The role of the human C-tactile system in affective somatosensation and pain

Jaquette Liljencrantz

Department of Clinical Neurophysiology
Institute of Neuroscience and Physiology
Sahlgrenska Academy at University of Gothenburg
Gothenburg, Sweden

UNIVERSITY OF GOTHENBURG

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jaquette.liljencrantz@neuro.gu.se

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For my mother

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ABSTRACT

Affective touch perception in humans is a complex construct of input from mechanoreceptive afferents, current homeostatic state and contextual factors. Previously, a relationship has been identified between the pleasantness perception of soft skin stroking and the firing rate of unmyelinated C-low-threshold mechanoreceptive afferents (C-LTMRs) known as C-tactile (CT) afferents in humans. This relationship is not seen for myelinated Aβ-LTMRs. The work in this thesis continued the basic characterization of CT response properties to pleasant touch by adding a thermal component to the stimulus. Using the electrophysiological technique of microneurography in combination with psychophysical testing we found a significant relationship between the hedonic evaluation of slow skin stroking stimuli and CT responses only for stimuli of skin-like temperature (i.e. not cooler or warmer temperatures), (**Paper I)**. This finding supports the role of CT afferents in pleasant touch, particularly relating to skin-to-skin contact between individuals and thus emphasizes the significance of CTs in signaling affective, interpersonal touch.

In patients with reduced density of thinly myelinated and unmyelinated afferent nerve fibers (hereditary sensory and autonomic neuropathy type V), gentle skin stroking (CT targeted touch) is perceived as less pleasant, even unpleasant. In addition, research in mice suggests a role for CTs in tactile allodynia. Here, in humans, we investigated the role of CTs in Aβ denervated patients and found no experimental tactile allodynia but a reduced C-touch sensation. These psychophysical findings were confirmed by fMRI data, comparing stroking in the allodynic to a control zone, and showed altered processing in the posterior insular cortex (primary cortical

receiving area for CTs) and reduced processing in medial prefrontal cortices (part of the hedonic network encoding C-touch). In neurologically intact subjects we found a greater drop in touch pleasantness for CT optimal compared to suboptimal (Aβ targeted) stimuli in the allodynic area but we did not find stimulus related differences in touch evoked pain. Thus, we conform to the canonical view of Aβ afferents mediating allodynic pain. We conclude that CT processing is altered but find no evidence for CTs signaling experimental tactile allodynia, (**Papers II and III**).

Other animal work has suggested that C-LTMRs exert a spinal inhibition on nociceptive signaling. Furthermore, C-LTMRs may release a protein with analgesic effects when activated and pharmacogenetic activation of C-LTMRs has positively reinforcing and anxiolytic behavioral effects. Here, we demonstrated a robust psychophysical reduction in experimental heat pain following CT targeted touch suggesting that activation of the CT system modulates pain perception also in humans (**Paper IV**).

In conclusion, the contribution of CTs to experimental tactile allodynia seems to be a reduced CT mediated hedonic processing and possibly also a loss of their pain inhibitory role. Thus, restoring normal CT function could be considered when investigating novel therapeutic strategies for neuropathic pain.

Keywords: touch, hairy skin, CT-afferents, microneurography, temperature, heat pain, experimental tactile allodynia, psychophysics, functional magnetic resonance imaging

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Vi människor har en unik uppsättning nerver i huden som långsamt leder signaler om hudberöring till ryggmärg och hjärna. I denna avhandling visas att dessa s.k. C-taktila (CT) nerver har unika egenskaper som gör dem specialiserade för att signalera mjuk och behaglig mellanmänsklig beröring (**Paper I**). Vi visar också att signaler i CT nerver lindrar smärta på ett effektivt sätt (**Paper IV**). Denna smärtmodulerande effekt kan försvinna vid neurologisk sjukdom vilket kan medföra att mjuk beröring istället upplevs som obehaglig (**Paper II-III**).

Upptäckten av CT nerver gjordes hos människa först 1990, hos djur redan 1939. Förståelsen för sambandet mellan CT nerver och behaglig beröring kom så sent som 2009. Sambandet var slående - det upplevda välbehaget samvarierade med intensiteten av impulser i CT nerverna. I denna avhandling åskådliggörs en ny dimension när också betydelsen av beröringens temperatur för upplevelsen undersöks. Våra resultat visar att CT fibrer är optimerade för hud-mot-hud beröring – det ska vara en mjuk, långsam hudstrykning av temperatur motsvarande hudens (varken kallare eller varmare) för mest effektiv stimulering av CT nerver. Vi tolkar detta fynd som att CT fibrer utgör ett medfött beröringssystem för signalering av kontakt människor emellan och som för oss närmare varandra.

Det välbehag och den trygghet som beröring utgör för oss människor kan också motverka smärta. Dessa effekter har tidigare studerats hos djur för CT fibrer. Nu har vi kunnat visa på denna effekt även hos människa – experimentell smärta upplevs som mindre smärtsam när den föregås av aktivering av vårt CT nervsystem.

Vad händer då med CT nerver vid sjukdom? Hos patienter med traumatisk nervskada, neurologisk sjukdom eller diabetes, som bl.a. slår ut dessa nerver, kan CT optimerad hudstimulering upplevas som obehaglig, ett tillstånd som kallas taktil allodyni. Genom att använda en experimentell modell för taktil allodyni hos människa har vi kunnat visa att CT signaleringen ändras. Resultaten pekar på att CT bidrar till taktil allodyni genom en avsaknad av signalerat välbehag och kanske även genom förlust av sina smärthämmande egenskaper. En möjlig framtida behandlingsstrategi vid taktil allodyni kan vara att stimulera CT funktion efter skada, t.ex. på farmakologisk väg.

Metoderna i avhandlingen inkluderar mätning av subjektiva upplevelser med så kallad psykofysisk metodik, registrering av nervsignaler från hudnerver med tekniken mikroneurografi och mätning av förändringar i hjärnans blodflöde med funktionell magnetresonansavbildning. Försöken är utförda på neurologiskt intakta försökspersoner och på patienter med väldefinierade nervskador.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Ackerley R*, Backlund Wasling H*, **Liljencrantz J**, Olausson H, Johnson R D, Wessberg J.
Human C-tactile Afferents Are Tuned to the Temperature of a Skin-Stroking Caress. *R.A. and H.B.W. contributed equally to this work.
Accepted for publication in The Journal of Neuroscience

II. **Liljencrantz J**, Björnsdotter M, Morrison I, Bergstrand S, Ceko M, Seminowicz D A, Cole J, Bushnell M C, Olausson H. Altered C-tactile processing in human dynamic tactile allodynia.
PAIN. 2013 Feb;154(2):227-34.

III. **Liljencrantz J**, Marshall A, Ackerley R, Olausson H. Discriminative and affective touch in human experimental tactile allodynia.
Accepted for publication in Neuroscience Letters

IV. **Liljencrantz J**, Strigo I, Ellingsen D M, Krämer H H, Lundblad L, Leknes S, Olausson H.
Pleasant touch modulates heat pain perception in humans.
Manuscript

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ABBREVIATIONS

In order of appearance:

LTMR	Low threshold mechanoreceptive
CT	C-tactile
SA	Slowly adapting
RA	Rapidly adapting
PC	Pacinian corpuscle
DRG	Dorsal root ganglion
WDR	Wide dynamic range
ADS	Activity-dependent slowing
HSAN-V	Hereditary sensory and autonomic neuropathy type V
fMRI	Functional magnetic resonance imaging
PET	Positron emission tomography
OFC	Orbitofrontal cortex
mPFC	Medial prefrontal cortex
pgACC	Pregenual anterior cingulate cortex
TRPV-1	Transient receptor potential vanilloid type 1
MRGPRB4	Mas-related G-protein-coupled receptor B4
TH	Tyrosine hydroxylase
VGLUT3	Vesicular glutamate transporter type 3
TAFA4	Gene encoding proteins of amino acids that contain conserved cysteine residues at fixed positions.
VAS	Visual analog scale
SF-MPQ	Short form-McGill pain questionnaire
GLM	General linear model
MVPA	Multivoxel pattern analysis
SVM	Support vector machine
FDR	False discovery rate
TDD	Tactile direction discrimination
AUC	Area under curve
WHO	World Health Organization
S1	Primary somatosensory cortex
IASP	International Association for the Study of Pain

1 INTRODUCTION

Touch consists not only of its well-known discriminative component but also of a social or affective one. The affective aspect of touch is a construct of many factors; the input from mechanoreceptive afferents, current homeostatic state as well as contextual factors (Craig 2002). The work in this thesis investigates how affective touch is modulated by temperature as well as by pain and closes in on pathophysiology through the study of altered touch percept following experimental tactile allodynia.

1.1 Human Aβ-low threshold mechanoreceptive afferents: discriminative touch

Most of the research on the human somatosensory touch system has been devoted to myelinated (Aβ) low threshold mechanoreceptive (LTMR) afferents. This system consists of large diameter fibers with rapid conduction velocities (approximately 50m s^{-1}) optimized for signaling immediate detection of and discriminative information about a touch stimulus. Aβ afferents are present throughout the skin, i.e. both in hairy and in glabrous skin. Aβ fibers can be subdivided further based on their electrophysiological response and adaptation characteristics. In hairy skin there are slowly adapting type I (SAI; Merkel end organs), slowly adapting type II (SAII; Ruffini end organs) and rapidly adapting type I (RA; hair, field, unknown end organs), and rapidly adapting type II (Pacini; Pacinian end organs) units (Vallbo et al. 1995). SA fibers discharge continuously to a constant mechanical stimulation, sending information to the brain that the current stimulus is still present on the skin. The RA fibers, instead, respond only to changes in mechanical stimuli, serving as a complementary function to signal that something new is happening on the skin (Johansson 1976, Vallbo et al. 1979). Similar unit types are present in the glabrous skin; SAI, SAII, and RAs of PC type but there is also another type of RA unit (Meissner end organ) that is present only in glabrous skin. Meissner's corpuscles and Merkel's disks are located near the surface at the dermal/epidermal boundary, i.e. superficially, whereas Pacinian corpuscles and Ruffini endings are located deeper within the dermis.

However, the focus of this thesis is on another less explored type of low threshold mechanoreceptive afferents with unmyelinated (C) axons and henceforth, I will concentrate on this slowly conducting touch system.

1.2 Animal C-low threshold mechanoreceptive afferents

Low threshold mechanoreceptors with C afferents were detected through a cat saphenous nerve preparation 75 years ago (Zotterman 1939). Gentle mechanical stimulation of these fibers elicited activity with long latency and afterdischarges (persisting discharges after stimulus cessation). C-low threshold mechanoreceptive (C-LTMR) afferents have since been identified in mice, rat, guinea-pig, rabbit, cat, pig and primate (Douglas et al. 1957, Iggo 1960, Bessou et al. 1971, Iggo et al. 1977, Kumazawa et al. 1977, Lynn et al. 1982, Shea et al. 1985, Sugiura et al. 1986, Leem et al. 1993, Liu et al. 2007, Seal et al. 2009, Obreja et al. 2010, Li et al. 2011, Abraira et al. 2013, Delfini et al. 2013, Vrontou et al. 2013). All studies report that C-LTMRs have a slow conduction velocity (approximately 1m s^{-1}) and respond to slowly moving stimuli. C-LTMRs cannot discriminate between blunt and sharp mechanical stimuli (Bessou et al. 1971) nor between inward versus outward stimulus movements (Iggo 1960, Bessou et al. 1971, Iggo et al. 1977) but respond to skin stretch (Kumazawa et al. 1977, Leem et al. 1993). C-LTMRs fatigue (decrease in response to repeated stimuli) easily (Iggo 1960, Bessou et al. 1971, Iggo et al. 1977, Lynn et al. 1982). C-LTMRs are incapable of following vibratory stimuli above 1 Hz whereas myelinated LTMRs follow vibration up and above 300 Hz (Bessou et al. 1971).

