, New York, USA, 2008.

Taylor DJ, McGillis SL, Greenspan JD. Body site variation of heat pain sensitivity. Somatosens Mot Res 1993;10(4):455-65.

Thunberg T. Förnimmelserna vid till samma ställe lokasirerad, samtidigtb pagaende köld och värmeretning. Uppsala Läkkfören Föhr 1896;2:489̇695.

Tominaga M, Caterina MJ. Thermosensation and pain. J Neurobiol 2004;61(1):3-12. doi: 10.1002/neu.20079

Torebjörk HE, Schady W, Ochoa J. Sensory correlates of somatic afferent fiber activation. Hum Neurobiol 1984;3(1):15-20.

Wilils WD JR. Temperature perception and pain. In: Greger R, Windhorst, U, editors. Comprehensive Human Physiology: From Cellular Mechanisms to Integration. Springer, Berlin, Germany, 1996. Vol.1. p.677-97.

Yarnitsky D. Quantitative sensory testing. Muscle Nerve 1997;20(2):198-204. DOI: 10.1002/(SICI)1097-4598(199702)20:2<198::AID-MUS10>3.0.CO;2-#

Yarnitsky D, Sprecher E. Thermal testing: normative data and repeatability for various test algorithms. J Neurol Sci 1994;125(1):39-45. doi:10.1016/0022-510X(94)90239-9

Zotterman, Y. Sensory Functions of the Skin of Primates with Special Reference to Man. Pergamon Press, Stockholm, Sweden. 1976. p. 263-400.

Zwart JA, Sand T. Repeatability of dermatomal warm and cold sensory thresholds in patients with sciatica. Eur Spine J 2002;11(5):441-6. doi: 10.1007/s005860100333