C-LTMRs, in contrast to nociceptors, do not respond to capsaicin (Foster et al. 1981, Kenins 1982, Seno et al. 1993) and in guinea-pig dorsal root ganglia (DRG) they lack immunoreactivity for both calcitonin gene-related peptide (CGRP) and for substance-P (Lawson et al. 1997, Lawson et al. 2002).

Despite C-LTMRs being found across various types of mammals they were for long a long period of time not found in humans and it was suggested that they had disappeared during evolutionary processes (Kumazawa et al. 1977).

1.2.1 Spinal processing and beyond

Based on findings in rats, guinea pigs and monkeys, it was shown that C-LTMRs project to the superficial lamina (I and II) of the spinal dorsal horn, mainly to the innermost part of lamina II (Kumazawa et al. 1977, Light et al. 1979, Sugiura et al. 1986, Lu et al. 2003). It was later found that the morphological properties of the C-LTMRs identified by Light *et al.* (1979) included vertical neurons (Grudt et al. 2002) with axons arborizing in lamina I (Maxwell et al. 2007) where they contact projection neurons (Lu et al. 2005). A more recent study in rats characterized the response properties of the lamina I spinal projection neurons that transmit tactile information from C-LTMRs to the brainstem and brain (Andrew 2010). These neurons respond not only to light touch but also to noxious stimuli. i.e. they are wide dynamic range (WDR) neurons with further projection to the contralateral brainstem parabrachial nucleus and via the ventral posterior and/or posterior triangular thalamic nuclei to the cortex (Andrew 2010).

1.3 Human C-tactile afferents: affective touch

About 25 years ago, using the electrophysiological technique of microneurography (see **3.3.1-4**), C-LTMRs were finally found to exist also in humans (Johansson et al. 1988, Nordin 1990, Vallbo et al. 1993). They are termed C-tactile (CT) afferents to distinguish them from C-LTMRs in mammals. However, CTs are believed to be the human homologue of C-LTMRs. They were first reported in the infra-orbital nerve (Johansson et al. 1988), and then in the supra-orbital nerve (Nordin 1990). Subsequently, a more general distribution became evident with CT afferents present also in the hairy skin of the arm and leg (Vallbo et al. 1993, Vallbo et al. 1999, Edin 2001, Wessberg et al. 2003). Although it is currently not possible to assess their density in human skin nerves, it is a recurring experience in microneurography that they are encountered as often as the Aβ afferents (Vallbo et al. 1999). Despite numerous recordings, CT afferents have never been found in the median nerve and are therefore unlikely to innervate the glabrous skin (Johansson et al. 1979, Johansson et al. 1979, Johansson et al. 1980, Johansson et al. 1980, Vallbo et al. 1984).

1.3.1 Electrophysiological response properties

The response properties of CT afferents have been identified using the technique of microneurography (see **3.3.1-4**). The similarities between CTs and C-LTMRs are striking. CTs respond to a low mechanical indentation force (< 5mN) (Vallbo et al. 1999), they respond to skin stretch, and they cannot discriminate between sharp and blunt probes (Nordin 1990, Vallbo et al. 1999). Their conduction velocity is approximately 1 m s^{-1}, as expected for unmyelinated afferents (Vallbo et al. 1999, Wessberg et al. 2003, Loken et al. 2009). They respond vigorously to slowly moving stimuli (Nordin 1990, Vallbo et al. 1999, Loken et al. 2009). The receptive field of CTs are round or oval consisting of one to nine small responsive spots (Nordin 1990, Wessberg et al. 2003). This receptive field structure is also consistent with animal observations indicating that the receptor is likely of free nerve ending type (Cauna 1973, Iggo et al. 1977, Messlinger 1996, Liu et al. 2007).

CTs exhibit maximum firing frequency (50 – 100 impulses s^{-1}) to stimuli that are clearly innocuous, such as gentle stroking with a soft brush (Vallbo et al. 1999, Wessberg et al. 2003, Loken et al. 2009). C-nociceptors also respond to light touch, although not to soft brush stroking, however their responses never exceed a few impulses to this type of stimuli (Vallbo et al. 1999). CT afferents have intermediate adaptation properties implying that they respond initially with a burst of high impulse rate which terminates after a few seconds of sustained indentation (Nordin 1990, Vallbo et al. 1999). CTs sometimes exhibit after-discharges (Wiklund Fernström 2004). Again similar to C-LTMRs CTs exhibit fatigue, although the recovery time seems to be variable across species with fatigue reported to range from 30 seconds in humans and up to 30 minutes in cats (Iggo 1960, Wiklund Fernström 2004). They can generally encode vibratory stimuli up to 1 Hz, but a small proportion of the afferents are sensitive to vibration up to 32 Hz (Wiklund Fernström 2002). Above 32 Hz CTs only respond with single spikes (Wiklund Fernström 2002). One CT unit has been studied with regard to activity dependent slowing (ADS) and displayed minimal ADS of approximately 1% at a 2 Hz tetanus (Campero et al. 2011).

The above properties suggest that CT afferents are poorly designed for signaling discriminative aspects of touch. Combining electrophysiological recordings with psychophysical observations shows that brush stroking with intermediate velocities (1-10 cm/s) is very effective in activating CT afferents, and that these stimuli are also rated as being most

pleasant (Loken et al. 2009). Indeed, there is a robust positive correlation between the firing rate of CT afferents and the perceived pleasantness of the touch (Loken et al. 2009). This relationship is not present between brush-stroking velocity and the firing rate of myelinated afferents (Loken et al. 2009). This study is critical for the hypothesis that the functional role of CT afferents is to encode affective touch perception in humans and thus promote social behavior (Morrison et al. 2010).

1.3.2 Patient studies - selective CT activation

The dual tactile innervations of human hairy skin is one of the main challenges in studying the human CT system as it is not possible to stimulate CT afferents without also activating Aβ afferents. However, studies of two unique patients (GL and IW) with complete Aβ de-afferentation have been crucial for collecting the information we have about CT afferents today (Olausson et al. 2002, Olausson et al. 2008, Bjornsdotter et al. 2009). These patients suffer from a rare sensory neuronopathy syndrome (see **3.2**), where large myelinated afferents are lacking but thinly myelinated and unmyelinated afferents are intact. Studies of GL and IW show that selective activation of CTs elicits a sympathetic skin response and evokes a faint sensation of pleasant touch with no qualities of pain and temperature and poor spatial localization (localizing stimuli on different body quadrants at slightly above chance) (Olausson et al. 2002, Olausson et al. 2008). In addition, both Aβ denervated participants have difficulties detecting 50 Hz vibratory stimuli which are known to give a poor excitation of CT afferents but a massive activation of Aβ afferents (Olausson et al. 2002, Wiklund Fernström 2002, Olausson et al. 2008).

1.3.3 Patient studies - lacking CTs

Another group of patients instead have a congenital selective loss of unmyelinated afferents (most likely including CT afferents) which is caused by a nerve growth factor beta gene mutation. Their condition has been classified as hereditary sensory and autonomic neuropathy type V (HSAN-V) with reduced density of thinly myelinated and unmyelinated afferent nerve fibers. These patients perceive gentle brush stroking, optimal for eliciting CT responses (1-10 cm/s), as less pleasant (even slightly unpleasant) compared to neurologically intact, matched controls. Thus, the perceptions of hedonic

aspects of dynamic touch are likely depending on intact CT afferent density (Morrison et al. 2011).

1.3.4 Cortical processing of CT input

Following the identification of these two unique patient populations (A-beta denervated and C-denervated) further studies have been conducted using functional magnetic resonance imaging (fMRI; see **3.4**). Selective CT stimulation in the patients lacking Aβ fibers demonstrates that CTs activate the contralateral posterior insular cortex (Olausson et al. 2002, Olausson et al. 2008) with forearm stimulation projecting anterior to thigh stimulation (Bjornsdotter et al. 2009). A similar somatotopic organization of the posterior insula is evident for noxious and cooling stimuli (Brooks et al. 2005, Hua et al. 2005, Henderson et al. 2007) suggesting that the human CT afferent system is organized in a similar manner as the pain- and temperature-mediating thin fiber systems. It thus seems plausible that CTs project through the lamina I spinothalamic pathway via the ventromedial posterior thalamic nucleus to the posterior insula (Craig 2002) akin to the pathway demonstrated for rats (see **1.2.1**) (Andrew 2010). Furthermore, brain imaging of CT-targeted touch in the patients lacking an intact CT system showed no activation of the posterior insular cortex. (Morrison et al. 2011).

Related fMRI and positron emission tomography (PET) studies have indicated other brain areas as being potentially involved in cortical CT processing: the orbitofrontal cortex (OFC) (a key-area for hedonic processing), the posterior superior temporal sulcus, the medial prefrontal cortex (mPFC), dorso anterior cingulate cortex, and the pregenual anterior cingulate cortex (pgACC) (Kringelbach et al. 2004, Gordon et al. 2011, Lindgren et al. 2012, McGlone et al. 2012, Ellingsen et al. 2013).

1.4 Molecular receptor mechanisms for CT afferents and C-LTMRs

Electrophysiologically, CTs are quite well characterized in humans but little is known about their receptor class and molecular properties. As described above, based on their receptive field properties it has been suggested that their end organ is a free nerve ending but their terminal morphology is currently unknown. Previous thesis work from our group has determined that CTs lack capsaicin sensitivity and hence Transient Receptor Potential Vanilloid type 1 channels (Wiklund Fernström 2004).

However, through work in rodents the molecular properties of C-LTMRs are gradually being elucidated. A population of unmyelinated sensory neurons in mice (Dong et al. 2001, Zylka et al. 2003), expressing the Mas-related G-protein-coupled receptor MRGPRB4 and exclusively innervating hairy skin have been identified. This finding was furthered through the use of a genetically encoded tracer revealing a MRGB4 subpopulation of unmyelinated, nonpeptidergic afferents in mice exclusively innervating hairy skin (Liu et al. 2007). The terminal structure of MRGPRB4 fibers are similar to the receptive fields structure defined in humans through microneurography (Wessberg et al. 2003). MRGPRB4 fibers were found to encircle and penetrate the necks of hair follicles (Liu et al. 2007). Using the technique of calcium imaging of the DRG and dorsal horn spinal projections in intact mice shows that these neurons are activated by gentle brushing of hairy skin, but not by noxious mechanical stimulation. In addition, pharmacogenetic activation of the MRGPRB4 neurons in freely behaving mice promotes conditioned place preference, indicating that such activation is positively reinforcing and/or anxiolytic (Vrontou et al. 2013). Thus, the CT system may be a potentially attractive target for the development of anxiolytic drugs.

The association with hair follicles was confirmed by another study (Li et al. 2011) who used genetic labelling in mice to identify sub-classes of LTMRs and to visualise their terminal endings in hairy skin and spinal cord. Each of the three hair follicle types (guard, awl/auchene, and zigzag) is innervated by a 'unique and invariant combination of LTMRs'. However, this group found that C-LTMRs are tyrosine hydroxylase positive (TH+) and do not express MRGPRB4 (Seal et al. 2009, Li et al. 2011, Abraira et al. 2013, Lou et al. 2013). TH+ neurons express the vesicular glutamate transporter type 3 (VGLUT3) in the DRG (Seal et al. 2009, Li et al. 2011, Lou et al. 2013) and is expressed widely in the nervous system (El Mestikawy et al. 2011). VGLUT3 lineage sensory neurons are divided into two groups depending on if they exhibit a transient or a persistent VGLUT3 expression (Lou et al. 2013). The VGLUT3-transient neurons are large- or medium-diameter myelinated mechanoreceptors whereas the VGLUT3-persistent neurons are small-diameter unmyelinated neurons containing two subtypes: TH^+ C-LTMRs that form the longitudinal lanceolate endings and TH^- neurons that form epidermal-free nerve endings. Electrophysiological recordings from VGLUT3-persistent neurons confirm that they are C-LTMRs

(Li et al. 2011). Recently, a novel specific marker of C-LTMRs has been identified: a chemokine-like secreted protein called TAFA4 which is predominantly co-expressed with VGLUT3 (Delfini et al. 2013). The authors speculate that upon activation C-LTMRs might release TAFA4 protein which has analgesic effects (Delfini et al. 2013).

The complexity of defining the receptor properties of C-LTMRs (let alone CTs) is evident and further studies are required to reconcile the contradictory results or alternatively to identify and characterize different subclasses of C-LTMRs.

1.5 The neurochemistry of affective touch

One question often raised in relation to the CT affective touch hypothesis is the potential role of the neuropeptide oxytocin. Oxytocin is known to be released during nurturing behavior, more specifically during gentle stroking touch (Uvanas-Moberg et al. 2005) which would typically activate CTs. Oxytocin is also released during other social interactions as well as during sex (Carter 1998, Panksepp 2006).

The combination of oxytocin treatment (nasal spray) and being touched by another human sharpens social evaluation of others with angry faces being perceived as *less* friendly and attractive, and neutral or happy faces being perceived as *more* friendly and attractive (Ellingsen et al. 2014). The touch experience itself is rated as most pleasant when presented with a happy face. These findings support the notion that oxytocin does indeed contribute to the interpretation of CT-related touch.

Pleasant touch is known to activate reward related brain areas such as the pgACC, OFC and mPFC (Rolls et al. 2003, Kringelbach et al. 2004, McCabe et al. 2008, Gordon et al. 2011, Grabenhorst et al. 2011, Lindgren et al. 2012, McGlone et al. 2012, Ellingsen et al. 2013, Liljencrantz et al. 2013) with known association to the opioid system, for example the pgACC exhibits a high density of opioidreceptors (Vogt 2005), and a role for this neurotransmitter system in affective touch seems likely. The endogenous opioid system of endorphins contributes to the liking component of a pleasant experience (Kringelbach et al. 2009). Endorphins are also released during social bonding (Dunbar 2010) and the endorphin system is activated by rewarding stimuli reducing both sympathetic activity and cortisol levels (Eisenberger 2012). Furthermore, monkeys spend far more time grooming than required for hygienic purposes alone, suggesting that this behavior has

an additional affective and social function stimulated by endorphin release (Dunbar 1997).

However, given the linkage between the opioidsystem and the serotonin, noradrenalin and dopamine systems these neurotransmittors are likely to also be involved in the pleasantness perception of affective touch.

1.6 C-LTMRs, CT afferents, and pain

Given the close proximity of nociceptive specific neurons in the superficial dorsal horn (Todd 2010), from which also C-LTMRs seem to have their spinal projections, (Sewards et al. 2002, Andrew 2010) a role for C-LTMRs/CTs in pain processing has often been speculated upon. The first study to implicate a role for C-LTMRs in pain suggested that C-LTMR targeted input may inhibit C-nociceptive messages in the dorsal horn of the rat (Lu et al. 2003). Using electrophysiology a specific inhibitory pathway was identified between substantia gelatinosa neurons receiving C-LTMR input and other substantia gelatinosa cells receiving nociceptive input (Lu et al. 2003). This unmyelinated circuit represents a potential pathway for C-LTMR impulses to suppress nociceptive impulses (Lu et al. 2003). This line of research has not been pursued further until recently, see below.

Meanwhile, C-LTMRs have instead been investigated in relation to dynamic tactile allodynia. Using a C-LTMR knock-out mouse model targeted against VGLUT3, which functionally disconnects signaling in C-LTMRs by preventing glutamate release (Seal et al. 2009), reduced mechanical hypersensitivity following inflammation, nerve injury and trauma. At the time of this study, VGLUT3 was thought to be specific for C-LTMRs, and thus a critical role for C-LTMRs in mechanical hypersensitivity was suggested (Seal et al. 2009). However, more recent evidence instead suggests that the VGLUT3 lineage sensory neurons are divided into two groups depending on if they exhibit transient or persistent VGLUT3 expression (Lou et al. 2013). VGLUT3-persistent neurons are likely to be C-LTMRs. A new analysis was performed in mice with a conditional knock-out of VGLUT3-persistent neurons and it demonstrated that both acute and chronic mechanical pain was largely, but not completely, unaffected. This finding thus argues against a role for C-LTMRs in allodynia (Lou et al. 2013).

New light has recently been shone on the question of C-LTMR suppression of nociceptors through the identification of the novel C-LTMR

specific marker TAFA4 (Delfini et al. 2013). To investigate the role of TAFA4 and C-LTMRs in pain a knock-in mouse model was generated, allowing the authors to genetically label TAFA4-expressing neurons while eliminating the TAFA4 protein. Following inflammation and nerve injury TAFA4-null mice show enhanced mechanical and chemical hypersensitivity. However, this effect is reversed by application of recombinant TAFA4 protein (Delfini et al. 2013). The authors speculate that upon activation, C-LTMRs might release both glutamate and TAFA4 with glutamate promoting mechanical hypersensitivity and TAFA4 instead preventing mechanical hypersensitivity. This suggestion also provides a potential explanation for the different findings regarding the functional knock-out of VGLUT3 (Seal et al. 2009) and the complete loss of C-LTMRs (Lou et al. 2013). TAFA4 could oppose the pain-promoting actions of glutamate release from C-LTMRs through the functional loss of glutamate release (Seal et al. 2009) and would then leave TAFA4 release unopposed and free to drive the resistance to hypersensitivity. However, in the case of a complete loss of C-LTMRs (Lou et al. 2013) both glutamate and TAFA4 are reduced leaving no net change in hypersensitivity. Strikingly, also in wild-type mice administration of TAFA4 reverses the effect of injecting an inflammatory agent (carrageenan) normally causing mechanical hypersensitivity. This finding suggests a potent analgesic role of TAFA4 and thus C-LTMRs in pain relief (Delfini et al. 2013). The topic of C-LTMRs in pain inhibition also ties back to the finding of pharmacogenetic activation of $MRGPRB4^{+}$ expressing neurons (thought to be C-LTMRs) promoting conditioned place preference in mice, indicating that such activation is positively reinforcing and/or anxiolytic (Vrontou et al. 2013) - mechanisms which also have an important role in pain modulation.

Nevertheless, the prevailing hypothesis regarding tactile allodynia is changed tactile signaling in the spinal cord (Woolf 1993, Campbell et al. 2006) following central sensitization were Aβ low-threshold mechanoreceptors signal to nociceptive neurons in the dorsal horn and, from there, to cerebral pain processing areas (Campbell et al. 1988, Koltzenburg et al. 1992, Torebjork et al. 1992, Woolf 1993, Iadarola et al. 1998, Wasner et al. 1999, Maihofner et al. 2003). This view is supported by human selective nerve block experiments demonstrating that tactile allodynia is abolished by compression or ischemic block of Aβ afferents (Gracely et al. 1992, Koltzenburg et al. 1992, Torebjork et al. 1992, Cervero et al. 1996, Landerholm et al. 2011). But, with regard to the mouse knock-out study

presented above, a role for not only Aβ but also CT afferents seems plausible. In humans, it has been demonstrated that ongoing muscle pain, induced by hypertonic saline muscle infusion, increases following CT-targeted stroking of the overlaying skin (Nagi et al. 2011). This effect survives compression block of myelinated cutaneous afferents suggesting that this type of allodynia is selectively mediated by CT afferents (Nagi et al. 2011).

2 SPECIFIC AIMS

The overall aim is to further the characterization of the human C-tactile afferent system and investigate its role in pain.

Paper I studied if human CT afferents are tuned to respond preferentially to stimuli with the mechano-thermal characteristics of a human caress.

Paper II studied if CT afferents have a role in human experimental tactile allodynia.

Paper III compared the integrity of discriminative and affective touch in human experimental tactile allodynia.

Paper IV investigated if CT-targeted pleasant touch modulates heat pain perception in humans.

3 METHODOLOGICAL CONSIDERATIONS

3.1 Ethics

All studies included in this thesis were approved by the local ethics committee of the medical faculty at the University of Gothenburg, Sweden. For **Paper II** the ethical review board at McGill University, Montreal, Canada also approved the procedures. All experiments were performed in accordance with the declaration of Helsinki. Informed written consent was obtained from all participants.

3.2 Participants

Healthy subjects were recruited by advertising. All participants were financially compensated in accordance with current university standards.
In **Paper I,** 20 healthy subjects participated in nerve recordings. Psychophysical data was obtained from another 30 healthy subjects. In **Paper II,** 43 healthy subjects and two unique Aβ denervated subjects (GL, age 60, female; IW, age 58, male) participated. Psychophysical data was collected from all participants, and 22 subjects including GL also participated in fMRI (see **3.4**). For the brain imaging part of the study only right handed participants were included.

GL and IW are diagnosed with a rare sensory neuronopathy (sensory ganglionopathy) syndrome leaving them without functional large-diameter myelinated somatosensory afferents (Sterman et al. 1980). GL became ill at age 31 and IW at age 19 (Cooke et al. 1985, Cole et al. 1992, Cole 1995, Forget et al. 1995). Clinical and electrophysiological examinations have been performed regularly and their condition has remained stable over the years. Using EEG and MEG, non-painful electrical stimuli of the peripheral nerves fail to produce sensory potentials or cortical evoked potentials (Caetano et al. 2010). Motor nerve conduction velocities and EMG findings are normal. GL and IW report intact temperature and pain perceptions, and thermal detection thresholds are normal or slightly reduced (Olausson et al. 2002, Cole et al. 2006). As typical for the neuronopathy syndrome, GL's and IW's sensory disturbances do not show a proximal-distal gradient, no patchy loss of light touch or movement/position sense, and no patchy loss of small fiber function (Camdessanche et al. 2009). A sural

nerve biopsy in GL demonstrates complete loss of Aβ afferents with preservation of small-diameter myelinated afferents (Forget et al. 1995). IW presented to neurology 12 years after his illness so a biopsy was not indicated. Initial clinical observations when GL and IW first presented suggested a total loss of tactile perception. However, it was later demonstrated that in two-alternative forced choice (2-afc) situations they can detect stimuli which effectively activates CT afferents (Olausson et al. 2008, Olausson et al. 2008).

In **Paper III,** 40 and in **Paper IV,** 44 healthy subjects participated.

3.3 Paper I - Stimuli and experimental design

With the aim to study if human CT afferents are tuned to the mechano-thermal characteristics of a human caress, axonal recordings, using the technique of microneurography (see **3.3.1-4**), were made from the left antebrachial cutaneous nerve. A rotatory tactile stimulator (see **3.3.5**) was used to move a mechano-thermal probe across the center of a unit's receptive field. The stroking velocities were 0.3, 1, 3, 10 or 30 cm s^{-1} at a force of 0.4 N. For each unit, three temperatures were tested; cool (18^{o}C), neutral (32^{o}C; i.e. typical human arm skin temperature (Arens 2006)), and warm (42^{o}C). The temperatures were presented in a pseudo-randomized block design, where three repeats of each velocity were given in a randomized order in each temperature block. The inter-stimulus-interval was 30 seconds to allow for recovery of the CT afferent response (Zotterman, 1939; Iggo, 1960; Bessou et al., 1971; Hahn, 1971; Iggo and Kornhuber, 1977; Nordin, 1990; Vallbo et al., 1999).

Psychophysical data were collected in a separate session. The mechano-thermal stroking was again delivered to the left forearm. The stimuli were presented in the same manner as in the mechano-thermal paradigm above and the participants rated each stimulus on a visual analog scale (VAS) with the endpoints *Unpleasant* and *Pleasant*. Subjects were prevented from seeing the tested extremity during tactile stimulation.

3.3.1 Nerve recordings and search procedure

Recordings from single afferents were sought through high-impedance, tungsten recording electrodes (FHC, Bowdoin, ME). When the tip of the electrode was located intrafascically the experimenter stroked the

participant's arm gently over the innervations territory to locate a single unit, thus, the sample was biased towards low-threshold mechanoreceptive afferents.

Single units were identified online by the spike detection algorithms of the data acquisition system (SC/ZOOM; Department of Physiology, Umeå University, Sweden) sampled at 12.8 kHz, band-pass filtered (0.2-4 kHz). The same device was used to record and store the data.

3.3.2 Unit identification

Units were classified as CT afferents when their spike configuration showed a major deflection in the negative direction (as expected for extracellular recordings from unmyelinated axons), long latency responses to mechanical stimulation, and monofilament force thresholds of $\leq$ 2.5 mN (Vallbo et al., 1993, 1999; Wessberg et al., 2003). The conduction velocity of CT units was estimated using a hand-held, blunt strain gauge device; responses were recorded to short, mechanical taps to the center of the unit's receptive field and the conduction velocity was calculated using the distance from this spot to the recording electrode (Vallbo et al., 1999). Unmyelinated afferents with monofilament thresholds above 5 mN were classified as nociceptors and were not further studied.

Myelinated A-fiber mechanoreceptive afferents were sub-classified as SAI, or SAII, or RA Pacinian, hair or field units according to their specific response and receptive field characteristic (Vallbo et al., 1995).

3.3.3 Data processing

Each recorded nerve impulse was inspected offline to verify the single-unit nature of all units with an offline pattern-matching algorithm, and the recorded nerve spikes were inspected in expanded time-scale using software implemented in MATLAB (The Mathworks, Natick, MA). Single spikes were time-stamped and the onset and offset of the probe movement were time-marked.

3.3.4 Statistical considerations

Descriptive statistics were gained about the mean firing frequency of individual units, and stroking velocity was transformed to $\log_{10}$ values. Statistical comparisons were made using SPSS (version 18: IBM, Armonk, NY) and significances were sought below the $P < 0.05$ level (P values are

given for significance to three decimal places). Regressions testing linear and quadratic models were used to investigate curve fitting of the data from individual units, and at the group level, for the stroking velocities, over each temperature. Multilevel mixed model analyses were conducted to uncover statistically significant main effects of the stroking velocity and temperature, using maximum likelihood estimation and a random intercepts model; differences between the levels of each variable were compared using Least Significant Difference tests. The firing frequency data for CTs were compared to the mean pleasantness ratings for each temperature using Pearson's correlation two-tailed tests.

3.3.5 Rotatory Tactile Stimuli (RTS)

A rotary stimulator (Dancer Design, Wirral, UK; Fig. 1A) was used to move a mechano-thermal probe (contact surface ~5 cm^2) across the center of a unit's receptive field. Two variables were changed: the stroking velocity and the temperature of the stimulus probe. The contact surface of the probe was a rounded, smooth metallic plate, warmed and cooled with a custom-designed thermode consisting of probe-mounted Peltier elements (Melcor CP Series thermoelectric module) interfaced to programmable control modules and thermocouples (Melcor PR-59, 0.05 ºC resolution, Laird Technologies, St. Louis, MO, USA). The probe was attached to an arm and central axle, which delivered different velocities of stroking stimuli. This robotic stimulator provided high-precision computer control over the velocities and temperatures at a calibrated normal force (0.4 N).

3.4 Paper II - Stimuli and experimental design

Aiming to study the contribution of CT afferents in human experimental tactile allodynia we established two zones, 7 cm apart, on the testing area (left forearm for psychophysics; left thigh for fMRI (see **3.4.2-5**); one control area and the other with heat capsaicin induced experimental tactile allodynia (see **3.4.1**). Effective stimulation of CT afferents (stroking velocity 3 cm s^{-1}; cotton swab or soft goat-hair brush; width 3mm) was delivered manually in the allodynic and in the control zones (stroking distance 9 cm, application force approximately 0.3 N). A total of 16 stimulations were delivered, 8 in each skin zone, pseudo-randomized order. Subjects were instructed to specify which of the paired stimuli was the most unpleasant (2-alternative forced

choice; 2-afc). GL and IW both reported a distinct difference in stroking sensation between the two zones but did not perceive unpleasantness. When asked to describe, both IW and GL independently used the words “weaker sensation” for stroking in the allodynic zone. Therefore, they were instead instructed to specify which of the paired stimuli gave the weakest sensation (2-afc).

A subset of subjects participated in fMRI of the same stimulus paradigm to be able to make inferences about differences in neuronal activity related to skin stimuli in the two zones. Following each stimuli, VAS ratings (with the endpoints *Unpleasant* and *Pleasant*) were collected. VAS data was not collected from GL as she could not manipulate the response unit due to her lack of proprioception. All participants completed the Short Form-McGill Pain Questionnaire (SF-MPQ) (Melzack 1987). Data was collected from 5 (median, range 3-5) consecutive fMRI runs (100 volume acquisitions) in each subject. Tactile stimuli, stroking over a 9 cm distance for 3 s, were delivered in the allodynic and control zones (8 stimuli/zone/run), pseudo-randomized order (inter-stimulus-interval 15 s). Timing guidance was provided through a visual display generated by a MATLAB (The MathWorks, Inc., Natick, MA, USA) script. Participants were instructed to focus on a fixation cross.

During all tactile stimulation subjects were prevented from seeing the tested extremity.

3.4.1 Heat capsaicin experimental model of tactile allodynia

The heat/capsaicin sensitization model was used to induce primary and secondary hyperalgesia (Petersen et al. 1999). In the model, a mild burn injury is induced after which capsaicin cream is applied to that same skin area. Primary hyperalgesia develops in the treated skin zone, and secondary hyperalgesia in the surrounding skin. In the secondary hyperalgesia zone light touch is perceived as unpleasant or painful (tactile allodynia) as a consequence of altered sensory processing in the central nervous system (Woolf 2011).

In detail, a Peltier thermode (3 x 3 cm, Medoc, TSA 2001, Thermosensory Analyzer, Rimat Yishai, Israel or 2.5 x 5 cm, Somedic, MSA Thermal Stimulator, Hörby, Sweden) was used to deliver a 45°C stimulus to the subject’s skin for 5 minutes after which capsaicin cream (Capsina, 0.075%, Hants, UK,) was applied to the same skin area for 30 minutes. All participants developed a visible flare. In pilot experiments, neurologically

intact subjects with ages up to 79 years were tested indicating that the model is effective in inducing flare and dynamic tactile allodynia also in older subjects (Zheng et al. 2000). Punctate hyperalgesia was mapped with a monofilament (calibrated indentation force 0.20 or 0.24 N).

3.4.2 Data acquisition fMRI

GL was scanned in Montreal, Canada, and neurologically intact subjects in Gothenburg, Sweden, with 8 channel headcoils in 3T MR scanners (Montreal, Siemens TrioTim; Gothenburg, Philips Achieva). A T1-weighted protocol was used to acquire anatomical scans, and a blood oxygen level dependent (BOLD) sensitive protocol with a T2*-weighted gradient-echo, echo-planar imaging sequence was used for functional scans (Montreal: single-echo, TR 2.9 s, TE 30 ms, flip angle 90°, 2.9x2.9x2.9 mm resolution; Gothenburg: double-echo (Poser et al. 2006), TR 3.1 s, TE 19 + 35 ms, flip angle 90°, 2.9x2.9x2.9 mm resolution). Planes were oriented 30° from the anterior–posterior commissure line. These settings resulted in an adequate OFC BOLD signal but the most superior part of the brain including the primary somatosensory cortex (S1) was not covered. For image reconstruction, a short multi-echo scan was acquired with TE 19, 36, 53, 70 and 87 ms following the double-echo acquisition (Poser et al. 2006).

3.4.3 Preprocessing

Data were processed in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). Functional scans were motion corrected, unwarped to remove variance caused by the combination of movement and susceptibility, and spatially normalized to MNI (Montreal Neurological Institute) space (using the supplied EPI template, voxel size 2x2x2 mm, tri-linear interpolation and 6 mm FWHM Gaussian kernel spatial smoothing). The multi-echo scan was then used to estimate the local T2* in each brain voxel (Posse et al. 1999). A weighted summation of the preprocessed double-echo images was performed using the normalized, estimated T2*-map (Posse et al. 1999).

3.4.4 General linear model (GLM) analysis

Each condition was modeled by one predictor convolved with the standard SPM8 hemodynamic response function. Fixed-effects analyses were performed in individual participants, and random effects analysis on a group

level. Critical cluster sizes (*k*) corresponding to a family-wise error rate of 0.05 corrected for the whole brain volume were calculated using a Monte Carlo simulation procedure with 1000 iterations (Slotnick et al. 2003). Individual level and group-level contrasts were thresholded at t = 2.34 (P = 0.01; *k* = 46), and t = 3.65 (P = 0.001; *k* = 16), respectively.

3.4.5 Multivoxel pattern analysis (MVPA)

Given the ongoing nociceptive input from the heat/capsaicin model during scanning, we expected the primary cortical receiving area for C-afferents i.e. the posterior insular cortex to be continuously activated. Nonetheless, if CT afferents are integral in tactile allodynia we would expect differences in this insular activation pattern in response to stimuli in the allodynic and control zones. To examine these fine-grained differences we applied multivoxel pattern analysis in a histologically pre-defined region-of-interest: the right (contralateral) posterior insular cortex (Kurth et al. 2010). This area is known to be activated by CT stimulation in humans (Olausson et al. 2002, Bjornsdotter et al. 2009, Morrison et al. 2011).

Following standard preprocessing (cf. above), MVPA specific preprocessing was performed using the Princeton MVPA Toolbox (www.pni.princeton.edu/mvpa): each voxel's response was normalized relative to the average of the time course within each scan. To account for hemodynamic delay, the condition labels were shifted by 2 volumes, after which linear trends were removed. Single trial estimates were formed by extracting the BOLD response corresponding to each of the stimuli.

Multivoxel patterns differentiating the conditions were identified using locally multivariate brain mapping (Bjornsdotter et al. 2011). A linear support vector machine (SVM) classifier (in the LS-SVM implementation; with fixed regularization parameter C = 1) was used to model the conditions (Suykens et al. 2001), and a leave-one-run-out cross-validation scheme was employed to robustly estimate individual voxel-wise SVM classification accuracies. Permutation testing was used to assess the significance of the classification accuracies (Nichols et al. 2002): the identical mapping procedure was iterated 999 times with different data label permutations to generate a probability distribution under the null hypothesis that there were no differences between the conditions. P-values were computed as the proportion of permuted values that were at least as large as

the true classification accuracy, and corrected for multiple comparisons by setting the false discovery rate (FDR) to $q < 0.05$.

3.5 Paper III - Stimuli and experimental design

We set out to examine the integrity of discriminative and affective touch in human experimental tactile allodynia. Two zones were established, 12 cm apart, on the left forearm: one control area and one with heat capsaicin induced experimental tactile allodynia (see **3.4.1**). Following model application, half of the subjects participated in tactile direction discrimination testing (TDD) (see **3.5.1**) and half in stroking evoked pleasantness and pain testing.

The stroking stimuli were delivered manually (soft goat's hair brush: 0.5cm wide, 3cm long) to the two zones (stroking distance 5cm, application force 0.3N). Two different stimulation velocities were used for preferential activation; 3cm s^{-1} for CT and 30cm s^{-1} for Aβ afferents (Loken et al. 2009, Gordon et al. 2011, Morrison et al. 2011, Bennett et al. 2013). To control for differences in stimulus duration, 10 consecutive strokes were applied at 30cm s^{-1} (10x30cm s-1). A single stroke stimulus of 30cm s^{-1} was also included. Ten stimuli of each type were delivered in a pseudo-randomized block design; subjects were allocated in a balanced design for the site of model application (i.e. proximal or distal forearm), zone where testing commenced (i.e. allodynic or control zone), and all stimulus sequences (although limited to a maximum of 4 consecutive identical stimuli). Subjects were prevented from seeing the tested extremity during tactile stimulation. Participants rated each stroking stimulus on one VAS with the endpoints *Unpleasant* and *Pleasant* (Essick et al. 1999) and another with the endpoints *No pain* to *Worst pain imaginable*. The areas of punctate hyperalgesia, tactile hypoesthesia and tactile allodynia were quantified after the main test protocols. All subjects completed the SF-MPQ (Melzack 1987).

3.5.1 Tactile direction discrimination (TDD)

A hand-held stimulator (half cylinder probe, contact surface of woven fabric, diameter 4mm x length 15mm, vertical load 16g, stimulus velocity 1cm s^{-1}) was used for the TDD testing (Loken et al. 2010). Participants were prevented from seeing the tested extremity during the TDD testing and were instructed to verbally report the direction (distal or proximal) after each probe

movement. The test started with a motion over an 18mm distance: three consecutive correct responses shortened the distance whereas one incorrect response increased it. The best (i.e. lowest) score obtainable was 18 points (Olausson et al. 1997, Loken et al. 2010). The paradigm consisted of 32 trials in each zone, pseudo-randomized order.

3.6 Paper IV - Stimuli and experimental design

Three different experimental designs (**Paper IV**: Fig. 1) were used to investigate the pain modulatory effect of a tactile stimulus preceding heat pain. Testing was performed either on the left thigh (experiment 1) or forearm (experiments 2 and 3). The pain stimulus consisted of an individually determined moderate heat pain (see **3.6.1**), and the tactile stimulus of either a 50 Hz vibratory stimulus or of brush stroking (soft goat's hair brush, 7 cm wide; proximal to distal stroking direction, manually delivered). Subjects were prevented from seeing the stimulated skin area through the use of a curtain. Participants performed continuous pain ratings of the heat pain on a VAS with the endpoints *No pain* and *Worst pain imaginable*. Three variables were extracted for each pain rating: area under the curve (AUC; sum of pain ratings), peak pain rating, and time to pain rating onset.

Experiment 1 (n=14) investigated heat pain with simultaneous tactile stimuli. Three conditions were compared: heat pain only, heat pain with simultaneous slow, soft brush stroking (optimal for eliciting a strong CT response) and heat pain with simultaneous skin vibration (inefficient CT but a highly efficient Aβ stimulus). Brushing was applied at a velocity of approximately 3 cm s^{-1}, proximal to distal direction, 10 cm distance. Vibration was applied at 50 Hz (4.0 cm x 1.2 cm x 0.7 cm of balsa wood connected to a piezo-element, Piezo Systems, Inc., Cambridge, Massachusetts). Each condition was repeated ten times in pseudo-randomized order, (**Paper IV**: Fig. 1A). The inter-trial-interval was 50s.

Experiment 2 (n=8) investigated temporal spacing of the heat pain and the CT targeted stimulus (slow brush stroking at 3 cm s^{-1} (Loken et al. 2009) over a distance of 12 cm, approximate indentation force 0.3 N). The duration of the brush stroking was either 8 or 20 s. The inter-stimulus-interval (i.e. from brush offset to pain onset), was 1, 5 or 10 seconds. Stimuli were delivered in a pseudo-randomized order with three repetitions of each of the six combinations of brush duration and ISI, (**Paper IV**: Fig. 1B). The inter-trial-interval was 40s.

Experiment 3 (n=22) compared the pain modulatory effect of CT targeted versus Aβ targeted touch stimuli. The inter-stimulus-interval was set to 1 second and the duration of the stroking was 12 seconds with a stroking distance of 18 cm. Different brush stroking velocities were used for preferential activation of CTs (slow, 3cm s^{-1}) and Aβs (fast, 30cm s^{-1}) (Loken et al. 2009, Gordon et al. 2011, Morrison et al. 2011, Bennett et al. 2013). Stimuli were presented in a pseudo-randomized order with seven repeats of each stimulus type, (**Paper IV**: Fig. 1C). The condition heat pain only was also included as a baseline. The inter-trial-interval was jittered (minimum 22 s, maximum 40 s and always with 40 s between subsequent heat pain stimuli). Subjects were also asked to complete questionnaires on mood state (State Trait Anxiety Inventory), psychiatric screening (Becks Depression Inventory, Toronto Alexithymia Scale) and a post task rating of touch pleasantness and intensity.

3.6.1 Experimental heat pain

Static heat pain stimuli were delivered using a Peltier thermode (3x3 cm, Medoc, TSA 2001, Thermosensory Analyzer, Rimat Yishai, Israel). The thermode was strapped onto the skin during the entire experimental session.

The thermode baseline temperature was set to 32-33°C and the rate of temperature change to 10°C s^{-1}. A moderately painful temperature (corresponding to a numeric rating of 4 on a scale with anchors 0 = *No pain*; 1 = *Pain threshold*; 10 = *Intense pain*) was tried out for each participant in a pre-testing session. The individually determined moderate heat pain stimulus was then used for the entire experimental session. The heat pain stimulus duration was 10 s in experiment 1 and 5 s in experiments 2 and 3. Participants were not informed that the same temperature was used for all stimuli in the experimental session, and they were instructed to focus on their experience of each individual heat pain stimulus and evaluate it uniquely.

4 SUMMARY OF RESULTS

4.1 Paper I. Human CT afferents are tuned to the temperature of a skin-stroking caress

We presented evidence that CTs discharge preferentially to slowly-moving stimuli at typical skin temperature.

4.1.1 Electrophysiological response properties

Eight CT units were tested with the mechano-thermal paradigm (see **3.3**). The CT units showed sensitivity to stroking velocity and temperature; their maximal mean firing frequency occurred at the stroking velocity of 3 cm s^{-1} and temperature of 32^{o}C (**Paper I**: Fig. 2A, B). There was a significant effect of temperature for all stroking velocities, apart from the fastest (30 cm s^{-1}). Stroking at the neutral temperature produced significantly higher CT mean firing frequencies than stroking at cool or warm ones (apart from at 3 cm s^{-1} where neutral was only significantly higher than cool; **Paper I**, Table 1).

Eight myelinated units (four hair, two SAI, one SAII and one field) were tested with the same mechano-thermal paradigm (see **3.3**). Given that CTs, as well as animal C-LTMRs, show a strong association with hairs (Nordin, 1990; Vallbo et al., 1993, 1999; Wessberg et al., 2003; Liu et al., 2007; Löken et al., 2009; Li et al., 2011; Lou et al., 2013; Vrontou et al., 2013) myelinated hair units provided the most interesting comparison. However, despite similar thermal conduction distances from the skin surface, the hair afferents showed no significant effect for temperature.

4.1.2 Psychophysics

Participants felt cool and warm sensations, whereas in the neutral temperature condition, they reported only minor temperature sensation. Significant main effects were found for the stroking velocity and temperature as well as for the interaction of velocity and temperature. There was a significant main effect of temperature from 0.3-10 cm s^{-1}, where stroking at the neutral temperature was always perceived as significantly more pleasant than at cool or warm temperatures (**Paper I**: Fig. 2D).

4.1.3 Correlations between afferent discharge and perceived pleasantness

Correlations were conducted between the CT and hair mean firing frequencies and the pleasantness ratings for corresponding temperatures. We found a significant correlation between the CT firing frequency and pleasantness ratings at the neutral temperature (**Paper I**: Fig. 2E). No significant correlations were found for the cool or warm CT firing frequency and pleasantness ratings comparisons, or between the hair unit firing frequency and pleasantness ratings.

4.2 Paper II. Altered CT processing in human dynamic tactile allodynia

The results suggested that experimental dynamic tactile allodynia is associated with reduced CT mediated hedonic touch processing but allodynic pain seemed to be signaled by Aβ afferents.

4.2.1 The heat capsaicin model of tactile allodynia

Using the SF-MPQ neurologically intact subjects described gentle stroking in the allodynic zone as *hot-burning*, *tender*, and *stabbing* (**Paper II**, Fig. 1A). Stroking in the control zone was perceived as neutral or pleasant by all subjects. VAS ratings confirmed that stroking in the allodynic zone was significantly less pleasant than stroking in the control zone. All participants, including the two unique patients GL and IW, developed a visible flare. Punctate hyperalgesia was mapped with a monofilament (calibrated indentation force 0.20 or 0.24 N), and was 9.7 cm^2 (median, range 1.1-32.0, n = 15) in neurologically intact subjects, and 31.0 cm^2 in IW (not mapped in GL due to time constraints).

4.2.2 Psychophysics

Healthy subjects reported tactile evoked pain following application of the heat capsaicin model of tactile allodynia whereas GL and IW did not. According to the patients, none of the descriptors from the SF-MPQ were applicable. Instead, patients reported their C-touch percept (faint sensation of pleasant touch) to be significantly weaker in the allodynic zone compared to untreated skin (**Paper II**, Fig. 1B, C).

4.2.3 Functional magnetic resonance imaging

In healthy subjects and in one of the Aβ denervated patients, fMRI indicated that stroking in the allodynic and control zones evoked different responses in the primary cortical receiving area for thin fiber signaling; the posterior insular cortex (**Paper II**, Fig. 4). In addition, when comparing stroking in the allodynic and the control zones we found reduced activation in the mPFC, a key area for CT hedonic processing, (**Paper II**, Fig. 2, 3).

4.3 Paper III. Discriminative and affective touch in human experimental tactile allodynia

We demonstrated that both discriminative and affective touch processing was affected in experimental allodynia. Tactile allodynia seemed to be signaled by Aβ afferents and CTs seemed to contribute with a reduced CT hedonic touch processing and possibly also through the loss of their normally pain inhibiting role.

4.3.1 The heat capsaicin model of tactile allodynia

The most common SF-MPQ descriptors selected for stroking in the allodynic zone were *hot-burning* (n=30), *tender* (n=22), and *stabbing* (n=10) (**Paper III**: Fig. 1). None of the descriptors were applicable in the control zone. All participants developed a visible flare.

4.3.2 Discriminative touch

The TDD accuracy was significantly lower in the allodynic zone compared to a control zone (**Paper III**: Fig. 2).

4.3.3 Affective touch

A significant decrease in pleasantness ratings was found when comparing stroking in the two zones for stroking at CT-optimal velocity and for single stroking at CT suboptimal velocity (**Paper III**: Fig. 3A; Table 1). However, no significant difference was found between the two zones for the duration controlled, repetitious stimuli at CT suboptimal (Aβ-targeted) velocity (**Paper III**: Fig. 3A; Table 1).

Tactile stimuli were rated as minimally painful for all touch conditions in the allodynic zone (**Paper III**: Fig. 3B; Table 1) but there was no significant difference in touch evoked pain between stimulus types.

4.4 Paper IV. Pleasant touch modulates heat pain perception in humans

The results suggested that CT afferents have a significant role in pain modulation in humans. The pain reduction was significant both when the CT targeted stimulation is simultaneous to as well as prior to the heat pain.

4.4.1 Experiment 1: Simultaneous heat pain and tactile stimuli

Simultaneous CT targeted slow brush stroking was significantly more effective in reducing pain ratings compared to simultaneous vibration (Aβ targeted) (**Paper IV**: Fig. 2A, B). This effect was seen for both AUC measurements and peak pain ratings. When normalized to the pain only condition, simultaneous slow skin stroking showed a 15% average decrease in peak pain ratings compared to the 3% average decrease seen for simultaneous vibration.

The AUC and peak pain ratings for heat pain only and heat pain with simultaneous vibration were not statistically separable. There were no significant effects for any of the conditions in time to pain rating onset (**Paper IV**: Fig. 2C).

4.4.2 Experiment 2: Temporal spacing of skin stroking and heat pain

The perceived pain reduction was more effective for a shorter time interval between the CT-targeted touch offset and the pain onset. All three output measurements, i.e. AUC, peak pain and time to pain rating onset were significantly lower for the 1 s compared to the 5 and 10 s ISIs (**Paper IV**: Fig. 3). For the AUC and the time to pain rating onset there was no significant difference related to the duration of the stimulus whereas the peak pain ratings where significantly lower for the longer duration touch stimuli (20 compared to 8 s duration) (**Paper IV**: Fig. 3B). There was no main effect of the interaction ISI and duration.

4.4.3 Experiment 3: Slow versus fast skin stroking preceding heat pain

CT targeted slow touch was significantly more effective in reducing pain perception of a subsequent nociceptive heat stimulus compared to Aβ targeted fast touch (**Paper IV**: Fig. 4A, B). This was true both for the AUC and for peak pain comparisons. When normalized to the pain only condition, slow stroking showed a 10% average decrease in peak pain ratings which was significantly larger compared to the 1% average decrease for fast stroking.

The AUC and peak pain ratings for pain preceded by fast brush stroking were not statistically separable from those of pain only. There was no significant effect for either type of brush stroking on the time to pain onset (**Paper IV**: Fig. 4C).

There was a significant negative correlation between CT related pain reduction and anxiety ratings as well as a positive correlation with calmness ratings (**Paper IV**: Fig. 5 A-C).

5 DISCUSSION

This thesis aims to further our understanding of the functional role of CT afferents by investigating their basic physiological properties in relation to thermal stimuli and entering investigations of their role in pain and pain modulation.

5.1 The tuning of CT afferents to human skin-to-skin touch

The work in this paper takes the role of CTs in human affective touch (Olausson et al. 2010) further by showing a tuning, not only to the velocity of a soft stroking human touch, but also to its thermal characteristics. More specifically we have shown that CTs respond vigorously to slow, stroking stimuli delivered at neutral, typical skin temperature (Arens 2006), and that the CT firing frequency correlated with hedonic ratings only at the neutral temperature. The CT firing frequency decreased when the moving tactile stimulus was set to warmer or cooler temperatures, compared to typical human skin temperature.

Our current finding relates to Harlow's studies on Rhesus monkeys demonstrating the significance of social touch and care for normal development through his experiments on social deprivation of young monkeys (Harlow 1958, Harlow et al. 1962). These theories have also been confirmed in the work of John Bowlby for the World Health Organization (WHO) in which he set out on a mission to support children who had been separated from their parents. Through this work he established theories on human attachment promoting the importance of touch as a responsive confirmation from the primary caregiver and its influence on a child's emotional development (Bowlby 1970, Bowlby 1973, Bowlby 1978). Also, almost needless to mention, is the importance of touch in our everyday interpersonal relationships.

However, at this point it feels necessary to acknowledge that one by no means can neglect the importance of other factors than CT firing rate in the contribution to human affective touch. Human touch is a construct of many factors including the input from mechano- and thermoreceptive afferents, current homeostatic state, and contextual factors (Craig 2002). For example, CTs have not been found in the glabrous skin of the hand, yet it is

commonly observed that glabrous skin touch is also perceived as pleasant. This has been studied through contrasting brain activation to slow brush stroking on the forearm (where CTs are present) to that of slow brush stroking in the palm (where CTs are not present). There is a significantly greater activation of the posterior insular cortex and mid-anterior OFC for brush stroking on the hairy skin of the forearm compared to the palm (McGlone et al. 2012). The opposite contrast (stroking on the arm minus stroking in the palm) shows a significant activation of somatosensory cortices. These differences are striking when adding that the psychophysical ratings show no differences in intensity or pleasantness. However, when presenting subjects with the touch-questionnaire *Touch Perception Task* (Guest et al. 2011) there is a significant difference for the two body sites; emotional descriptors are rated higher on the forearm and sensory discriminatory descriptors are rated higher in the palm (McGlone et al. 2012). These findings are consistent with the hypothesis that CT targeted touch from hairy skin is processed in limbic cortical areas and represent an innate non-learned process. In contrast, pleasant touch from glabrous skin, mediated by Aβ afferents, is processed in somatosensory cortex and likely represents an analytical process dependent on previous tactile experiences (McGlone et al. 2012), nonetheless equally pleasant.

Yet, there is a robust positive correlation between the firing rate of CT afferents and the perceived pleasantness of touch (Loken et al. 2009) suggesting a more specific functional role for CT afferents in human pleasant touch perception compared to myelinated afferents (Loken et al. 2009). The CT-pleasantness correlation was confirmed by our current study, again highlighting the difference between the physiological responses of CTs and myelinated hair afferents, this time with respect to their firing at different stroking velocities and temperatures. Both afferents types are primarily mechanoreceptive afferents yet CTs showed thermo-modulatory effects whereas hair afferents showed no modulation to variations of the stroking temperature. Anatomically, CTs and animal C-LTMRs are associated with hairs (Nordin 1990, Vallbo et al. 1993, Vallbo et al. 1999, Wessberg et al. 2003, Liu et al. 2007, Loken et al. 2009, Li et al. 2011, Lou et al. 2013, Vrontou et al. 2013), and are thus probably located at a similar depth in the skin as hair afferents (Liu et al. 2007, Li et al. 2011, Lou et al. 2013). Despite similar thermal conduction distances from the skin surface, the hair afferents showed no significant effect for temperature (Hunt et al. 1960).

5.1.1 Other thermoreceptive afferents

The effect of temperature on CTs and C-LTMRs has been previously studied but only by using separately-applied mechanical and thermal stimuli; a response is sometimes seen to rapid cooling but not to warming (Iggo 1960, Bessou et al. 1971, Hahn 1971, Iggo et al. 1977, Vallbo et al. 1999, Wiklund Fernström 2004, Seal et al. 2009). In contrast, microneurographical recordings in humans have shown other types of afferents that are sensitive to cooling; C-cool (Hämäläinen 1979, Konietzny 1984, Campero et al. 1996, Campero et al. 2001, Campero et al. 2009, Campero et al. 2010) and Aδ-cool (Campero et al. 2009). Temperature afferents are often spontaneously active and respond well to rapid thermal changes by increasing their firing rate and continue to fire down to 0℃. Some C-cold receptors also respond, paradoxically, to heating of the skin starting at 40℃ (Campero et al. 2001). Both fiber types (C and Aδ) are unresponsive to mechanical stimulation. Human unmyelinated warm fibers are spontaneously active at skin temperatures with frequency of discharge increasing with increasing temperatures up into the noxious range (Konietzny et al. 1977, Schmelz et al. 2010). They too are unresponsive to mechanical stimuli and their activity is inhibited by cooling. Additionally, C-polymodal nociceptors are activated by both cooling (<20^{o}C (Campero et al. 1996) and heating (>37^{o}C (Schmidt et al. 1997). Therefore, it seems likely that different types of human thermoreceptive afferents will be activated by the thermo-mechanical (18, 32, 42℃) stimuli in our study and therefore contribute to the percept, although, perhaps not at the fastest stimulus velocity due to the short contact time.

5.1.2 Temperature perception

We decided to choose and compare the neutral stroking temperature (comparable to skin-to-skin contact in social touch) with cool and warm stroking temperatures that were outside the boundary of typical skin temperature (21-37^{o}C; (Arens 2006)), but which were not considered painful. Stroking around typical skin temperature produced the highest CT firing frequencies. It would obviously have been advantageous to include more stroking temperatures; however, this would not have been feasible. The experiments were difficult as there were many steps where they can fail e.g. finding the nerve, gaining CTs in locations that we could test, and holding a stable recording for up to an hour. Adding more temperatures would have jeopardized the comparability across velocities and temperatures, as it was

unlikely that we could have maintained a stable CT recording for testing all the velocities over a broader set of temperatures. Further, our data showed that the temperature effect was subtle; CTs primarily provided mechanical sensory input, but we showed that they also exhibit thermal modulation.

Our data showed that when CT activity decreased (through non-optimal stroking velocity and/or temperature), the perceived pleasantness of the stimulus also decreased, providing support for the CT affective touch hypothesis. However, the correlation between CT firing and pleasantness was only found at the neutral temperature, demonstrating the effect of other factors than CT firing on pleasantness. It has previously been shown that the perceived pleasantness of a thermal stimulus is dependent on core and skin temperature; cool stimuli are preferred during warm core temperature and warm stimuli during cool core temperature. However, temperature discrimination itself relies solely on peripheral signals and is independent of the affective quality (Cabanac 1971, Marks et al. 1974).

5.2 CT afferents in experimental tactile allodynia

The first studies on induced experimental allodynia were probably performed by the German medical officer Goldscheider while he was working on the Western Front during World War One (Goldscheider 1916, Goldscheider 1917). He originally termed it “hyperalgesie” and it was later through the International Association for the Study of Pain (IASP) that the condition acquired its current term: tactile allodynia (1979). Tactile allodynia is a disabling symptom of neuropathic pain where normally innocuous tactile stimuli produce pain. People with tactile allodynia typically experience a burning, tender sensation during soft stroking of the affected skin (Rasmussen et al. 2004). Even a very light stimulus, such as a patient’s garment brushing against the skin, can evoke allodynia. The prevailing hypothesis is changed tactile signaling in the spinal cord (Woolf 1993, Campbell et al. 2006) following central sensitization where Aβ-LTMRs signal to nociceptive neurons in the dorsal horn and from there to cerebral pain processing areas (Campbell et al. 1988, Koltzenburg et al. 1992, Torebjork et al. 1992, Woolf 1993, Iadarola et al. 1998, Wasner et al. 1999, Maihofner et al. 2003). This view is based on human selective nerve block experiments demonstrating that tactile allodynia is abolished by compression or ischemic block of Aβ afferents (Gracely et al. 1992, Koltzenburg et al. 1992, Torebjork et al. 1992, Cervero et al. 1996, Landerholm et al. 2011).

However, these theories were established just as CTs were discovered in humans (Johansson et al. 1988, Nordin 1990) and CTs were thus not taken into account. Over the last few years the role of CTs in mechanical hypersensitivity has been explored but the various findings point in different directions; some studies point towards a prominent role in which CTs signal allodynia whereas others entertain evidence of a more subtle modulatory, yet significant role.

5.2.1 Evidence of CT afferents signaling experimental tactile allodynia

The first study to investigate the role of C-LTMRs used a VGLUT3 functional knockout mouse model (see **1.5**) (Seal et al. 2009). Direct recording in the DRG from VGLUT3 neurons confirmed that they were C-LTMRs. This study demonstrated that the functional loss of C-LTMRs impair mechanical pain sensation, and in particular the mechanical hypersensitivity to normally innocuous stimuli that accompanies inflammation, nerve injury and trauma (Seal et al. 2009).

Following the C-LTMR knock-out study, a human study demonstrated that muscle pain, induced by hypertonic saline muscle infusion, increases following slow stroking of the overlaying skin (Nagi et al. 2011). The effect survives compression block of myelinated cutaneous afferents leading the authors to conclude that allodynia is selectively mediated by CT afferents (Nagi et al. 2011).

However, there are also numerous studies showing the opposite, i.e. tactile allodynia being abolished by compression or ischemic block of Aβ afferents (Gracely et al. 1992, Koltzenburg et al. 1992, Torebjork et al. 1992, Cervero et al. 1996, Landerholm et al. 2011). Further, Aβ denervated participants do not develop experimental, tactile evoked pain (Treede et al. 1993). Therefore, given this discrepancy, we also set out to examine the contribution of CTs in human dynamic tactile allodynia using the heat/capsaicin experimental model (Petersen et al. 1999).

5.2.2 Evidence of altered CT processing in experimental tactile allodynia

We were able to induce tactile allodynia in neurologically intact subjects but not in the two subjects lacking Aβ afferents confirming a previous report of intracutaneous injection of capsaicin in one of these patients (Treede et al. 1993). Following application of the heat/capsaicin model, GL and IW developed pain and flare (Treede et al. 1993), to the same extent as

neurologically intact subjects. All participants were prevented from seeing the stimulated skin areas, but GL and IW were "blinded" to a greater extent since the CT system only allows for a very crude spatial localization (Olausson et al. 2008). Hence, in the two patients, any differences in perception from the two zones must be based on non-spatial cues.

The novelty of our observation lies in the finding that the Aβ denervated subjects reported a reduced C-touch sensation (faint sensation of pleasant touch familiar to both subjects) to stroking in the allodynic zone (**Paper II**). For the Aβ denervated as well as the neurologically intact subjects, fMRI confirmed differences in cortical processing between stroking in the allodynic and control zones.

Both GL and neurologically intact subjects showed reduced processing in mPFC as well as altered processing in the posterior insular cortex when comparing the two zones. Since mPFC has been implicated in a hedonic network of brain areas for CT mediated affective touch (Kringelbach et al. 2004, Gordon et al. 2011) and the posterior insular cortex is the primary receiving cortical area for CT signaling (Olausson et al. 2002, Bjornsdotter et al. 2009), it seems fair to suggest that the allodynic condition is associated with reduced hedonic C-touch processing following subcortical alteration of CT signaling. However, the sensation of allodynic pain seems to require Aβ signaling.

In parallel with our study, it was found that VGLUT3 neurons can be divided into two groups depending on transient or persistent VGLUT3 expression (Lou et al. 2013). The VGLUT3-transient neurons are myelinated whereas the VGLUT3-persistent neurons are unmyelinated mechanoreceptors. In mice with a conditional knock-out of VGLUT3-persistent neurons both acute and chronic mechanical pain is largely, but not completely, unaffected. This suggests, in line with the canonical view, that VGLUT3-transient neurons, i.e. myelinated cutaneous afferents, may control mechanical hypersensitivity (Lou et al. 2013). This finding thus argues against a role for C-LTMRs in signaling allodynia (Lou et al. 2013) and is more in line with the finding of altered CT-touch in the two sensory neuronopathy patients (**Paper II**).

5.2.3 Both discriminative and affective touch processing was affected in allodynia.

In **Paper II** we identified a disturbance in both Aβ and CT afferent processing; we therefore proceeded to investigate the integrity of these two systems in neurologically intact subjects (**Paper III**).

TDD testing evaluates Aβ function with high sensitivity and specificity (Loken et al. 2010). Our findings showed a consistent and significant decrease in TDD accuracy in the allodynic zone. It seems unlikely that distraction by the capsaicin could explain the difference in TDD scores since the ongoing pain from the treated skin area was the same after testing in the allodynic and the control zones. Following capsaicin injection, there is numbness and reduced tactile detection in an area surrounding the allodynic zone (Magerl et al. 2004). This is explained in terms of pain-induced inhibition of non-nociceptive somatosensory input, i.e. tactile peripheral input is re-routed resulting in cross-talk into nociceptive pathways (Magerl et al. 2004). Physiological alteration of somatosensory processing supporting this inhibition has been demonstrated at the level of the spinal cord (Dougherty et al. 1998), the thalamus (Bruggemann et al. 1998), and the contralateral primary somatosensory cortex (Apkarian et al. 1992). We did not find a significant correlation between the degree of perceived hypoesthesia and reduction in TDD accuracy (Magerl et al. 2004). However, another method for quantifying the area of hypoesthesia (e.g. tactile detection thresholds using monofilaments) may have been more sensitive (Kauppila et al. 1998). Two point discrimination (TPD) and other measures of tactile acuity are typically reduced in chronic pain conditions with (and without) allodynia (Hollins et al. 1998, Moriwaki et al. 1999, Maihofner et al. 2006, Moseley 2008, Lewis et al. 2012, Stanton et al. 2013). Chronic pain patients may have a re-organization of their somatosensory cortex and the extent of this re-organization seems related to their pain intensity as well as their reduced tactile acuity (Flor et al. 1995, Flor et al. 1997, Maihofner et al. 2004, Pleger et al. 2005). Further, as the pain diminishes the tactile acuity increases (Nathan 1960, Maihofner et al. 2004, Pleger et al. 2005).

We present further evidence suggesting affected CT processing in experimental allodynia since the greatest drop in pleasantness ratings was seen for CT targeted stroking (3cm s^{-1}). This may indicate an altered processing of CT information (Liljencrantz et al. 2013), but does not indicate that CT afferents drive allodynia (Seal et al. 2009, Nagi et al. 2011).

In the allodynic zone, the pleasantness ratings for the CT targeted stroking dropped to those of the Aβ targeted stroking suggesting that the CT processing was suppressed (Delfini et al. 2013). A similar finding of reduced pleasantness ratings has been observed in CT-denervated patients (Morrison et al. 2011). For stroking at 10x30cm s^{-1} there was no change in pleasantness ratings likely due to low CT firing in the control zone as well. The low firing is due to the repeated stimulation which may fatigue CTs, almost to the point of inexcitability (Nordin 1990). We found no differences in touch evoked pain when comparing CT and Aβ targeted stimulation. The pain ratings were minimal although the heat capsaicin model developed as expected and subjects chose pain descriptors from the SF-MPQ accordingly. One explanation for not finding any differences in pain ratings across the stimulus conditions could be that our rating scale was too crude in its endpoint (*Worst pain imaginable*) to detect fine-grained differences. An evident next step is to investigate differences in pain ratings between patients with diagnosed tactile allodynia and model induced touch evoked pain. Another explanation (again supporting the canonical view) is that tactile evoked pain is solely mediated by Aβ afferents gaining access to pain signaling pathways, and that the Aβ afferent input varied less across our testing conditions than did the CT input.

The details of the anatomical and functional reorganization of the dorsal horn during central sensitization are controversial (Campbell et al. 2006). Following capsaicin-induced C-fiber injury in rats, Aβ afferents may sprout (from their normal terminations in lamina III-VI) and connect to lamina II, a region that normally receives only C-fiber input (Mannion et al. 1996), but see (Bao et al. 2002) for an alternative view. Another proposed mechanism is injury-induced unmasking (disinhibition) of polysynaptic low-threshold input to lamina I nociceptive output neurons (Keller et al. 2007). Such unmasking may be rapid enough to account for the acute onset of allodynia in the heat/capsaicin model. Although C-LTMRs activate nociceptive (WDR) lamina I projection pathways of the dorsal horn in rats, a C-LTMR specific pathway has yet not been observed (Andrew 2010). It may however be that a lamina I WDR pathway to the posterior insular cortex terminates differently than a postulated pathway signaling the normal C-touch sensation. In this scenario, noxious stimulation may suppress CT signaling through the C-touch spinal pathway (resulting in the reduced perception of brush stimuli in the allodynic zone described by the Aβ denervated subjects), whereas signaling through the WDR pathway is

enhanced. Hence, suppressed signaling in the C-touch pathway and increased signaling in the WDR pathway may contribute to the allodynic condition. Thus, for CT afferents which are suggested to signal touch pleasantness through the spinothalamic tract there might be a gating resulting in a significant decrease in pleasantness perception (Craig 2002, Andrew 2010) to prioritize the nociceptive information from the periphery. However, since fMRI does not allow distinction between bottom-up or top-down effects we cannot exclude that the mechanisms for capsaicin-induced alteration of CT signaling seen in **Paper II** may be altogether located at a supraspinal level.

As in all studies involving patients, the alternative possibility of the patients having undergone compensatory plastic changes that prevent them from perceiving pain following CT – lamina I WDR – posterior insular activation must be considered. However, we consider this less likely since GL and IW only have slightly reduced or normal pain perception, suggesting that they have largely intact pain systems (Olausson et al. 2008).

5.2.4 Evidence of CT afferents having a pain modulatory role in tactile allodynia

Recently, it was suggested that the central terminations of A- and C-LTMRs are somatotopically organized in a unifying pattern in lamina IIiv – IV of the mouse dorsal horn with projections through the dorsal column to the somatosensory cortices (Li et al. 2011, Abraira et al. 2013). Although, yet not investigated it seems conceivable that this dorsal horn integration of A- and C-LTMR signaling is affected in the allodynic condition. Such an altered integration could be reflected in reduced Aβ-fiber mediated discriminative touch sensation (Magerl et al. 2004). A previous electrophysiological study in rats suggests that C-LTMR targeted input may inhibit C-nociceptive messages in the dorsal horn (Lu et al. 2003), (see **1.5** and **5.3**). This unmyelinated circuit represents a potential pathway for innocuous C-LTMR impulses to suppress nociceptive impulses (Lu et al. 2003). A disruption of this circuit due to central sensitization may cause a loss of the nociceptive balancing effect of C-LTMRs and thus the notion that normalizing/restoring CT function may be a treatment strategy for tactile allodynia (Craig 2002, Seal et al. 2009, Andrew 2010, Delfini et al. 2013, Lou et al. 2013) is introduced.

A new hypothesis for the role of CT afferents in human dynamic tactile allodynia may be formulated. Namely, that the contribution

of CT afferents is a decrease in hedonic signaling and a decrease in pain inhibitory mechanisms disrupting body homeostasis and thus enabling touch evoked pain. Upon activation, C-LTMRs might release TAFA4, and this protein acts to prevent mechanical hypersensitivity (Delfini et al. 2013). Following inflammation and nerve injury TAFA4-null mice show enhanced mechanical and chemical hypersensitivity which is reversed by application of recombinant TAFA4 protein (Delfini et al. 2013) i.e. the restoration of normal C-LTMR functional signaling. Thus it seems fair to speculate that restoring normal CT processing may be a novel therapeutic strategy against neuropathic pain.

5.3 CT afferents and pain modulation

The concept of touch inhibiting pain was proposed as the gate control theory almost 50 years ago (Melzack et al. 1965). The theory proposes that cells in the substantia gelatinosa (SG) act as a control system to modulate the excitatory incoming afferent patterns. The inhibitory effect exerted by the SG on the nociceptive fibers is increased by stimuli activating Aβ afferents. However, when this theory was proposed in 1965 human touch was thought to be signaled solely by Aβ afferents (Kumazawa et al. 1977). As mentioned earlier it took until 1990 for CTs to be found in humans and a pain modulatory role also for them might now be suggested.

In the manuscript (**Paper IV**) we addressed this question and showed that the stimulation of CT afferents may have analgesic effects. CT targeted touch was effective in reducing pain perception of a simultaneously or subsequently applied nociceptive heat stimulus. When CT targeted touch was applied simultaneously with heat pain we found a significant pain reduction, however, it might be argued that the CT targeted touch acted as a distracter from the heat pain as opposed to a specific role for CTs in pain reduction. However, there are three observations supporting a specific role for CTs. First, we did not observe any analgesic effect for the Aβ targeted stimuli which could also be considered a distraction. This is particularly noteworthy since Aβ targeted stimuli was considered more salient as reflected in the higher intensity ratings for this stimuli compared to intensity ratings of CT targeted touch. Secondly, we found a pain reduction not only when the CT targeted touch was applied simultaneously with the painful stimulation but also when the two stimuli were separated in time. Thirdly, the reduction in pain ratings was more pronounced for a longer compared to a

shorter lasting CT stimulation. Therefore, it seems likely that in addition to unspecific mechanisms, such as shift of attention, there was an analgesic contribution from a mechanism that was dependent on CT signaling. The CT related pain reduction was found to be negatively correlated with anxiety ratings and positively correlated with ratings of calmness which could suggest cortical mechanisms for the CT related analgesia.

In addition, there are animal studies which give resonance to our human findings. C-LTMR targeted input may inhibit C-nociceptive messages in the dorsal horn (Lu et al. 2003) (see **1.5)**. The spino-cortical projection pathways for human CT afferents remains unknown but for C-LTMRs in rats there is a similar projection as the nociceptive pathway, i.e. from superficial lamina (I-II) and onward along the spinothalamic tract (Andrew 2010) (see **1.2.1**). Functional brain imaging of CT stimuli suggests that the primary cortical receiving area for CTs is the posterior insular cortex which would be consistent with a spinothalamic projection for CTs in humans as well (Olausson et al. 2002, Bjornsdotter et al. 2009, Morrison et al. 2011). In contrast, human Aβ afferents project from deeper laminae (III-IV) and along the dorsal column to the primary and secondary somatosensory cortices. However, recent evidence suggests a high level of integration of Aβ and C-LTMRs already at the dorsal horn level in mice (Li et al. 2011, Abraira et al. 2013) thus potentially enabling modulatory mechanisms.

Other recent evidence suggests that C-LTMRs, when activated, may release a chemokine-like secreted protein called TAFA4 that has analgesic effects (Delfini et al. 2013) (see **1.5)**. In addition, pharmacogenetic activation of Mas-related G protein-coupled receptor B4 (MRGPRB4+) expressing neurons, thought to be C-LTMRs, promotes conditioned place preference in mice, indicating that such activation is positively reinforcing and/or anxiolytic (Vrontou et al. 2013) (see **1.5)**. This result ties into our current finding of correlations between CT related pain reduction and state ratings, broadening for mechanistic speculations beyond the spinal cord. Since CTs are optimized to signal caress like touch which provides a sense of support and reassurance, and that the CT firing correlates significantly with the perceived pleasantness of the stimulus (Loken et al. 2009), CT stimuli may decrease pain perception in the same way as positive pictures (Kenntner-Mabiala et al. 2005), beautiful music (Roy et al. 2008), pleasant odors (Villemure et al. 2003, Villemure et al. 2009), sweet tastes (Dum et al. 1984, Reboucas et al. 2005), and positive expectations (placebo)

(Ellingsen et al. 2013). By providing a pleasant opponent sensation pain processing can be modulated; a concept known as pleasure-related analgesia (Leknes et al. 2008). In humans, psychological/emotional pain modulation is likely mediated by endogenous opioids activating descending pain inhibitory pathways from the periaqueductal grey (PAG) and rostral ventral medulla (Fields 2000) but also through direct opioid effects on cortical areas such as the anterior cingulate cortex (Zubieta et al. 2001, Petrovic et al. 2002). Endogenous opioid activity is disrupted both during sad mood (Zubieta et al. 2003) and in chronic pain patients (Willoch et al. 2004). In humans there is co-morbidity between chronic pain and depression often involving anhedonia (Marbach et al. 1981). Replicating the current study in various patient groups could be way to explore potential disturbances in endogenous pain modulation.

5.4 Summary

The significant relationship between the hedonic evaluation of the stroking stimuli at neutral temperature and the CT responses supports the role of CT afferents in affective, pleasant touch, particularly relating to skin-to-skin contact between individuals. These findings emphasize an evolutionary significance of CTs in signaling affective touch (**Paper I**).

Experimental tactile allodynia was associated with reduced CT mediated hedonic processing. We based this conclusion on the reduced C-touch sensation perceived by the Aβ denervated patients (**Paper II**) as well as the finding of the greatest drop in pleasantness ratings in the area of experimental allodynia for CT targeted (3cm s^{-1}) compared to Aβ targeted stroking (**Paper III**). Additionally, we found a significant decrease in TDD accuracy in the allodynic zone, indicating that also Aβ processing was affected (**Paper III**). However, the lack of tactile evoked pain in the Aβ denervated subjects (**Paper II**) and the lack of differences in touch evoked pain between CT optimal and suboptimal stimuli (**Paper III**) is consistent with the canonical view that Aβ afferents are necessary for allodynic pain (Campbell et al. 1988, Gracely et al. 1992, Koltzenburg et al. 1992, Torebjork et al. 1992, Treede et al. 1993, Cervero et al. 1996, Wasner et al. 1999, Maihofner et al. 2003, Landerholm et al. 2011).

The known positive reinforcement and anxiolytic effects of touch between caregiver and children, between partners or friends, and in patient care, support our finding of a robust reduction in pain following CT

targeted touch (**Paper IV**). The precise mechanisms are as yet unknown but possible mechanisms include pain relief through inhibition of dorsal horn nociceptive projections although cortical mechanisms also seem likely to play a role.

6 CONCLUSIONS

Paper I

We conclude that CT afferents are uniquely tuned mechanoreceptive afferents responding optimally to tactile stimuli with the specific characteristics of a gentle caress delivered at typical skin temperature. This provides a peripheral mechanism for signaling pleasant skin-to-skin contact in humans, which promotes inter-personal touch and affiliative behavior.

Paper II

We conclude that experimental tactile allodynia is associated with reduced CT mediated hedonic processing, following subcortical alteration of CT signaling, but that intact Aβ signaling is required for allodynic pain to develop.

Paper III

We conclude that tactile direction discrimination is less accurate and that the perceived pleasantness of soft brush stroking is decreased following experimental tactile allodynia. Thus, both discriminative and affective aspects of touch are affected by this condition.

Paper IV

We conclude that experimental heat pain in humans can be effectively reduced by a preceding CT optimal touch stimulus perhaps through inhibition of dorsal horn nociceptive projections and/or cortical mechanisms through pleasure analgesia possibly mediated by endogenous opioids.

7 FUTURE PERSPECTIVES

Directions to take from **paper I** would be to investigate also static thermal stimuli in humans since this is the type of thermal stimuli explored in previous animal studies. Testing a wider range of stimulus temperatures including the nociceptive range would also be an obvious step. Regarding the pleasantness perception seemingly encoded by CTs already from the periphery one could use skin microdialysis to search for substances released during CT activation; one might speculate that a role for endogenous opioids and/or endocannabinoids might be found. Immunohistochemical characterization of the human CT receptor structure would be an important first step towards defining molecular mechanisms for C-touch and may have critical implications for future drug development.

The work in **papers II and III** relies heavily on an experimental model aiming to mimic the complex clinical condition of dynamic tactile allodynia. A direct comparison between the perceptual experience of the experimentally induced change in touch sensation and the clinical condition would provide insight into the accuracy of the model. Investigating patients with the clinical condition using our touch protocol with 5 different skin stroking velocities (0.3, 1, 3, 10, 30 cm^{-s}) and VAS pleasantness ratings could be important. A finding similar to that in HSAN-V patients (i.e. a generally reduced pleasantness perception and reduced difference to the various stimulus velocities) could provide further support for reduced CT processing during tactile allodynia.

For **paper IV** an evident continuation is fMRI of the same stimulus protocol to seek out the presence of a potentially cortical explanation for the finding of reduced pain perception following CT stimulation. Investigating this paradigm in chronic pain patients as well as in psychiatric patients would provide further insights as one might anticipate a loss of their CT pain modulatory function.

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